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Beam attenuation and chlorophyll concentration as alternative optical indices of phytoplankton biomass

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ABSTRACT

Chlorophyll has long functioned as the prominent field metric for phytoplankton biomass, but its variability can be strongly influenced by (even dominated by) physiological shifts in intracellular pigmentation in response to changing growth conditions (light, nutrients, temperature). The particulate beam attenuation coefficient (c_p) may offer an alternative optical measure of phytoplankton biomass that is readily assessed *in situ* and relatively insensitive to changes in intracellular pigment content. Unlike chlorophyll, however, c_p is not uniquely associated with phytoplankton and varies as well with changes in inorganic, detrital, and heterotrophic particles. In open ocean environments, particles in the size range of ~0.5 to 20 μm (i.e., within the phytoplankton size domain) dominate c_p. Multiple field studies have indicated that the ratio of c_p to chlorophyll (i.e., c_p^*) registers first-order changes in algal physiology, suggesting that c_p covaries with phytoplankton carbon biomass. Here we use approximately 10,000 measurements of c_p and fluorescence-based chlorophyll estimates (Chl_{Fl}) to evaluate the correspondence between these two phytoplankton biomass proxies. Our study focuses on a region of the eastern equatorial Pacific where mixed layer growth conditions are relatively homogeneous, thereby constraining phytoplankton chlorophyll:carbon ratios and allowing chlorophyll to function as a reliable measure of phytoplankton biomass. Over our 6600 km transect, c_p was exceptionally well correlated with Chl_{Fl} ($r^2 = 0.93$). Our results contribute additional support for c_p as a viable index of phytoplankton carbon biomass in the open ocean.

1. Introduction

Chlorophyll (Chl) concentration is a uniquely algal trait of the water column and accordingly has been a central metric of phytoplankton biomass in the field. One particularly attractive attribute of chlorophyll as a biomass[4] index is its accessibility through optical measurements of stimulated fluorescence, allowing essentially continuous assessments without the inherent complications (technique or interpretation) of filtration or microscopic approaches. Chlorophyll concentration, however, is far from being a sole function of phytoplankton biomass and varies as well with taxonomic composition and mean physiological state of the algal assemblage. Indeed, physiological adjustments can

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4. Unless stated otherwise, phytoplankton biomass refers throughout the text to total carbon biomass of the phytoplankton assemblage (see Appendix I for further discussion).

even dominate chlorophyll variability over vast expanses of the ocean (Behrenfeld *et al.,* 2005). Fluorescence-based assessments of chlorophyll biomass can additionally suffer from changes in fluorescence yields, which in high-light environments are often exceptionally variable over even a single diurnal period.

A subset of the aforementioned issues compromising chlorophyll as a biomass measure also bestow upon it particularly useful information. Specifically, if the component of chlorophyll concentration due to biomass is removed, the remaining variability contains the signature of changing growth conditions (i.e., an index of physiological variability) (Behrenfeld and Boss, 2003). With such an optical index of the rate component of photosynthesis, estimates of phytoplankton biomass can be converted with greater fidelity into estimates of net primary production (NPP). To retrieve this 'physiological component' from chlorophyll data, an alternative measure of phytoplankton carbon biomass is needed that is insensitive to intracellular changes in pigmentation. For field measurements, the most likely optical candidate to fulfill this requirement is the particulate beam attenuation coefficient (c_p). Related measures may also be available from space (Behrenfeld *et al.,* 2005).

Beam-attenuation at 660 nm is essentially a measure of light scattering by suspended particles, because attenuation by dissolved material over the pathlength of the measurement is negligible at this wavelength in oceanic environments. After subtracting the invariant contribution of pure seawater, the remaining variability is consequently referred to as the particulate beam attenuation coefficient (c_p). Particles in the 0.5 to 20 μm size class (Morel, 1973; Stramski and Kiefer, 1991; DuRand and Olson, 1996; Boss *et al.,* 2001) dominate c_p for open ocean type size distributions where particle volume per logarithmic size bin is approximately constant (Sheldon *et al.,* 1972; Stramski and Kiefer, 1991). This range encompasses the lower portion, and numerically dominant component, of the phytoplankton size domain, lending to a direct dependence of c_p on phytoplankton biomass. However, c_p also registers changes in inorganic, detrital, and heterotrophic particles, thus compromising its utility as a phytoplankton biomass index when these contributions are significant and variable (e.g., suspended sediments, coccolithophore blooms).

Interestingly, far more effort has been invested into relating c_p to total particulate organic carbon (POC) than to phytoplankton carbon. This is in part due to the practical difficulty of separating phytoplankton carbon from other non-algal forms. Filtration-based POC data often reveal significant, if not remarkable, correlations with c_p (Gardner *et al.,* 1993, 1995; Walsh *et al.,* 1995; Loisel and Morel, 1998; Bishop, 1999; Bishop *et al.,* 1999; Claustre *et al.,* 1999). Since the particle size domain dominating c_p more closely matches that of phytoplankton than POC, a somewhat improved relationship might be anticipated between c_p and phytoplankton carbon. Regional differences have been reported for the slope of the c_p -POC relationship (see summary in Fennel and Boss, 2003), implying that universal relationships between c_p and either POC or phytoplankton carbon may not exist. These differences have also given rise to controversies regarding their real - versus methodological-origins (e.g., Bishop, 1999; Gardner *et al.,* 2003).

Relatively few studies have directly compared phytoplankton carbon to POC in the upper ocean (e.g., Eppley *et al.,* 1992; DuRand and Olson, 1996; Gundersen *et al.,* 2001; Oubelkheir *et al.,* 2005). Results from such studies indicate that the ratio of phytoplankton carbon to POC only varies between about 25% and 40% across seasons, biomass levels, and ocean basins. This range includes the influence of technical differences in estimating phytoplankton carbon and is even more constrained within methodologically consistent data sets. DuRand and Olson (1996) found phytoplankton to contribute essentially a constant fraction of POC across seasons near Bermuda. Such results suggest that community responses are rapid enough over much of the open ocean to cause phytoplankton biomass changes to be well matched by changes in the other components comprising POC (bacteria, detritus, small grazers). This first-order coupling in part reflects similar potential growth rates of dominant ecosystem components and is less likely in systems where development of large grazer biomass lags that of phytoplankton growth.

Additional indirect evidence supporting c_p as a phytoplankton carbon index comes from the frequently observed consistency between c_p:Chl ratios (c_p*) and anticipated physiological behavior across significant gradients in light, nutrients, and temperature. For example, depth-dependent changes in c_p* typically follow expected changes in intracellular pigmentation resulting from photoacclimation (Kitchen and Zaneveld, 1990; Mitchell and Kiefer, 1988; Mitchell and Holm-Hansen, 1991; Fennel and Boss, 2003). Recently, Behrenfeld and Boss (2003) synthesized data from a range of oceanographic regions and demonstrated spatial and temporal correlations between mixed layer c_p* and independent ^{14}C-based indices of physiological variability. In a related study, Behrenfeld *et al.* (2005) found that remote sensing based ratios of light scatter to chlorophyll exhibited spatial and temporal variations with light, nutrients, and temperature that were highly consistent with dependencies observed in the laboratory.

Direct comparisons between c_p and phytoplankton biomass are nearly nonexistent. In one of the few studies conducted, DuRand and Olson (1996) used flow-cytometry data to demonstrate that "changes in c_p can be almost completely accounted for by the changes in attenuation by phytoplankton" (p. 902). More recently, Green *et al.* (2003) and Green and Sosik (2004) reported that eukaryotic phytoplankton in particular were the dominant contributors to particle scattering coefficients (b_p, which dominates c_p). Oubelkheir *et al.* (2005), on the other hand, found phytoplankton to contribute a smaller fraction to c_p.

Here we take a somewhat different approach to investigating c_p as a potential index of phytoplankton biomass: we evaluate c_p, chlorophyll, POC, and Coulter-based particle abundance in a region of the eastern equatorial Pacific Ocean where mixed layer growth conditions (i.e., nutrient stress, temperature, light) are relatively homogeneous over a broad area. Physiological shifts in intracellular pigmentation are constrained under such conditions, making chlorophyll a more robust index of phytoplankton biomass than in highly variable environments. We hypothesize that if c_p likewise tracks changes in phytoplankton abundance, then the correlation between c_p and chlorophyll for our study site should be improved relative to previous analyses encompassing data from a wide range of growth conditions. Our analysis is based on approximately 10,000 c_p-chlorophyll pairs collected

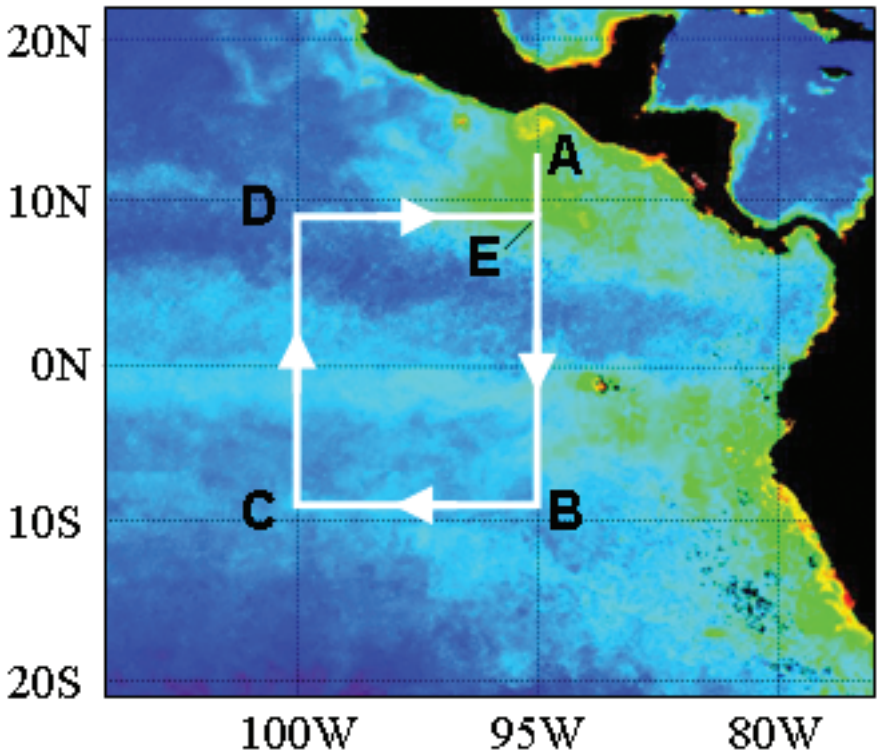

Figure 1. Geographic location of the 2003 *Ronald Brown* cruise in the eastern equatorial Pacific. Direction of travel during the 6600 km transect was from (A) 12N, 95W to (B) 8S, 95W to (C) 8S, 110W to (D) 8N, 110W and then back to (E) 8N, 95W. Background image is SeaWiFS ocean color data for November 2003.

along a 6600 km transect, where chlorophyll was assessed from stimulated fluorescence and filtration-based extraction measurements.

2. Methods

Measurements were conducted on the R.V. *Ronald Brown* during a Tropical Atmosphere Ocean (TAO) cruise between 28 October and 17 November, 2003. Sample collection commenced at 12N, 95W, proceeded along the 95W line to 8S, followed 8S to 110W, continued along the 110W line to 8N, and then returned to 8N, 95W (Fig. 1). Vertical profiles of temperature, salinity, and pressure to 1000 m were collected at each latitudinal degree on the 95W and 110W lines, along with Niskin bottle water samples from 0, 10, 25, 40, 60, 100, 150, and 200 m. Discrete 500 ml seawater samples were collected from the 8 Niskin bottles for determination of chlorophyll concentrations. Samples were gently filtered through Whatman GF/F® filters, which were then placed in glass scintillation vials with 10 ml of 90% acetone and stored in a freezer for 24 to 36 h.

Chlorophyll concentrations were determined from the acetone extracts using a calibrated Turner Designs® fluorometer. Downwelling photosynthetically active solar radiation (PAR) was measured at 15 s intervals with a Licor cosine-collecting light sensor (Model LI-1400). Ship position was recorded every 15 s with a Garmin global positioning satellite system receiver (Model GPSmap 76-Am).

Beam attenuation (c) at 532 nm and 660 nm was determined using flow-through seawater from the ship's clean seawater supply (intake at 3 m depth) plumbed through two 25 cm WetLabs C-star® beam transmissometers equipped with flow tubes. Both red and green wavelength measurements were made to permit comparison of classic beam attenuation at 660 nm and at 532 nm. We denote them hereafter as $c_p(660)$ and $c_{pg}(532)$, respectively, to highlight the fact that at 660 nm particles dominate the measurement of transmission relative to water while at 532 nm both particles and dissolved materials may contribute significantly. Transmissometer windows and flow tubes were cleaned each night with alcohol and distilled water and then, after a thorough rinsing and drying, the instruments were re-calibrated with Milli-Q® water. All the measurements were blanked with water calculated as: $c_{pg} = c_{measured} - c_w$, where c_w is the attenuation coefficient determined daily with Milli-Q. Seawater from >1000 m was also periodically collected from deep bottle casts and used for additional instrument calibration tests and to verify the stability of our Milli-Q water over the course of the study. Beam attenuation data were recorded every 15 s.

Coincident with each vertical profiling station, samples were collected from the flow-through seawater system for particulate organic carbon concentration (POC) and particle enumeration. For POC determinations, 600, 1000, and 2000 ml samples were collected and filtered through pre-combusted 24 mm Whatman GF/F® filters. Intercepts calculated through linear regression analysis of data from the three different volumes (average $r^2 > 0.99$) were used as one approach for estimating the 'blank' value. A second 'blank' estimate was made for each station by collecting the filtrate from the 1000 ml sample and then re-filtering it through a new pre-combusted GF/F filter. All four filters were individually sealed in aluminum foil sleeves and placed in liquid Nitrogen for the remainder of the cruise and transport back to the lab. Prior to laboratory analysis, filters were dried at 50°C and then packed into 7x5 mm Nickel sleeves which had been pre-combusted in a muffle furnace at 900°C for one hour. Carbon and nitrogen concentrations were then determined using an Exeter Analytical, Inc. Model 440 CHN/O/S Elemental Analyzer following manufacturer's protocols. Acetanilide of known weight was used as the standard for comparison with samples, with one Acetanilide standard and one blank analyzed after every dozen samples. For particle enumeration, 25 ml flow-through seawater samples were collected, dispensed into Beckman-Coulter Accuvette® containers, and then analyzed for particle concentration using a Beckman-Coulter Multisizer™ 3 particle counter fitted with a 70 μm aperture tube (which permits particle enumeration between 1.5 to 42 μm).

Determining proper blanks has been a concern since the first POC measurements (Gardner *et al.,* 2003). Considerable evidence indicates that significant adsorption of

dissolved organic carbon (DOC) onto filters occurs and must be accounted for. During the current study, we found filtrate-based 'blanks' to be poorly correlated ($r^2 = 0.07$) with regression-based 'blank' values. Application of the filtrate-based 'blanks' commonly yielded POC concentrations that varied clearly with the volume of seawater filtered, while regression-based 'blanks' gave consistent POC values for all three sample volumes. Regression-based 'blanks' also indicated a slight positive correlation ($r^2 = 0.26$) with total particulate material, while no such relationship was found for filtrate-based 'blanks'. Based on these findings, regression-based 'blanks' were chosen over filtrate-based 'blanks' for calculation of POC. On average these blank values were 26% of total carbon concentration measured for the 1000 ml sample (standard deviation = 10%).

Chlorophyll fluorescence was monitored with a bench-top Fast Repetition Rate fluorometer (FRRf) (Kolber and Falkowski, 1993; Falkowski and Kolber, 1995). Seawater from the ship's flow-through system was plumbed in darkness to a 1 cm^3 custom quartz flow cell and then exposed to a rapid sequence (1 ms spacing) of blue-light flashes. Fluorescence induction was recorded from an initial dark-adapted level (F_0) to the maximum level (F_m). Photochemical yield was calculated as: $F_v/F_m = (F_m - F_0)/F_m$ and is referred to herein simply as an index of physiological changes in the phytoplankton assemblages. Diurnal fluorescence values exhibited strong light-dependent depressions each day resulting from non-photochemical quenching (NPQ) processes, so comparisons between c_p and fluorescence-based chlorophyll estimates (Chl_{Fl}) were restricted to nighttime data. Excluding data from the non-iron limited northwest corner of our survey area (see below), comparison of nighttime F_m data with coincident discrete chlorophyll data (i.e., the Turner fluorometer data described above) revealed a good correlation ($r^2 = 0.75$), that improved to $r^2 = 0.89$ by removal of a single outlier at 4N, 95W. Based on this comparison, Chl_{Fl} ($\mu g\ L^{-1}$) was estimated from F_m as: $Chl_{Fl} = 0.01\ F_m$. Certainly, this conversion factor is instrument dependent and should not be applied outside the current study. A more precise fluorescence-to-chlorophyll conversion (which is beyond the scope of the current study) would require characterization of nutritional and taxonomic conditions, as well as consideration of any potentially significant hysteresis in NPQ induction and recovery.

Mixed layer depth (MLD) was computed as the depth where density increased by 0.125 kg m^{-3} relative to surface values. Resultant MLD values were similar to values achieved using a temperature criterion of 0.5°C following Kara *et al.* (2003). This criteria is the same as that employed for the National Oceanic and Atmospheric Administration (NOAA) world ocean data base (Monterey and Levitus, 1997).

For our analyses, type-I regressions were used to quantify relationships between various components of the particle assemblage (c_p, chlorophyll concentration, POC, particle number). While this approach is most consistent with previously published works (thus more compatible), it is noteworthy that in all these comparisons uncertainties exist in both the dependent and independent variables. Strictly speaking, therefore, type-II regressions are most appropriate, as the square differences between both variable values to the regression line are minimized in type-II analyses (Laws, 1997). Although regression

coefficients differ between type-I and type-II fits, both approaches yield identical coefficients of determination (r^2).

a. Additional data sets

During our study, a strong relationship between c_p and Chl_{Fl} was observed. To put this result in a more global context, we compare our results with data from a variety of other ocean areas. For this comparison, we used c_p and filtration-based chlorophyll data from the Hawaii Ocean Time-series (HOT) and Bermuda Atlantic Time Series (BATS) programs, the North Atlantic Bloom Experiment (NABE), the Equatorial Pacific (EqPac) study, and JGOFS Southern Ocean program. The HOT, BATS, NABE, and EqPac data were exactly the same as used in our previous study (Behrenfeld and Boss, 2003). The Southern Ocean data set was obtained from the web site: http://usjgofs.whoi.edu/jg/dir/jgofs/southern/ and included all c_p - chlorophyll pairs for the 11 studies included at this website (designations: nbp 1 - 4, 4a, 5, and 8 and rr-kiwi 6 - 9).

3. Results

One of the dominant environmental factors responsible for physiological variability in phytoplankton carbon to chlorophyll ratios in the field (thus, c_p:Chl_{Fl} data) is growth irradiance (Behrenfeld *et al.,* 2003). Throughout our study region, water column density profiles indicated a shallow surface mixed layer (Fig. 2A,B) and vertical chlorophyll profiles exhibited homogeneous surface layers that rarely exceeded 10 to 25 m, with significant vertical structure at greater depths (Fig. 2C). Daylengths were also constrained to between only 12.5 and 13.3 h and midday downwelling PAR was generally between 1500 and 2200 μmol photon m^{-2} s^{-1} (Fig. 3A). Daily mixed layer growth irradiances for the region were thus relatively high and invariant. Under such conditions, light-dependent changes in carbon to chlorophyll (C:Chl) ratios within the mixed layer are expected to be minimal.

A second dominant factor controlling phytoplankton C:Chl ratios is nutrient stress (Behrenfeld *et al.,* 2003). While manipulative experiments were not conducted to directly identify the type or extent of nutrient limitation, we did observe over a bulk of the study region the large nocturnal decreases in F_v/F_m (typically $> 40\%$) indicative of iron-limiting growth conditions (Behrenfeld and Kolber, 1999), with the exception of the northwest corner of the survey area (approximately 7N, 110W to 8N, 104W; marked by yellow triangles in Fig. 3A) where nocturnal decreases were only ~20%. Particulate carbon: nitrogen (C:N) ratios were also highly constrained, with a median value of 5.8 μg μg^{-1} and standard deviation of only 0.5 μg^{-1}. These attributes again point toward a reasonably homogeneous region with respect to physiology, such that variability in c_p and Chl_{Fl} might be expected to largely reflect changes in phytoplankton abundance.

Over the entire transect, $c_{pg}(532)$ ranged from 0.012 to 0.297 m^{-1} (factor of 25 variability), while $c_p(660)$ ranged from 0.014 to 0.245 m^{-1} (factor of 17 variability) (Fig. 3A). Beam attenuation values at 532 nm and 660 nm were very well correlated ($r^2 = 0.98$) in

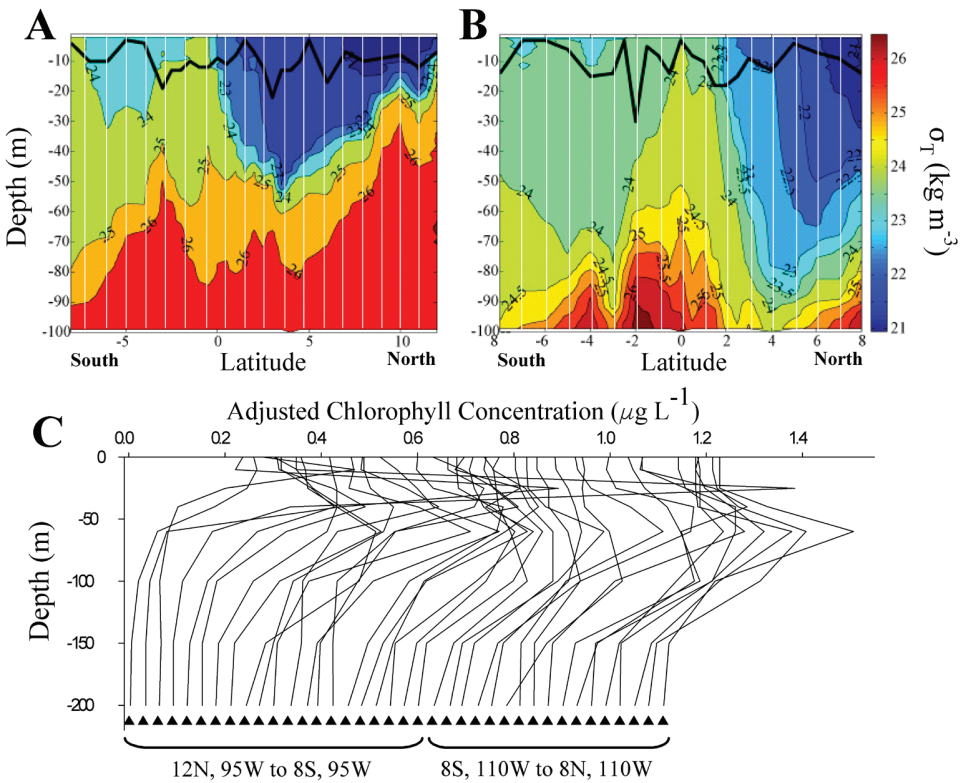

Figure 2. Vertical structure in density and chlorophyll concentration. Density contours (Φ_T : kg m^{-3}) between (A) 12N and 8S along the 95W longitudinal line and (B) 8S and 8N along the 110W longitudinal line (Fig. 1). Heavy black line in (A) and (B) indicates mixed layer depth (MLD), computed as the depth where density increased by 0.125 kg m^{-3} relative to the surface value. (C) Vertical profiles of chlorophyll concentration at each latitudinal degree along the 95W and 110W longitudinal lines shown in Figure 1 (labeled at bottom). Discrete samples were collected at 8 depths between the surface and 200 m (see Methods). A value of 0.3 μg Chl L^{-1} has been sequentially added to separate each profile. For reference, the upright triangle below each profile points to the true value of 0 μg Chl m^{-3} for each profile.

approximately a linear manner [$c_{pg}(532) = 1.1212\ c_p(660) + 0.0035$]. On average, $c_{pg}(532)$ was 6% higher than $c_p(660)$. The most significant deviation from this general relation occurred between 10N and 11N along the 95W line (marked in Fig. 3A with a red triangle), where $c_{pg}(532)$ averaged 19% higher than $c_p(660)$ [which contributed to the overall greater range in the $c_{pg}(532)$ versus $c_p(660)$ data]. While a definitive explanation for this deviation was not resolved, we did find that the ratio of the two attenuations did not correlate with size distribution parameters (e.g. median size of particles or PSD slope (Boss *et al.,* 2001)). Conceivably, an increase in colored dissolved organic matter (cDOM) could have contributed to the anomaly, as c_{pg} at 532 nm is more strongly influenced by cDOM

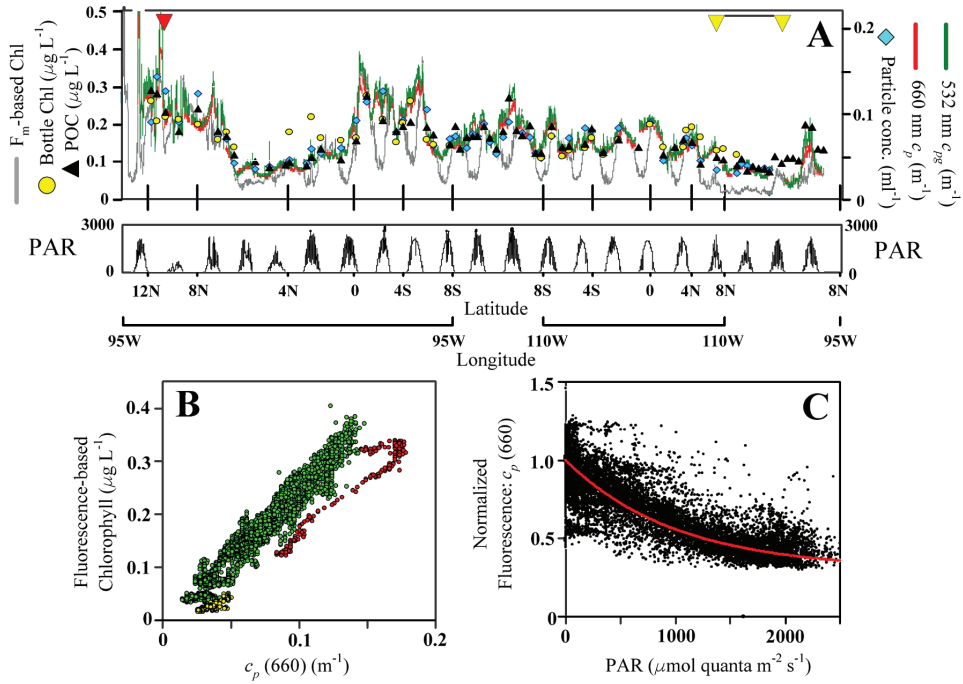

Figure 3. (A) Particulate beam attenuation coefficients (c_p) (m^{-1}: right axis) measured at 532 nm (green line) and 660 nm (red line), fluorescence-based (Chl_{Fl}) (gray line) and filtration-based (yellow dots) chlorophyll concentrations ($\mu g\ L^{-1}$: left axis), Coulter-based concentration of particles with diameters between ~1.5 and 40 μm (right axis) (blue diamonds), and total particulate organic carbon concentration (POC: black triangles: left axis: $\mu g\ L^{-1}$) along the entire transect shown in Figure 1. Particle number has been divided by 9.8×10^5 to scale with c_p and POC has been divided by 294 to scale with chlorophyll. Coincident values of photosynthetically active radiation (PAR: μmol quanta $m^{-2}\ s^{-1}$) are shown as a black trace in the lower inset. Latitudinal and longitudinal coordinates are provided along the x-axis of the PAR inset. Note that the data are not equally spaced because the ship periodically stopped to service buoys of the TAO array. For additional reference, note that the discrete chlorophyll, particle number, and POC samples were collected at each latitudinal degree. (B) Correlation between nocturnal c_p(660) and Chl_{Fl} ($r^2 = 0.93$, n = 9,647). Red dots correspond to data collected between 10N and 11N along the 95W line (identified in A by red arrow at top of figure). Yellow dots correspond to the northwest corner of the survey area where a shift in diel patterns of photochemical yields (F_v/F_m) and ratios of c_p:Chl_{Fl} suggest a shift in limiting nutrient (region bracketed in A by yellow arrows). (C) Changes in diurnal fluorescence yields resulting from physiological nonphotochemical quenching protective mechanism. Fluorescence:c_p values for each diurnal cycle were normalized to unity at sunrise and are plotted here against coincident PAR (μmol quanta $m^{-2}\ s^{-1}$). Red line is a fit to the data described by: $\Delta f.y. = 0.3 + 0.7 \exp^{-0.001\ PAR}$, where $\Delta f.y.$ = fractional change in fluorescence yield due to NPQ (range: 0.3 to 1.0) and PAR = μmol photon $m^{-2}\ s^{-1}$

absorption than at 660 nm due to the rapid exponential decrease in cDOM absorption with increasing wavelength (e.g, Jerlov, 1976).

Consistent with the region's constrained mixed layer growth conditions (Fig. 2), both c_{pg}(532) and c_p(660) were well correlated ($r^2 = 0.93$) with nighttime Chl_{Fl} (Fig. 3A,B). As

anticipated, data from the 10N to 11N sector (Fig. 3B − red circles) and the northwest corner of the survey area (Fig. 3B − yellow circles) deviated from this general c_p-Chl_{Fl} trend (from here on, c_p denotes $c_p(660)$). The later deviation is consistent with a physiological shift in phytoplankton C:Chl ratios associated with a transition between iron-limiting and nitrogen-limiting conditions, as indicated by the coincident loss of the diagnostic nocturnal F_v/F_m decreases (Behrenfeld and Kolber, 1999). During the day, non-photochemical quenching (NPQ) caused significant and regular deviations between c_p and F_m that exhibited roughly an exponential relationship with incident downwelling PAR (Fig. 3A,C).

As c_p is a size-sensitive measure of light scatter by all particles, with particular sensitivity to the 0.5 to 20 μm range, its capacity to function as a phytoplankton biomass index relies heavily on the conservative nature of particle assemblages. When particle composition and/or size distribution varies significantly, the relationship between c_p and phytoplankton biomass degrades. For our equatorial Pacific region, the strong correlation between c_p and Chl_{Fl} (Fig. 3B) is consistent with conserved characteristics of the particle assemblage. In addition, we found station c_p values to be highly correlated with Coulter-based particle number ($r^2 = 0.77$) (Fig. 4A) and filtration-based POC concentrations ($r^2 = 0.75$) (Fig. 4B). The fraction of the particle assemblage contributing to Coulter (1.5 to 42 μm) and POC (roughly all particles > 0.7 μm) data differs from that dominating c_p, so their strong correlations with c_p are again indicative of conservative particle size distributions (*see* Appendix II for additional analyses). Interestingly, regression analysis of c_p versus particle number indicated an intercept through the origin (Fig. 4A), while the c_p versus POC relationship suggested an intercept of 7.6 μg C L^{-1} (Fig. 4B). This c_p -POC intercept is at the upper end of the range in intercepts reported by Loisel and Morel (1998) (line 'd' in Fig. 4B), Claustre *et al.* (1999) (line 'b' in Fig. 4B) and Gardner *et al.* (2003) (lines 'a', 'c', and 'e' in Fig. 4B), while the slope of our c_p-POC relationship is very consistent with these earlier reports (Fig. 4B). For the same station data, c_p and chlorophyll concentration were well correlated ($r^2 = 0.83$) (Fig. 4C) and consistent with earlier data from the equatorial Pacific (line 'f' in Fig. 4C).

We additionally assessed from the Coulter counter data variability in the slope of particle size distributions and quartile sizes (as in Boss *et al.,* 2001). None of these parameters were found to correlate with the biogeochemical variables or their value normalized to POC ($r^2 < 0.05$). In other words, scatter in the relationships between beam attenuation, POC, particle number, and chlorophyll (Fig. 3B, 4) could not be accounted for by changes in size distribution.

4. Discussion

Despite its essential role in ocean biogeochemical cycles and ecosystem functioning, phytoplankton carbon biomass is rarely assessed in the field and approaches to its measurement are varied and controversial. Lack of routine phytoplankton biomass determinations seriously compromises any attempt to establish chlorophyll concentration or particulate beam attenuation (c_p) as the more robust biomass index. Nevertheless, an

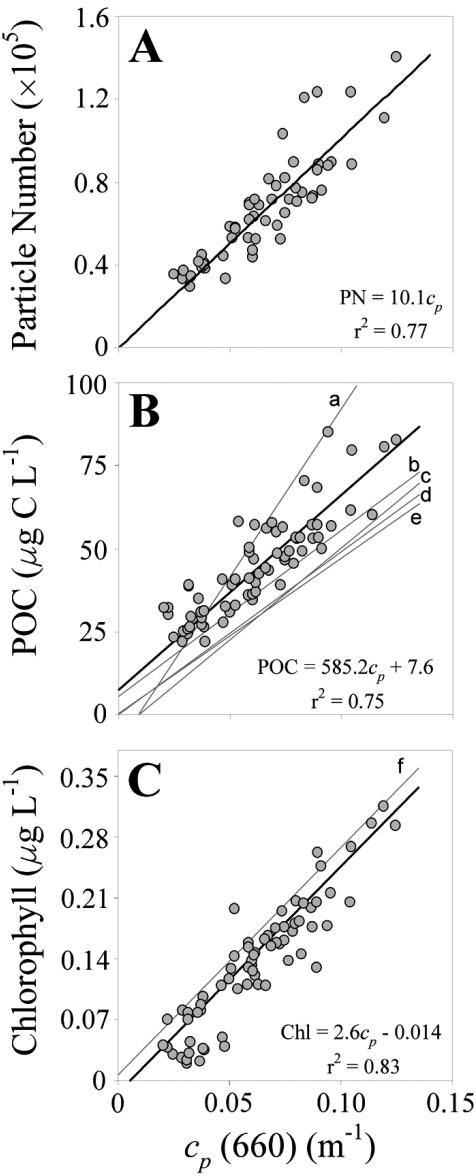

Figure 4. Comparison of station-based particulate beam attenuation coefficients (c_p) measured at 660 nm (m^{-1}) and coincident measurements of (A) Coulter-based concentration of particles with diameters between ~1.5 and 40 μm ($\times 10^5$ ml^{-1}), (B) total particulate organic carbon concentration (POC) (μg L^{-1}), and (C) fluorescence-based chlorophyll concentration (μg L^{-1}). Bold lines in each panel indicate fit to data, with corresponding equation and coefficient of determination (r^2) provided at the bottom right of the panel. The bold line in (C) is based on the full data set shown in Figure 3B. Light solid lines in (B) and (C) represent relationships from the following previously published studies: (a, c, e) = Gardner *et al.* (2003) cruises TTN-008, TTN-012, and TTN-007 respectively; (b) = Claustre *et al.* (1999); (d) = Loisel and Morel (1998), (f) = EqPac data as described by Behrenfeld and Boss (2003).

ever-growing body of observational data is developing that (largely indirectly) supports c_p as a useful proxy of phytoplankton biomass:

1. phytoplankton abundance generally dominates c_p in the few cases where both variables are measured (DuRand and Olson, 1996; Green *et al.,* 2003; Green and Sosik, 2004),
2. c_p:chlorophyll ratios (c_p*) vary through the water column in a manner consistent with photoacclimation to the vertical light gradient (Kitchen and Zaneveld, 1990; Mitchell and Kiefer, 1988; Mitchell and Holm-Hansen, 1991; Fennel and Boss, 2003),
3. c_p* covaries with ^{14}C-based determinations of light-saturated photosynthesis over a wide range of growth conditions (Behrenfeld and Boss, 2003),
4. satellite-based light scattering:chlorophyll ratios behave in a manner consistent with laboratory-based physiological relationships between phytoplankton Chl:C ratios and changes in growth rate, light, and temperature (Behrenfeld *et al.,* 2005), and
5. when measured in a region where growth conditions are highly constrained, c_p and chlorophyll concentrations are tightly coupled ($r^2 = 0.93$), indicating that both indices are reliably tracking changes in phytoplankton biomass (current study).

The conservative nature of open ocean particle size distributions (Bader, 1970; Stramski and Kiefer, 1991; Kiefer and Berwald, 1992; Twardowski *et al.,* 2001) and the reasonably constrained contribution of phytoplankton to total particulate carbon (Eppley *et al.,* 1992; DuRand and Olson, 1996; Gundersen *et al.,* 2001; Oubelkheir *et al.,* 2005) (however, see also Bricaud *et al.,* 1998; Claustre *et al.,* 1999) contribute to these consistencies between c_p and phytoplankton biomass. Nevertheless, c_p is not uniquely algal in origin and will poorly reflect phytoplankton biomass under certain conditions (e.g., high coccolith or suspended sediment conditions). On the other hand, few ocean areas exist where mixed layer growth conditions are sufficiently stable that acclimation to light and nutrient stress does not have a significant influence on the relationship between chlorophyll concentration and phytoplankton carbon biomass.

a. Beam attenuation, chlorophyll concentration, and phytoplankton carbon biomass

The relationship between the variable component of light absorption (particularly chlorophyll absorption) and particulate light scattering coefficients (b_p) (or c_p) is an issue of long-standing interest and has a variety of practical implications, including the interpretation of ocean color and its relation to ecosystem processes. Historically, pigment versus light scattering analyses have involved the assemblage of measurement data from a wide range of ocean areas and depths (e.g., Morel, 1980; Gordon and Morel, 1983; Loisel and Morel, 1998). This approach has advantages of yielding a large dynamic range in the two variables and a comprehensive representation of environmental conditions. The drawback, however, is that the approach makes it exceedingly difficult to partition variability in the chlorophyll-scattering relationship into that due to shifts in the composi-

tion of the particle assemblage and that caused by physiological adjustments in intracellular pigmentation. It is in this vein that the current study stands apart.

Our underlying premise was that physiologically-based ambiguities in chlorophyll as an algal biomass index can be minimized by restricting measurements to a region of relatively homogeneous growth conditions. Under the perfect scenario of completely uniform light, nutrients, temperature, and taxonomy, cellular pigmentation would be a constant and thus chlorophyll concentration would perfectly correlate with phytoplankton carbon biomass. Scatter in the chlorophyll-c_p relationship (Fig. 5A) would then provide a quantitative measure of the amount of variance in phytoplankton carbon that can be explained by variations in c_p (i.e., 93% in the current study). The remaining 7% unexplained variance would presumably reflect light scattering by non-algal components varying independently of phytoplankton abundance. In the real world, however, perfectly uniform physiological conditions do not exist and even in the eastern equatorial Pacific a finite uncertainty remains in relating chlorophyll estimates to phytoplankton carbon (thus contributing a portion of the variance in our chlorophyll-c_p relationship (Fig. 5A)). These uncertainties include physiological responses to light and nutrient stress, as well as inaccuracies in relating fluorescence to chlorophyll concentration (e.g., we observed a mild hysteresis in the recovery time-course of nonphotochemical quenching that suppressed fluorescence yields during early evening). Accordingly, our results suggest that c_p actually correlated with phytoplankton biomass to better than 93%, assuming independent behavior of factors degrading chlorophyll and c_p relationships with phytoplankton carbon.

The exceptional correspondence between chlorophyll and c_p observed during the current study was not found in our five selected published data sets (Fig. 5B-F) (see Methods) (note that restriction of the Southern Ocean data to a comparable range as our equatorial data set decreases the r^2 from 0.91 to 0.52). Instead, data from relatively stable oligotrophic systems (BATS and HOT) exhibited no significant relationship between chlorophyll and c_p whatsoever (Fig. 5B,C). In our earlier study (Behrenfeld and Boss, 2003), we used independent ^{14}C-based assessments to demonstrate that chlorophyll:c_p ratios at HOT and BATS correlate with physiological variability. Thus, results in Figures 5B and 5C are indicative of ecosystems where phytoplankton biomass and c_p are temporally stable, while physiological responses to changing growth conditions are largely responsible for variations in chlorophyll concentration. Our other three historical data sets (Fig. 5D-F) are more similar to the current study, in that they all exhibit roughly linear chlorophyll-c_p relationships dominated by variability in phytoplankton biomass. For the NABE and EqPac data (Fig. 5D,E), chlorophyll:c_p ratios have again been shown to track physiological variability (Behrenfeld and Boss, 2003) and further suggest that degradation of the c_p-biomass relationship can be small relative to physiological adjustments in intracellular pigmentation.

If data from all six studies in Figure 5 are combined, the environmental conditions represented become reminiscent of the diversity found in earlier chlorophyll-scattering data sets. Based on the preceding discussion though, we would now emphasize the role of physiology on both the observed scatter and overall shape of such relationships. Morel

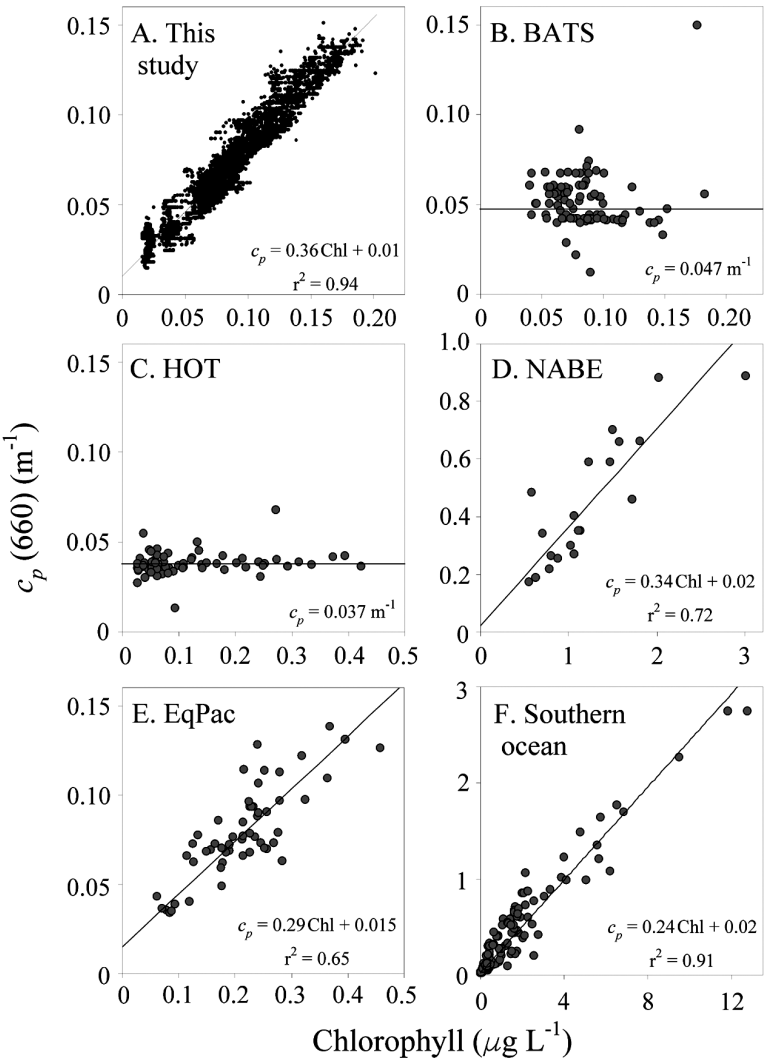

Figure 5. Comparison of chlorophyll:c_p relationships from a variety of ocean environments based on the following studies: (A) the current study using night-time fluorescence based chlorophyll data as indicated by the green symbols in Figure 3B, (B) the Bermuda Atlantic Time Series (BATS) program, (C) the Hawaii Ocean Time-series (HOT) program, (D) the North Atlantic Bloom Experiment (NABE), (E) the Equatorial Pacific (EqPac) study, and (F) the Joint Global Ocean Flux Study (JGOFS) Southern Ocean program. Solid lines in panels A and D-F indicate fits to data, with the corresponding equation and coefficient of determination (r^2) provided at the bottom right of the panel. Solid lines in B and C correspond to median c_p(660) values for the given data set, with value provided at bottom right of each panel.

(1980) presented an analysis of b_p and chlorophyll data that was soon expanded upon by Gordon and Morel (1983). These studies yielded non-linear relationships of the form: $b_p = A[Chl]^d$, where A and d are empirical coefficients and $d < 1$. This type of equation was

again employed by Voss (1992) and Loisel and Morel (1998) to describe relationships between chlorophyll and c_p, and could easily be adopted for the Southern Ocean data shown in figure 5F. This curvature has been interpreted as reflecting variations in the contribution of non-algal particles to scattering. However, we propose that an equally valid interpretation is that the curvature results from an unavoidable correlation between physiology and biomass. Specifically, increases in light attenuation coefficients (K_d) accompanying increases in chlorophyll cause a general decrease in acclimation light levels that stimulate a rise in phytoplankton pigmentation (i.e., increased biomass = increased K_d = decreased light = increased pigmentation = increased Chl:c_p). Additional ecosystem characteristics may also covary with phytoplankton biomass and contribute to the apparent curvature in chlorophyll-scattering relationships. For example, an increase in mean phytoplankton cell size with increasing biomass (e.g., Bricaud *et al.,* 2004) would accordingly influence the mean particle-specific attenuation coefficient in a biomass-dependent manner.

b. Broad-scale patterns in light-scattering-chlorophyll relationships

Establishing relationships between particulate scattering coefficients and phytoplankton carbon biomass provides an avenue for characterizing global variability in phytoplankton physiology through satellite retrievals of scattering:chlorophyll ratios (Behrenfeld and Boss, 2003; Behrenfeld *et al.,* 2005). While approaches to retrieving c_p from water leaving radiance data are being pursued (e.g., Roesler and Boss, 2003), the closest current remote sensing product to c_p is particulate backscattering coefficients (b_{bp}) (e.g., Garver and Siegel, 1997). Behrenfeld *et al.* (2005) analyzed satellite chlorophyll and b_{bp} data from the Sea-viewing Wide Field of View Sensor (SeaWiFS) and found regional Chl:b_{bp} ratios to vary in a manner consistent with anticipated physiological responses of phytoplankton to changing light, nutrient, and temperature conditions. In their analysis, they described the global relationship between chlorophyll concentration and b_{bp} as being biphasic, with a 'physiology dominated regime' where chlorophyll exhibited significant variability while b_{bp} was relatively constant and a 'biomass dominated regime' where b_{bp} and chlorophyll covaried over a similar range (see their Fig. 1).

Unlike the regionally-integrated monthly-mean satellite chlorophyll-b_{bp} data, none of the chlorophyll-c_p data shown in Figure 5 exhibit an intra-regional biphasic pattern. Instead, the data sets appear to belong either to the 'physiology dominated regime' (HOT and BATS) or the 'biomass dominated regime' (NABE, Southern ocean, equatorial Pacific). The biphasic nature of the satellite chlorophyll-b_{bp} data may therefore be a consequence of the regional binning procedure applied by Behrenfeld *et al.* (2005) causing an unavoidable separation of regions into functional groups. In other words, the 'physiology dominated regime' emerges because stable oligotrophic regions yield consistently lower average chlorophyll values than the upwelling or strongly seasonal regions that comprise the 'biomass dominated regime.' An interesting similarity between the field and satellite data is that in both cases regression analysis of data from 'biomass dominated

regimes' yields positive intercepts as chlorophyll biomass goes to zero, indicating a scattering contribution from background non-algal particles to both b_{bp} and c_p.

An additional common aspect of the satellite (Behrenfeld *et al.,* 2005) and field (Fig. 5) chlorophyll-scattering relationships is that both indicate a median particulate scattering coefficient for the 'physiology dominated' data that is clearly higher than the intercept suggested by regression analysis of 'biomass dominated' data. This apparent 'floor' in phytoplankton biomass in relatively stable environments (Fig. 5B,C) may be associated with a zooplankton grazing threshold (Lessard and Murrell, 1998). If so, then physical (as opposed to ecosystem) constraints may largely be responsible for continuation of the linear chlorophyll-c_p relationship in 'biomass dominated' regions below this 'stable-environment c_p threshold' (e.g., Fig. 5F). Such physical factors may include strong seasonality in light and surface mixing (export and dilution) or a temporal lag between deep-water upwelling and the development of a stable ecosystem biomass.

5. Conclusions

Beam attenuation and chlorophyll concentration represent alternative metrics of phytoplankton carbon biomass. Neither is perfect. Taxonomic differences and physiological responses to environmental forcings can drive changes in phytoplankton chlorophyll:C ratios that exceed an order of magnitude, both in culture and in the field. While it may be feasible to account for the light- and temperature-dependent components of this variability, taxonomic dependencies and nutrient stress effects remain poorly constrained in natural populations. These issues have stymied progress in the general description of ocean productivity for decades, a problem that has only been exacerbated by the general lack of field measurements of phytoplankton carbon biomass.

In this paper, we compare total particulate volume and cross sectional area, beam attenuation, particulate organic carbon and chlorophyll data collected in the eastern equatorial Pacific to investigate relationships between light scattering properties and phytoplankton biomass. We find very good correlations among the measured variables and, furthermore, find that the ratios between them are consistent with expectations based on laboratory cultures and optical theory (Appendix II). These results, along with previous evidence linking physiological variability to changes in chlorophyll:beam attenuation ratios (Behrenfeld and Boss, 2003), suggest that uncertainties in the conversion of beam attenuation to phytoplankton carbon in the open ocean are bounded and can be smaller than those associated with deriving phytoplankton biomass from chlorophyll.

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APPENDIX I

When discussing characteristics of particle assemblages, an understanding of terminology is important. In the current paper, we consider the term 'abundance' to mean the number of particles, irrespective of size or volume. In contrast, we consider 'biomass' to be a measure of the total carbon content within a given particle group, accounting for both the abundance and carbon content of the particles. Thus, 'phytoplankton biomass' refers to the total carbon content of all phytoplankton within a given volume of water. Oceanographers have developed a wide range of techniques for quantifying phytoplankton concentrations, including microscopy, flow cytometry, analysis of pigment or chlorophyll mass, fluorescence, electronic cell counts (e.g., Coulter counter), and analysis of particulate organic carbon or nitrogen mass. Depending on the application (e.g., heat budgets, primary production estimates, trophic dynamics, energy flow in an ecosystem model), one estimate of phytoplankton concentration may be more or less relevant than another. In all cases, additional information about the particle assemblage is necessary to derive the true phytoplankton carbon biomass.

APPENDIX II

In order to increase our confidence in the quality of the data and its interpretation we performed a few additional calculations. First, if the particles enumerated by the Coulter Counter are to explain the bulk of our optical data, then the average attenuation efficiency factor (i.e., the ratio of the beam attenuation to the total cross sectional area of the counted particles) should be around two and not larger than 3.2 (Van de Hulst, 1957, Fig. 32). Figure A1 illustrates that observed values of this efficiency factor were all within the expected range, suggesting that the bulk of the particles contributing to beam attenuation have been sized.

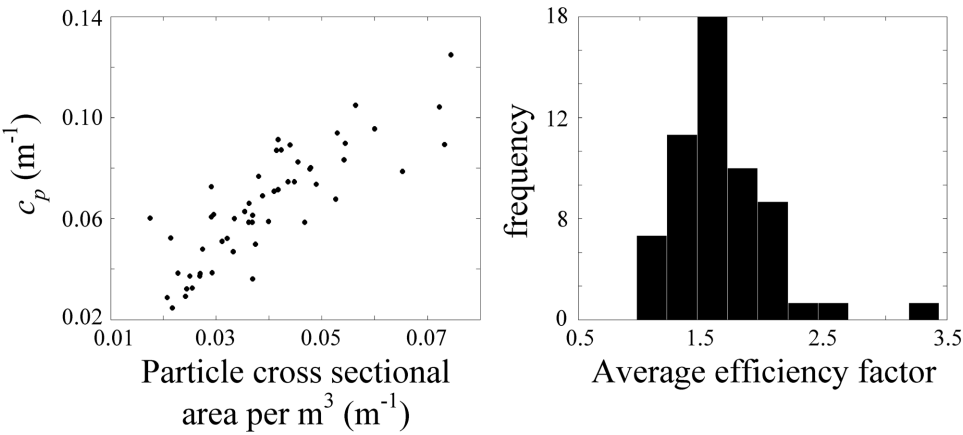

Figure A1. Beam attenuation versus cross-sectional area (left panel) and a frequency distribution of their ratio (right panel), the average attenuation efficiency factor.

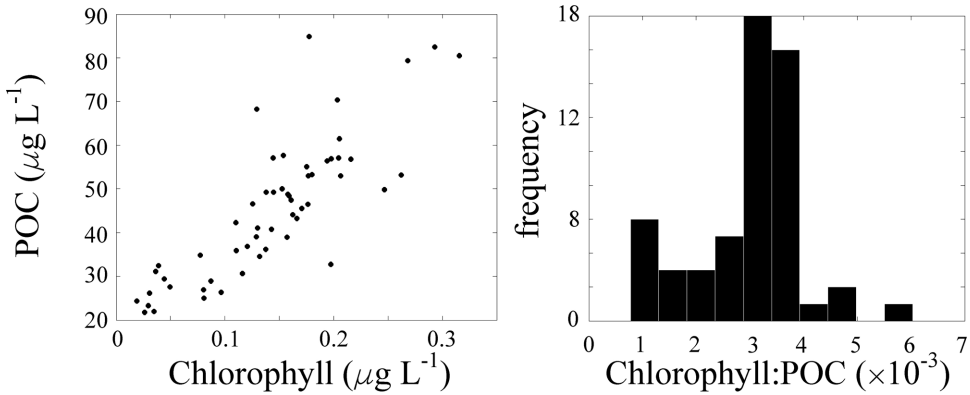

Figure A2. POC vs chlorophyll *a* (left panel) and a frequency distribution of the ratio of chlorophyll to POC (right panel).

Second, it has been observed that 25% to 40% of observed POC is due to phytoplankton (see introduction). One would therefore expect the slope between chlorophyll and POC to be bounded within the range of Chl:C variability observed in phytoplankton cells, with a correction factor associated with the phytoplankton carbon to POC ratio. As shown in figure A2, we find the median ratio of chlorophyll to POC to be about 0.003 μg Chl μg C^{-1}. Assuming that 33% of the POC is due to phytoplankton, the Chl:C ratio of these cells will be on average 0.01 μg Chl μg C^{-1}, consistent with high-light adapted cells (e.g. Fig. 1 of Cloern *et al.,* 1995).

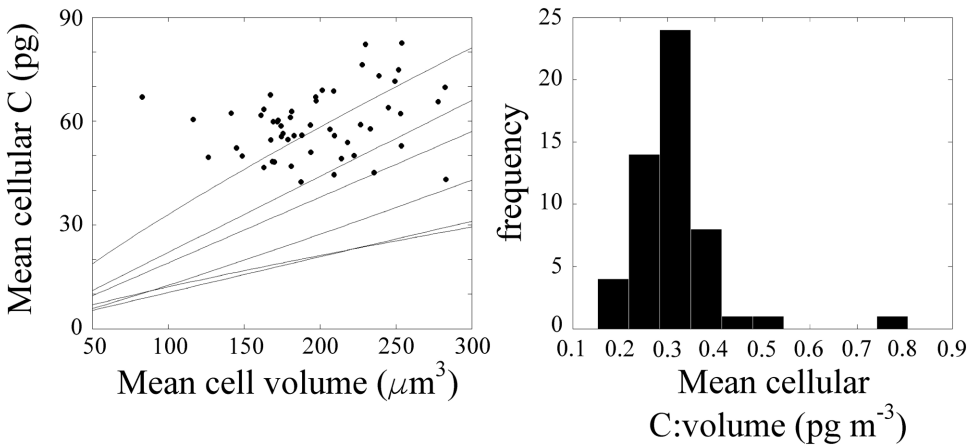

Figure A3. Averaged cellular POC as function of averaged cell volume overlaid with six published regressions (as compiled in Table 1 in Davidson *et al.*, 2002), including Bacteria (Fagerbakke *et al.*, 1996), Diatoms (Motagnes *et al.*, 1994; Menden-Deuer and Lessard, 2000), photosynthetic nanoflagelates (Motagnes *et al.*, 1994), heterotrophic nanoflagelates (Borsheim and Bratbak, 1987), Dinoglagellates (Menden-Deuer and Lessard, 2000) and Ciliates (Putt and Stoecker, 1989).

Finally, measurements of cell number, volume and POC can be compared to provide information about the average carbon per volume of the observed particles. The amount of organic carbon in cellular material is bounded by laboratory work. In Figure A3, we compare our POC measurements normalized by Coulter Counter particle numbers to cell volume. The observed values are consistent with observation, although slightly elevated. This elevation likely reflects the fact that POC represents particles larger than 0.7 μm (GFF), while the Coulter Counter threshold was 1.5 μm.

REFERENCES

Bader, H. 1970. The hyperbolic distribution of particle sizes. J. Geophys. Res., *75,* 2822–2830.

Behrenfeld, M. J. and E. Boss. 2003. The beam attenuation to chlorophyll ratio: an optical index of phytoplankton photoacclimation in the surface ocean? Deep-Sea Res., *50*, 1537–1549.

Behrenfeld, M. J., E. Boss, D. A. Siegel and D. M. Shea. 2005. Carbon-based ocean productivity and phytoplankton physiology from space. Global Biogeochem. Cycles, *19*, GB1006, doi:10.1029/2004GB002299.

Behrenfeld, M. J. and Z.S. Kolber. 1999. Widespread iron limitation of phytoplankton in the south Pacific Ocean. Science, *283*, 840–843.

Behrenfeld, M. J., O. Prasil, M. Babin and F. Bruyant. 2004. In search of a physiological basis for covariations in light-limited and light-saturated photosynthesis. J. Phycology, *40*, 4–25.

Bishop, J. K. B. 1999. Transmissometer measurements of POC. Deep-Sea Res. I., *46*, 353–369.

Bishop, J. K. B., S. E. Calvert and M. Y. S. Soon. 1999. Spatial and temporal variability of POC in the northeast Subarctic Pacific. Deep-Sea Res. II, *46*, 2699–2733.

Børsheim, K.Y. and G. Bratbak. 1987. Cell volume to carbon conversion factors for a bacterivorous Monas sp. enriched from seawater. Mar. Ecol. Prog. Ser., *36*, 171–175.

Boss, E., W. S. Pegau,W. D. Gardner, J. R. V. Zaneveld, A. H. Barnard., M. S. Twardowski, G. C. Chang and T. D. Dickey. 2001. The spectral particulate attenuation and particle size distribution in the bottom boundary layer of a continental shelf. J. Geophys. Res., *106*, 9509–9516.

Boss E., M. S. Twardowski and S. Herring. 2001. Shape of the particulate beam attenuation spectrum and its inversion to obtain the shape of the particulate size distribution. Appl. Optics., *40*, 4885–4893.

Bricaud, A., H. Claustre, J. Ras and K. Oubelkheir. 2004. Natural varaibility of phytoplankton absorption in oceanic waters: influence of the size structure of algal populations. J. Geophys. Res., *109*, C11010, doi:10.1029/2004JC002419.

Bricaud, A., A. Morel, M. Babin, K. Allali and H. Claustre. 1998. Variations of light absorption by suspended particles with chlorophyll *a* concentration in oceanic (case 1) waters: Analysis and implications for bio-optical models. J. Geophys. Res. *103*, 31033–31044.

Claustre, H., A. Morel, M. Babin, C. Cailliau, D. Marie, J-C Marty, D. Tailliez and D. Vaulot. 1999. Variability in particle attenuation and chlorophyll fluorescence in the tropical Pacific: Scales, patterns, and biogeochemical implications. J. Geophys. Res., *104*, 3401–3422.

Cloern, J. E., C. Genz and L. Vidergar-Lucas. 1995. An empirical model of phytoplankton chlorophyll:carbon ratio: the conversion factor between productivity and growth rate. Limnol. Oceanogr., *40*, 1313–1321.

Davidson, K., E. C. Roberts and L.C. Gilpin. 2002. The relationship between carbon and biovolume in marine microbial mesocosms under different nutrient regimes. Eur. J. Phycol., *37*, 501–507.

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.

Eppley, R.W., F. P. Chavez and R.T. Barber. 1992. Standing stocks of particulate carbon and nitrogen in the Equatorial Pacific at 150°W, J. Geophys. Res., *97*, 655–661.

Fagerbakke, K. M., M. Heldal and S. Norland. 1996. Content of carbon, nitrogen, oxygen, sulphur and phosphorus in native aquatic and cultured bacteria. Aquat. Microb. Ecol., *10*, 15–27.

Falkowski, P.G. and Z. Kolber. 1995. Variations in chlorophyll fluorescence yields in phytoplankton in the world oceans. Aust. J. Plant Physiol., *22*, 341–355.

Fennel, K. and E. Boss. 2003. Subsurface maxima of phytoplankton and chlorophyll: Steady state solutions from a simple model. Limnol. Oceanogr., *48*, 1521–1534.

Gardner, W. D., S. P. Chung, M. J. Richardson and I. D. Walsh. 1995. The oceanic mixed-layer pump. Deep-Sea Res. II., *42*, 757–775.

Gardner, W. D., M. J. Richardson, C. A. Carlson, D. Hansell and A.V. Mishonov. 2003. Determining true particulate organic carbon: bottles, pumps and methodologies. Deep-Sea Res. II., *42*, 655–674.

Gardner, W. D., I. D. Walsh and M. J. Richardson. 1993. Biophysical forcing on particle production and distribution during a spring bloom in the North Atlantic. Deep-Sea Res. II., *40*, 171–195.

Garver, S. A. and D. A. Siegel. 1997. Inherent optical property inversion of ocean color spectra and its biogeochemical interpretation: I. Time series from the Sargasso Sea. J. Geophys. Res., *102*, 607–18,625.

Gordon, H. and A. Morel. 1983. Remote assessment of ocean color for satellite visible imagery. A review. Lecture Notes on Coastal and Estuarine Studies, R. T. Barber, C. N. K. Mooers, M. J. Bowman, and B. Zeitzschel, eds., Springer-Verlag, 1–114.

Green, R. E. and H. M. Sosik. 2004. Analysis of apparent optical properties and ocean color models using measurements of seawater constituents in New England continental shelf surface waters. J. Geophys. Res., *109*, doi:10.1029/2003JC001977.

Green, R. E., H. M. Sosik and R. J. Olson. 2003. Contributions of phytoplankton and other particles to inherent optical properties in New England continental shelf waters. Limnol. Oceangr., *48*, 2377–2391

Gundersen, K., K. M. Orcutt, D. A. Purdie, A. F. Michaels and A. H. Knap. 2001. Particulate organic carbon mass distribution at the Bermuda Atlantic time-series Study (BATS) site. Deep-Sea Res. II, *48*, 1697–1718.

Jerlov, N. G. 1976. Marine Optics, Elsevier, 231 pp.

Kara, A. B., P. A. Rochford and H. E. Hurlburt. 2003. Mixed layer depth variability over the global ocean. J. Geophys. Res., *108*, doi:10.1029/2000C000736.

Kiefer, D. A. and J. Berwald. 1992. A random encounter model for the microbial planktonic community. Limnol. Oceanogr., *37*, 457–467.

Kitchen, J. and J. R. Zaneveld. 1990. On the noncorrelation of the vertical structure of light scattering and chlorophyll *a* in case I waters. J. Geophys. Res., *95*, 20,237–20,246.

Kolber, Z. and P. G. Falkowski. 1993. Use of active fluorescence to estimate phytoplankton photosynthesis *in situ*. Limnol. Oceanogr., *38*, 1646–1665.

Laws, E. A. 1997. Mathematical Methods for Oceanographers: An Introduction, Wiley-Interscience, NY, 343 pp.

Lessard, E. J. and M. C. Murrell. 1998. Microzooplankton herbivory and phytoplankton growth in the northwestern Sargasso Sea. Aquatic Microbial Ecol., *16*, 173–188.

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

Menden-Deuer, S. and E. J. Lessard. 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol. Oceanogr., *45*, 569–579.

Mitchell, B. G. and D. A. Kiefer. 1988. Variability in pigment specific particulate fluorescence and absorption spectra in the northeastern Pacific Ocean. Deep-Sea Res., *35*, 665–689.

Mitchell, B. G. and O. Holm-Hansen. 1991. Bio-optical properties of Antarctic Peninsula waters: differentiation from temperate ocean models. Deep-Sea Res., *38*, 1009–1028.

Montagnes, D. J. S., J. A. Berges, P. J. Harrison and F. J. R. Taylor. 1994. Estimating carbon,

nitrogen, protein and chlorophyll *a* from volume in marine phytoplankton. Limnol. Oceanogr., *39*, 1044–1060.

Monterey, G. and S. Levitus. 1997. Seasonal variability of mixed layer depth for the World Ocean. NOAA Atlas, NESDIS 14, Washington DC, 100 pp.

Morel, A. 1973. Diffusion de la lumière par les eaux de mer: Résultats expérimentaux et approche théorique. In: Optics of the Sea, AGARD Lecture Ser. 61, 3.1.1–3.1.76.

——1980. In-water and remote measurements of ocean color. Boundary-Layer Meteorol., *18*, 177–201.

Oubelkheir, K., H. Claustre, A. Sciandra and M. Babin. 2005. Bio-optical and biogeochemical properties of different trophic regimes in oceanic waters. Limnol. Oceanogr., *50*, 1795–1809.

Putt, M. and D. K. Stoecker. 1989. An experimentally determined carbon:volume ratio for marine 'oligotrichous' ciliates from estuarine and coastal waters. Limnol. Oceanogr., *34*, 1097–1103.

Roesler, C. and E. Boss. 2003. A novel ocean color inversion model: retrieval of beam attenuation and particle size distribution. Geophys. Res. Lett., *30(9)*, 10.1029/2002GL016366

Sheldon, R., A. Prakash and W. Sutcli. 972. The size distribution of particles in the ocean. Limnol. Oceanogr., *17*, 327–340.

Stramski, D. and D. Kiefer. 1991. Light scattering by microorganisms in the open ocean. Prog. Oceanogr., *28*, 343–383.

Twardowski M. S., E. Boss, J. B. Macdonald, W. S. Pegau, A. H. Barnard and J. R. 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*, 14,129–14,142.

Van de Hulst, H. C. 1957. Light Scattering by Small Particles, Wiley, NY, 470 pp.

Voss, K. J. 1992. A spectral model of the beam attenuation coefficient in the ocean and coastal areas. Limnol. Oceanogr., *37*, 501–509.

Walsh, I. D., S. P. Chung, M. J. Richardson and W. D. Gardner. 1995. The diel cycle in the integrated particle load in the equatorial Pacific: A comparison with primary production. Deep-Sea Res. II, *42*, 465–477.

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