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Effect of temperature on diatom volume, growth rate, and carbon and nitrogen content: Reconsidering some paradigms

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Abstract

We examined the response of diatoms to naturally experienced temperatures and tested these hypotheses: (1) diatoms follow the rule that organism size decreases with increasing temperature; (2) diatom growth rate follows a Q_{10}-like response; (3) diatom carbon (C) and nitrogen (N) content per unit volume (V) decrease with increasing size, and changes in temperature affect this relationship; and (4) diatom C : V is the same as that of other phytoplankton. We also present, as predictive equations, relationships between (1) growth rate, temperature, and size; (2) C content and V; and (3) N content and V. Eight diatoms and two flagellates were acclimated for approximately five generations and grown for approximately five more generations at five temperatures (9–25°C) on a 14 : 10 light : dark cycle at ~50 μmol photons m^{-2} s^{-1}. Growth rate, cell V, and C and N content per cell were measured; relationships between these parameters and temperature were determined. For five diatoms and both flagellates, cell V decreased with increasing temperature; cells decrease by ~4% of their mean V per °C. Growth rate appeared to increase linearly with temperature in all cases. The literature suggests that a linear response is the rule, not the exception. Temperature did not significantly affect C or N per V of diatom species. When all diatoms were considered, both C and N per V decreased with increasing cell size; our data support the argument that diatoms differ from other protists in this respect, but the difference is less pronounced than stated in previous reports.

As diatoms are indisputably a major component of many food webs, estimating their abundance, biomass, and growth rate has been, and will be, an essential component of marine studies. Like all organisms, diatoms are influenced by ambient temperatures, a point that has long been accepted (e.g., Eppley 1972; Goldman and Carpenter 1974). There is now an increasing awareness of global-warming impacts and other anthropogenic and natural changes in marine systems. Concomitantly, there is a need to better understand the influence of temperature on phytoplankton in general and on diatoms specifically.

This study improves our ability to assess the effect of temperature change on diatoms by making estimates of how their size, biomass, and growth rate vary over naturally occurring ranges. Furthermore, three biological paradigms are examined: the rule of diminishing size with increasing temperature (Atkinson 1994); the Arrhenius/Q_{10} relationship (e.g., Cossins and Bowler 1987); and the difference in carbon : volume (C : V) ratio between diatoms and other phytoplankton (Strathmann 1967).

Paradigm i—Reviews by Atkinson (1994, 1995) indicated that for ectotherms, size decreases with increasing temperature. One of the few exceptions to this rule was the diatom *Phaeodactylum tricornutum* (Atkinson 1994), but there are other conflicting reports for diatoms: V may decrease (Jørgensen 1968; Margalef 1969; Olson et al. 1986), increase (Durbin 1977; Thompson et al. 1992), or show no clear trend with temperature (Yoder 1979; Verity 1981; Sournia 1982). Clearly, this phenomenon needed further investigation, as changes in diatom size can affect sedimentation rate, feeding pressures by zooplankton, and autotrophic production.

Paradigm ii—Phytoplankton growth rate increases with temperature, and an exponential (Arrhenius/Q_{10}) relationship is usually assumed between temperature and growth rate (e.g., Eppley 1972; Raven and Geider 1988). However, work on other protists indicates that, for single species, this relationship is often linear (e.g., Montagnes and Lessard 1999). Such relationships are used in food-web models and in the scaling of allometric relationships. Thus, if accurate predictions are to be made, there is a need to determine if the temperature-growth relationship is exponential or linear.

Paradigm iii—Although diatom V may vary with temperature, C per cell may not change at the same rate (Durbin 1977; Raven and Geider 1988), i.e., C : V ratio may not be constant. Thus, production in terms of C (and possibly N) and quality of diatoms as food may not parallel temperature-induced changes in cell V. Furthermore, Strathmann (1967) and Menden-Deuer and Lessard (2000) indicated that the diatom C : V ratio differs from that of other phytoplankton. Data collected by Montagnes et al. (1994) suggest that this might not be the case; we reconsider this contradiction.

To interpret the impact of temperature on diatoms, we have examined cell size, specific growth rate, and biomass (i.e., C and N) under a range of naturally experienced temperatures. The following hypotheses were tested: (1) diatoms are not an exception to the rule that organism size decreases with increasing temperature; (2) diatom growth rate follows a Q_{10}-like response, as seen for many ectotherms (e.g., Ep-

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Table 1. The species used in this study and the geometric shapes used to estimate their volume. Mean cell volume is the average volume calculated over the experimental temperature range. Slope is the relationship between growth rate (μ day^{-1}) and temperature (°C), presented in Fig. 1.

Species	Shape	Mean volume (μm^3)	Slope (μ vs. °C)
Diatoms			
Coscinodiscus sp.	cylinder	1,500,000	0.024
Cyclotella cryptica	cylinder	700	0.020
Ditylum brightwelii	triangular prism	16,400	0.037
Phaeodactylum tricornutum	two cones × 1.5*	80	0.058
Skeletonema costatum	cylinder	200	0.036
Thalassiosira eccentrica	cylinder	60,000	0.014
Chaetoceros simplex var. *calcitrans*	cylinder	160	—†
Thalassiosira weissflogii	cylinder	1,960	—†
Flagellates			
Isochrysis galbana	prolate sphereoid	80	0.010
Rhodomonas salina	prolate sphereoid	300	0.061

* 2 × a cone produces the volume of half a prolate spheroid; *Phaeodactylum tricornutum* volume was approximated as two ''rounded cones'' and is thus 1.5 times the double-cone shape.
† Insufficient data were available to determine the slope (*see* Methods).

pley 1972; Cossins and Bowler 1987; Raven and Geider 1988); (3) diatom C and N content per unit V decreases with increasing size, and changes in temperature will affect this relationship; and (4) diatom C : V ratio does not differ from that predicted for other phytoplankton.

Besides testing these theories, we also present relationships between (1) growth rate, temperature, and size; (2) C content and V; and (3) N content and V, as predictive equations. These equations will aid the numerical modeling of marine systems and the determination of rate processes from field studies.

Materials and Methods

Stock cultures—Phytoplankton (Table 1) stock cultures were maintained in 100-ml glass flasks in f/2 media (Guillard 1972) at 16°C on a 14 : 10 light : dark (LD) cycle between 10 and 100 μmol photons m^{-2} s^{-1}. Cultures were transferred weekly and were maintained in exponential phase.

Acclimation, experimental batch culture conditions, and sample size—Phytoplankton species were initially acclimated to experimental light and temperature conditions in 50-ml tubes in f/2 media. Light was provided on a 14 : 10 LD cycle at ~50 μmol photons m^{-2} s^{-1}. Each culture was acclimated, in exponential phase, in a tube for about five generations; 5–10 ml of each culture was then inoculated into three 500-ml spherical, flat-bottomed, glass flasks containing 375 ml of f/2. Thus, by serial dilution, the cultures were maintained in exponential phase.

The three 500-ml flasks were placed in a temperature-regulated water bath (±1°C) and exposed to light at ~50 μmol photons m^{-2} s^{-1}; cells were allowed to grow in exponential phase for a further three to five generations. All subsequent measurements (*see below*) were made independently on each of these three flasks (i.e., at each temperature, for each species, $n = 3$). All cultures were mixed at least twice a day.

Growth rate calculations—Fluorescence of cultures was measured daily (model 10-005R fluorometer, Turner Designs), and growth was calculated as the slope of *ln* fluorescence versus time, providing an estimate of specific growth rate (μ day^{-1}). Such growth rate estimates, using fluorescence, are identical to those based on cell numbers when measured during exponential growth phase, when cell numbers are sufficiently low to avoid self-shading. For the large species *Thalassiosira eccentrica* and *Coscinodiscus* sp., which sink rapidly and cannot be measured in fluorometer tubes, cells were counted and growth rate was determined by change in numbers. After experimental cultures were allowed to grow for approximately five generations, they were harvested, in mid-log phase, for cell V and C and nitrogen (N) quota analyses.

Cell volume—Live cells were examined. Using an inverted microscope and 10-ml settling chambers, 100 randomly selected cells were measured. For each species, two linear dimensions were measured, and V was calculated assuming a standard geometric shape (Table 1). Measurements were made with an ocular micrometer or an image analysis system connected to the microscope. For the two motile flagellate species, only active cells were measured.

Carbon and nitrogen—Concurrent with the sampling of cultures for cell V analysis, 5- to 30-ml samples were filtered through precombusted (450°C, 4 h), 13-mm-diameter Whatman GF/F filter papers. Total C and N of these filters was determined by elemental analysis (NC 2500, CE Instruments). Blanks were run routinely by omitting the filtration step and were used to correct for background measurements. Cell abundance in these samples was determined by triplicate counts with 1-ml Sedgewick-Rafter chambers. These

data were then used to calculate C and N per cell, and cell V data were used to calculate C and N per cubic micrometer.

Data presentation and treatment—Least-squares regression analysis was performed using functions in Sigmaplot (V. 5, SPSS). Statistical tests followed those described by Zar (1984). Data were examined to determine if they followed a linear or positive-exponential relationship by methods outlined by Montagnes and Lessard (1999). Curves were fit to data where growth rate was positive and had increased with temperature, and more than two temperatures were examined. All three replicates at each temperature were used in this analysis; thus, a total of 9–15 measurements were used to fit each curve (*see Fig. 1*). The shape of the line was assessed by a nonlinear curve-fitting program (Sigmaplot, V. 5, SPSS) to fit data to the equation $\mu = a \times t^b + c$ (where μ is growth rate, t is temperature, and a, b, and c are constants); the automatic initial-parameter estimator function in Sigmaplot was used. The program provides an asymptotic standard error for estimated parameters. The error term associated with ''b'' was used to test the null hypothesis $b = 1$ (one-way t-test, $\alpha = 0.05$) against the alternate hypothesis $b > 1$. If the null hypothesis could not be rejected, the line was considered linear; if $b > 1$, it was considered positively exponential. Note that failing to reject the null hypothesis ($b = 1$) is the conservative approach, and it is possible that other, more complex relationships might fit the data. We have taken the parsimonious approach, i.e., that a linear relationship is the simplest fit to the data if it is not possible to provide evidence for an exponential fit.

When b was not significantly different from unity, least-squares linear regression was used to fit a line through the data. The slope of the response of growth rate (μ day^{-1}) to temperature (°C) for several other marine planktonic diatoms was also examined (Table 2).

To determine if C and N per cell V varied as a function of cell V, allometric relationships were determined following methods outlined by Strathmann (1967), Montagnes et al. (1994), and Menden-Deuer and Lessard (2000). Using C as an example, this method examines the slope of the relationship between log C per cell and log-cell V. If the slope differs from 1, then there is an allometric relationship. Several studies have shown this slope to be <1 (*see* Menden-Deuer and Lessard 2000), indicating that large cells have less C per unit V than small cells.

Results

Cell volume as a function of temperature—For five of the eight diatom species and both flagellates, there was a significant decrease in cell V with increasing temperature (Fig. 1, top panels). For *Coscinodiscus* sp., when all cells were considered, cell V increased with increasing temperature (Fig. 1F). However, this trend was driven by a few large (>300 μm in diameter) cells: 0% of cells between 10 and 17°C, 9% of cells at 20°C, and 15% of cells at 25°C were >300 μm. When the large cells were excluded, there was no significant relationship between cell V and temperature (Fig. 1F, open squares at 20 and 25°C).

We examined the log–log relationship between the absolute slope of cell V versus temperature and mean cell V (determined over the range of experimental temperatures) for all diatoms and flagellates that showed a significant decrease in V with temperature (Fig. 2). The slope of this regression was not significantly different from unity (t-test, $\alpha = 0.05$), indicating that regardless of size, cells decrease by a constant exponential rate with temperature. For all seven species, the average value of the absolute slope divided by the mean V of a species was 0.039 ± 0.008 (SE), indicating that cells decrease by ~4% of their mean V per °C.

Growth rate as a function of temperature—In all cases, there was an increase in specific growth rate with increasing temperature (Fig. 1, bottom panels); however, for *Thalassiosira weissflogii* (Fig. 1E), this increase occurred only between 9 and 12°C and was followed by a decline in growth rate between 12 and 25°C. Other species also decreased in growth rate after reaching a maximum (Fig. 1B,C,F,G,J).

Growth rate did not exponentially increase with temperature in any case (Fig. 1, bottom panels). For all species tested (Table 2), the exponent ''b'' for the relationship $\mu = a \times t^b + c$ (where μ is growth rate, t is temperature, and a, b, and c are constants) could not be shown to be greater than unity ($\alpha = 0.05$, power of tests ranged from 0.73 to 0.99). When least-squares linear regression was applied to the relationship between growth rate and temperature for our diatom data, the mean slope was 0.032 μ °C^{-1}. The mean slope of this response was also determined for data collected from the literature (Table 2). The mean slope ±SE for all data on diatoms (Table 2) was 0.056 ± 0.006, suggesting that the linear relationship between growth rate (μ day^{-1}) and temperature (°C) has a slope ~0.05–0.06.

Carbon and nitrogen per unit volume as a function of temperature—When all diatoms were considered, there was no significant relationship ($\alpha = 0.05$) between change in temperature and C or N per unit V (Fig. 3). However, because of unusually high levels of C and N in *Rhodomonas salina* at 8°C, there was an influence of temperature on flagellate C and N levels.

Carbon and nitrogen per cell as a function of volume—Diatom C and N per unit V did not vary with temperature (*see* ''Carbon and nitrogen per unit volume as a function of temperature''). Thus, data for all temperatures were combined to determine diatom allometric relationships for C and N (Fig. 4). For both relationships, the slope of the line was significantly less than unity ($\alpha = 0.05$), indicating that C and N per unit V are size-dependent. The parameters for the equations, illustrated in Fig. 4, are presented in Table 3.

Discussion

Cell volume as a function of temperature—Although diatom cell size has been observed to decrease with increasing temperature (Jørgensen 1968; Margalef 1969; Olson et al. 1986), there is some evidence to the contrary (Durbin 1977; Fawley 1984; Thompson et al. 1992), and other studies do not indicate a clear trend (Strathmann 1967; Yoder 1979; Verity 1981; Sournia 1982). However, many of these studies

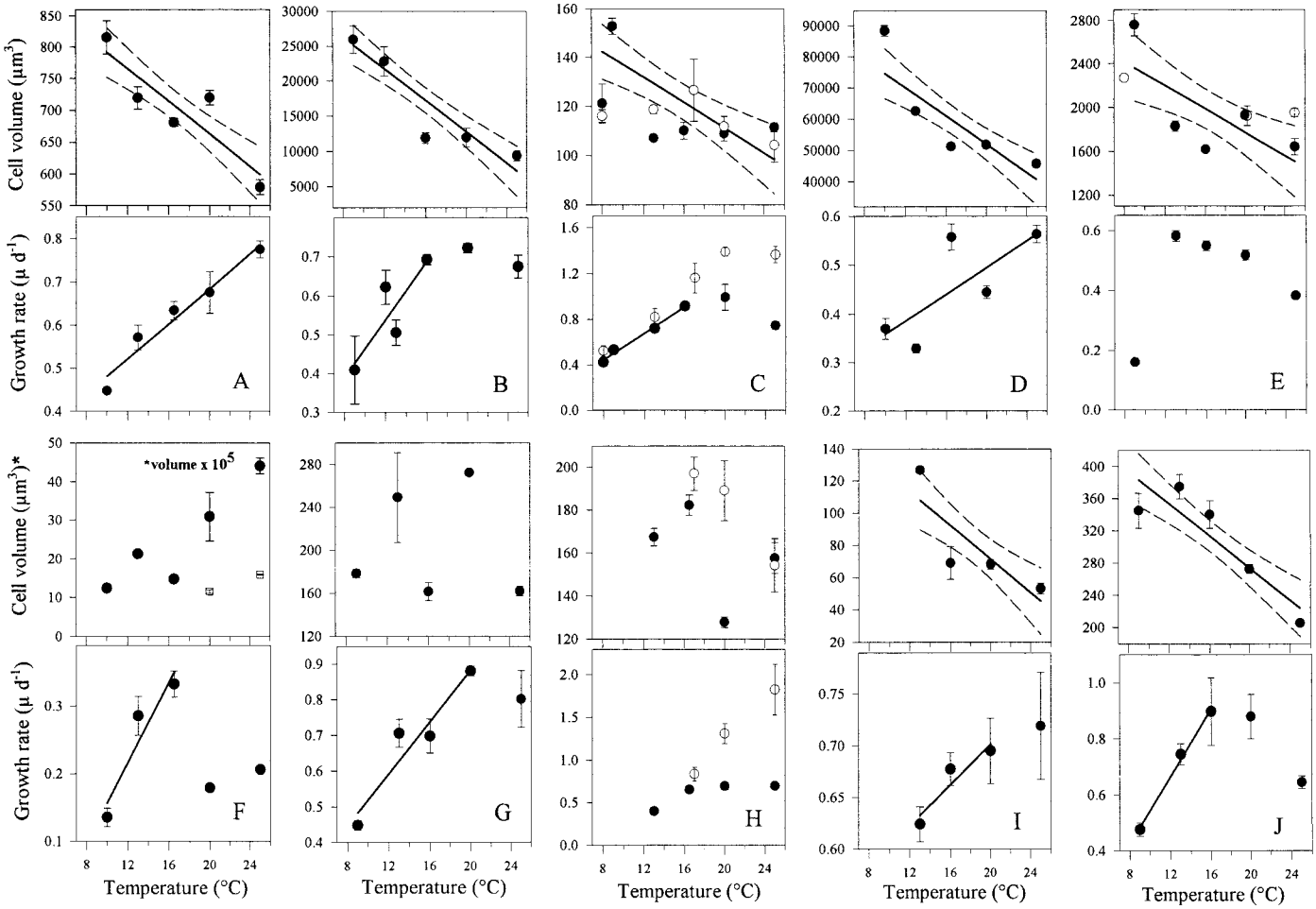

Fig. 1. The relationship between cell V (top panels) and growth rate (bottom panels) for eight diatoms (A–H) and two flagellates (I,J). Solid circles represent the mean of three values; error bars are 1 SE; (A) *Cyclotella cryptica,* (B) *Ditylum brightwelii,* (C) *Phaeodactylum tricornutum,* (D) *Thalassiosira eccentrica,* (E) *Thalassiosira weissflogii,* (F) *Coscinodiscus* sp., (G) *Skeletonema costatum,* (H) *Chaetoceros simplex* var. *calcitrans,* (I) *Isochrysis galbana,* and (J) *Rhodomonas salina.* Regression lines (solid lines) were determined for V versus temperature data where a significant relation existed (*see* Results); dashed lines are the 95% confidence intervals. Open circles are data from experiments conducted by D. Franklin (unpubl. data) under conditions identical to those described in Methods, except that constant light was applied at ~130 μmol photons m^{-2} s^{-1}; regressions do not include open data circles. Open squares (F, top panel) represent data where large cells (>300 μm in diameter) are excluded (*see* Materials and Methods as well as Discussion for details).

were not specifically aimed at examining cell V, and there are potential errors associated with some of the data. To our knowledge, the present study represents the first systematic examination of this phenomenon, comparing the response of cell V to temperature of several diatom species. For most species examined, there was a decrease in size with increasing temperature (Fig. 1). Furthermore, the two flagellates followed the same trend. These data support a trend exhibited not only by phytoplankton (Margalef 1969) but one also exhibited by most ectotherms (Atkinson 1994).

However, our data do show exceptions to the rule: we lack an explanation as to why *Chaetoceros simplex* var. *calcitrans* (Fig. 1H) did not decrease in size with increasing temperature, but there may be reasons for the anomalous trend exhibited by *Coscinodiscus* sp. and *Skeletonema costatum* (Fig. 1F,G). *Coscinodiscus wailesii* shows a sudden increase in cell V when switched to higher temperatures (Nagai et al. 1995). This enlargement is vegetative rather than induced by sex, the commonly recognized means by which diatoms increase in size after repeated divisions reduce cell size and viability (Edlund and Stoermer 1997). Such vegetative increases occur in several species; specifically, they occur in laboratory and natural populations of *C. wailesii* (*see* Nagai et al. 1995 and references within) and laboratory populations of *S. costatum* (Gallagher 1983). Vegetative enlargement is interpreted as a bypass of sexual reproduction for short-term competitive advantage (Gallagher 1983; Edlund and Stoermer 1997). In this study, large cells occurred only at the highest temperatures (Fig. 1F), and the percentage of large cells increased with temperature. The presence of these cells, although deviating from the trend shown by the other diatoms, supports the concept that vegetative cell enlargement may be induced by temperature change alone and not the combination of light and temperature (*see* Nagai et al. 1995).

Table 2. The response of growth rate (μ day^{-1}) to temperature for marine and estuarine diatoms and two flagellates; E, exponential response; L, linear response. Where necessary, irradiance was converted to μmol photons m^{-2} s^{-1} by values in Parsons et al. (1984).

Species	Slope (μ day^{-1} vs. °C)	Source	Curve shape	Comments
Diatoms				
Coscinodiscus sp.	0.024	this study	L	grown at ~50 μmol m^{-2} s^{-1}, 14:10 LD
Cyclotella cryptica	0.020	this study	L	grown at ~50 μmol m^{-2} s^{-1}, 14:10 LD
Ditylum brightwelii	0.037	this study	L	grown at ~50 μmol m^{-2} s^{-1}, 14:10 LD
Phaeodactylum tricornutum	0.058	this study	L	grown at ~50 μmol m^{-2} s^{-1}, 14:10 LD
Phaeodactylum tricornutum	0.046	Fawley (1984)	L*	grown at 54 μmol m^{-2} s^{-1}, 14:10 LD
Phaeodactylum tricornutum	0.077	Fawley (1984)	L*	grown at 208 μmol m^{-2} s^{-1}, 14:10 LD
Phaeodactylum tricornutum	0.055	Thompson et al. (1992)	L†	grown at 220 μmol m^{-2} s^{-1}, 24:0 LD
Phaeodactylum tricornutum	0.066	Li and Morris (1982)	L†	grown at saturating, continuous light
Skeletonema costatum	0.036	this study	L	grown at ~50 μmol m^{-2} s^{-1}, 14:10 LD
Skeletonema costatum	0.064	Jørgensen (1968)	L†	grown at ~400 μmol m^{-2} s^{-1}
Skeletonema costatum	0.067	Suzuki and Takahashi (1995)	L†	grown at 200 μmol m^{-2} s^{-1}, 24:0 LD
Skeletonema costatum	0.107	Langdon (1988)	L†	maximum predicted growth
Skeletonema costatum	—	Yoder (1979)	E‡	grown at subsaturating light
Thalassiosira eccentrica	0.014	this study	L	grown at ~50 μmol m^{-2} s^{-1}, 14:10 LD
Thalassiosira nordenskioeldii	0.027	Suzuki and Takahashi (1995)	L†	grown at 100 μmol m^{-2} s^{-1}, 24:0 LD
Thalassiosira pseudonana	0.098	Thompson et al. (1992)	L†	grown at 220 μmol m^{-2} s^{-1}, 24:0 LD
Thalassiosira fluviatilis	—	Hobson (1974)	E†	grown at saturating light
Chaetoceros sp.	—	Lomas and Gilbert (1999)	E‡	grown at 150 μmol m^{-2} s^{-1}, 14:10 LD
Chaetoceros simplex	0.098	Thompson et al. (1992)	L†	grown at 220 μmol m^{-2} s^{-1}, 24:0 LD
Chaetoceros gracilis	0.098	Thompson et al. (1992)	L†	grown at 200 μmol m^{-2} s^{-1}, 24:0 LD
Detonula confervacea	0.036	Suzuki and Takahashi (1995)	L†	grown at 100 μmol m^{-2} s^{-1}, 24:0 LD
Pseudo-nitzschia pseudodelicatissima	0.093	Lundholm et al. (1997)	L†	grown at ~50 μmol m^{-2} s^{-1}, 16:8 LD
Nitzchia americana	—	Miller and Kamykowski (1986)	E†	Based on three points, grown at 30 μmol m^{-2} s^{-1}
Rhizosolenia fragilissima	0.037	Ignatiades and Smayda (1970)	L†	grown at *t* maximum light levels
Flagellates				
Isochrysis galbana	0.010	this study	L	grown at ~50 μmol m^{-2} s^{-1}, 14:10 LD
Rhodomonas salina	0.061	this study	L	grown at ~50 μmol m^{-2} s^{-1}, 14:10 LD

* From a regression presented in a figure.
† Visually interpreted, by us, from a graph derived from a table in a paper or from data read from a graph.
‡ Stated in the published paper.

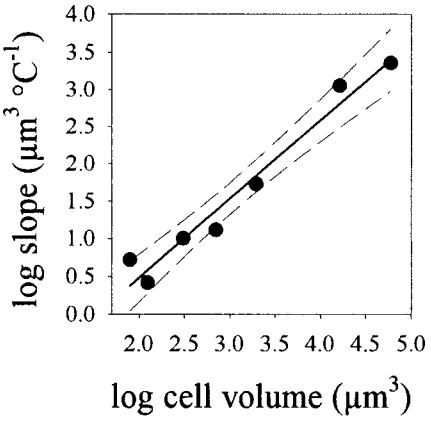

Fig. 2. The relationship between the log-absolute slope of cell V versus temperature and the log-mean cell V (determined over the range of experimental temperatures) for all diatoms and flagellates that showed a significant decrease in V with temperature (regression lines in Fig. 1). The solid line is the regression through the data, and dashed lines are the 95% confidence intervals.

There is also one notable report of a diatom being an exception to the rule: *P. tricornutum* increased in size with temperature (Fawley 1984; Atkinson 1995). This study, however, indicated that *P. tricornutum* decreased in size with increasing temperature, although this relation was weak (Fig. 1C). What are the reasons for these differing results? Using an ocular micrometer, Fawley (1984) measured 10 cells per temperature and found width, but not length, changed with temperature. Using image analysis, we measured 100 cells in three replicates at six temperatures and found that length (data not shown) and V (Fig. 1C) significantly decreased with increasing temperature, but width did not significantly change with temperature (data not shown). *P. tricornutum* is small, and width changes are ~1 μm. Such changes would be difficult to measure with an ocular micrometer, and their accuracy would be improved by image analysis. Furthermore, we measured more cells than Fawley (1984); thus, our estimates are likely more accurate and precise. We also used a larger range of temperatures (8–25°C) compared to 14–23°C employed by Fawley (1984) and Atkinson (1995). However, our regression was strongly influenced by points

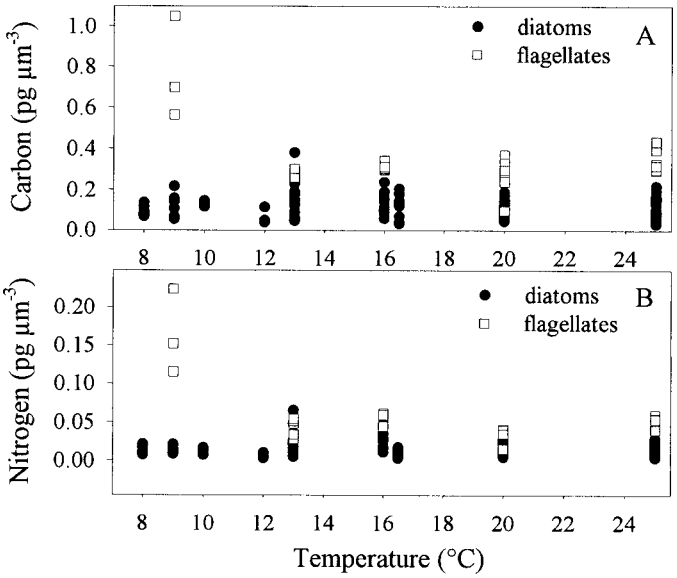

Fig. 3. Relationships between (A) C per unit V and temperature and (B) N per unit V and temperature for eight diatoms and two flagellates (Table 1). For both panels, solid circles represent mean values for each diatom species, and open squares represent flagellates.

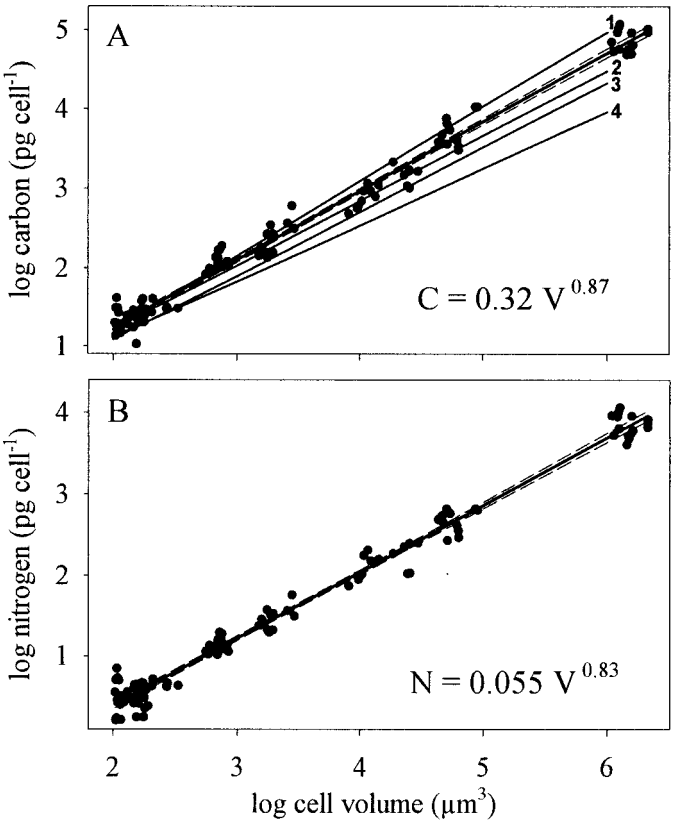

Fig. 4. Log–log relationships between (A) C per cell and cell V and (B) N per cell and cell V. Circles represent diatoms from this study only. The solid lines with dashed confidence intervals (95%) are the least-squares fit through the data. The numbered lines are regressions obtained from (1) Menden-Deuer and Lessard (2000), for all protists; (2) Blasco et al. (1982), for diatoms; (3) Menden-Deuer and Lessard (2000), for diatoms only; and (4) Strathmann (1967), for diatoms. *See Table 3* for parameters of these lines.

below 14°C, and this may further explain the difference in results.

Although there are specific exceptions to the rule, we conclude that diatoms, in general, are not an exception to the rule that organism size decreases with increasing temperature (Atkinson 1994). However, the specific exceptions are intriguing, and their anomalous behavior can help elucidate unique adaptive strategies, e.g., the vegetative increase in size of *Coscinodiscus* sp. at high temperatures.

Why do diatoms decrease in size with increasing temperature?—Is this trend real, or is it an artifact of the decrease in cell size attributed to the diatom cell cycle (*see* Edlund and Stoermer 1997)? First, all of our cultures were grown for a similar number of generations rather than for a discrete time. Thus, if size reduction occurred only because of repeated divisions, the effect would have been similar at all temperatures. Second, visual inspection of cell size distribution at single temperatures indicated a normal rather than a skewed distribution of cell sizes (data not shown); this suggests that no progression to smaller cells occurred within a single treatment. Third, stock cultures were maintained at ~16°C; diatoms introduced to conditions below this temperature increased in size (Fig. 1); we might then extend the argument to suggest that the decrease in size at higher temperatures was due to increased temperature. Finally, a number of diatoms maintain a constant cell size even after numerous generations (Edlund and Stoermer 1997).

Table 3. A comparison of the parameters of the formula $\log y = \log a + \log x \cdot b$ from several sources (Fig. 4), where y is carbon (C) or nitrogen (N) as pg cell^{-1}, x is cell volume (μm^3), and a and b are constants. Values are presented ± standard error.

Material	Taxa	a	b	r^2	n	Source
y = C	diatoms	−0.420±0.039	0.850±0.010	0.984	112	this study
y = N	diatoms	−1.187±0.039	0.809±0.011	0.982	112	this study
y = C	planktonic protists	−0.665	0.939*	0.96	91	Menden-Deuer and Lessard (2000)
y = C	diatoms	−0.541	0.811*	0.97	94	Menden-Deuer and Lessard (2000)
y = C	diatoms	−0.314	0.712*	—	—	Strathmann (1967)
y = C	diatoms	−0.423	0.817*	0.98	6	Blasco et al. (1982)†

* Values that significantly differ from the estimate made in this study (t-test, α = 0.05).
† Calculated from table 1 in Blasco et al. (1982), as parameters in Blasco et al. (1982, table 3), were derived from model II regressions.

Consequently, we conclude that diatom size change was primarily induced by temperature; similar changes occurred for the two flagellates.

It is not the intention of this work to discuss extensively why ectotherms follow the temperature-size rule; the reader is directed to Atkinson (1994) and Atkinson and Sibly (1997). However, we will speculate briefly on some trends. First, one possible benefit of the decrease in size of phytoplankton with increasing temperature is that sinking rate might be reduced. Water viscosity decreases as temperature rises, potentially increasing sinking rate. Following Stokes' Law, as diatoms shrink, they reduce their sinking rate. Size reduction may thus be an adaptive response to limit sinking rate. However, the smaller vacuole size and higher frustule : cytoplasm of small diatoms may increase sinking rate; hence, reduced size may not reduce sinking (Walsby and Reynolds 1980). Diatoms may also avoid sinking by decreasing their density, but no temperature-induced change in C or N per unit V occurred (Fig. 3). This implies that cell density, of these major cell constituents, may not change with temperature. We are at present investigating the potential impact of temperature-induced size reduction on sinking by comparing size changes of benthic and planktonic diatoms (Franklin, Muehlig-Hoffman, and Montagnes, 2000, British Phycological Society, winter meeting, poster presentation; Franklin et al. unpubl. data).

Data from this study can only be used to speculate on physiological reasons for temperature-induced size reduction of diatoms. Von Bertalanffy (1960) suggested that temperature-induced changes in the balance between anabolic and catabolic processes alter organism size; Atkinson and Sibly (1996, 1997) have supported this argument. The general concept is appealing: if cell composition remains constant, and cells shrink, then there must be an imbalance between cellular gain and loss terms. This relationship would be complicated by metabolic expenditures as cells change in growth rate with increasing temperature, but this does not alter the general model of change in gains and losses. This concept is supported by the relative change in several processes with changing temperature (*see* Raven and Geider 1988; Atkinson and Sibly 1997). Comparing the relative temperature dependence of anabolic and catabolic processes is an ambitious but potential direction of future research. Furthermore, the trend in which all cells seem to reduce by ~4% their mean cell V per °C provides a guideline to this relationship. We are presently investigating if this phenomenon of a 4% change is universal for all protists (Montagnes et al. unpubl. data, Montagnes et al. in press).

Growth rate as a function of temperature—It is well established that growth rate increases with increasing temperature (Cossins and Bowler 1987). Typically, this response is assumed to be exponential, but there is a history of reports suggesting it can be linear (*see* Cossins and Bowler 1987). Thus, the paradigm that protist growth rate increases exponentially with increasing temperature (e.g., $Q_{10} = 2$ or follows an Arrhenius relationship: Goldman and Carpenter 1974; Harris 1986) may not hold true (e.g., Ahlgren 1987; Thompson et al. 1992; Weisse and Montagnes 1998; Montagnes and Lessard 1999).

Our examination of the response of the specific growth rate (μ) of diatoms to increases in temperature suggests a linear relationship. The data (Fig. 1; Table 2) indicate that the relationship cannot be shown to deviate significantly from linearity, and visual interpretation of other responses from the literature (Table 2) suggests that a linear relationship is common.

The mean slope of this response was between 0.05 and 0.06 μ °C^{-1} (Table 3). However, the data in Table 2 include species growing under a variety of light regimes, and the response would be higher if only light-saturated responses were examined, i.e., closer to 0.07–0.08 μ °C^{-1}. An upper value of 0.07 μ °C^{-1} is similar to that derived for the linear relationship between planktonic ciliate growth rate and temperature (Montagnes and Lessard 1999). Possibly, this is a constant, common to many protists; we are at present pursuing this hypothesis (Montagnes et al. unpubl. data, in press).

It might be argued that an exponential response would occur at higher light intensities, as there are interactive effects of light and temperature (Harris 1986; Raven and Geider 1988), and at low light levels, there is possibly no effect of temperature on growth (e.g., Harris 1986). However, data on *P. tricornutum* reflecting the growth-temperature response over a range of light intensities indicated that the relationship remains linear, regardless of light intensity (Fawley 1984). Similarly, several of the linear responses reported in Table 2 were conducted at saturating light levels. The underlying theory for an exponential response of physical and chemical rate processes to temperature is thoroughly described elsewhere (e.g., Cossins and Bowler 1987), and this logic has then been applied to the growth rates of many protists. However, growth rate is undoubtedly a combination of chemical and physical processes, the sum of which might not yield an exponential response. There is clearly a need to further test, at a physiological level, the linearity of the growth response to temperature at saturating light levels. Unfortunately, it is not a simple task to pick a single saturating light level, as there can be interaction between light and temperature on photoinhibition of growth (Raven and Geider 1988).

Our observations (Fig. 1) are purely empirical. We do not postulate a physiological basis for the effect of temperature on growth rate; this may be done in subsequent studies. We simply indicate that, given the limitations of our data and of data in the literature, intraspecific change of diatom growth rate with temperature appears to follow a linear response. Thus, use of an exponential response to scale growth data to a single temperature (e.g., for allometric relationships or for food-web modeling) may be inappropriate. Until an exponential response is clearly demonstrated, we propose to err toward parsimony and consider the relationship between specific growth rate (μ) and temperature to be linear.

Predicting growth rate from cell volume and ambient temperature—To determine diatom growth rate from ambient temperature and cell V, we have developed two multiple regressions by performing iterative fits on the data (Sigmaplot 5.0, SPSS). In the first regression, only the diatom data where growth was not inhibited at high temperatures were

considered; these data were used to follow relationships, examining optimal growth, that exist in the literature (e.g., Montagnes 1996). However, natural conditions may include high temperatures that inhibit growth; thus, the entire diatom data set was also used to determine a more robust, predictive relationship.

On the basis of the assumption that growth rate is inversely related to the log of cell V (e.g., Banse 1982; Harris 1986) and linearly related to temperature (this study), we have established the relationship $\mu = 0.544 + 0.0206 \times T - 0.0864 \times \log V$, where μ is growth rate (day^{-1}), T is temperature (°C), and V is volume (μm^3) (Fig. 5a); this relationship included only data where growth increased with temperature. This regression was significant ($P < 0.001$), parameters of the equation have coefficients of variation ~10%, and the adjusted R^2 for the fit was 0.69; these statistics indicate a relatively good predictive ability of the equation. The second equation, which used all the data, was based on Berthelot's temperature relationship (rate = A × B^T, where T is temperature, and A and B are constants; Cossins and Bowler 1987), growth rate being inversely related to the log of cell V, and an interaction between temperature and cell V; this predictive model was also chosen because it kept the number of parameters to a minimum while maintaining low error terms associated with these parameters. The second equation was $\mu = -2.40 \times 0.85^T - 0.0056 \times \log V \times T + 1.10$ (Fig. 5b). This regression was significant ($P < 0.001$), parameters of the equation have coefficients of variation ranging between 3 and 20%, and the adjusted R^2 for the fit was 0.63; these statistics also indicate a relatively good predictive ability.

Similar fits relating temperature and cell V to growth have been used for other protists (*see* Montagnes 1996) and can be used in numeric models to determine growth rates or to scale data for use in allometric relationships (e.g., Banse 1982; Tang 1995; Tang and Peters 1995). Several formulae have been used to develop such fits. We have attempted to choose the simplest equations that adequately describe the relationship. Note that these equations are predictive and are restricted to conditions where nutrients are replete and light intensity is ~50 μmol photons m^{-2} s^{-1}. Light will alter growth rate (and photosynthesis) and may interact with temperature effects (Goldman and Carpenter 1974; Falkowski and Raven 1997; Thompson 1999). Our choice of light intensity was intentional: predictive equations of phytoplankton growth often use data where light is saturating (e.g., Tang 1995). However, saturating light levels for diatoms are likely the exception rather than the rule under natural conditions. Thus, if only one light level is used, it may be more appropriate to examine subsaturating levels similar to those experienced in the water column. Nutrient levels may also interact with temperature to alter growth rate (Goldman and Carpenter 1974); thus, our models are not exhaustive and should be applied with prudence.

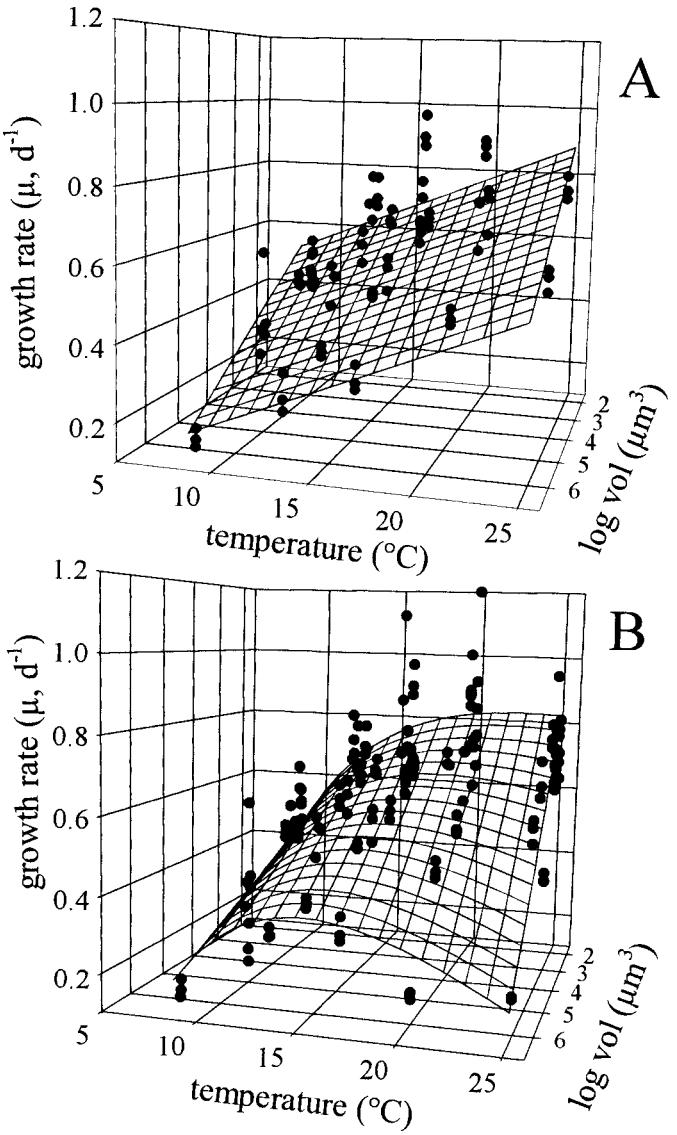

Fig. 5. Two predictive models indicating the relationship between diatom growth rate (μ day^{-1}), temperature (T), and cell V. Circles represent growth rates, and grids represent the best fit to the data. (A) Only diatom data where growth was not inhibited at high temperatures were considered: $\mu = 0.544 + 0.0206 \times T - 0.0864 \times \log V$. (B) The entire diatom data set was used: $\mu = -2.40 \times 0.85^T - 0.0056 \times \log V \times T + 1.10$. *See* "Predicting growth rate from cell volume and ambient temperature" for a further explanation.

Carbon and nitrogen per unit volume as a function of temperature—Contradictory reports exist concerning temperature effects on diatom C and N levels. Harris (1986), relying on the literature (Goldman 1977; Goldman and Mann 1980), indicated that C and N levels per diatom (cell quota) follow a U-shaped pattern with increasing temperature: quotas were high at the ends of the temperature range for growth and were low where temperature-stimulated growth was at a maximum. Thompson et al. (1992) studied five diatom species: three exhibited no response, while two exhibited a U-shaped response in C and N quotas. Thompson (1999) also indicated a significant effect of temperature on C and N quotas of *Thalassiosira pseudonana.* These data may indicate a trend, but their interpretation, in terms of C and N per unit V, is complicated by several factors: the work re-

viewed by Harris (1986) and the study by Thompson (1999) did not examine cell V changes, and light and nutrients were poorly controlled in the work reviewed by Harris (1986). Likewise, data in Thompson et al. (1992) indicated fluctuations in diatom V, which may have altered the C and N per unit V relationship with temperature.

Other reports are also confusing or contradictory. C per V in five diatoms measured between 10 and 20°C was not affected by temperature (Strathmann 1967). By contrast, between 5 and 20°C, C and N per unit V significantly increased with temperature for *Leptocylindrus danicus* (Verity 1981), and there was a peak in C at a mid-temperature (15–25°C) for *Chaetoceros curvisetum* but no effect on N per unit V (Furnas 1978). For *Thalassiosira nordenskioeldii,* Durbin (1977) suggested that C and N per unit V were higher at 0°C than at 10°C but, in the same work, indicated for C and implied for N that these differences were not significant. Furthermore, protein (and presumably N) increased in *S. costatum* between 7 and 20°C (Jørgensen 1968). Clearly, this relationship needed further examination.

Data from this study suggest that, for diatoms, there is no relationship between C and N per unit V and temperature. In contrast, the flagellate *R. salina* differed from the diatoms at low temperatures (Fig. 3A,B). The lack of change in either C or N per unit V allowed the use of data from all temperatures, for all diatoms to determine C and N to V relationships (Fig. 4A,B). Furthermore, this lack of dependence of C and N per unit V on temperature suggests that researchers collecting samples from natural waters ~8–25°C can use the relationships presented below (Fig. 4A,B). However, our work was conducted under nutrient-replete conditions, and this response may not hold true under limiting conditions. Thus, as for many lab studies, this relationship must be applied with some caution.

Carbon and nitrogen per cell as a function of volume—Several relationships between C or N and V have been developed for phytoplankton (e.g., Strathmann 1967; Blasco et al. 1982; Montagnes et al. 1994); for a review of these relationships for protists in general, *see* Menden-Deuer and Lessard (2000).

The much-cited work by Strathmann (1967) indicated that diatoms have a lower C : V than other phytoplankton, and this relationship is strongly size-dependent. Montagnes et al. (1994) questioned this difference but compared only 3 diatom species to 30 other phytoplankton species. Menden-Deuer and Lessard (2000) reviewed the literature and indicate that the diatom C : V relationship significantly differed from that of other protists but not to the extent predicted by Strathmann (1967). Below, diatom data from this study are compared to that from Strathmann (1967), Blasco et al. (1982), and Menden-Deuer and Lessard (2000).

Significant differences ($\alpha = 0.05$) exist between the slope of log C versus log V obtained from this study and that from the others: the slope from this study is lower than that for ''all other protists'' but is higher than other values for diatoms (Fig. 4; Table 2). Our slope is also significantly less than unity, supporting indications that C : V of diatoms decreases with increasing cell size (Strathmann 1967; Blasco et al. 1982; Menden-Deuer and Lessard 2000).

Data for *Ditylum brightwelii* are worth noting; our data lie near the regression. Strathmann (1967) indicated the C : V of *D. brightwelii* was unusually low; this was supported by Menden-Deuer and Lessard (2000), who removed these data from their C versus V regression. Our C : V values for *D. brightwelii* are more than fivefold higher than those presented by Strathmann (1967). In this study, V measurements for *D. brightwelii* were based on a triangular prism rather than a cylinder, which was used by Strathmann (1967); this would theoretically increase the C content by ~1.57, if all else was constant. Menden-Deuer and Lessard (2000) found that a similar correction did not bring Strathmann's data in line with other values. We have not found this to be the case and have included *D. brightwelii* in the analysis.

This study supports the hypothesis that, likely due to large vacuoles, diatoms possess less C per unit V than other phytoplankton (*see* Strathmann 1967; Menden-Deuer and Lessard 2000). However, it indicates that this difference is not as great as Strathmann (1967) suggested. There are several possible reasons for the difference between responses. Data presented by Strathmann (1967) and Menden-Deuer and Lessard (2000) were collected from studies that used a wide variety of methods, several of which may bias the results. Furthermore, as the relationship presented by Menden-Deuer and Lessard (2000) was based on literature data and was influenced by Strathmann's data, it is not surprising that their line deviates from ours.

Some methods applied in previous studies use fixatives or electronic particle counters, both of which will underestimate cell size and consequently increase C : V and N : V (*see* Montagnes et al. 1994). Furthermore, we followed methods outlined in Montagnes et al. (1994), who chose conditions that attempted to mimic those often experienced in situ: an LD cycle, rather than 24 h continuous light, and light at ~50 μmol photons m^{-2} s^{-1}, rather than saturating levels; these conditions may also alter C : V and N : V. However, we have considered only one diatom species greater than 10^5 μm^3; it is possible that inclusion of other larger diatoms would alter the relationship.

Given that (1) the data from this study lack biases caused by combining various methods, (2) our findings indicate that C and N per V are temperature invariant, and (3) the experiments were conducted under simulated in situ conditions, we suggest that the equations for C : V and N : V presented in this study are more applicable than those previously presented. We recommend use of these equations by plankton ecologists who estimate biomass from diatom cell V and by modelers who require conversion factors (e.g., for developing allometric relationships and assessing food-web dynamics). However, both nutrients and light may alter these relationships, and the caveat that neither of these parameters was varied must be recognized.

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