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Analytical phytoplankton carbon measurements spanning diverse ecosystems

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ABSTRACT

The measurement of phytoplankton carbon (C_{phyto}) in the field has been a long-sought but elusive goal in oceanography. Proxy measurements of C_{phyto} have been employed in the past, but are subject to many confounding influences that undermine their accuracy. Here we report the first directly measured C_{phyto} values from the open ocean. The C_{phyto} samples were collected from a diversity of environments, ranging from Pacific and Atlantic oligotrophic gyres to equatorial upwelling systems to temperate spring conditions. When compared to earlier proxies, direct measurements of C_{phyto} exhibit the strongest relationship with particulate backscattering coefficients (b_{bp}) (R^2=0.69). Chlorophyll concentration and total particulate organic carbon (POC) concentration accounted for ~20% less variability in C_{phyto} than b_{bp}. Ratios of C_{phyto} to Chl a span an order of magnitude moving across and within distinct ecosystems. Similarly, C_{phyto}:POC ratios were variable with the lowest values coming from productive temperate waters and the highest from oligotrophic gyres. A strong relationship between C_{phyto} and b_{bp} is particularly significant because b_{bp} is a property retrievable from satellite ocean color measurements. Our results, therefore, are highly encouraging for the global monitoring of phytoplankton biomass from space. The continued application of our C_{phyto} measurement approach will enable validation of satellite retrievals and contribute to an improved understanding of environmental controls on phytoplankton biomass and physiology.

1. Introduction

The direct measurement of phytoplankton carbon (C_{phyto}) in the field has long been recognized as critical for understanding plankton dynamics (e.g. growth, production) in the marine environment (Sutherland, 1913; Eppley, 1968; Laws, 2013). Accurate estimates of C_{phyto} at local, regional, and global scales provide a means to observe phytoplankton standing stocks and seasonal or inter-annual biomass trends relative to environmental variability. Despite this ongoing need, the direct assessment of C_{phyto} has proven an elusive goal. Key to this challenge has been an inability to isolate phytoplankton from the pool of other particulate forms of carbon that are abundant in seawater (zooplankton, detritus, bacteria, etc.). Consequently, proxy measurements or conversions of properties related to phytoplankton have been heavily relied upon to evaluate C_{phyto}.

Historical biomass proxies have included cell counts (Sutherland, 1913), color indices (Reid et al., 1998), chlorophyll (Kreps and Verjbinskaya, 1930), adenosine triphosphate (ATP) (Sinclair et al., 1979), proportions of particulate organic carbon (Strickland, 1960), and cell volume (Strathmann, 1967; Verity et al., 1992; Montagnes et al., 1994), all of which require conversions to biomass estimates. Single cell elemental analysis (Heldal et al., 2003) has been performed and has potential, but thus far is very limited in the number of cells that have been analyzed from a sample. Some of the proxy estimates are uniquely tied to phytoplankton (e.g. cell counts, pigments) while others are clearly influenced by non-phytoplankton organisms and non-living seawater constituents (e.g. ATP, POC). Nevertheless, these estimates have greatly influenced our understanding of phytoplankton abundance and community dynamics, as well as the interpretation of long-term trends in phytoplankton properties (Reid et al., 1998; Mcquatters-Gollop et al., 2011). More thorough discussions of these proxies and their relationships with C_{phyto} can be found elsewhere (e.g. Banse, 1977; Geider et al., 1997; Graff et al., 2012).

A more recent alternative approach to assessing C_{phyto} has been through analyzing variability in light scattering properties, specifically the particulate beam attenuation coefficient (c_p) and the

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particulate backscatter coefficient (b_{bp}). An appealing aspect of this approach is that these light scattering properties can be continuously measured in situ (Behrenfeld and Boss, 2003, 2006; Huot et al., 2007) and b_{bp} can be retrieved from space (Behrenfeld et al., 2005; Siegel et al., 2005; Westberry et al., 2008). These optical properties have the additional important advantage that they vary with particle abundance but, unlike chlorophyll, do not register physiological variability associated with light and nutrient conditions (i.e., variability in cellular Chl:C ratios). However, a drawback of the optical indices of C_{phyto} is that a portion of the scattered light is due to non-algal particles. The severity of this issue has not been quantified because, like all the other C_{phyto} proxies, direct comparisons with analytical field measurements of biomass have not been possible.

A solution to the long standing C_{phyto} problem was introduced in Graff et al. (2012) where a method was described for isolating the phytoplankton community from field samples and then assessing C_{phyto} of the sorted sample through elemental analysis. The approach employs sorting flow-cytometry to identify phytoplankton and separate them from non-algal particles. As demonstrated in Graff et al. (2012), the resultant sorted sample is highly representative of the in situ phytoplankton community. Carbon measured in the sorted sample is converted to in situ C_{phyto} using a cell count ratio calculated from cell counts from the sorted and whole seawater samples. In the original report (Graff et al., 2012) only a small set of samples collected off the Oregon Coast were collected to demonstrate the field application of the approach.

Here, we report analytical measurements of C_{phyto} covering a diversity of open ocean conditions, ranging from Pacific and Atlantic oligotrophic gyres to equatorial upwelling systems and temperate waters. We compare our direct phytoplankton biomass estimates to simultaneous measurements of b_{bp}, c_p, Chl a concentration and total particulate organic carbon (POC). From this comparison, we provide a new parameterization for the relationship between C_{phyto} and b_{bp}, the latter property being the biomass proxy exhibiting the best correlation with C_{phyto}. The new relationship can be applied to satellite retrievals of b_{bp} and resultant C_{phyto} estimates employed, for example, in the Carbon-based Productivity Model (CbPM) (Westberry et al., 2008), to re-evaluate global variability in ocean net primary production. Validated assessments of C_{phyto} will also help discern underlying drivers of change (e.g. nutrients, light environment, etc.) in historical field and satellite chlorophyll records (Boyce et al., 2010, 2014; Siegel et al., 2013).

2. Material and methods

Field samples were collected during two cruises (Fig. 1). The first cruise took place in the Equatorial Pacific Ocean (EPO) in conjunction with the National Oceanic and Atmospheric Administration's (NOAA) Tropical Atmospheric Ocean (TAO) project aboard the NOAA Vessel Ka'imimoana from 7 May to 28 June 2012 (Fig. 1). The second field effort was part of the 22nd Atlantic Meridional Transect (AMT-22) on the RSS James Cook from 10 October to 24 November 2012 (Fig. 1). During each cruise, surface optical properties were continuously measured and discrete samples were collected for C_{phyto}, high-pressure liquid chromatography (HPLC) pigments and POC.

2.1. Analytical C_{phyto} measurements

Collection and elemental analyses of C_{phyto} samples, hereto referred to as direct or analytical C_{phyto}, were made following a modified procedure first described in Graff et al. (2012). Surface samples were collected from seawater flow-through lines on both cruises and from a Niskin rosette when possible on the EPO cruise. Comparative tests (data not shown here) were performed during the TAO cruise to compare samples collected using a Niskin and flow-through lines. No differences in the abundance or community composition of the samples were apparent. Samples for sorting and collecting phytoplankton cells were processed on a BD Biosciences Influx Cell Sorter (BD ICS) flow-cytometer. The BD ICS was aligned and calibrated for cell sorting before whole seawater was collected. Sample lines were flushed for approximately 30 min prior to sample collection and cell sorting to remove potential contamination from the fluorescent beads used for alignment and calibration and the detergents in which they are stored. Our BD ICS is equipped with a 488 nm (blue) excitation laser, fluorescence collection at 530 nm and 692 nm, side scatter detection, and forward scatter detection for resolving small particles (down to 0.2 μm). A 100 μm nozzle tip was used for all samples. Gates for selecting and sorting cells were drawn to select for the scatter and fluorescence signatures of all phytoplankton groups present in the samples. Up to 4 mL of whole seawater were sorted at sea for each sample. Sort times ranged from less than 1 h up to 4 h, resulting in 0.75–1 mL samples composed of sorted phytoplankton cells suspended in artificial sheath fluid (deionized water and sodium chloride) (Graff et al., 2012). A sample of sheath water was collected from the nozzle following each sort and used to correct carbon values for sorted samples for the dissolved organic contribution from the sheath fluid. All samples were immediately frozen in liquid nitrogen (LN) and stored at −80 °C or in LN until they were analyzed.

Analysis of sorted phytoplankton and sheath fluid samples was performed on a Shimadzu TOC-N analyzer using manual injection techniques. Manual injection methods require less volume compared to automated protocols, which use a portion of a sample for filling and rinsing the sample lines and syringe. Due to sample salinity, ceramic wool in the combustion column was replaced with platinum wire beads (Carlson et al., 2004). The Shimadzu TOC-N provides an integrated area under a response curve for each sample. The difference in area between the sorted phytoplankton sample and the sheath fluid is attributed to C_{phyto}. Each sample analyzed was first acidified to a pH < 2.0 and sparged for 10 min with 75 mL min^{-1} of ultra pure air to remove inorganic carbon. A minimum of three 100 μl replicate injections per sample were analyzed and the mean area was calculated using 2–3 of the replicates with the lowest coefficient of variation (average CV for field samples = 0.047). Instrument accuracy and precision were checked throughout the day and prior to sample analysis with deionized water and a seawater reference standard (~40–44 μmol L^{-1} carbon) from the Consensus Reference Materials Project hosted at the Hansell Lab at the University of Miami.

Phytoplankton carbon in the sorted sample was determined by subtracting the mean area of the sheath fluid sample from the mean area of the sorted phytoplankton sample and converting to biomass using the instrument calibration. The areas for the sheath samples (~18–20 μM) are generally half of the values obtained when analyzing the standard reference material and well above the detection limits of the instrument. Measured signals for sorted samples containing cells are higher than their paired sheath sample values. The additional signal area due to cells is a variable fraction of the sheath signal and is dependent upon the concentration and types of cells in the sorted sample. The low coefficient of variation for each sample (median = 0.04) provides the resolution required to determine the difference between the sheath and sorted samples. Cell counts for the whole seawater samples and for each sorted sample were determined on the BD ICS. In situ C_{phyto} was calculated by dividing the carbon attributed to the phytoplankton portion of the sorted sample by the cell concentration ratio between the sorted and natural samples (Graff et al., 2012). *Synechococcus* cell count ratios were used here as they are easily discernible as a group from both noise and other cell types based on fluorescence and scatter properties and were present in all samples.

To calibrate the Shimadzu TOC-N, natural phytoplankton were used as a standard material. Whole seawater was collected 25 miles