Carbon to volume relationship of *Isochrysis galbana* (Prymnesiophyceae) during cell divisions

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**Abstract:** Dependency of carbon cell quota (C) on cell volume (V) was studied for the Prymnesiophyte *Isochrysis galbana*. Changes in the cell volume and carbon quota during cell divisions were examined at 25°C in a continuous culture with an enriched f/2 medium under a 12/12 h light/dark cycle at an irradiance of 45, 150, and 365 μmol m⁻² sec⁻¹. Carbon quota estimates followed the relation log C=b log V+log a, where b is the slope and log a is the intercept of the relationship. Significant relationships between carbon quota and cell volume during cell divisions were obtained at the three irradiances tested. The value of the slope and the intercept ranged from 0.59 to 0.98 and from 0.22 to 0.64, respectively, however, they were not significantly different between the three irradiances. The relationship between carbon quota to cell volume during cell divisions also corresponded well to previously published carbon quota to cell volume relationship values within the size range of nanophytoplankton ranging from 2 to 20 μm, even though the irradiances used in the experiments differed from those studied previously. Due to the close similarity in cell size and division manner between the majority of nanophytoplankton communities and *I. galbana*, the results of the present study can be applied to natural assemblages of nanophytoplankton to estimate their carbon biomass in the euphotic layer regardless of sampling time on a given day.

**Key words:** allometry, carbon quota, cell division, cell volume, irradiance

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**Introduction**

Phytoplankton is currently responsible for about 45% of global primary production (Falkowski & Raven 2007). However, the biomass of phytoplankton amounts to less than 1% of the total photosynthetic biomass on our planet. Estimating phytoplankton biomass has been one of the most essential components to understand plankton ecology. The difficulty in estimating the amount of phytoplanktonic carbon is due to the co-occurrence of other matter (e.g., bacteria and detritus) with significant fractions of non-phytoplanktonic carbon, so that living phytoplanktonic carbon cannot be accurately determined from total particulate carbon. Carbon is the principal structural component of phytoplankton and is important for community biomass and bioenergetics. Estimates of phytoplankton biomass in terms of carbon are usually made using empirically derived carbon quota to cell volume relationships (e.g. Mullin et al. 1966, Strathmann 1967). Therefore phytoplankton community structure can be quantified from estimated biomass (Ishizaka et al. 1997, Shalapyonok et al. 2001, Cermeño et al. 2005). However, analyzing the community structure of phytoplankton depends on the accuracy of the determination of carbon quota and cell volume relationships.

The first detailed study of the relationship between carbon quota and cell volume was made by Mullin et al. (1966). However, the relationships described to date have mainly been reported for phytoplankton larger than 20 μm. Since a substantial contribution to biomass by small phytoplankton such as nanoplankton (2–20 μm) in oceanic areas has been recognized (e.g., Malone 1980), the carbon to volume relationship has been studied extensively for nanophytoplankton under only one irradiance condition (Verity et al. 1992, Montagnes et al. 1994). The slopes of carbon quota to cell volume for nanophytoplankton have been found to be greater in this size fraction than in earlier work on phytoplankton larger than 20 μm (Mullin et al. 1966).

Cell volume and carbon quota is known to change during cell division. The carbon quota increases during light
periods and decreases during dark periods under light and nutrient saturated conditions in laboratory experiments (e.g. Kohata & Watanabe 1989, Varela & Harrison 1999) except for diatom species (Berges et al. 1995). Little is known about the dependency of carbon quota on cell volume during cell division (DuRand & Olson 1998). The changes in cell volume and carbon quota during cell division have not been considered when interpreting carbon quota to cell volume relationships in natural assemblages of nanophytoplankton (Verity et al. 1992, Montagnes et al. 1994), and collection of field samples for carbon estimation has been conducted without an awareness of the changes in cell volume and carbon quota during cell division. The carbon quota to cell volume relationship should be determined during cell division to obtain precise biomass measurements.

Allometric modeling i.e. size scaling, is a powerful approach for ecological research concerning biological oceanography (e.g. Banse 1976, Taguchi 1976). Cell volume and carbon quota have been used to estimate growth and respiratory rates in phytoplankton (e.g. Banse 1976, Tang 1995). Allometric models deal with cell volume and/or carbon quota, and those relationships are assumed to be constant regardless of the environmental conditions (e.g. Tang 1995). If this constancy indeed holds, volume measurements should be useful for ecological studies.

For the experiments on carbon quota to cell volume relationships regulated by cell division, we chose the Prymnesiophyte Isochrysis galbana as a model species because it is classified as an open-ocean eukaryotic nanophytoplankter based on its size and the division pattern of its cells (Nelson & Brand 1979, Ohii et al. 2002). We measured cell volume and carbon quota under a 12/12 h light/dark cycle for 48 hours at three light intensities. We discuss how our results can aid in the understanding of the dependency of cell quota on the cell volume during cell division and the interpretation of the carbon quota to cell volume relationship of the nanophytoplankton community in the field by comparing our results with published data.

Materials and Methods
Culture conditions and sampling
Isochrysis galbana Parke (NEPCC633) was obtained from The North East Pacific Culture Collection (NEPCC) at the University of British Columbia. It is free-living ellipsoidal flagellate cell with a mean equivalent spherical diameter of 5.1 μm, and a mean volume of 68 μm³. The cultures were grown in a continuous-culture system in a glass, double-walled 1 L volume vessel as described by Laws & Banister (1980). Cultures were kept at a temperature of 25°C by immersion in circulating water baths, stirred continuously by a magnetic Teflon-coated stir bar at 200 revolutions min⁻¹ and bubbling sterile air through the culture. Photosynthetically active radiation was determined by a scalar quantum sensor (Model QSL 100, Biospherical Instruments, USA) at 45, 150, and 365 μmol m⁻² sec⁻¹. The light cycle was maintained at a 12/12 h light/dark cycle by cool-white fluorescent tubes (Panasonic, Osaka, Japan). The growth medium was the natural seawater f/2 formulation (Guillard & Ryther 1962). The base for the medium was coastal surface seawater (salinity of 35.0) collected off the Manazuru coast (35°09′49″N, 139°10′33″E). Seawater was pre-filtered through 0.2 μm membrane filter (Millipore, USA) with added nutrients, trace metals, and vitamins at f/2 concentrations. Cultures were continuously diluted with fresh media at a fixed rate with a peristaltic pump to maintain stationary phase (Tokyo Rikakikai Co. Ltd., Tokyo, Japan). The culture was acclimatized to each light condition for two weeks.

Cell numbers were determined once a day to confirm synchronized and steady growth. Dilution rates were recorded daily by measuring culture overflow volumes. Once steady growth was confirmed, the daily growth rate (μ) was calculated using the equation:

$$\mu = \frac{V}{V_c}$$

where V is the volume of fresh media and V_c is the culture volume. Daily growth rates were 0.12 d⁻¹ under 45 μmol m⁻² sec⁻¹, 0.45 d⁻¹ under 150 μmol m⁻² sec⁻¹, and 0.53 d⁻¹ under 365 μmol m⁻² sec⁻¹ in the present study. Samples were collected for the determination of cell densities, cell volumes, and particulate organic carbon every two hours for 48 hours to repeat the one day cell division cycle. Aseptic techniques were used to minimize bacterial contamination throughout the incubation experiment.

Cell densities and volumes
Samples for the determination of cell density and cell volume were preserved with 2% formaldehyde. Cell density of 200–600 cells was measured under a microscope with a hemocytometer (Erma Inc., Tokyo, Japan) at a magnification of 200×. Cell size of 50–100 cells was measured under a microscope at a magnification of 1000× with an ocular ruler calibrated with a micrometer. Cell volumes (V) were calculated assuming I. galbana is an ellipsoid, by the formula:

$$V = \frac{\pi}{6} r_1 r_2^2$$

where π refers to the circular constant, and r_1 and r_2 are the long and short radius of ellipsoid, respectively (Hillebrand et al. 1999).

Since we kept cells with formaldehyde, we conducted time-series experiments to determine when shrinkage occurred due to preservatives. We corrected the fixed cell volume to the fresh one, by multiplying the fixed volume by a factor of 1.34 (Iwasawa et al. 2009).
Particulate organic carbon

Triplicate subsamples were filtered onto Whatman GF/A glass fiber filters pre-combusted at 500°C for 2 h for the analysis of particulate organic carbon (POC). POC was analyzed on an elemental analyzer (Fison, Model EA-1108) calibrated using acetonilide as the standard (Nagao et al. 2001). Cellular carbon quotas were calculated by dividing POC by cell density. Cellular carbon densities were calculated by dividing carbon quota by cell volume.

Mathematical and statistical analyses

The light and dark maximum or minimum deviation from the mean of the entire 48 hours measures the amplitude of the change during cell divisions at the experimental irradiances. Differences in the averages and amplitudes of the variables during cell division among the different irradiances were analyzed using ANOVA. Standard errors of averages and amplitudes were calculated by dividing standard deviation by the square root of the number of data points.

The periodicity of the rhythm was analyzed by the sine function based on Kieding et al. (1984) and modified as follows:

\[ Y = A + B \sin \left( \frac{(t + C)\pi}{12} \right) \]

where \( Y \) is the estimate of a variable during cell division (e.g. carbon quota), \( A \) is the average, \( B \) is the amplitude of the sine function, \( t \) is time and \( C \) is the difference between values at \( t=0 \) and \( t=24 \). A nonlinear least-squares regression analysis was used to test the degree of fitness of the estimated value to the observed value. Since the amplitude might be related to the average, in order to standardize the change during cell division in the amplitude of both cell volume and carbon quota, the magnitude of change during cell division (MCD) was calculated for the first 24 hours and the second 24 hour-period as follows:

\[ \text{MCD} = \frac{\text{Max} - \text{Min}}{\text{Min}} \times 100 \text{ (%)} \]

where Max is the maximum value and Min is the minimum value of each variable, respectively. The MCD allows direct comparisons between cell volume and carbon quota.

Log transformation

Apparent linear relationships between cell volume (V) and carbon quota (C) were evaluated with least-squares regression model I of the form:

\[ \log C = b \log V + \log a \]

where \( b \) is the slope and \( a \) is the intercept of the relationship between \( \log V \) and \( \log C \). Confidence intervals and standard errors around regression slopes and intercepts, and their significance, were calculated according to Sokal & Rohl (1995). Comparisons of regression lines among irradiances were conducted by ANCOVA (Snedecor & Cochran 1989).

Published data

Previously published data on maxima and minima in either cell volume or carbon quota during cell divisions of nanophytoplankton were obtained from the following sources: Eppley & Coatsworth (1966), Kohata & Watanabe (1986, 1989), Berdalet et al. (1992), DuRand & Olson (1998), Varela & Harrison (1999), DuRand et al. (2002), and Ohi et al. (2002, 2003). From these sources, we selected the data giving both the cell volume and carbon quota during cell division: Kohata & Watanabe (1989), DuRand & Olson (1998), Varela & Harrison (1999) and DuRand et al. (2002). The cell volumes and carbon quotas were then transformed to log scale. When the change in cell volume and carbon quota during cell division had a slope, the slope was corrected to zero to obtain a maximum and minimum.

Results

Change in cell volume and carbon quota during cell division

Cell volume and carbon quota of Isochrysis galbana increased during the light period and decreased during the dark period at the three irradiances (Figs. 1 and 2). Averages of cell volume and carbon quota over light and dark cycles differed significantly among the three irradiance levels (ANOVA; \( p<0.001 \)), and decreased with increasing irradiance as a decaying exponential function (\( p<0.001 \) for cell volume, \( p<0.01 \) for carbon quota; Fig. 3A). Significant linear relationships between the amplitudes of cell volume and carbon quota were obtained with irradiance (\( p<0.05 \) for cell volume, \( p<0.01 \) for carbon quota; Fig. 3B). The magnitude of change during cell division (MCD), a synonym for amplitude of cell volume and carbon quota, increased linearly when irradiance ranged from 45 to 365 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \) (\( p<0.001 \); Fig. 3C). The slopes of the MCD of carbon quota and cell volume to irradiance were not significantly different (ANCOVA, \( p>0.05 \)).

Carbon quota to cell volume relationship during cell division

Significant relationships between carbon quota and cell volume during cell division were obtained at the three light intensities (\( p<0.01 \) for 45 and 150 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \); \( p<0.001 \) for 365 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \); Fig. 4). The slopes of the line with 95% confidence intervals were 0.59±0.40 pgC \( \mu \text{mol}^{-3} \), 0.66±0.39 pgC \( \mu \text{mol}^{-3} \), and 0.98±0.27 pgC \( \mu \text{mol}^{-3} \) under 45, 150, and 365 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \), respectively. The intercepts of the line with 95% confidence intervals were 0.22±0.79, -0.0098±0.74, and -0.64±0.48 under 45, 150, and
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365 μmol m$^{-2}$ sec$^{-1}$, respectively. The slope and the intercept were not significantly different among the light treatments (ANCOVA, $p>0.05$).

**Discussion**

The changes in the cell volume and carbon quota during cell division were consistent with previous studies conducted on nanophytoplankton such as Prymnesiophyceae (Varela & Harrison 1999), Prasinophyceae (Kohata & Watanabe 1989, DuRand et al. 2002), and Chlorophyceae (DuRand & Olson 1998). The use of steady state cultures grown in continuous culture compared to batch cultures allowed us to measure the change of cell volume and carbon quota during cell division at a given growth irradiance. Generally, the changes in cell volume and carbon quota within a one day cycle are characterized by a daytime increase due to photosynthesis and a nighttime decrease due to cell division. However, even within the nanophytoplankton size range, some diatoms display a distinctively different cell division pattern (Nelson & Brand 1979, Chisholm & Costello 1980, Berges et al. 1995, Needoba & Harrison 2004). Berges et al. (1995) showed the change in cell volume over one day in the diatom *Thalassiosira pseudonana* had two apparent peaks due to cell division over both the light and dark periods, while the carbon quota exhibited a similar change to other phytoplankton taxa. Even if one
deals with the change of cell volume and carbon quota during cell division in the natural nanophytoplankton community in the ocean, careful consideration needs to be paid to the community structure.

A variable range of carbon quota values can be found in the literature even for the same species (Falkowski et al. 1985, Montagnes et al. 1994, Fujiki (personal communication; Fig. 5). For example, the carbon quota was 6.97 pgC cell\(^{-1}\) for *I. galbana* at 16°C under 20–60 \(\mu\text{mol m}^{-2} \text{sec}^{-1}\) on a 14/10 h light/dark cycle with batch culture (Montagnes et al. 1994), whereas the carbon quota in the present study was 23.8 pgC cell\(^{-1}\) for the same species at 25°C under 45 \(\mu\text{mol m}^{-2} \text{sec}^{-1}\) on a 12/12 h light/dark cycle with continuous culture. The growth rate in Montagnes et al. (1994) was 0.32 d\(^{-1}\), whereas it was 0.12 d\(^{-1}\) in the present study. The growth stage when cells were harvested in Montagnes et al. (1994) and Fujiki & Taguchi (2002) was mid- or late-logarithmic, whereas it was in the stationary phase in the present study. Cellular biochemical components such as the protein, carbohydrate and lipid contents (main components in the carbon quota) are relatively higher in the stationary phase than in the logarithmic phase (Brown et al. 1996). Different culture conditions and difference in the growth stage when cells were harvested between Montagnes et al. (1994) or Fujiki & Taguchi (2002) and the present study might be the reason for the relatively higher...
carbon quota in the present study. Since cells divide faster with increasing irradiance, the average cell volume and carbon quota decreased, but their amplitudes increased in the present study (Fig. 3A and B). This pattern was consistent with data provided by Fujiki (personal communication). Carbon quotas in Fujiki (personal communication) changed from 23.6 to 10.6 pgC cell\(^{-1}\) with increasing irradiance from 25 to 750 \(\mu\text{mol m}^{-2}\text{sec}^{-1}\) under a 12/12 h light/dark cycle with the same species at the same temperature as in the present study under semi-continuous batch culture (Fig. 5). The stage of harvesting in Fujiki & Taguchi (2002) was mid- or late-logarithmic phase. Different culture methods and differences in the growth stage when cells were harvested between Fujiki & Taguchi (2002) and the present study might be the cause for the relatively higher carbon quotas observed in the present study. Opposite patterns have been reported for the relationship between carbon quota and irradiance with the same species at the same temperature as in the present study (Falkowski et al. 1985). Carbon quotas in Falkowski et al. (1985) increased from 10.3 to 20.0 pgC cell\(^{-1}\) with increasing irradiance from 30 to 600 \(\mu\text{mol m}^{-2}\text{sec}^{-1}\) under 24 h continuous light with the same species under a day-night light cycle (Lacour et al. 2012). A similar nitrogen-limited condition can be found at the sea surface in the euphotic layer where limited nitrogen usually reduces the growth of phytoplankton, and light is saturated under the light/dark cycle. Future studies are needed to investigate the carbon quota to cell volume relationship under nitrogen-limited condition and under a light/dark cycle to determine if the present relationship between cell volume and carbon quota is held under those conditions.

The response of MCD estimated using cell volume and/or carbon quota showed a hyperbolic relationship when the published experimental data were included (Eppley & Coatsworth 1966, Kohata & Watanabe 1986, 1989, Berdalet et al. 1992, DuRand & Olson 1998, Varela & Harrison 1999, DuRand et al. 2002, Ohi et al. 2002, Ohi et al. 2003; \(p<0.001\)); Fig. 6). The initial slope that was obtained was almost linear, particularly for the irradiance range employed in the present study. The initial slope was not different from the slope of the relationship between irradiance and cell volume or carbon quota (Fig. 3C). Analysis of the diel observations published for five different species (Pyramimonas parkeae, Nannochloris sp., Emiliania huxleyi, Micromonas pusilla, Isochrysis galbana) and including our results yields a significant regression line (\(p<0.001\)); Fig. 7).

\[
\log C = 0.97 \log V - 0.58
\]
This relationship could be characterized as phylogenetic allometry (Gould 1966). What kind of relationship exists between MCD and the slope of carbon quota to cell volume during cell division? Generally, the degree of leaning of the linear function indicates the ratio of an increment of y to an increment of x. When applied to the present study, an increment of x or y means the amount of change in carbon quota or cell volume within a day, in other words, the MCD of carbon quota or cell volume. Because both carbon quota and cell volume change in a similar manner during cell division, the slope of the carbon quota to cell volume relationship may not vary significantly with irradiance, even though the MCD of carbon quota and cell volume changes hyperbolically with irradiance. Investigations on the carbon quota to cell volume relationship during cell division of nanophytoplankton, over a wide range of cell volumes, will help in understanding the ecology of nanophytoplankton communities. Although the carbon quota to cell volume relationship applies for the size range of nanophytoplankton occurring in the ocean, the species composition should be taken into consideration, particularly diatoms due to their characteristic manner of cell division in the euphotic layer (e.g. Needoba & Harrison 2004).

The present results indicate that the relationship between cell volume and carbon quota during cell division can be fitted to a single line within the nanophytoplankton size range. The results of the present study also suggest that light intensity might not affect this carbon quota to cell volume relationship. If such a trend is a general feature for nanophytoplankton in the natural environment, this would suggest that estimating biomass from the carbon quota to cell volume relationship may be justified in some oceanic situations—regardless of the sampling time and the depth of the euphotic layer. Since the cell size and division manner of *J. galbana* is similar to that of the majority of nanophytoplankton, the results of the present study provide important information for the interpretation of the carbon quota to cell volume relationship in other nanophytoplankton in the ocean.

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