Light backscattering properties of marine phytoplankton: relationships to cell size, chemical composition and taxonomy

ROBERT D. VAILLANCOURT*, CHRISTOPHER W. BROWN1, ROBERT R. L. GUILLARD AND WILLIAM M. BALCH
BIGELOW LABORATORY FOR OCEAN SCIENCES, WEST BOOTHBAY HARBOR, ME 04575 AND 1 OFFICE OF RESEARCH AND APPLICATIONS, NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION, COLLEGE PARK, MD 20742-2465, USA

*PRESENT ADDRESS: LAMONT-DOHerty EARTH OBSERVATORY OF COLUMBIA UNIVERSITY, 61 ROUTE 9W, PALISADES, NY, 10964, USA

CORRESPONDING AUTHOR: vaillanc@ldeo.columbia.edu

Spectral backscattering coefficients were determined for 29 species of cultured marine plankton representing 12 classes using a fixed-angle backscattering meter. Using a multi-angle scattering meter, the volume scattering function was measured and a proportionality constant (χ) between χ = C14 and bb was determined as 0.82 (± 0.01 SE), less than the χ value of 1.08 reported for natural waters. Backscattering efficiencies (at 440 nm) of cultures varied between 0.0023 and 0.081 and showed little spectral variation. Plankton backscattering coefficients at 510 nm showed the lowest variability between species when normalized to particulate organic carbon (POC) [4 × 10−6 (± 57% SD) m2 mg POC−1 at 510 nm], more when normalized to chlorophyll a (Chl a) [8 × 10−4 (± 112% SD) m2 mg Chl a−1 at 510 nm], and the greatest when normalized to cell number concentration [9 × 10−13 (± 238% SD) m2 mg cell−1 at 510 nm]. There were large variations in the relationships between Chl a, POC and backscattering within and between species. The dinoflagellates were the most efficient backscatterers, owing to their high POCi and D. The diatoms were mid-range in Qbb because the presence of the vacuole decreased POCi. The cyanophytes, eustigmatophytes and heterotrophic bacteria were the least efficient scatterers owing to their small cell sizes. Comparison of experimental Qbb to those predicted by Mie’s scattering model that represents a phytoplankton cell as a homogeneous sphere probably overestimates the value of the real refractive index for these species. Scattering models that consider more complex cellular structure are likely to provide better closure with experimental results.

INTRODUCTION

Understanding the component of the radiation field that is scattered in the upward direction and re-emerges from the ocean is essential to interpreting satellite ocean color data. This quantity is expressed as reflectance R, and is proportional to the quotient b/b [a + b] where a and b (units m−1) are the coefficients of absorption and backscattering, respectively (Gordon et al., 1975; Morel and Prieur, 1977). Through this analytical expression, the color of the emergent radiation measured via airborne, satellite or in situ sensors can be related to the concentrations (C) of the optically-active constituents using the mass-specific optical coefficients. For example, backscattering b = C · b, where C is the mass-specific backscattering coefficient in units of m2 mg−1. In surface waters that do not have appreciable terrigenous input of materials, the optically active constituents are water, living (bacteria, phytoplankton, heterotrophic plankton) and non-living (i.e. detritus) organic particles, and their derived dissolved substances. Surface waters in shallow bays or overlying continental shelves can show significant scattering from resuspended silts, clays and other inorganic particles that are brought to the surface by the action of tidal and coastal currents and winds (Chang and Dickey, 2001). When appreciable air injection occurs, air bubbles may also contribute significantly to light scattering (Terrill et al., 2001).

To determine the mass concentration of chlorophyll (derived from phytoplankton cells), or carbon biomass (derived from all planktonic organisms or organic detritus),
using spectral reflectance data requires an accurate and comprehensive database of mass-specific coefficients for backscattering and absorption for a representative group of suspended particulate and dissolved substances. A large database of mass-specific absorption coefficients exists for micro-algae and marine heterotrophic bacteria (Kiefer et al., 1979; Bricaud et al., 1983; Morel and Bricaud, 1986; Sathyendranath et al., 1987; Bricaud et al., 1988; Morel and Ahn, 1990; Balch and Kilpatrick, 1992; Stramski and Mobley, 1997; Stramski et al., 2001). By comparison, the database of measured backscattering coefficients for aquatic micro-organisms is poor owing to the inherent difficulty in measuring scattering at sufficient angular and spectral range.

The measurement of optical backscattering requires that the intensity of scattered light across the entire reverse hemisphere (180°>θ>90°) be assessed, and is technically very demanding and difficult. Consequently, the measurement of ‘true’ backscattering has not yet been accomplished. Early attempts at measuring some component of the backscattering by plankton utilized techniques that measured essentially a ‘diffuse backscattering’ (Kiefer et al., 1979). Later, Bricaud et al. used a similar optical arrangement and applied geometric corrections to account for the shallow collection angle of the measurement apparatus, as well as corrections for the fluorescence emission by chlorophyll (Bricaud et al., 1983). Using ‘reasonable values’ of the phytoplankton real refractive index and Mie scattering models they extrapolated the radiant flux into the portion of the reverse hemisphere that was not measured, thus attaining the complete backscattering coefficient for several species of marine phytoplankton. This backscattering measurement technique was later refined by applying a more precise geometric correction. In this approach the imaginary, n’i, and real, n, components of the complex refractive index (m = n – ni) were determined from measured phytoplankton absorption and attenuation coefficients, cell-size distribution and cell-number concentrations of suspensions of cultured marine phytoplankton and heterotrophic bacteria (Bricaud and Morel, 1986). These data were used as input parameters in a forward Mie model for homogeneous spheres (Bohren and Huffman, 1983) to derive the particle’s volume scattering function (VSF) and backscattering (Morel and Ahn, 1990; Ahn et al., 1992). A modified version of this approach (Stramski et al., 1988) was also used to develop extensive libraries of VSFs and backscattering efficiencies for many types of marine particles including viruses, heterotrophic and photosynthetic bacteria, and eukaryotic phytoplankton (Stramski and Kiefer, 1991; Stramski and Mobley, 1997; Stramski et al., 2001). The validity of this approach requires that backscattering can be adequately modeled by simple representations of phytoplankton cells as spherical homogeneous spheres (Bohren and Huffman, 1983), an assumption that has repeatedly been called into question (Zielinski et al., 1986, 1987; Zaneveld and Kitchen, 1995; Mishchenko et al., 2000). Nevertheless, the backscattering data of Stramski et al. represent the most comprehensive database of backscattering coefficients for marine particles to date (Stramski et al., 2001).

Others have measured the VSF of cultured phytoplankton and other marine particles using multiple-angle scattering meters (hereafter MASMs) designed to collect photons scattered in the reverse hemisphere at small collection angles (Sugihara et al., 1982; Zielinski et al., 1986, 1987; Krolik et al., 1992; Witkowski et al., 1994, 1998; Balch et al., 1996, 1999, 2000; Voss et al., 1998). Since most MASMs are unable to measure the volume scattering at angles greater than about 150°, these methods (as well as the one presented here—see Method section) have relied on interpolations of the measured VSF to 180° to estimate the backscattering coefficient (Sugihara et al., 1982; Balch et al., 1996, 1999, 2000; Voss et al., 1998). Also, backscattering measurements using MASMs have traditionally lacked the spectral resolution of those made by spectrophotometer, measuring at most several wavelengths.

Here, we report spectral backscattering coefficients of 28 phytoplankton species and one strain of marine heterotrophic bacterium cultured in the laboratory. The backscattering coefficients were calculated from scattering measurements made at a single backward solid angle using the Hydroscat-6 scattering meter (Hobi Labs, CA) and then extrapolated to the entire backward hemisphere. Independent measurements were made of phytoplankton VSFs to calibrate the Hydroscat-6 and to evaluate the efficacy of using measurements at a single backscattering angle to calculate the total backscattering coefficient. In addition, measurements of cell-number density, particulate organic carbon (POC) and chlorophyll a (Chl a) were made on the same cultures to calculate mass-specific coefficients. Together, these experimentally-derived optical properties represent a unique data set that can be used to assess analytically the mass-specific coefficients for a diverse collection of marine plankton and to assess the veracity of some theoretical light-scattering models. Tentative conclusions can also be made regarding species-specific and class-specific differences in backscattering efficiencies to aid in detecting and identifying phytoplankton blooms from space and airborne ocean color sensors.

**METHOD**

**Culturing of micro-organisms**

Monospecific cultures representing 28 species from 11 marine algal classes (Table I) were purchased from the
Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP; W. Boothbay Harbor, ME, USA). All species were grown in batch culture using Boothbay Harbor sea water (salinity = 32 p.p.t., except for *C. polylepis*, salinity = 27 p.p.t.) that had been previously autoclaved, nutrient-enriched according to the L1 or L2 media recipes (Guillard, 1995), and then filter-sterilized twice. The second filtration was aseptically dispensed into the sterile culture flasks. Two liter glass Fernbach flasks were filled with 1.0 L of growth medium and inoculated with primary stocks of healthy cultures. Cultures were grown under banks of cool white fluorescent bulbs at a light:dark cycle of 12 h:12 h at a nominal photosynthetically active irradiance of $5 \times 10^{15}$ quanta cm$^{-2}$ s$^{-1}$. To promote the growth of high cell densities the irradiance was increased to $20 \times 10^{15}$ quanta cm$^{-2}$ s$^{-1}$ at about the mid-point of their growth curve, and the flasks were bubbled with sterile-filtered, CO$_2$-enriched air. Population growth was followed by daily measurements of *in vivo* Chl $a$ fluorescence (Brand and Guillard, 1981). Cells were harvested for measurements at late exponential phase, identified as the linear region of the log$_{10}$ fluorescence versus time curve. All measurements were completed during the light phase of the culture’s photoperiod as described below. Variations because of the diel photic cycle were kept to a minimum by harvesting and measuring at approximately the same time each day. All cultures were tested for the presence of heterotrophic bacteria and contaminated cultures were removed from the experiment. The heterotrophic bacterium was isolated

### Table I: List of phytoplankton taxa examined in the study and their physical, biochemical and backscattering properties

<table>
<thead>
<tr>
<th>Class</th>
<th>Name and CCMP #</th>
<th>ESD (μm)</th>
<th>Chl $a$ (pg cell$^{-1}$)</th>
<th>Carbon (pg cell$^{-1}$)</th>
<th>$Q_{440}$</th>
<th>$Q_{470}$</th>
<th>$Q_{510}$</th>
<th>$Q_{620}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Fragilariophyceae</td>
<td><em>Fragillaria pinnata</em> (395)</td>
<td>3.6</td>
<td>0.09</td>
<td>25.6</td>
<td>0.0129</td>
<td>0.0116</td>
<td>0.0086</td>
<td>0.0088</td>
</tr>
<tr>
<td>B Coscinodiscophyceae</td>
<td><em>Thalassiosira oceanica</em> (1005)</td>
<td>6</td>
<td>0.25</td>
<td>36.7</td>
<td>0.0050</td>
<td>0.0045</td>
<td>0.0035</td>
<td>0.0030</td>
</tr>
<tr>
<td>C Coscinodiscophyceae</td>
<td><em>Minutisculus polymorphus</em> (497)</td>
<td>2.9</td>
<td>0.09</td>
<td>10.2</td>
<td>0.0112</td>
<td>0.0108</td>
<td>0.0080</td>
<td>0.0067</td>
</tr>
<tr>
<td>D Coscinodiscophyceae</td>
<td><em>Thalassiosira pseudonana</em> (1015)</td>
<td>4.0</td>
<td>0.24</td>
<td>13.9</td>
<td>0.0038</td>
<td>0.0043</td>
<td>0.0033</td>
<td>0.0029</td>
</tr>
<tr>
<td>E Coscinodiscophyceae</td>
<td><em>Thalassiosira weissflogii</em> (1336)</td>
<td>13</td>
<td>3.50</td>
<td>212</td>
<td>0.0210</td>
<td>0.0188</td>
<td>0.0146</td>
<td>0.0096</td>
</tr>
<tr>
<td>F Coscinodiscophyceae</td>
<td><em>Chaetoceros calcitrans</em> (1315)</td>
<td>6.0</td>
<td>0.11</td>
<td>11.6</td>
<td>0.0029</td>
<td>0.0026</td>
<td>0.0018</td>
<td>0.0014</td>
</tr>
<tr>
<td>G Coscinodiscophyceae</td>
<td><em>Thalassiosira rotula</em> (1647)</td>
<td>18</td>
<td>3.74</td>
<td>407</td>
<td>0.0086</td>
<td>0.0077</td>
<td>0.0054</td>
<td>0.0045</td>
</tr>
<tr>
<td>H Cryptophyceae</td>
<td><em>Guillardia theta</em> (327)</td>
<td>5.4</td>
<td>0.35</td>
<td>22.6</td>
<td>0.0031</td>
<td>0.0033</td>
<td>0.0029</td>
<td>0.0032</td>
</tr>
<tr>
<td>I Cryptophyceae</td>
<td><em>Hemiselmis virescens</em> (443)</td>
<td>3</td>
<td>0.09</td>
<td>30.5</td>
<td>0.0363</td>
<td>0.0336</td>
<td>0.0258</td>
<td>0.0217</td>
</tr>
<tr>
<td>J Cryptophyceae</td>
<td><em>Rhodomonas lens</em> (739)</td>
<td>7.0</td>
<td>0.97</td>
<td>103</td>
<td>0.0113</td>
<td>0.0111</td>
<td>0.0095</td>
<td>0.0100</td>
</tr>
<tr>
<td>K Dinophyceae</td>
<td><em>Amphidinium carterae</em> (1314)</td>
<td>9</td>
<td>1.40</td>
<td>168</td>
<td>0.0133</td>
<td>0.0123</td>
<td>0.0101</td>
<td>0.0093</td>
</tr>
<tr>
<td>L Dinophyceae</td>
<td><em>Heterocapsa triquetra</em> (449)</td>
<td>13</td>
<td>5.10</td>
<td>793</td>
<td>0.0380</td>
<td>0.0335</td>
<td>0.0263</td>
<td>0.0262</td>
</tr>
<tr>
<td>M Dinophyceae</td>
<td><em>Alexandrium tamarense</em> (1771)</td>
<td>31</td>
<td>25.4</td>
<td>3040</td>
<td>0.0208</td>
<td>0.0188</td>
<td>0.0148</td>
<td>0.0150</td>
</tr>
<tr>
<td>N Dinophyceae</td>
<td><em>Katothrioum rotundatum</em> (1542)</td>
<td>7.1</td>
<td>1.72</td>
<td>159</td>
<td>0.0399</td>
<td>0.0369</td>
<td>0.0274</td>
<td>0.0245</td>
</tr>
<tr>
<td>O Dinophyceae</td>
<td><em>Gymnodinium simplex</em></td>
<td>7.6</td>
<td>0.75</td>
<td>255</td>
<td>0.0812</td>
<td>0.0772</td>
<td>0.0643</td>
<td>0.0681</td>
</tr>
<tr>
<td>P Raphidophyceae</td>
<td><em>Heterosigma akashiwo</em> (452)</td>
<td>11</td>
<td>5.70</td>
<td>348</td>
<td>0.0183</td>
<td>0.0179</td>
<td>0.0141</td>
<td>0.0125</td>
</tr>
<tr>
<td>Q Chlorophyceae</td>
<td><em>Dunaliella tertiolecta</em> (1320)</td>
<td>5.6</td>
<td>0.91</td>
<td>46.5</td>
<td>0.0076</td>
<td>0.0075</td>
<td>0.0074</td>
<td>0.0054</td>
</tr>
<tr>
<td>R Chlorophyceae</td>
<td><em>Nannochloris atomus</em> (508)</td>
<td>3.1</td>
<td>0.04</td>
<td>11.6</td>
<td>0.0109</td>
<td>0.0107</td>
<td>0.0095</td>
<td>0.0067</td>
</tr>
<tr>
<td>S Eustigmatophyceae</td>
<td><em>Nannochloropsis</em> sp. 1 (531)</td>
<td>2.9</td>
<td>0.01</td>
<td>6.62</td>
<td>0.0039</td>
<td>0.0039</td>
<td>0.0032</td>
<td>0.0030</td>
</tr>
<tr>
<td>T Prymnesiophyceae</td>
<td><em>Isochrysis galbana</em> (1323)</td>
<td>4</td>
<td>0.14</td>
<td>21.6</td>
<td>0.0041</td>
<td>0.0042</td>
<td>0.0036</td>
<td>0.0031</td>
</tr>
<tr>
<td>U Prymnesiophyceae</td>
<td><em>Chrysochromulina polypleis</em> (1757)</td>
<td>6</td>
<td>1.41</td>
<td>111</td>
<td>0.0257</td>
<td>0.0235</td>
<td>0.0188</td>
<td>0.0175</td>
</tr>
<tr>
<td>V Prymnesiophyceae</td>
<td><em>Pavlova sp.</em> (616)</td>
<td>3.2</td>
<td>0.26</td>
<td>28.5</td>
<td>0.0236</td>
<td>0.0206</td>
<td>0.0153</td>
<td>0.0136</td>
</tr>
<tr>
<td>W Prymnesiophyceae</td>
<td><em>Prasinococcus capsule</em> (1192)</td>
<td>2.2</td>
<td>0.01</td>
<td>5.93</td>
<td>0.0055</td>
<td>0.0054</td>
<td>0.0050</td>
<td>0.0043</td>
</tr>
<tr>
<td>X Prasinochyphyceae</td>
<td><em>Micromonas pusilla</em> (1545)</td>
<td>1.4</td>
<td>0.01</td>
<td>2.07</td>
<td>0.0072</td>
<td>0.0073</td>
<td>0.0065</td>
<td>0.0058</td>
</tr>
<tr>
<td>Y Prasinochyphyceae</td>
<td><em>Pyrocococcus</em> provasoli (1203)</td>
<td>2.0</td>
<td>0.03</td>
<td>3.39</td>
<td>0.0061</td>
<td>0.0057</td>
<td>0.0057</td>
<td>0.0037</td>
</tr>
<tr>
<td>Z Cyanophyceae</td>
<td><em>Synechococcus elongatus</em> (1629)</td>
<td>2.5</td>
<td>0.02</td>
<td>2.51</td>
<td>0.0032</td>
<td>0.0031</td>
<td>0.0024</td>
<td>0.0019</td>
</tr>
<tr>
<td>AA Pelagophyceae</td>
<td><em>Pelagococcus subviridis</em> (1429)</td>
<td>2.6</td>
<td>0.03</td>
<td>9.18</td>
<td>0.0244</td>
<td>0.0218</td>
<td>0.0161</td>
<td>0.0145</td>
</tr>
<tr>
<td>AB Pelagophyceae</td>
<td><em>Pelagomonas calceolata</em> (1756)</td>
<td>1.9</td>
<td>0.04</td>
<td>2.25</td>
<td>0.0033</td>
<td>0.0029</td>
<td>0.0030</td>
<td>0.0026</td>
</tr>
<tr>
<td>AC Unknown</td>
<td>Heterotrophic bacterium</td>
<td>0.5</td>
<td>0</td>
<td>0.149</td>
<td>0.0038</td>
<td>0.0041</td>
<td>0.0039</td>
<td>0.0035</td>
</tr>
</tbody>
</table>

Letter D denotes cultures used in VSF measurements (see Method).
as a contaminant of a _Pelagococcus subviridis_ culture (CCMP 1429) by streaking an aliquot onto nutrient-enriched agar plates. A single bacterial colony was aseptically transferred to filtered sea water enriched with Bacto-tryptone and Bacto-dextrose (1 g L\(^{-1}\) each) and two 2 L cultures were grown to high cell density by rotating flasks at 22°C.

**Backscattering measurements of cultures**

Measurements of spectral backscattering were made on all cultures using a Hydroscat-6 (HOBI Labs, henceforth abbreviated HS6), a six-wavelength submersible fixed-angle (141°) backscattering meter (Maffione and Dana, 1997). Only four of the six HS6 wavelengths are reported here (440, 470, 510, and 620 nm) owing to instrument malfunction at the 590 nm channel (R. Maffione, personal communication) and possible contamination by chlorophyll fluorescence at the 670 nm channel (M. J. Perry, personal communication). In addition, measurements of the VSF at a single wavelength (514 nm) at 15 angles (35–144°) were made on a subset of nine of the cultures, using a Wyatt Dawn multi-angle laser light-scattering photometer (Wyatt Technology Corp.).

HS6 measurements were performed in a 40 L black plastic trash can filled with ~20 L of sea water or Milli-Q water. The HS6 was suspended above the container so that the instrument face (with optical windows) was immersed approximately 1 cm below the air–water interface, which was 25 cm from the container’s bottom. Precautions were taken, such as shrouding the container and HS6, to prevent extraneous light from entering the container. The backscattering from container sides and bottom was determined by lowering the HS6 into the sample container filled with 0.2 µm-filtered MilliQ water such that the instrument’s faceplate was within 8 cm of the bottom. The instrument was then raised in steps and the backscattering measured until no further changes in instrument readings were seen. Backscattering reached minimum values and remained constant with the HS6 faceplate positioned ≥18 cm above the bottom of the container. All subsequent readings on phytoplankton cultures were made with the faceplate of the HS6 suspended 25 cm above the container bottom.

To measure the backscattering of phytoplankton cultures, the container was filled with 17.5 L sea water that had been filtered twice through 0.2 µm filters, the second filtration performed immediately prior to the measurement. Small bubbles that formed on the instrument and container sides were removed every few minutes by gently brushing them away with a soft rubber squeegee. After a steady clean-seawater baseline had been established, 25–100 mL samples of culture were added, and the mixture was stirred to distribute the cells evenly and obtain a stable backscattering reading. The entire 1.1 L culture was added incrementally in this fashion in nine to 15 separate additions. Mean backscattering cross-sections were determined from linear regression of the backscattering coefficient and cell concentration following appropriate corrections for path-length attenuation (discussed later). The water temperature in the bucket before and after cell additions was not significantly different from the growth temperature of the cultures.

A Dawn multi-angle laser light-scattering photometer measures β(θ) at 15 discrete angles, including θ = 140°, which is close to the centroid collection angle of 141° of the HS6 meter. Phytoplankton cultures were placed in 15 mL glass scintillation vials and placed in the center well of the Dawn photometer. A rotating, flea-sized stirring bar kept the phytoplankton homogenized in suspension during the period of measurement. Several serial dilutions of a concentrated phytoplankton culture were sequentially measured (with 0.2 µm-filtered sea water as baseline) and mean scattering cross-sections were determined from linear regressions of the scattering coefficient at each angle and cell concentration. From the VSF we calculated the backscattering coefficient (independently of the bₙ determined by the HS6) by fitting a curve to the θ = 45°, 90° and 135° data using the Beardsley–Zaneveld (BZ) function (Beardsley and Zaneveld, 1969), performing a trapezoidal integration of the θ = 90° to 135° values, and applying the BZ function to extrapolate to θ = 180° (Balch et al., 1999). The BZ curve fitting procedure has been shown by Gordon (Gordon, 1976) to estimate backscattering to within 1.5% of the ‘bₙ real’ provided by ‘complete’ VSFs from Petzold (Petzold, 1972). Additionally, we have found trapezoidal integration of the 15 angles measured by the Dawn photometer, plus the extrapolated bₙ from the BZ function for angles greater than the Dawn photometer is able to measure (＞144°), agree to within <5% of bₙ estimated using only the BZ function. Regular checks of the instrument with 0.02 µm-filtered distilled water showed the error in the volume scattering at 90° was 2.09 × 10⁻⁵ m⁻¹ sr⁻¹ (less than the theoretical value for pure water). Lastly, error introduced by imperfections in the glass walls of the sample vials was minimized by careful selection of a set of ‘optically-clean’ vials prior to culture experiments. A set of vials was chosen which produced minimal aberrations in optical signals using 0.02 µm filtered Milli-Q water. Typical precision for Milli-Q water using a ‘good’ vial and rotating it in the sample compartment between scattering readings was ±3% (coefficient of variation of the mean, n = 5).

Because it was impossible to eliminate all particles in our ‘clean’ sea water (nominal filter cut-off 0.2 µm), the baseline scattering values represent the combined effects of the
presence of small particles and pure sea water. The VSF of filtered sea water shows enhanced forward-angle scattering with respect to the more symmetrical shape of pure sea water VSF, but this was lower than that predicted for pure sea water in the backward directions (Figure 1a). The ~4-fold enhanced scattering in the forward angles ($\theta \sim 65^\circ$) can be attributed to the presence of particles that remained in the sea water even after repeated filtrations, and is seen even in the clearest ocean waters (Petzold, 1972; Mobley, 1994). At $\theta \sim > 65^\circ$, however, the scattering for filtered sea water is less than that predicted for pure sea water by $\leq 2$-fold, and cannot be explained by the presence of small particles, and is attributed to measurement error. Backscattering by clean sea water measured with the HS6 was enhanced at all wavelengths by approximately twice the theoretical values (Figure 1b) and was between two and four times less than the VSF measured in the clearest open ocean water by Petzold (Petzold, 1972). On average, the Dawn photometer VSF and HS6 $b_b(140)$ values for algal cultures were ~8- and 4.5-times higher than those of the

Fig. 1. (a) The volume scattering function at 514 nm for filtered (0.2 μm) sea water (symbols) measured with the Dawn VSF meter prior to each of the culture analyses. The symbols are the mean and the error bars are the standard deviation. The dotted line is the VSF for pure sea water, normalized to the experimental data at 90°, calculated from equation 3.28 in Mobley (Mobley, 1994). The dashed line is the VSF of open ocean sea water from the Tongue of the Ocean, Bahamas from Petzold (Petzold, 1972). (b) Backscattering spectra for filtered sea water measured with the HS6 meter. The symbols are the mean $b_{b,w}$ of 29 experimental seawater samples (± SD) after multiple 0.2 μm filtrations (with capsule filter) prior to addition of the algal cultures. The experimental data were best-fit to the power equation, $b_{b,w} = 7.2E3 \lambda^{-3.1}$, $R^2 = 0.90$, $n = 94$. The dotted line indicates $b_b$ for pure sea water calculated as one-half the published values for the total scattering coefficient and varied with the wavelength as $\lambda^{-4.32}$ (Mobley, 1994).
clean water, respectively, and showed high signal-to-noise ratios (Table II).

The spectral backscattering function can be expressed as

$$b_b(\lambda) = \frac{2}{C_25} \left( \frac{1}{C_25} \right) \sin \frac{1}{C_18} \left[ \text{units: m}^{-1} \right]$$ (1)

where the volume scattering function, $b_\nu(\theta, \lambda)$, is integrated across the reverse hemisphere. Because the HS6 is a fixed-angle scattering meter, only photons scattered within a solid angle with a nominal centroid angle of $\theta_c = 141^\circ$ (with respect to the direction of propagation of the incident photon) were collected by the photo-detector. The backscattering coefficient (uncorrected for path-length attenuation, denoted by an apostrophe) is linearly-related to the scattering coefficient at the nominal backscattering angle, $b_\nu(\theta_c)$ through the constant $\chi$ (Maffione and Dana, 1997):

$$b'_b = \frac{2\pi}{C_25} \chi \beta(\theta_c) \left[ \text{units: m}^{-1} \right]$$ (2)

and can be expanded to include the contributions from water and particles separately (Boss and Pegau, 2001):

$$b'_b = b'_w + b'_p = 2\pi \left[ \chi_w \beta_w(\theta_c) + \chi_p \beta_p(\theta_c) \right] \left[ \text{units: m}^{-1} \right]$$ (3)

At the nominal scattering angle of the HS6 instrument ($141^\circ$) there is a sizeable difference in the values of $\chi_w$ and $\chi_p$, depending on the relative contributions of pure sea water and particles (Boss and Pegau, 2001). Accordingly, the correct value of $\chi$ in our determinations probably varied proportionally with the increasing concentrations of phytoplankton culture that were added stepwise to the filtered sea water. We used a simplified approach here and subtracted $b_w(\theta_c)$ from $b_b(\theta_c)$ to obtain the volume scattering of the particulate fraction, $b_p(\theta_c)$. A mean value of $\chi_p$ was then determined empirically from the relationship between the $b_p(141^\circ)$ and $b'_b$, both determined with the Dawn VSF meter, on nine representative species of the 29 listed in Table I. A mean value of $\chi_w$ was determined similarly, using the filtered sea water blanks prior to measuring each of the phytoplankton cultures.

### VSF and determination of $\chi$ for algal cultures

The VSF of cultured phytoplankton, plotted as differential scattering cross-sections on a per cell basis, exhibited pronounced forward lobes, reached minima near 110–120° (Figure 2a). In general, the data approximated the BZ functions very well, but departed slightly from the measured $b_p(140^\circ)$ and $b_p(144^\circ)$ values, particularly for the largest species examined, *Alexandrium tamarense* (mean $D = 31$ μm). In the samples for which the fitted line departed from the measured VSF, the backscattering may have been slightly underestimated.

Volume scattering of eight phytoplankton species was measured on both the HS6 and the Dawn photometers to determine the internal consistency of the two instruments. The two instruments measure VSFs at similar wavelengths and angles ($\lambda_{\text{DAWN}} = 514$ nm, $\lambda_{\text{HS6}} = 510$ nm; $\theta_{\text{DAWN}} = 140.1^\circ$; $\theta_{\text{HS6}} = 141^\circ$). While there is some scatter for

<table>
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<th>Table II: Summary of bio-optical measurements</th>
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<td>Chlorophyll [μg L⁻¹]¹</td>
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</tr>
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</tr>
<tr>
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<tr>
<td>HS6, at max. chlorophyll</td>
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<tr>
<td>Dawn, at max. chlorophyll</td>
</tr>
</tbody>
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¹Chlorophyll concentrations for scattering measurements. N.D. = no data.
²Mean values at highest chlorophyll concentration, divided by the value of clean seawater.
³Mean value divided by the standard deviation of the mean.
Fig. 2. (a) Volume scattering functions for nine species of phytoplankton measured on the Dawn VSF meter. Since the VSF for filtered sea water has been subtracted from the VSF of the cell suspension, the data points (symbols) represent the particulate beta values. The VSF was divided by the cell concentration to yield the $\beta_p(\theta)$ on a per-cell-basis (units of $m^2$ cell$^{-1}$ sr$^{-1}$). Each VSF curve represents the mean of 10 replicate determinations performed at 200 Hz. The dashed lines are functions fit according to Beardsley and Zaneveld (Beardsley and Zaneveld, 1969) to each set of $\beta_p(\theta)$ data at $35, 90$ and $135^\circ$. (b) Log–log plot of volume scattering cross-sections measured on the HS6 and Dawn photometers for eight phytoplankton species. The dashed line denotes the 1:1 relationship and units are $m^2$ sr$^{-1}$ cell$^{-1}$. (c) Relationship between $b_p$, determined using the Beardsley–Zaneveld (BZ) function on the VSF data of (a) and the $\beta_p(141)$. Each value is the average of five serial dilutions. The slope estimate of the type II linear regression ($=0.92$) is equivalent to the $\chi_a$ parameter in Equations 2 and 3. Species designations apply to symbols in all three figures.
a few of the algal clones, the $b(\psi)$ for five of the eight phytoplankton clones fall within a factor of 2 along the 1:1 line, indicating reasonable agreement between the two methods across nearly four orders of scattering magnitude (Figure 2b). With due consideration of the limitations and assumptions of this comparison (see Discussion), we felt reasonably confident in the internal consistency of the volume-scattering measurements of the two instruments to use the Dawn $b'(\psi)$ to determine $w$-parameters for the HS6 that are appropriate for sea water and phytoplankton cultures. A mean value of $w_p = 0.82 \pm 0.01$ (± SE), with lower and upper 95% confidence limits of 0.79 and 0.85 (Figure 2c) was determined, and used to convert the HS6 $b_p(141\text{ nm})$ to $b_b$ (Equation 2). The seawater blank $\chi_w$ value averaged for these nine cultures was $1.06 \pm 0.02$ (± SE), with lower and upper 95% confidence limits of 1.01 and 1.10 (not shown). This mean $\chi_w$ value was used to convert the baseline seawater HS6 $b(141\text{ nm})$ values to total clean-water backscattering values, which were then subtracted from the total backscattering values to arrive at particle $b_b$. The VSF at the wavelength of the Dawn’s laser light (514 nm) were used to derive $b_b$ at all four HS6 wavelengths.

**Corrections for HS6 path-length attenuation**

The measured backscattering values from the HS6 must be corrected for the light attenuation along the paths of the detector and source beams. The uncorrected backscattering coefficients $b'_b$ were adjusted using independent spectrophotometric measurements of total (including water) absorption $a_b$, and scattering $b_b$ (wavelength excluded for brevity), such that $b_b = b'_b \Omega$ and where:

$$\Omega = k_0 + k_1 (0.4b_b + a_b) + k_2 (0.4b_b + a_b)^2$$

(units: dimensionless).

The HS6 constants $k_0$, $k_1$, and $k_2$ are wavelength-dependent and instrument-specific coefficients determined during factory calibration. Independent attenuation $\epsilon$ and absorption $a$ measurements at 1 nm resolution were made on the same cultures immediately prior to the HS6 measurements using a pair of identical Campspec scanning spectrophotometers. One spectrophotometer was equipped with an integrating sphere to measure absorption, while the other was outfitted with a field-stop at the entrance to the photo-detector in order to measure $\epsilon$, and $b$ was determined by difference.
Values for pure seawater absorption and scattering, taken from Pope and Fry (Pope and Fry, 1997), were added to particulate $a$ and $b$ to arrive at $a_i$ and $b_i$.

The light scattering measured with the HS6 was linear with cell density for the range of cell densities used for most culture measurements. For some samples, however, the uncorrected backscattering became non-linear at higher cell concentrations. These non-linearities were removed when the attenuation correction (Equation 4) was applied. The corrected and uncorrected $b_i$ values (shown for Isochrysis galbana, Figure 3) diverged as the concentration of algal biomass increased. The degree of divergence is indicated by the value of $\Omega$ (Equation 4), which is equivalent to the ratio $b_i/b_i'$. The chlorophyll concentrations for $I. \text{galbana}$ at which the true $b_i$ value diverged from the measured value by 10% (i.e. $\Omega = 1.1$) was 4.9 mg m$^{-3}$ (Figure 3). The chlorophyll concentrations at which $\Omega = 1.1$ varied widely between species, ranging between 1 and 33 mg m$^{-3}$ (mean ±SD, 10 ± 8 mg m$^{-3}$; $n = 32$), as a result of differences in chlorophyll specific attenuation coefficients. Converting these threshold concentrations to their corresponding attenuation values (using the relation $c660 = c660 \times [\text{Chl} \ a]$, where $c660$ is the chlorophyll-specific attenuation value at 660 nm in m$^2$ mg Chl $a^{-1}$, and [Chl $a$] is the threshold Chl $a$ concentration in mg m$^{-3}$), collapsed the variability in the mean threshold value to 0.7 ± 0.1 m$^{-1}$ (± SD). Above this threshold Chl $a$ (or attenuation) level, absorption and scattering within the sample volume would have resulted in an underestimate of the true backscattering >10% if the correction had not been applied. After the path-length correction was applied, the backscattering-Chl $a$ relationships were highly linear, with $R^2$ values of 0.99 or greater for all species (data not shown).

Phytoplankton chlorophyll, POC, size and abundance determinations

Chl $a$ was determined by high performance liquid chromatography (HPLC) analysis following the procedure described previously (van Heukelem and Thomas, 2000). Culture samples were filtered onto GF/F filters prior to each day’s optical measurements, and frozen in cryotubes in liquid nitrogen until analysis time. Phytoplankton samples for CHN analysis were filtered onto pre-combusted (400°C, 24 h) GF/F filters and stored at −20°C. POC concentrations were determined on an Integra CN continuous-flow isotope ratio mass spectrometer. Cell-size frequency distributions were determined by image analysis of cells preserved in glutaraldehyde (0.3% v/v) and stained with the cytoplasm stain Proflavin to a final concentration of 5 µg mL$^{-1}$. We filtered the cells onto 25 mm black polycarbonate filters with pore size of 0.8 µm, or with 0.2 µm pores for cells smaller than ~2 µm diameter. The cells were visualized under epifluorescence microscopy and images were digitized in RGB color (Sieracki and Viles, 1990). The digital images were analysed using the MIND2 method (Sieracki and Viles, 1998) and the equivalent spherical diameters were calculated from cell biovolumes (Sieracki et al., 1989). Approximately 200 cells were measured per culture. A careful comparison of cell sizes of preserved and live cells from the same cultures showed no systematic difference in size because of preservation in glutaraldehyde (linear regression analysis yielded $R^2 = 0.87$, slope = 0.94, for $n = 11$ when the line was forced through the origin). For five of the cultures, the size frequency distributions were determined on live cells using an imaging flow cytometer, the FlowCam® (Sieracki et al., 1998). The size frequency distributions were binned into five intervals of equivalent spherical diameter $D$ to $D + dD$, whose relative abundances were scaled to the total cell concentration. Phytoplankton cell abundances were determined by microscopic counts of Lugol-preserved aliquots of cultures. Depending on approximate cell abundance, the counting chamber used was either a hemacytometer (improved Neubauer) or a Sedgwick Rafter cell. We counted a minimum of 200 cells for each sample and obtained an average coefficient of variation for all cell counts of 18% (range 5–39%).

Treatment of optical data

The mean efficiency factors for backscattering by the population of phytoplankton cells, $Q_{bb}$ (in the mean value theorem sense), were calculated by numerical integration from their respective $b_i$ (m$^{-1}$) coefficients and the cell size distributions by:

$$Q_{bb} = b_i \left[ \frac{\pi}{4} \int_0^\infty F(D)D^2d(D) \right]^{-1}$$  \[units : \text{dimensionless}\]  \(5\)

where $F(D)d(D)$ was the number of cells per cubic meter in the diameter interval $D$ to $D + dD$ per cell ([Morel and Ahn, 1991] equation 1]. The Chl $a$-specific and POC-specific backscattering coefficients, $b_i^{ma}$, were calculated from $Q_{bb}$ and the cell size frequency distributions following:

$$b_i^{ma} = \left( \frac{3}{2} N \right)Q_{bb} \int_0^\infty F(D)D^2d(D) \int_0^\infty F(D)D^3d(D) \left[ \int_0^\infty F(D)D^3d(D) \right]^{-1}^{-1}$$  \[units: m^2 mg^{-1}\]  \(6\)
where the superscript \( m \) denotes Chl \( a \) or POC, and \( X \) denotes the mean internal cellular concentrations for the population (in the mean value theorem sense), in units of \( \text{mg m}^{-3} \) of cell volume (Ahn et al., 1992).

**RESULTS**

**Phytoplankton spectral backscattering coefficients**

The backscattering efficiencies, \( Q_{bb} \), at four wavelengths for six of the 29 cultures, along with their respective cell-size distributions, are shown in Figure 4. The complete \( Q_{bb} \) dataset is presented in Table I. \( Q_{bb} \) was always higher in the blue than the red and had maxima at 440 nm with a few exceptions. Maximum \( Q_{bb} \) was found at 470 nm instead of at 440 nm for several species (Guillardia theta, Thalassiosira pseudonana, Isochrysis galbana, Micromonas pusilla, and the heterotrophic bacterium). The \( Q_{bb} \) minima were always at the longer wavelengths, 510 and 620 nm. The range of \( Q_{bb} \) values at 440 nm was 0.0029 for the diatom Chaetoceros calcitrans to 0.0812 for the dinoflagellate Gymnodinium simplex. \( Q_{bb} \) spectra were fitted with the power model \( Q_{bb} = A \lambda^{-Y} \), where \( A \) is a scaling constant and \( Y \) is the hyperbolic slope value (Figure 4). Slope values of these curves for all 29 species ranged from \(-0.0046 \) to \(-2.32 \). For those curve fits that had an \( R^2 \) value of 0.9 or greater, the mean ± SD \( Y \) value was \(-1.4 ± 0.5 \). No relationship was found between the \( Y \) parameter and any index of cell size or cell mass.

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**Fig. 4.** Backscattering efficiency \( (Q_{bb}) \) factors for six of the 29 cultures examined at four wavelengths, determined on the HS6. The fitted lines are the non-linear least squares regression using a power law function, \( Q_{bb} = A \lambda^{-Y} \). Inset: Cell-size distributions for the experimental cultures shown. The symbol representations, and the equation parameters for the fitted lines are as follows: ■, Alexandrium tamarense, \( A = 6, Y = -0.9, R^2 = 0.69 \); ○, Hemiaulus chui, \( A = 427, Y = -1.5, R^2 = 0.93 \); ◊, Fragilaria pinnata, \( A = 1270, Y = -1.9, R^2 = 0.95 \); ▼, Prasinococcus capulatus, \( A = 1, Y = 0.9, R^2 = 0.96 \); ▲, Synecococcus elongatus, \( A = 42, Y = -1.6, R^2 = 0.93 \); triangle with cross, heterotrophic bacterium, \( A = 0.04, Y = -0.4, R^2 = 0.64 \).
**Backscattering related to cell size and POC content**

The POC-specific backscattering coefficient, \( b_b \) (in units of \( m^2 \text{ mg}^{-1} \) POC), is linked to its efficiency factor \( Q_b \) through the relationship \( Q_b = \frac{2}{3} POC_i \times D \times b_b \), where \( POC_i \) is the internal POC concentration (in units of \( \text{mg m}^{-3} \) of cell volume), and \( D \) is cell diameter in meters (Morel and Bricaud, 1986). The efficiency of backscattering can be thought of as the proportion of the photon flux that is incident on the cross-sectional area of the cell that is scattered in the reverse hemisphere. This relationship is shown graphically for the experimental values of \( Q_b \), \( POC_i \) and \( D \) for all the species individually (Figure 5a), and separately for all species grouped into taxonomic classes (Figure 5b).

Implicit in this relationship is that the \( Q_b \) increases as cell equivalent spherical diameter \( (D) \) and carbon density.

---

**Fig. 5.** (A) Measured backscattering efficiency factors plotted against the product of the mean internal cell POC concentration and diameter, for each of the 29 cultures. The dashed line is the type II least squares regression line, for which the equation is shown. The slope value \( (5.0 \times 10^{-6} \text{ m}^2 \text{ mg}^{-1}) \) approximates the mean POC-specific backscattering coefficient for phytoplankton. Letters within colored symbols designate species names listed in column 1 of Table I. (B) \( Q_b \) for taxonomic classes. The symbol designations are: 1, diatoms; 2, dinoflagellates; 3, raphidophytes; 4, eustigmatophytes; 5, chlorophytes; 6, cryptophytes; 7, cyanobacteria; 8, pelagophytes; 9, prymnesiophytes; 10, prasinophytes; 11, heterotrophic bacterium. Error bars denote one standard deviation of the mean when number of species within class >1.
increase. Figure 5 shows variation in $Q_{mb}\times10^{-5}$ of as much as two-fold at a given POC $i \times D$ value, and this is attributable to measurement error and species-specific variations in POC-specific backscattering. For the species cultured here, the most efficient backscatterers were the dinoflagellates *Heterocapsa triquetra* (‘L’), *Kakodinium rotundatum* (‘N’), and the cryptophyte *Hemiselmis virescens* (‘T’). In contrast, the dinoflagellate *A. tamarense* (‘M’) had a similar POC $i \times D$ value to *K. rotundatum* (‘N’; 6.04 versus 5.97 $\times 10^{-5}$ mg m$^{-2}$, respectively), but their $Q_{mb}\times10^{-5}$ values differed by a factor of nearly two (Table I, Figure 5a). Factors other than carbon density and size were probably driving the difference in backscattering efficiency, as the POC-specific backscattering (at 510 nm) also differed by a factor of two (3.81 versus 7.83 $\times 10^{-5}$ m$^{-2}$ mg POC$^{-1}$, respectively for *A. tamarense* and *K. rotundatum*). Cell shape, internal structure and the presence of non-POC cell components (poly-phosphate bodies, calcite, silica cell walls, etc.) probably also contributed to the $Q_{mb}$ variations observed. Linear regression analysis showed that 32% of the variation in $Q_{mb}\times10^{-5}$ could be attributed to these non-POC related factors (Figure 5a). The three other backscattering wavelengths examined in this study (440, 470 and 620 nm) showed very similar relationships to POC and $D$ as are shown in Figure 5, as would be expected considering the relative constancy of the backscattering spectra (Figure 4).

When considered on the basis of taxonomic class (Figure 5b), the most efficient backscatterers (i.e. highest $Q_{mb}$) were the dinoflagellates (symbol #2, $n = 5$), cryptophytes (#6, $n = 3$), rhaphidophytes (#3, $n = 1$) and the Prymnesiophytes (#9, $n = 3$). The least efficient backscatterers were the cyanobacterium *Synechococcus elongatus* (#7, $n = 1$) and the heterotrophic bacterium (#11, $n = 1$). Allometric relationships for the phytoplankton reveal that the dinoflagellates as a group have higher internal carbon concentrations than other phytoplankton groups (Moal et al., 1987), however, in our study POCi concentrations for the dinoflagellates were in the middle of the range for all the taxa examined (mean POCi = 611 $\pm$ 335 fg $\mu$m$^{-3}$) but were offset by their relatively large cell thicknesses (mean $D = 7–31$ $\mu$m). The diatoms possessed a lower mean $Q_{mb}\times10^{-5}$ value than the dinoflagellates, because of their smaller thicknesses (mean $D = 3–18$ $\mu$m) and the fact that their cell volume is largely vacuole-containing cell sap, they possess relatively small POCi concentrations (mean POCi = 384 $\pm$ 315 mg m$^{-3}$). The order in Figure 5b must be viewed with caution, however, owing to the relatively small number of species examined from each class (sometimes only one) and the large amount of variation observed within a class. For example, within the diatoms we observed nearly a 10-fold variation in $Q_{mb}\times10^{-5}$ between *Chaetoceros calcitrans* (0.0026) and *Thalassiosira weissflogii* (0.0188) (Table I) owing to the relatively high POCi concentrations and larger diameter for *T. weissflogii* (4 times greater POCi and $\sim$2 times greater cell diameter). The cyanophyte *S. elongatus* and the heterotrophic bacteria were the least efficient backscatterers. Although they possessed moderate to high POCi concentrations (POCi = 382 and 1710 fg m$^{-3}$, respectively), this was offset by their relatively small cell thicknesses (mean $D = 0.5$ and 2.2 $\mu$m, respectively).

Another way of viewing the relationship between cell size and backscattering properties is to ask what is the backscattering observed when, for a constant number of cells, the mean cell size (expressed either as POC cell$^{-1}$ or cell diameter) varies up or down? For this purpose the backscattering data are represented as optical cross-sections by normalizing $b_{s}$ (m$^{-1}$) to the cell number concentration, and are plotted against cell POC (Figure 6a) or cell diameter (Figure 6b). The trend shown in Figure 6 is that as cells become larger, either by increasing cell diameter or POC content (which are to some extent co-dependent) the mean ‘per-cell’ backscattering increases proportionally. In this study, the four species with the highest backscattering cross-sections were the dinoflagellates *A. tamarense* (‘M’), *H. triquetra* (‘L’), *K. rotundatum* (‘O’), and the diatom *T. weissflogii* (‘E’). The species with the lowest backscattering cross-sections were the smallest species, the heterotrophic bacterium (‘AC’), the Pelagophyte *Pelagomonas calceolata* (‘AB’), and the cyanobacterium *S. elongatus* (‘Z’). It must be kept in mind, though, that viewing the trends in Figures 6a and b in this way ignores the contributions of other structural components to backscattering such as cell shape, and non-POC components such as inorganic polyphosphate deposits, diatom silica cell walls, and extracellular organic matrices (such as in *Prasinothecaeus capsulatus*). Nevertheless, the high coefficients of determination in the POC and $D$ versus backscattering cross-section relationships ($r^2$ of 0.96 and 0.85, respectively) indicate that the variations in cell-specific backscattering could be attributed primarily to POC and secondarily to cell diameter. The slope and intercept values for the other three wavelengths (440, 470 and 620 nm) differed by only a few per cent from those shown for the 510 nm channel in Figure 6.

It is instructive also to normalize the backscattering coefficient to Chl $a$, and ask the question, for a given concentration of bulk chlorophyll, how would the backscattering coefficient of a hypothetical water body vary if the pigment it contained was packaged into cells of varying sizes, shapes, and possessing a variety of intracellular and extracellular structures? The optical and biochemical data are arranged in Figure 7 so as to emphasize this relation-
ship. Generally speaking, in the phytoplankton there is an inverse relationship between cell size and POC and cell size and the ratio POC:Chl \(a\), as is shown for our dataset (Figure 7a,b). The inevitable result of this relationship is that backscattering normalized to Chl \(a\) increases as cells decrease in size (and increase their POC:Chl \(a\)), but only in those species that also increase their POC relative to Chl \(a\). For our experimental cultures, the highest Chl \(a\)-specific backscattering was observed in the species *P. subviridis* (‘AA’), *H. virescens* (‘I’), *G. simplex* (‘O’), *Nannochloropsis* sp. (‘S’), and *P. capsulatus* (‘W’), all of which had POC:Chl \(a\) ratios exceeding 250 (Figure 7b). Considering what is known about the environmental dependence of the POC:Chl \(a\) ratio it is likely that this relationship will vary not only with phytoplankton species composition then, but also with light and nutrient conditions (discussed later).

We calculated the frequency distributions of POC-, Chl \(a\)- and cell-specific backscattering for our data set to determine which biomass normalization best constrained the variability in the backscattering coefficient (Table III). There was the greatest dispersion of backscattering coefficients when expressed on a per cell basis. Cell-specific optical coefficients (optical cross-sections) had coefficients of variation of 234–247%, reflecting the large differences in cell mean geometrical cross-sections across the range of species in our study (Table I). The variability in the

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**Fig. 6.** Backscattering cross-sections at 510 nm plotted against mean cell (a) POC, and (b) diameter for each of the 29 cultures. The dashed lines are the best-fits to the power function \(Y = Ax^b\) and coefficients of determination for each relationship.
backscattering values was reduced to between 107 and 116% when the coefficients were normalized to Chl a concentration, and reduced further to between 52 and 57% when normalized to POC concentration (Table III). The greater dependence of backscattering on cell POC compared to diameter is also seen in the higher statistical dependence found in the regression analysis of $b_b$ and POC as compared to $b_b$ and $D$ (Figure 6). There was no significant relationship found between backscattering cross-section and Chl a per cell (not shown), and this probably reflected the greater linkage of Chl a content to photoadaptation rather than its scaling to cell size.

### Table III: Mean backscattering coefficients [± coefficients of variation (CV)] determined for the microbial cultures and normalized to cell number, chlorophyll a, and particulate organic carbon (POC)

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<td>470</td>
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<tr>
<td>510</td>
<td>9 (234)</td>
</tr>
<tr>
<td>620</td>
<td>9 (247)</td>
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Fig. 7. Cell diameters plotted against (a) the internal POC concentration and (b) the ratio POC:Chl a, for all species in this study, individually. The symbol color represents the magnitude of the Chl a-specific backscattering coefficient at 510 nm (see bar legend). Letters within colored symbols designate species names listed in column 1 of Table I.
DISCUSSION

Use of a multi-angle scattering meter to measure backscattering

Our results, in part, demonstrate the use of a multi-angle scattering meter to measure the backscattering coefficient of microbial cultures in the laboratory. The HS6 measures the light intensity scattered at a fixed angle of 141° and estimates the scattered intensity in the entire reverse hemisphere based on the proviso of a co-varying relationship. The validity of our results relies, in part, on the accuracy of this relationship. Oishi (Oishi, 1990), using values of VSF for natural waters compiled from the literature, and Mie-modeled VSF of sea water containing particles following Junge-type size distributions, concluded that the most significant relationship was found between $b(120°)/b_p$ and $b_p$. Maffione and Dana (Maffione and Dana, 1997) re-examined the data of Oishi (Oishi, 1990) and settled on $b(141°)/b_p$ as having the strongest relation to $b_p$. Both their modeled and observed data demonstrated that, while the magnitudes of the VSF in the backward angles was highly variable compared to the forward angles, the relative variations of $b(\theta)$ within the domain $100° \leq \theta \leq 150°$ were minimal. The co-variation allowed, to a first approximation, the assumption of a constant $b(141°)/b_p$ ratio, termed $\chi$ (Maffione and Dana, 1997). This ratio was determined to be 1.08, and is typically used as the default transform function for the HS6. Boss and Pegau (Boss and Pegau, 2001) examined the contribution of molecular scattering of sea water and particle assemblages separately and determined that $\chi = \chi_p \approx \chi_m$ between approximately $\theta = 115$ and $123°$, in agreement with Oishi (Oishi, 1990). They suggested that when a scattering angle outside these boundaries is used, the value of $b_m(\theta)$ should be removed from total $b(\theta)$ prior to calculating $b_p$. Because our HS6 measurements of the cell-specific scattering coefficient were determined as the slope of the regression of $b(141°)$ and cell concentration, the value for $b_m(141°)$ was effectively removed from $b_p(141°)$, and is equivalent to the $y$-intercept at zero cell concentration.

Fig. 8. Relationship between internal POC concentration and cell diameter for this study (closed circles) and literature values (all other symbols). Error bars for Mullins et al. (Mullins et al., 1966) data are one standard deviation of the means.
We independently determined $\chi$ because our cultures possessed particle size distributions that were relatively monodisperse and differed from the hyperbolic ‘Junge type’ distribution assumed in the Mie models of others (Oishi, 1990; Maffione and Dana, 1997; Boss and Pegau, 2001). Our $\chi_w$ value determined for 0.2 $\mu$m filtered sea water was 1.06 $\pm$ 0.01 ($\pm$ SE) and was larger than the mean $\chi_p$ value of 0.82 $\pm$ 0.02 ($\pm$ SE) determined for nine phytoplankton cultures (Figure 2b). This is in contrast to the results of Boss and Pegau (Boss and Pegau, 2001) who predicted a low value of $\chi_w$ that increased with increasing particle loads. The discrepancy can be resolved if one considers that our filtered seawater blank was not ‘pure sea water’ and probably contained colloidal-sized particles that changed the shape of the VSF and increased backscattering, as is predicted by Mie theory (Morel and Ahn, 1991; Stramski and Kiefer, 1991; Ulloa et al., 1994). Our $\chi_p$ value for laboratory cultures was lower than the $\chi$ values determined for natural particle assemblages by other groups (Maffione and Dana, 1997; Boss and Pegau, 2001), and suggests that the assumption of a constant relationship between $\beta(140^\circ)$ and $b_0$ might be violated under some conditions in nature, such as when monospecific blooms of algae dominate the water’s optical properties.

Our empirically-derived $\chi$-parameter was determined using a Dawn VSF meter the laser line of which was 514 nm, and applied to the $\beta(140^\circ)$ values determined at four different wavelengths (440, 470, 510 and 670 nm) by the HS6. Unfortunately, the VSF measurements on the Dawn meter were made several days following the backscattering measurements on the HS6, and it is probable that changes in cell optical characteristics occurred during this time. This is likely to have reduced the accuracy of the $\chi$ parameter to some unknown degree. Additionally, we do not know how $\chi$ varied as a function of wavelength across the particle size-wavelength domain we encountered for our cultures, and therefore cannot state unequivocally that our $\chi$ value, derived at a green wavelength, can be used to derive $b_0$ at the two blue and one red wavelength of the HS6 (the fourth HS6 wavelength of 510 nm is very close to the Dawn’s 514 nm laser line). According to the Mie calculations of Oishi (Oishi, 1990), however, for a realistic range of size parameter (0.2–200) and complex refractive indices, the $b_0/\beta(0)$ ratio remained relatively constant at 6.77–6.98. This range of size parameters is equivalent to particle diameters between 0.025 and 25 $\mu$m at 700 nm, and 0.045 and 45 $\mu$m at 400 nm, and overlaps the particle sizes and wavelength ranges used in our experiments. In addition, VSF measurements made in a variety of water types indicate that the $b_0/\beta(0)$ ratio is remarkably constant and similar to the values predicted by Mie calculations for wavelengths between 440 and 800 nm [see Table 3 in (Oishi, 1990)]. For these reasons we feel confident that, to a first approximation, our empirically-determined $\chi$-parameter was valid for all wavelengths.

The Dawn’s laser light is vertically linearly polarized and, strictly speaking, does not measure $\beta(0)$, which requires by definition the use of an unpolarized beam of light (Bohren and Huffman, 1983). The error introduced into the calculation of the $\chi$-parameter by the use of the Dawn photometer is unknown. However, we can constrain this error to a factor of $\leq 2$, with our comparison of the phytoplankton VSFs measured on the Dawn and Hydroscat photometers (Figure 2). Because the phytoplankton cultures used in this instrument comparison had aged several days between measurement on each instrument, optical changes associated with cell growth could be responsible for some of the deviation of the data from the 1:1 line. If we assume that the growth-related optical changes resulted in departures away from the 1:1 line only, and not towards it, then it is reasonable to assume that the points closest to the 1:1 line represent the true inter-instrument error, and possibly the error associated with the use of a polarized incident beam in the Dawn photometer. Additionally, the good agreement between the $\beta(0)$ measured on the Dawn and HS photometers proves that the two measurements are internally consistent, but not that either is the true $\beta(0)$ value. A more robust estimation of the $\chi$-parameter for converting scattering angles made at a single angle in the backward direction to the entire reverse hemisphere to estimate the backscattering coefficient will require accurate measurements of phytoplankton VSFs to be made using an unpolarized incident beam.

Comparisons to other measured and modeled values of backscattering

Generally speaking, the published backscattering values for phytoplankton and bacterial cultures have been determined using two different approaches. The most commonly used approach utilizes a combination of direct measurements of particle spectral attenuation, absorption, particle number concentration and particle size distribution, and may also include some ‘incomplete’ measure of spectral backscattering utilizing an integrating sphere attached to a spectrophotometer (Bricaud et al., 1983; Bricaud and Morel, 1986; Morel and Ahn, 1990; Stramski and Kiefer, 1991; Ahn et al., 1992; Stramski and Mobley, 1997; Subramaniam et al., 1999; Stramski et al., 2001). These approaches rely on the assumptions inherent in the homogeneous sphere Mie model to determine by inverse methods the real and imaginary indices of refraction. These values are then used, along
with the particle-size distribution and number concentration, to derive, again by Mie models, the particle VSF and backscattering coefficient, either completely (Stramski and Kiefer, 1991; Stramski and Mobley, 1997; Stramski et al., 2001), or for a portion of the backward hemisphere that is not measured by the integrating sphere technique (Bricaud et al., 1983; Bricaud and Morel, 1986; Morel and Ahn, 1990; Ahn et al., 1992; Subramaniam et al., 1999). The other class of methods involves the use of MASMs at one to several wavelengths and/or scattering angles (Sugihara et al., 1982; Witkowski et al., 1998; Voss et al., 1998; Balch et al., 1999, 2000). These methods rely on direct measurements of scattering at one or several discrete small angles in the reverse hemisphere and then extrapolation over the entire backward direction to determine $b_j$ with the use of curve-fitting procedures, or by empirically-determined relationships between $\beta(\theta)$ and $b_j$. Each approach has its inherent advantages but each also suffers from methodological limitations such as the lack of spectral and angular resolution in MASMs, and the theoretical limitations inherent in the over-simplifying representation of living cells as homogeneous spheres (Bohren and Huffman, 1983).

Backscattering spectra of phytoplankton measured with the integrating sphere and spectrophotometer are typically lower in magnitude and exhibit larger spectral variations than the backscattering spectra determined by MASMs or by the reverse modeling approach. Values of $Q_{bs,550}$ for marine phytoplankton measured on a spectrophotometer by Bricaud et al. (Bricaud et al., 1983) and later by Ahn et al. (Ahn et al., 1992), were all within the range 0.0002–0.005 (median value = 0.0016), and their backscattering spectra had distinct suppressions within absorption bands. The $Q_{bs,550}$ values for non-pigmented heterotrophic flagellates, ciliates and bacteria using this method were generally lower, between 0.0006 and 0.001 (Morel and Ahn, 1991). In contrast, the $Q_{bs}$ values reported in this study were generally higher (median $Q_{bs,510} = 0.0086$, low to high 0.0018–0.064 at 510 nm, Table I) than those measured by the spectrophotometric method for similar types of cultured phytoplankton (Bricaud et al., 1983; Morel and Ahn, 1991; Ahn et al., 1992) and closer to values reported by investigators using MASMs (Balch et al., 1996, 1999; Voss et al., 1998). Backscattering efficiencies determined by the reverse modeling approach were lowest. Using previously published values of $\sigma_{bs,510}$ and mean $D$ (Stramski et al., 2001), determined for a similar suite of 16 cultured marine phytoplankton species, we calculated a median $Q_{bs,510}$ value $(4*\sigma_{bs,}/\pi D^2)$ of 0.0008, or 10 times lower than our median $Q_{bs,510}$ value, and only a factor of two less than those determined by the spectrophotometric method of Ahn et al. (Ahn et al., 1992). The values of $\sigma_{bs,510}$ from Stramski et al. (Stramski et al., 2001) showed a similar relationship to cell size as that demonstrated in Figure 6b, in particular a strong ($R^2 = 0.88$) relationship to $D^{0.9}$, but with a lower $y$-intercept of $6 \times 10^{-16} \text{m}^2 \text{cell}^{-1}$. This suggests that while both our and Stramski’s data indicate a dependence of backscattering on the square of the mean cell diameter (i.e. mean geometric cross-section), for a given cell diameter, our backscattering cross-section values are on average higher. While it is tempting to attribute this difference to inadequacy of the homogeneous sphere model, we cannot rule out differences in growth conditions of the phytoplankton cultures between the two studies resulting in differences in cell carbon density, as this parameter seems to be the strongest predictor of backscattering in phytoplankton (Figure 6a).

Our backscattering estimates, as those of other MASM results, lacked the spectral variations predicted by the theory of anomalous dispersion. The relatively small number of wavelengths measured with the HS6, and the wide spectral bandwidths (10 nm) probably do not provide the spectral resolution required to observe these $b_j$ variations. Anomalous dispersion theory predicts that variations in the real component of refractive index, and thus scattering, will occur within wavelength regions with sharp variations in absorption, such as on the edges of absorption peaks (van De Hulst, 1957; Bohren and Huffman, 1983). These theoretical predictions have been reportedly shown in phytoplankton backscattering spectra measured with the spectrophotometric method that appear as inverse absorption spectra (Kiefer et al., 1979; Bricaud et al., 1983; Morel and Ahn, 1990; Ahn et al., 1992). Backscattering spectra derived from reverse Mie modeling methods (Stramski and Mobley, 1997; Stramski et al., 2001) show similar depresions within the strong red and blue absorption peaks, but lack the pronounced inverse correlations in the less-strongly absorbing regions of the spectrum. In contrast, phytoplankton backscattering spectra measured with the HS6 and other MASMs, because they lack the spectral resolution, are spectrally invariant and increase monotonically from long to short wavelengths [Figure 4, and see Figure 6 in Voss et al. (1998), and Table 4 in Balch et al. (1999)]. The phytoplankton backscattering spectra of Voss et al. (Voss et al., 1998) were fitted to a power law function, with exponents varying between near zero and $-2.7$, a similar range reported for our data ($0.0046$ to $-2.32$). Several of our $Q_{bs}$ spectra show a peak at $Q_{bs,470}$ and a decrease at the adjacent $Q_{bs,440}$ ($T. pseudonana$, G. theta, I. galbana and M. pusilla, Table I), which may indicate an anomalous dispersion effect in a wavelength region that is strongly absorbing by chlorophyll.

Our VSF measurements on nine phytoplankton species using the Dawn meter (Figure 2a) show VSF shapes...
similar to those reported for a variety of cultured phytoplankton (Zielinski et al., 1986, 1987; Witkowski et al., 1994, 1998; Volten et al., 1998). Features that all phytoplankton VSFs have in common are a strong peak in the forward direction and minimum values in the angular range of ~110–130°. Volten et al. (Volten et al., 1998) observed scattering in the forward direction that was as much as two orders of magnitude greater, and scattering in the backward angles (ca. >130°) that was several-fold higher than that at the minimum angles. Both these features are also seen in the VSFs of our nine species (Figure 2a). Volten et al. (Volten et al., 1998) also observed a drop in scattering at forward angles <45° in two species of Microcystis, and attributed this anomaly to the presence of gas vacuoles in these species. However, we also observed a similar feature in our culture of Synechococcus elongatus, a cyanophyte (like Microcystis) that does not possess gas vacuoles (Figure 2a). Possibly, the anomalous feature in the forward angles is related to structural components other than gas vacuoles.

**Relationship of backscattering to POC**

It is consistent that backscattering was most constrained when normalized to POC concentrations because protein constitutes a significant and sometimes dominant component of algal cell POC (Aas, 1996). Of the four main classes of biologically important molecules (proteins, carbohydrates, lipids and nucleic acids) the real refractive index is known to vary proportionally and most significantly with the cellular concentration of protein (Barer and Joseph, 1954). The remainder of the variation in the POC- or Chl a-normalized backscattering was probably the result of variations in cell size and shape, and of the arrangement of external and internal cell components.

It is possible that our cultured phytoplankton were made unusually carbon-dense from the CO₂-enrichment procedure we used to obtain high cell densities (see Method), as has been shown before for cultured phytoplankton (Zondervan et al., 2002). Our reported carbon cell densities are between 97 and 1710 fg C μm⁻³ (median POCi = 640 fg C μm⁻³). The largest carbon density value is for the heterotrophic bacterium that was grown in carbon-rich broth; the highest POCi value for phytoplankton was 1367 fg C μm⁻³. These values are greater than what is considered typical (~400 fg C μm⁻³ or less) for phytoplankton cells that are composed primarily of carbohydrate and protein, and have a high water content (Aas, 1996; Morel and Ahn, 1990), but nevertheless are consistent with POCi values reported for some similarly-sized heterotrophic bacteria and flagellates (Sin et al., 1998), and phytoplankton (Taguchi, 1976; Falkowski and Owens, 1980; Moal et al., 1987) (Figure 8). There are also many reported cases of carbon densities that are lower than ours for similar-sized phytoplankton and heterotrophic bacteria (Stramski and Morel, 1990; Morel and Ahn, 1991; Verity et al., 1992; Stramski, 1999). We are confident that the high values of carbon density are not the result of cell shrinkage from exposure to glutaraldehyde, which has been shown to sometimes cause significant changes in cell volume (Verity et al., 1992; Menden-Deuer et al., 2001). Comparisons of cell sizes measured on live versus glutaraldehyde-preserved cells showed no systematic change in cell size with preservation for our cultures (data not shown). The range of POCi and D values culled from the literature is shown in Figure 8 along with our data to emphasize the source of the variation in backscattering found in nature, as is evident in the relationships between D, POCi and backscattering demonstrated in Figures 5 and 6.

The reduced variability we observed when the algal scattering coefficients were normalized to POC instead of particle number or Chl a (Table III) suggests precise estimates of in situ POC concentrations can be derived from in situ or remote optical measurements (Balch et al., 1999; Stramski et al., 1999). In situ backscattering includes contributions of non-chlorophyllous particles, such as heterotrophic micro-organisms (bacteria, heterotrophic nanoflagellates, ciliates) and non-living organic detritus derived from the breakdown of micro-organisms. Mie models suggest that these optically ‘soft’ submicrometer particles may also contribute significantly to the total backscattering in the sea (Morel and Ahn, 1991; Stramski and Kiefer, 1991; Ulloa et al., 1994). Future laboratory experiments should expand the database of particle optical properties to include these submicrometer particles, specifically the non-living detrital particles (Stramski et al., 2001). Because the single-particle optical cross-sections vary directly in proportion with the geometrical cross-section for micro-organisms ranging in size from heterotrophic bacteria to large phytoplankton (Figure 6), it is possible that this relationship can be extrapolated to smaller particle sizes in order to include the submicrometer organic particles. This is likely considering that the backscattering cross-section for the heterotrophic bacterium with mean D = 0.53 μm (symbol ‘AC’ in Figure 6) fell on the regression line with the larger phytoplankton. Answering this question will require that more accurate and routine measurements be made of the smallest particle size fractions as well as contemporaneous POC and optical measurements to better identify and constrain these relationships.

We have observed that backscattering normalized to Chl a will vary with the POC:Chl a ratio (Figure 7). Allometric relationships in the phytoplankton have revealed a tendency for smaller cells to be more highly
concentrated with respect to carbon [Figure 7a, and see also (Taguchi, 1976; Blasco et al., 1982; Thompson et al., 1991)]. Accordingly, we would expect that a phytoplankton population composed of smaller cells would backscatter more light than one composed of larger cells, for an equal amount of chlorophyll present, owing to this intrinsic inverse relationship between POC and \( D \). The exception to this would be if the small phytoplankters possessed a low POC:Chl ratio, such as during nutrient stress (Moal et al., 1987; Langdon, 1988). Since the cultures used in this study were harvested, and their optical properties measured, during exponential phase of batch culture, we are confident that the backscattering properties reported here represent those of these species grown under optimal nutrient conditions. Variations of 12–66% in phytoplankton cell POC and 13–70% in protein cell quotas have been related to nutrient stress in cultures (Moal et al., 1987), and variations in growth irradiance have been shown to result in changes of 23–72% in cell volume, and 26–72% in POC (Thompson et al., 1991). Both studies also showed variations in POC and \( D \) of similar magnitude between species within taxonomic groups. Additionally, changes in chemical composition related to the diel cycle of light and dark have been shown to result in changes of as much as 20% in the POC:Chl ratio, and 5% in cell diameter in a diatom culture (Stramski and Reynolds, 1993). It should be kept in mind, then, that variations in the cells’ physical properties related to environmental effects or the diel light–dark cycle would be likely to cause commensurate changes in backscattering efficiency, and change the taxonomic order of \( Q_{bb} \) shown for our data.

**Comparison to Mie backscattering models**

We compare our measured phytoplankton backscattering values with the Mie model for homogeneous spheres (Bohren and Huffman, 1983), which represents a particle (i.e. phytoplankton cells) as spherical and homogeneously constructed. We derived Mie solutions of \( Q_{bb} \) for a range of \( a \) that spans the range measured for our experimental cultures. By varying iteratively the real (\( n \)) and imaginary (\( n' \)) refractive indices (\( m = n - i n' \)) we arrive at solutions that bracket the measured \( Q_{bb} \). Our goal here is not to derive Mie solutions that exactly mimic our measured \( Q_{bb} \) spectra but rather to constrain broadly the upper and lower limits using a very simplistic representation of cell construction. Our results show that phytoplankton cells that possess the backscattering properties we measured are best represented by homogeneous spheres with \( n \) between 1.05 and 1.15 with \( >90\% \) of the determinations falling below the \( m = 1.1 - i 0.0 \) line (Figure 9). If we include absorption,

![Figure 9](image_url)
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represented as an $n'$ value of 0.005 when $n = 1.10$, we observe a reduction in $Q_{bb}$ at relative sizes above about 10. These values of $n$ are significantly higher than published values for phytoplankton. The few direct measurements of the refractive index of phytoplankton by immersion methods report $n$ values for phytoplankton between 1.06 and 1.08 (McCrone *et al.*, 1967; Hodgson and Newkirk, 1975). In addition, values of $n$ determined by inversion of the Mie solution to absorption, attenuation or VSF vary widely [reviewed in (Aas, 1996)] but are generally also lower than the values we derived from inversion of the Mie solution to backscattering. The preponderance of evidence then suggests that the homogeneous sphere model does not adequately reproduce the backscattering of plankton cells.

Similar conclusions regarding the utility of the homogeneous sphere model for backscattering and angular light scattering have been reached by others (Sugihara *et al.*, 1982; Quinby-Hunt *et al.*, 1989; Zaneveld and Kitchen, 1992; Zaneveld and Kitchen, 1995; Volten *et al.*, 1998; Stramski and Piskozub, 2003). Quinby-Hunt found that the model that best reproduced angular light scattering intensities and polarization states was one that represented the phytoplankton cell as a double concentric sphere [the ‘coated sphere model’ of Bohren and Huffman (Bohren and Huffman, 1983)] with different values of $m$, rather than as a homogeneous sphere (Quinby-Hunt *et al.*, 1989). Zaneveld and Kitchen showed that backscattering predicted by a multi-layered sphere was ~3 times higher than that predicted by the homogeneous sphere model for given values of bulk refractive index and particle size distribution (Zaneveld and Kitchen, 1995). In particular, the magnitude of the backscattering was most sensitive to the thickness of the outer layer (Kitchen and Zaneveld, 1992). The importance of the cell wall in light scattering was also recognized by Sugihara *et al.* (Sugihara *et al.*, 1982) who presented phase functions for *Chlorella* that were virtually identical before and after physical disruption of the cell membrane and removal of the cell interior leaving only the cell walls or ‘ghosts’. The authors concluded that the structure of the cell walls determined the volume-scattering characteristics of phytoplankton. In contrast, Witkowski *et al.* reported that internal cell structures were more important in determining the pattern of angular light scattering in algal cells (Witkowski *et al.*, 1998). Stramski and Piskozub used Monte Carlo radiative transfer simulations to derive backscattering ratios for two species of cultured phytoplankton that were several-fold higher than $b_0$ ratios based on Mie-scattering calculations for homogeneous spheres (Stramski and Piskozub, 2003). More detailed examinations of the structure of phytoplankton cell walls and organelles are warranted to help constrain the variations in their dimensions as well as in the relative solid contents and POCi and their impact on the refractive index (Stramski, 1999; Aas, 1996).

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