The carbon and chlorophyll content of phytoplankton from various nutrient regimes

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Abstract. We have measured the relationship between chlorophyll and carbon in phytoplankton cultures and natural populations of phytoplankton using simple standard extraction procedures without correction for degradation products. During exponential growth of four cultures of unicellular and colonial green algae and one diatom, chlorophyll varied from 1.22 to 6.08% of the phytoplankton carbon content (\(x = 3.03\%\)). The chlorophyll content of the cultures was lower during nitrogen and phosphorus deficiency. Results from natural populations of phytoplankton from eutrophic lakes and from a coastal marine station gave values mostly ranging from 1.5 to 3.7% chlorophyll, corresponding to carbon/chlorophyll ratios of 27-67. If only a rough estimate of the phytoplankton carbon biomass is required, a simple, efficient extraction procedure can be used without any corrections for degradation products.

Introduction

In the literature a large number of methods are available to measure chlorophyll from phytoplankton (e.g. Marker et al., 1980). Pigment extracts are measured directly by means of spectrophotometric or fluorometric procedures (Strickland and Parsons, 1972), and techniques are often included to correct for presence of phaeopigments (Jensen and Liaaen-Jensen, 1959; Lorenzen, 1967; Jeffrey, 1981). Chlorophyll values are often used to identify changes in the distribution of algae in vertical profiles (Hobbie et al., 1972; Holm-Hansen et al., 1976) or in lake classification. However, chlorophyll values are also used to calculate phytoplankton carbon biomass assuming a fixed carbon to chlorophyll ratio (Riemann et al., 1982; Søndergaard et al., 1988). A number of older reports in the literature, mostly based on laboratory cultures of phytoplankton, have demonstrated upper and lower limits on what one might expect in carbon/chlorophyll relationships (e.g. Parsons, 1961; Parsons et al., 1961).

Unfortunately, few attempts have been made to evaluate the consequences of applying more sophisticated chlorophyll procedures to estimate phytoplankton carbon biomass.

The objective of this study was to measure the relationship between chlorophyll and carbon in phytoplankton under different growth conditions, using a simple standard chlorophyll technique. Results from a number of unicellular and colonial phytoplankton cultures were compared with those from various aquatic environments.

Methods and materials

Batch cultures of the following algae were used in the investigation: Selenastrum capricornutum Printz, Pediastrum duplex Mayen, Chlorella pyrenoidosa Chick,
Scenedesmus acutus Meyer, and the diatom Phaeodactylum tricornutum. The algae were cultivated in a modified Østerlind medium (Steemann Nielsen and Wium-Andersen, 1970) in containers as described by Jørgensen and Steemann Nielsen (1961). Illumination was 4.3 mW cm⁻² (Philips TL 20 W/32) for 12 h. alternating with 12 h darkness. The temperature was 20°C. Haemocytometer counts were used daily to check the growth of the cultures.

Phosphorus- and nitrogen-depleted cells were obtained by transferring the algae to media either without phosphate or without nitrogen. Sampling was commenced when growth decreased, usually after 5–9 days, and phosphate or nitrate was added 3 days later.

**Organic carbon**

Triplicate volumes of algal suspensions were filtered through 25 mm precombusted GF/C filters. Prior to analysis, filters were dried in a desiccator over P₂O₅, combusted at 770°C and analysed in a Perkin Elmer CHN analyser (240).

**Chlorophyll**

Triplicate volumes of algal culture suspensions were filtered through 25 mm GF/C filters. The filters were extracted in absolute methanol for 20 h without homogenization and measured spectrophotometrically (Beckman 240) without correction for degradation products (Riemann and Ernst, 1982). To calculate the concentration of chlorophyll a in methanol, a specific absorption coefficient of 77.9 1⁻¹ g⁻¹ cm⁻¹ was used (Riemann, 1978a).

During 1987 water samples were taken from eutrophic Frederiksborg Slotssø. Samples were mixed from 1, 3, 5 and 7 m depths. Cell dimensions were measured of the various species and calculated cell volumes were converted into carbon assuming 0.225 pg carbon μm⁻³ for mixed populations of phytoplankton and 0.24 pg carbon μm⁻³ for cyanobacteria (Reynolds, 1984).

Chlorophyll a was extracted from the natural populations of phytoplankton using 96% ethanol according to Jespersen and Christoffersen (1987).

Water samples were taken from 24 large enclosures situated in Roskilde Fjord, Denmark, during three periods in 1986. The enclosures were manipulated by the addition of benthic suspension feeders, planktivorous fish and nutrients. Some of them had contact with the sediment. Details of the enclosures as well as the analytical procedures are presented elsewhere (Riemann et al., 1988).

**Results and discussion**

In the batch cultures, chlorophyll (expressed as a percentage of the total organic carbon) varied from low values during N deficiency (\(\bar{x} = 1.28\%\), range 0.72–1.25%) and P deficiency (\(\bar{x} = 1.52\%\), range 0.80–3.05%) to higher values during exponential growth (\(\bar{x} = 3.03\%\), range 1.22–6.08%) (Figure 1).

When chlorophyll a versus carbon from exponential growth were plotted, a correlation coefficient of \(R^2 = 0.96\) was obtained from Selenastrum, Chlorella and Phaeodactylum (Figure 2). The slope was significantly larger than zero (\(P < 0.001\)). The intercept on the ordinate indicated a higher content of carbon per chlorophyll unit from Scenedesmus and Pediasstrum than from the unicellular algae. The line obtained from the unicellular algae was significantly different (Student’s \(t\) test, \(P < 0.01\)) from the line obtained from the colonial algae.

Similar changes in the ratio of carbon to chlorophyll in cultures were found by Holm-Hansen (1970). He found the lowest values and the widest variation (5 to 17-fold) during nitrogen deficiency of Monochrysis, Dunaliella and Skeletonema, whereas during exponential growth a maximum of 3-fold variation in the chlorophyll content was found with Monochrysis and Dunaliella.
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addition or removal of large quantities of nutrients. In a more detailed analysis for degradation products. Results from the exponential growth gave chlorophyll values of between 1.0 and 4.7% of the organic carbon (mean 2.9%), but lower values were found from the nutrient-depleted cultures.

Earlier reports showed that phytoplankton chlorophyll, expressed as a percentage of the organic matter, varied 10-fold (Riley, 1941; Gilibrecht, 1952; Banse, 1956; Krey, 1958). These variations were not species-specific, but represented also changes in nutrient and light regimes, growth rate and temperature, etc. (Strickland, 1960; Parsons, 1961; Parsons et al., 1961; Sakshaug and Holm-Hansen, 1977).

Despite these variations in the phytoplankton chlorophyll content, it is common in many deterministic aquatic models to use a single conversion factor to estimate the carbon content (e.g. Andersen and Jacobsen, 1979; Jørgensen et al., 1981). The choice of conversion factor has been based on a single reference (Andersen and Jacobsen, 1979) or on the average of results from selected papers (Jørgensen et al., 1981).

Whatever the basis for this conversion factor, we believe that chlorophyll data should not be the only information used in estimating phytoplankton carbon biomass from natural populations. Only in situations where one or two species dominate the population can the chlorophyll data give reasonable estimates of phytoplankton carbon biomass (Riemann et al., 1982).

Nevertheless, many aquatic models give reasonable predictions of a number of events, even when phytoplankton carbon biomass is calculated from chlorophyll data. Such models or submodels include vertical profiles (Hobbie et al., 1972; Holm-Hansen et al., 1976), in which variations in phytoplankton composition and growth are often limited. Others include models of lake classification or predictions of broad changes in lake phytoplankton by the addition or removal of large quantities of nutrients. In a more detailed analysis of the carbon flux, however, a simple factor for the estimation of chlorophyll is inadequate.
When applying a chlorophyll/carbon conversion factor a major question is whether extreme nutrient deficiency is common in eutrophic freshwater systems. Inflow from sewage plants, precipitation, runoff from agricultural fields and high microbial remineralization of nutrients all suggest fast replenishment of nutrients in many eutrophic aquatic ecosystems. Because periods of extreme nutrient deficiency are short in eutrophic environments, results from the extremely nutrient-depleted cultures may justly be eliminated.

Phytoplankton chlorophyll and carbon were also measured in samples from the eutrophic Frederiksborg Slotssø. The seasonal distribution of cell volume of different groups of phytoplankton revealed that diatoms and green algae dominated in the spring and early summer and cyanobacteria in the late summer and early fall (Figure 3). A plot of chlorophyll a versus phytoplankton carbon, estimated from phytoplankton volumes, gave two straight lines. The first regression line, covering the period 21/4-20/7, gave $r^2 = 0.99$ ($n = 8$), and the average percentage of chlorophyll was 3.7% (Figure 4a). The second regression line covered the period 10/8-5/10, where *Aphanizomenon* dominated. The correlation coefficient $r^2 = 0.94$ ($n = 5$) and the average percentage of chlorophyll was 2.0% (Figure 4a).

During the development, peak and decline of the bloom in Mossø, chlorophyll a expressed as a percentage of organic carbon varied from 2.8% at the peak of the bloom to 1.5% after the crash (Figure 4b). Assuming that the detrital carbon intercept on the ordinate (338 mg C L$^{-1}$) was constant for all samples, the average percentage of chlorophyll was 2.6% ($SE = 0.5$).

Finally, carbon/chlorophyll values were obtained from marine enclosures. A log/log plot of chlorophyll versus particulate carbon (corrected for bacterial plankton, flagellate plankton and zooplankton biomasses and for the intercept on the ordinate) revealed that the line was not significantly different from one, but significantly ($P > 0.01$) differed from zero (Figure 5). On average 2.6% of the carbon was chlorophyll. During the months of June and September 1986 the same plots revealed that lines were significantly different from one (unpublished data). Slopes indicated an average value of 3.3% from June and 8.3% from September.

Our results indicate that calculations of phytoplankton carbon biomass from chlorophyll data in eutrophic environments may require a conversion factor between 16 and 83 (corresponding to 1.2-6.1% chlorophyll). The major part of our data from natural populations suggests that this carbon/chlorophyll ratio most likely lies between 27 and 67. A further narrowing of the boundaries of this ratio would require determination of the particulate carbon pools present in the water, as well as details of the composition and growth of the phytoplankton.

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**Fig. 4.** (a) A plot of chlorophyll versus phytoplankton carbon estimated from phytoplankton volumes from eutrophic Frederiksborg Slotssø during 1987. (b) A plot of organic carbon versus chlorophyll a from the spring diatom in population in Lake Mossø during 1980 (data from Riemann et al., 1982).

**Fig. 5.** A plot of chlorophyll versus particulate carbon (corrected for bacterial-carbon, flagellate-carbon and zooplankton-carbon (data from Riemann et al., 1988).
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