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Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton

Susanne Menden-Deuer[1] *and Evelyn J. Lessard*
School of Oceanography, University of Washington, Box 357940, Seattle, Washington 98195

Abstract

Cellular carbon and nitrogen content and cell volume of nutritionally and morphologically diverse dinoflagellate species were measured to determine carbon to volume (C : vol) and nitrogen to volume (N : vol) relationships. Cellular C and N content ranged from 48 to 3.0×10^4 pgC cell^{-1} and 11 to 2,656 pgN cell^{-1} for cells ranging in volume from 180 to 2.8×10^5 μm^3. C and N density in dinoflagellates decreased significantly with increasing cell volume. C : N ratios ranged from 3.44 to 6.45. C : vol and N : vol in dinoflagellates are significantly related as expressed by the equations pgC cell^{-1} = $0.760 \times$ volume$^{0.819}$ and pgN cell^{-1} = $0.118 \times$ volume$^{0.849}$. Previously published data were combined to compare C : vol relationships in different phylogenetic protist groups, including chlorophytes, chrysophytes, prasinophytes, and prymnesiophytes. Our analysis indicated differences between the C : vol relationships available for ciliates. A new C : vol relationship for diatoms was established (pgC cell^{-1} = $0.288 \times$ volume$^{0.811}$). Dinoflagellates are significantly more C dense than diatoms. Except for diatoms, we found few significant differences between C : vol relationships of different phylogenetic groups. Consequently, one C : vol relationship for taxonomically diverse protist plankton excluding diatoms was determined (pgC cell^{-1} = $0.216 \times$ volume$^{0.939}$). In the combined data set, carbon density was not constant but decreased significantly with increasing cell volume. Using constant C : vol conversion factors for plankton over large size ranges will cause systematic errors in biomass estimates.

The carbon biomass of planktonic organisms is a fundamental parameter in ecosystem models and biogeochemical carbon budgets. Temporal and spatial variability in total and export primary production can be quantified and predicted only if the carbon content of the major planktonic organisms is known. Carbon is the principal structural component of both heterotrophic and phototrophic organisms and is the basis for community-wide as well as group-specific comparisons of biomass and bioenergetics. Estimates of carbon biomass of planktonic organisms are usually made by converting microscopic size measurements to cell volumes, which are then converted to carbon biomass using empirically or theoretically derived carbon to volume ratios. Carbon to volume (C : vol) relationships have been reported for several planktonic groups, such as heterotrophic bacteria (e.g., Nagata and Watanabe 1990), diatoms (Strathmann 1967), phototrophic nanoplankton (Verity et al. 1992), various phytoplankton (Montagnes et al. 1994), ciliates (Verity and Langdon 1984; Putt and Stoecker 1989), and copepods (e.g., Uye 1982). However, no empirically derived C : vol relationship has been determined for dinoflagellates.

Dinoflagellates are a significant group of planktonic protists that includes toxic and bloom-forming species, as well as potentially important grazers on diatoms and other plankton. Dinoflagellates are nutritionally diverse; species can be phototrophic, heterotrophic, or mixotrophic. They are distinguished morphologically as thecate species, which have an organic coating of cellulose-like plates, and athecate species, without these plates. Measurements of cellular carbon content are available for some phototrophic species but, with the exception of *Noctiluca scintillans* (e.g., Nakamura 1998) and *Oxyrrhis marina* (Pelegri et al. 1999), no empirical measurements for heterotrophic species have been reported. Cellular carbon content for photosynthetic dinoflagellates is usually determined through equations derived from C : vol regression analyses, which include few or no dinoflagellate species (Strathmann 1967) or by applying a constant theoretical value (e.g., Smetacek 1975). Cellular carbon for heterotrophic dinoflagellates is generally estimated using a constant C : vol ratio based on limited measurements (e.g., Lessard 1991). None of these estimates are based on a systematic study of dinoflagellate cellular carbon as a function of cell size, morphological differences, or nutritional mode.

To fill this gap, we determined the cellular carbon content and volume of 20 morphologically and nutritionally diverse dinoflagellate species. In addition to providing an empirical volume to biomass conversion for dinoflagellates, we were interested in comparing the C : vol relationships between phylogenetic groups. We specifically addressed the following questions: What is the relationship between carbon and nitrogen content and cell volume of marine dinoflagellates? Are thecate dinoflagellates more C dense than athecate species? Do photosynthetic and heterotrophic species differ in their C : vol relationships? Do dinoflagellates differ in their C : vol relationship from other phylogenetic groups?

[1] Corresponding author (smenden@ocean.washington.edu).

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Table 1. Source (culture collection or origin of wild-type isolate) and presence or absence of a theca of dinoflagellate species analyzed in this study.

Species	Source	Thecate/Athecate
Phototrophs		
Alexandrium catenella (Whedon and Kofoid) Balech	East Sound, Washington	thecate
Amphidinium asymmetricum Kofoid and Swezy	NEPCC* 67	athecate
Amphidinium carterae Hulburt	UW Botany UTEX LB 1002	athecate
Amphidinium carterae Hulburt	NEPCC 629	athecate
Ceratium fusus (Ehrenberg) Dujardin	Case Inlet, Washington	thecate
Ceratocorys horrida Stein	UW Botany UTEX LB 2499	thecate
Glenodinium foliaceum Stein	UW Botany UTEX LB 1688	thecate
Glenodinium sp.	UW Botany UTEX LB 1625	thecate
Gymnodinium sanguineum Hirasaka	CCMP† 1740	athecate
Gymnodinium simplex (Lohmann) Kofoid and Swezy	NA	athecate
Prorocentrum micans Ehrenberg	NEPCC 33	thecate
Scrippsiella trochoidea (Stein) Loeblich	NEPCC 15	thecate
Heterotrophs		
Bernardinium sp.	Georges Bank	athecate
Noctiluca scintillans (Macartney) Ehrenberg	Admiralty Inlet, Washington	athecate
Oxyrrhis marina Dujardin	Norway	athecate
Protoperidinium conicum (Gran) Balech	Twanoh, Washington	thecate
Protoperidinium depressum (Bailey) Balech	Admiralty Inlet, Washington	thecate
Protoperidinium excentricum (Paulsen) Balech	Copalis Beach, Washington	thecate
Protoperidinium oceanicum (Vanhöffen) Balech	Copalis Beach, Washington	thecate
Protoperidinium pellucidum (Bergh) Schütt	Admiralty Inlet, Washington	thecate

* North East Pacific Culture Collection.
† Provasoli-Guillard National Center for Culture of Marine Phytoplankton.

Methods

Culturing conditions—Phototrophic dinoflagellates were cultured at 18°C in F/2 medium (Guillard 1975) in 500–1,000-ml polycarbonate bottles. Cultures were grown on 16 : 8 light : dark (LD) cycle, at 50 μmols photons m^{-2} s^{-1} provided by cool and warm white lights. Cultures were transferred every 12 d to maintain exponential growth. One species, *Ceratium fusus*, failed to grow at 18°C in standing flasks and was maintained at 12°C on a plankton wheel at 1 rotation per minute (rpm) (all other conditions as above).

The heterotrophic dinoflagellates *Protoperidinium* spp. and *N. scintillans* were fed the diatom *Ditylum brightwellii* and maintained at 12°C in F/2 medium at 30 μmols photons m^{-2} s^{-1} on a plankton wheel at 1 rpm. *O. marina* and *Bernardinium* sp. were fed the cryptophyte *Rhodomonas lens* and maintained at 18°C in 250-ml Erlenmeyer flasks at 30 μmols photons m^{-2} s^{-1}. Dinoflagellate species and their origin are listed in Table 1.

Microscopic analysis—Cell counts were performed on 5–10-ml aliquots, fixed to a final concentration of 1% glutaraldehyde, stained with 4′,6-diamidino-2-phenylindole (DAPI), and drawn onto a 0.2-μm black polycarbonate filter (Poretics). A minimum of 300 cells were counted using a Zeiss standard epifluorescence microscope. Cells were harvested during exponential growth phase. Dinoflagellate cultures were not axenic. Different antibiotics were tested to eliminate bacterial contaminants; however, dinoflagellate growth was inhibited by the addition of antibiotics, whereas bacterial concentrations were unaffected. Therefore, the bacterial concentrations were monitored prior to harvest. Bacterial carbon was estimated using 25 fg C $cell^{-1}$ (Bell 1993). In exponentially growing dinoflagellate cultures, bacterial carbon contributed $<1\%$ to the total and was considered insignificant.

Dinoflagellate cell volume was determined from linear measurements made with a Zeiss Axiovert microscope equipped with a digitizer pad and Microbiota software (Roff and Hopcroft 1986). Microscopic analysis of live cells was performed because fixatives can cause varying degrees of shrinkage (e.g., Verity et al. 1992; Leakey et al. 1994; Montagnes et al. 1994; Stoecker et al. 1994). Cells also were preserved to determine the effect of fixation on dinoflagellate cell volume (data not shown). Measurements of live cells were made immediately before cultures were harvested for CHN filter preparation or ^{14}C isolations. All live-cell measurements were made on 10-ml aliquots in a settling chamber using transmitted light. If necessary, cells were immobilized by adding nickel sulfate to a final concentration of 0.003%; nickel sulfate did not appear to alter cell shape or size during the time of measurement. Only cells in planar view were measured. Generally 100–200 cells were measured; when cultures were not sufficiently dense, a minimum of 30 cells were measured. Linear measurements were converted to cell volume using geometric formulae. Microscopy limits the measurements per cell to two dimensions, requiring assumptions to be made about the third dimension. To overcome this constraint, a subset of cells had been measured before the experiment to determine the aspect ratio of the species (i.e., length : width : depth ratio). These ratios

were used to estimate the third dimension in the volume calculations.

Carbon content—To determine cellular carbon content, two approaches were used, CHN measured on cells collected on a filter and ^{14}C labeling of individual cells.

CHN analysis—Glassware and GF/F filters were precombusted for 4 h at 450°C. Dinoflagellate cells were harvested during the exponential growth phase, filtered onto 25-mm GF/F filters, and rinsed with 10-ml F/2 medium. Filters were placed in precombusted aluminum foil sleeves, dried for 1 h at 60°C, and stored at −20°C until further analysis. Five replicates were prepared for each species. Blanks to correct for contribution of filter and media background were prepared at the same time.

For the heterotrophic species, *O. marina* and *Bernardinium* sp., cells were harvested after the prey biomass was grazed to <1% of total carbon (based on 58 pgC cell^{-1} for *R. lens*, unpubl. data). Heterotrophic dinoflagellates of the genus *Protoperidinium* and *N. scintillans* did not grow to sufficient densities to apply the filtration method described above. Moreover, prey cells were not completely grazed and a considerable amount of detritus was observed. Therefore, 500–1,000 dinoflagellate cells were isolated from their phytoplankton prey using a micropipette, rinsed a minimum of three times in sterile F/2 medium, and placed on a GF/F filter. A total of five replicates were prepared for each species. Blank filters were prepared by adding medium from the last rinse to a GF/F filter. Particulate organic carbon was determined by dry combustion in a Control Equipment Corporation (CEC) 440-SHA elemental analyzer, calibrated with acetanilide. There was no difference in the carbon signal from precombusted blank GF/F filters and GF/F filters with added medium; therefore, the results were pooled to provide one background value to subtract from filters. The nitrogen signal from the F/2-medium blanks was higher than those of GF/F filter blanks. Therefore, the higher medium signal was used as the basis for the background correction.

^{14}C uniform labeling analysis—A second method to determine cellular carbon content was applied because heterotrophic dinoflagellates could not be separated completely from their prey during filter preparation for CHN. Isolating individual cells, as done for *Protoperidinium oceanicum* and *N. scintillans*, in sufficient numbers to exceed the detection limit of CHN analysis (9.5 μgC) was labor intensive. Instead, cellular carbon of individual cells was measured by uniformly labeling cells with radioactive carbon (^{14}C) (Welschmeyer and Lorenzen 1984; Putt and Stoecker 1989). As photosynthetic cells grow in medium labeled with ^{14}C–bicarbonate, they incorporate the radioactive carbon and become uniformly labeled over time (i.e., the ^{14}C signal of cells and medium become equal). At equilibrium, the specific activity of the medium (^{14}C medium) and the specific activity of a cell (^{14}C cell) can be converted to cellular carbon content by

$$\mu\text{gC cell}^{-1} = \frac{^{14}\text{C (cell)}}{^{14}\text{C (medium)}} \times \text{DIC} \times 1.05$$

$$= \frac{\text{DPM cell}^{-1}}{\text{DPM ml}^{-1}} \times \mu\text{gC ml}^{-1}$$

where DPM are disintegrations per minute, DIC is dissolved inorganic carbon, and 1.05 is a fractionation factor describing the inequality in uptake of $^{14}C/^{12}C$ (Welschmeyer and Lorenzen 1984). This method can be applied to heterotrophic species where grazer-specific activity reaches equilibrium with prey cells (Putt and Stoecker 1989). $NaH^{14}CO_3^-$ (ICN Biochem) was added to the medium to a final concentration of 0.1 μCi ml^{-1}. The prey diatom, *D. brightwellii*, was incubated in labeled medium in 50-ml glass tubes with polypropylene screw caps. Cell density and the specific activity of the medium and cells were monitored until no further increase in cellular specific activity was observed. Heterotrophic dinoflagellates were added to the uniformly labeled diatoms by single cell isolation to minimize carryover of unlabeled prey. Specific activity of the media, prey, and predator cells was monitored daily. Once predators were uniformly labeled, about 30 dinoflagellates of similar size were isolated individually, rinsed twice in unlabeled medium, and added to 7-ml scintillation vials. The specific activity of the last rinse was monitored to ensure no labeled organic C was carried over. To remove inorganic ^{14}C, 0.2 ml of 0.2 N perchloric acid was added to each scintillation vial and left to outgas for 1 h. Four milliliters of Biofluor were added to each vial, then samples were vortexed for 10 s and counted on a low-level liquid scintillation counter (Packard 22350CA). Medium specific activity remained constant throughout the course of the experiment. DIC was determined manometrically using a purge and trap procedure (Kroopnick 1985). The DIC concentration of the F/2 medium used in the radiolabeling experiment was 20.79 μg C ml^{-1}.

Previously published C : vol data—Previously published data for carbon content and volume of plankton were obtained from the following sources: Parsons et al. (1961) table 1 using wet oxidation C (omitting *Tetraselmis maculata* and *Monochrysis lutheri* as recommended by Strathmann 1967); Mullin et al. (1966) table 1; Strathmann (1966) table 3; Verity and Langdon (1984) table 1; Moal et al. (1987) tables 1 and 4 using exponentially growing cells only; Verity et al. (1992) tables 2 and 3. Montagnes et al. (1994) kindly provided their original data. When the same species was measured in different analyses, we treated these as independent measurements, rather than pooling the data. Sarcodines were not included in this analysis, as appropriate biovolume data are not available (Michaels et al. 1995). Data for live cells were used where available (Parsons et al. 1961; Montagnes et al. 1994). For other data, fixed cell volumes were used but no correction was applied (Mullin et al. 1966; Strathmann 1966; Moal et al. 1987; Verity et al. 1992).

Data analysis—The distribution of cell sizes of most species was not normal, presumably due to differences in growth and life cycle stages of individual cells. To normalize data, volume and carbon measurements were $\log_{10}$ transformed prior to analysis (Sokal and Rohlf 1981). All data analysis was performed on $\log_{10}$ transformed data, and all figures are presented in a log–log format. Least-squares regression analysis was used to determine the C : vol relationships. For consistency, regression equations are also ex-

Table 2. Linear dimensions (μm) and volume (μm^3), C and N quota (pg cell^{-1}), density (pg μm^{-3}), and C : N ratios (pg pg^{-1}) of dinoflagellate species analyzed in this study. ND, no data; C.V., coefficient of variation.

Species	Length	Width	Volume	C.V.	C content	C.V.	C density	N content	C.V.	N density	C : N ratio
Phototroph											
A. catenella		28.8	13,027	0.37	2,316.25	0.15	0.178	507.15	0.10	0.039	4.57
A. asymmetricum	48.6	32.9	28,058	0.26	4,131.51	0.08	0.147	724.20	0.06	0.026	5.70
A. carterae	12.9	8.2	470	0.39	95.46	0.04	0.203	17.14	0.05	0.036	5.57
A. carterae	15.8	10.6	960	0.38	259.49	0.01	0.270	44.35	0.02	0.046	5.85
C. fusus	335.5	23.1	47,435	0.24	3,305.63	0.06	0.070	727.75	0.06	0.015	4.54
C. horrida	61.0	50.5	84,811	0.28	14,368.47	0.12	0.169	2,656.04	0.05	0.031	5.41
G. foliaceum		24.7	8,163	0.31	1,645.26	0.18	0.202	312.90	0.13	0.038	5.26
Glenodinium sp.	12.9	7.8	422	0.36	125.31	0.03	0.297	19.42	0.04	0.046	6.45
G. sanguineum	69.5	47.8	88,099	0.41	4,449.91	0.15	0.051	910.19	0.07	0.010	4.89
G. simplex	8.1	6.4	180	0.35	47.76	0.10	0.265	10.62	0.03	0.059	4.50
P. micans	41.6	27.2	16,303	0.22	2,735.08	0.11	0.168	795.57	0.14	0.049	3.44
S. trochoidea	28.1	23.8	8,474	0.23	2,010.97	0.08	0.237	399.19	0.06	0.047	5.04
Heterotroph											
Bernardinium sp.	25.6	10.7	1,579	0.32	222.89	0.02	0.141	48.43	0.02	0.031	4.60
N. scintillans	302.8	274.1	1.2×10^7	0.37	35,339.56	0.26	0.003	ND			
O. marina	29.1	16.9	4745	0.56	469.48	0.04	0.099	89.09	0.02	0.019	5.27
P. conicum	45.6	46.2	50,721	0.35	2,717.31	0.20	0.054	ND			
P. depressum	86.2	70.3	278,883	0.30	30,222.32	0.68	0.108	ND			
P. excentricum	46.1	41.2	24,181	0.35	5,531.41	0.26	0.229	ND			
P. oceanicum	97.0	61.4	124,459	0.27	9,147.03	0.16	0.073	ND			
P. pellucidum	53.2	60.7	105,667	0.34	8,133.87	0.66	0.077	ND			

pressed in the log format, but can easily be converted based on the following relationship: $y = a\ \text{vol}^b \Leftrightarrow \log y = b \log \text{vol} + \log a$, with y = pgC cell^{-1}, b = slope, and a = y-intercept of the regression equation.

Much discussion has surrounded the question whether model I (fixed independent variable) or model II (independent variable measured with error) is the appropriate regression analysis for this application. Laws and Archie (1981) argue that most oceanographic data are best analyzed using model II, since frequently both variables are measured and have error; therefore, one of the assumptions of parametric regression analysis is violated. This is also true for the data presented here; however, the sources for both errors are not correlated. Furthermore, as Montagnes et al. (1994) note, the ultimate goal of this analysis is to provide a conversion to predict cellular carbon from volume measurements. In this case, model I analysis is permissible and gives reliable results (Sokal and Rohlf 1981).

Tests of significant differences between regression coefficients (slope) and elevations (y-intercept) of the C : vol regression equations were made using Student's t-test as outlined by Zar (1996). Tests for differences between y-intercepts were only performed if no significant difference between slopes was found. Tests for differences between predictions of regression equations were performed using a paired t-test, where the variance of each predicted C value was used as the basis for the error determination (Zar 1996). The significance of density dependence on size was determined from the regression equation and not the density ratios, due to the pitfalls of determining statistics based on ratios of dependent variables (Atchley et al. 1976; Berges 1997). *N. scintillans* has a uniquely large vacuole, resulting in an unusually low C : vol ratio. Owing to its size, it was an influential outlier in the regression analysis and significantly affected the regression coefficients. Therefore, it was omitted from the analysis and figures.

Results and discussion

Volume measurements—All data presented here are based on size measurements performed on live cells. Average live-cell volume (μm^3) ranged from 180 to 2.79×10^5 μm^3, (Table 2). There was considerable variation in cell volume within species; the coefficient of variation (CV) averaged 33% (ranging 22–56%). This is comparable to other studies, which have reported volume measurement CV to be 25–30% and 49% (Putt and Stoecker 1989 and Verity et al. 1992, respectively). This size variability appears to be intrinsic to cultured protists and is probably due to differences in life cycle and growth status of individual cells, which even in clonal cultures are not necessarily synchronized.

All volumes were computed using the geometric formula for either a sphere, a cylinder, or a prolate spheroid (length $>$ width $\geq$ depth). Cell volumes were also determined according to composite geometric bodies as recommended by Edler (1979) and Hillebrand et al. (1999). However, we found that the difference between volumes based on simple (e.g., sphere) or composite shapes (e.g., cylinder plus two cones) was very small. For example, species of the genus *Protoperidinium* possess large antapical spines, requiring a minimum of two additional measurements per cell (i.e., spine length and width). The volume of these spines constituted $<$1% of the total volume. Considering the intrinsic

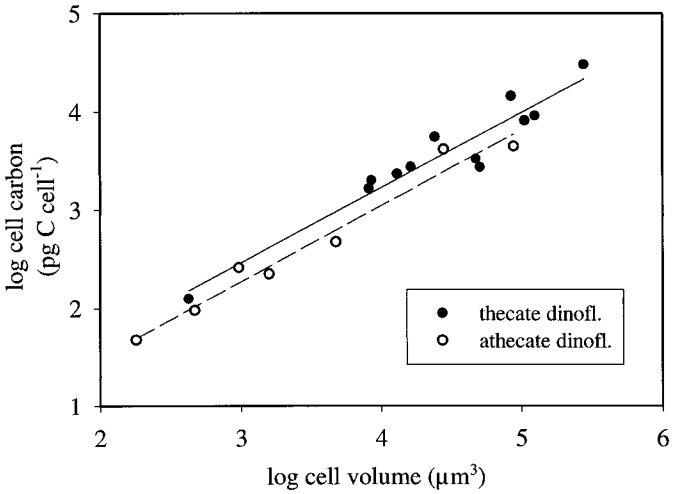

Fig. 1. Log carbon (pg cell^{-1}) to log volume (μm^3) regressions for thecate and athecate dinoflagellate species. Regressions in all figures are lines of best fit, as determined by model I regression analysis of $\log_{10}$ transformed data. Regression statistics are given in Table 3.

error of microscopic volume measurements and the sometimes inaccurate designation of composite shapes, the additional effort does not seem justified. Image analysis techniques (e.g., Sieracki et al. 1989; Verity et al. 1992) can be used to improve the accuracy of volume measurements for cells with relatively simple shapes (i.e., where width is a good proxy for depth). In the future, three-dimensional imaging techniques (e.g., confocal and deconvolution microscopy) could provide standard conversion factors for volume estimates made based on linear measurements. Until these techniques are routinely available, we recommend using simple geometric formulae that require few linear measurements, and empirically determined aspect ratios, to estimate cell volume.

Carbon content—Cellular carbon content for dinoflagellates ranged from 48 to 3.02×10^4 pgC cell^{-1}, and carbon density ranged from 0.054–0.297 pgC μm^{-3} (Table 2). The CV of cellular carbon content averaged 8% (range 1–17%) for the CHN method. Carbon cell^{-1} measured with the radiolabeling method had an average CV of 45% (range: 20–68%, Table 2). The higher variance was a reflection of the variability in carbon content between individual cells. In contrast, the CV of the CHN data provided a measure of the variance between the average C cell^{-1} between the five replicate filters, where each filter is loaded with hundreds of cells.

Nitrogen content and C : N ratios—Nitrogen measurements were obtained for 14 dinoflagellate species. Cellular nitrogen content ranged from 11 to 2,656 pgN cell^{-1}, with an average CV of 6% (range 2–14%), and nitrogen density (pgN μm^{-3}) ranged from 1.54×10^{-2} to 4.88×10^{-2} pgN μm^{-3} (Table 2). C : N ratios ranged from 3.44 to 6.45 (Table 2). The two heterotrophic species measured had very similar C : N ratios to the phototrophs. The C : N ratios for dinoflagellates in this study are in the same range as previously published C : N ratios for dinoflagellates, as well as other plankton grown under nutrient replete conditions (Verity et al. 1992; Montagnes et al. 1994; Flynn et al. 1996*a*,*b*). Nutrients, temperature, and light conditions can affect C : N ratios; the C : N ratio can increase significantly when cells are subjected to nutrient stress (DeYoe and Suttle 1994; Flynn et al. 1994). Growth stage can also influence a cell's chemical composition. For instance, Moal et al. (1987) found a reduction in carbon, nitrogen, and protein density of more than 50% after the shift from exponential to stationary phase. All species analyzed here were grown under nutrient or prey-replete conditions and harvested during exponential growth. Our data suggest that dinoflagellate C : N ratios do not differ from other protist plankton, and that heterotrophic and photosynthetic species have similar C : N ratios.

C : vol relationships for morphologically and nutritionally diverse dinoflagellates—Many species of dinoflagellates are covered with cellulosic plates, which comprise the theca (e.g., Dodge 1985). Species lacking these plates are referred to as athecate, or naked. Owing to this cellulosic coating, thecate dinoflagellates have long been hypothesized to be more carbon dense than other plankton (Smetacek 1975; Edler 1979). We tested this hypothesis by comparing the C : vol relationships of species with and without a theca, irrespective of nutritional mode (Fig. 1, Table 3). There was no significant difference in the slopes of the regression lines between the two groups. If thecate species were more carbon

Table 3. Results of model 1 least-squares regression of $\log_{10}$–transformed dinoflagellate carbon (pgC) and volume (μm^3) data from this study. Presented are the slope and y-intercept of the regression equations, the 95% confidence intervals, the square of the correlation coefficient r, and n, the number of data points included. All slopes are significantly different from zero ($p \leq 0.001$). The cellular carbon content can be determined from volume based on the equation log pgC (or pgN) cell^{-1} = log a + $b \times$ log V (μm^3), where log a is the y-intercept and b is the slope.

Data	log a	95% C.I.	b	95% C.I.	r^2	n
Dinoflagellate C	−0.119	0.401	0.819	0.096	0.95	19
Dinoflagellate N	−0.928	0.465	0.849	0.121	0.95	14
Thecate dinoflagellate C	0.175	0.735	0.764	0.164	0.91	12
Athecate dinoflagellate C	−0.050	0.585	0.774	0.164	0.97	7
Phototrophic dinoflagellate C	−0.076	0.462	0.817	0.118	0.96	12
Heterotrophic dinoflagellate C	−0.547	1.232	0.900	0.270	0.94	7

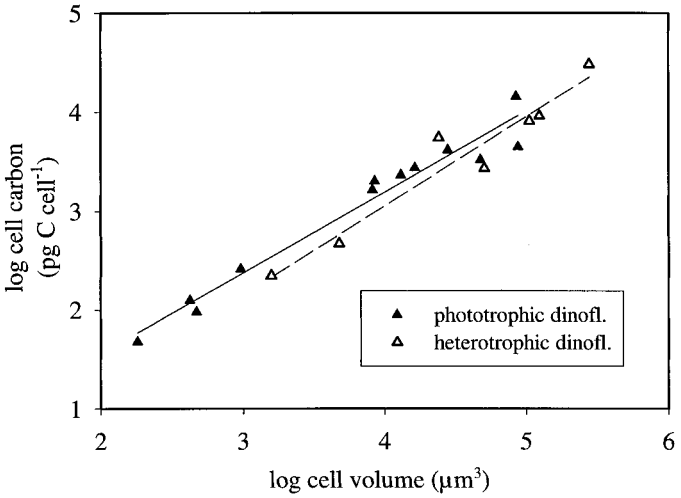

Fig. 2. C : vol relationships for phototrophic and heterotrophic dinoflagellate species. Regression statistics are given in Table 3.

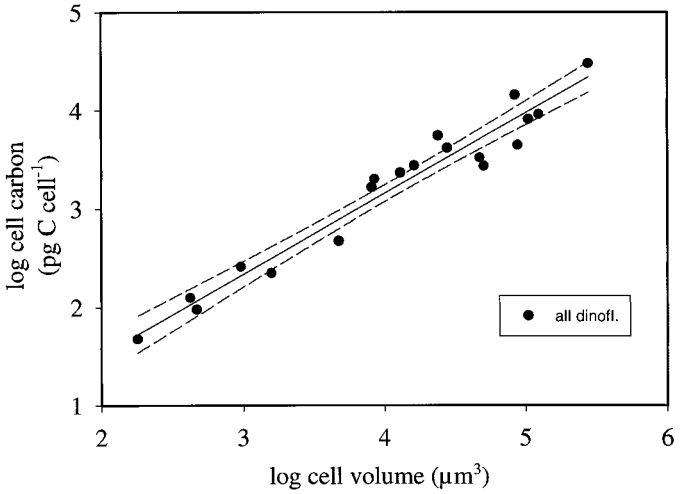

Fig. 3. C : vol relationship for all dinoflagellate species analyzed in this study. Solid line represents line of best fit, and dashed lines are 95% confidence intervals. Regression statistics are given in Table 3.

dense than athecate species, a higher elevation (*y*-intercept) of the thecate equation would be expected. There was no significant difference between the *y*-intercepts (*but see discussion below*).

The significance of heterotrophic dinoflagellates in marine food webs has only recently received attention (Hansen 1991; Lessard 1991). No previous analysis had examined the C : vol relationship of heterotrophic dinoflagellates (Fig. 2, Table 3). We hypothesized that phototrophic species would be more carbon dense than heterotrophs due to the presence of chloroplasts and the lack of potentially less dense food vacuoles. The slopes and *y*-intercepts of the regression equations of these groups were not significantly different (Fig. 2, Table 3), failing to support our hypothesis.

Although no statistically significant differences were found for either comparison, inspection of the C : vol relationships (Figs. 1 and 2) showed distinctly separated regression lines for each group. Further analysis indicated that for both comparisons, statistical power of the tests was low (<30%, much less than the desired 80%). Therefore, failure to detect statistical differences with this type of analysis is not conclusive. With the high level of species-specific variance, the analysis of even several hundred species might not resolve the question of differences between groups using the *t*-test for differences between regression coefficients. As an alternative test for differences, we compared predictions of the C : vol regressions over the same range of volumes (*see Methods*). The C : vol relationship for thecate species predicted significantly more C cell^{-1} ($p < 0.0001$) than athecate species, on average 35% (range 32–37%); the relationship for phototrophic species predicted on average 25% more C cell^{-1} than for heterotrophic species ($p = 0.003$, range 4–48%). These results suggest that both morphology and nutritional mode influence carbon density, but that other factors contribute to a high species-specific variance, which prohibit a distinction between the subgroups based on individual factors. Instead, a species' C : vol ratio may be a composite of many characteristics, of which only morphology and nutritional mode were considered here. The choice of which C : vol conversion to use should be based on the aim of the study and the resolution desired. If the analysis specifically examines nutritionally or morphologically distinct dinoflagellates, we recommend using the conversion equation available for that group.

One C : vol relationship for dinoflagellates—In order to compare dinoflagellates as a group to other protist plankton, a single regression for all dinoflagellate species from our study was determined. Cell volume and carbon were significantly related by the regression equation log pgC cell^{-1} = $-0.119 + 0.819 \times$ log vol. (Fig. 3, Table 3). We compared our results to the C : vol relationship obtained based on combining previously published dinoflagellate C : vol data (Parsons et al. 1961; Mullin et al. 1966; Moal et al. 1987; Verity et al. 1992; Montagnes et al. 1994) and found no significant difference (Table 3), although our regression predicts on average 10% more carbon cell^{-1}. These results differ considerably from previously suggested constant C : vol conversion factors for dinoflagellates, which range from 0.10 to 0.14 pgC μm^{-3} (Smetacek 1975; Edler 1979; Lessard 1991; Hansen et al. 1997). These constant conversion factors underestimate the biomass of small species and overestimate the biomass of large species when compared to the empirically derived C : vol relationship for dinoflagellates.

Comparison to C : vol relationships in other phylogenetic groups—Strathmann (1967), Verity and Langdon (1984), Putt and Stoecker (1989), and this study constitute the only analyses that examined the C : vol relationship of a specific phylogenetic group. The majority of C : vol analyses combine data for mixed plankton from various phylogenetic groups (Parsons et al. 1961; Mullin et al. 1966; Moal et al. 1987; Verity et al. 1992; Montagnes et al. 1994). We combined data from these analyses and established C : vol relationships for groups represented by four or more separate measurements. For many groups, these are the first estimates

Table 4. Results of model 1 least-squares regression of $\log_{10}$–transformed carbon (pgC) and volume (μm^3) data compiled from 1—Parsons et al. (1961), 2—Mullin et al. (1966), 3—Strathmann (1966), 4—Verity and Langdon (1984), 5—Moal et al. (1987), 6—Putt and Stoecker (1989), 7—Verity et al. (1992), 8—Montagnes et al. (1994), and 9—this study. Listed are the slope and y-intercept of the regression equations, with slopes significantly different from zero marked * $p \leq 0.05$ or ** $p \leq 0.001$, the 95% confidence intervals, the square of the correlation coefficient *r*, and *n*, the number of data points included. The cellular carbon content can be determined from volume based on the equation $\log \text{pgC cell}^{-1} = \log a + b \times \log V$ (μm^3), where $\log a$ is the y-intercept and *b* is the slope.

Data	log *a*	95% C.I.	*b*	95% C.I.	r^2	*n*	Reference
Protist plankton*	−0.665	0.132	0.939**	0.041	0.96	91	1, 2, 4, 5, 7–9
Protist plankton <3,000 μm^3	−0.583	0.158	0.860**	0.060	0.86	128	1–3, 5, 7–9
Diatoms	−0.541	0.099	0.811**	0.028	0.97	94	1–3, 5, 8
Diatoms >3,000 μm^3	−0.933	0.465	0.881**	0.093	0.94	26	1–3, 5, 8
Chlorophytes	−1.026	0.343	1.088**	0.136	0.98	8	1, 2, 5, 7, 8
Chrysophytes	−1.694	0.854	1.218*	0.345	0.98	5	1, 8
Dinoflagellates	−0.353	0.287	0.864**	0.074	0.95	34	1, 2, 5, 7–9
Prasinophytes	−0.545	0.368	0.886**	0.199	0.96	4	7, 8
Prymnesiophytes	−0.642	0.490	0.899**	0.217	0.84	17	2, 5, 7, 8
Loricate ciliates	−0.168	0.322	0.841**	0.071	0.99	9	4
Aloricate ciliates†	−0.639		0.984				6

* Excluding diatoms.
† Based on predicted C values using Putt and Stoecker's (1989) regression equation.

of a C : vol regression equation. These C : vol relationships should be viewed with some caution. The data used were collected by different investigators over the last 30 yr. Therefore, differences in methods used to measure carbon and volume may contribute to the observed variability. For example, Parsons et al. (1961) and Mullin et al. (1966) used wet oxidation, whereas more recent studies used dry combustion methods, although the differences appear small (Parsons et al. 1961; Strathmann 1967). Furthermore, data for both live and fixed cells were included in this analysis, this could have affected the results. It has been shown that fixation can alter cell volume, resulting in shrinking or swelling. Fixation effects however, appear to be species specific, and the magnitude and direction of cell volume change is dependent on type and strength of fixative (e.g., Verity et al. 1992; Leakey et al. 1994; Montagnes et al. 1994; Stoecker et al. 1994; Menden-Deuer, unpubl. data). Therefore, corrections for fixation effects cannot be made reliably.

Phytoflagellates: Published data were combined to determine C : vol relationships for chlorophytes, cryptophytes, chrysophytes, prasinophytes, and prymnesiophytes. Significant regressions (i.e., slopes significantly different from zero) were obtained for all but the cryptophytes ($p < 0.05$, Table 4). Only the regressions for chrysophytes and chlorophytes were significantly different from that for dinoflagellates ($p < 0.05$). The chrysophyte and chlorophyte regressions were based on data from only three and two different species respectively, and may not be reliable. Although the available data are limited, they suggest that different classes of flagellates have similar C : vol relationships. Further work on chlorophytes and chrysophytes is needed.

Diatoms: We examined whether dinoflagellates are more carbon dense than diatoms based on the data used by Strathmann (1966) and some new values for diatoms (Moal et al. 1987; Montagnes et al. 1994). Strathmann (1967) observed that his diatom C : vol relationship was significantly different from the C : vol relationship for diatoms determined by Parsons et al. (1961) and Mullin et al. (1966). We combined all available diatom data (Parsons et al. 1961; Mullin et al. 1966; Moal et al. 1987; Montagnes et al. 1994) and contrasted the resulting C : vol regression with Strathmann's (1967); the two equations were significantly different ($p = 0.001$). The lower biomass prediction made by Strathmann's (1967) C : vol regression is primarily due to data for a single species, *D. brightwellii*, which falls well below the regression line (his fig. 2). Strathmann (1967) suggested that his volume estimate (cylinder) may have overestimated the volume for *D. brightwellii*, which is triangular in cross section. We determined the volume of *D. brightwellii* as a triangular bar based on Strathmann's (1966) size data. Although this decreases *D. brightwellii's* volume by 50% and the data lie closer to the regression, Strathmann's (1967) diatom regression remains significantly different from the one for all other diatoms ($p = 0.018$). When *D. brightwellii* is omitted from the data set, the two C : vol relationships for diatoms no longer differ significantly. *D. brightwellii* shows a distinct departure from the general C : vol relationship in diatoms that cannot be explained by errors in volume estimation or methodology. Allometry by necessity ignores inherent species-specific variability in order to provide average estimates and grounds for comparison. The example of *D. brightwellii* is a reminder that C : vol relationships are useful general trends but that individual species can deviate significantly from the general regression.

We determined a new C : vol regression based upon all C : vol data available for diatoms omitting *D. brightwellii* (Table 4). Compared to Strathmann's (1967) equation, this new C : vol regression predicted 20% less C for small diatoms (<30 μm^3) and 5 to 60% more C as diatom volume increases from 300 to 10^6 μm^3. Comparing our dinoflagellate regression to the new C : vol relationship for diatoms, we found that dinoflagellates are significantly denser than diatoms ($p = 0.027$). However, this was only the case for cells

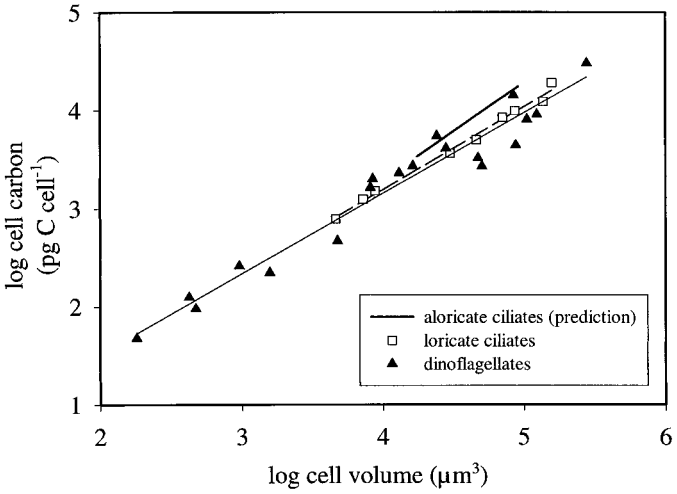

Fig. 4. C : vol relationships for loricate ciliates, determined by Verity and Langdon (1984), for aloricate ciliates by Putt and Stoecker (1989) and for dinoflagellates from this study. Regression statistics are given in Tables 3 and 4.

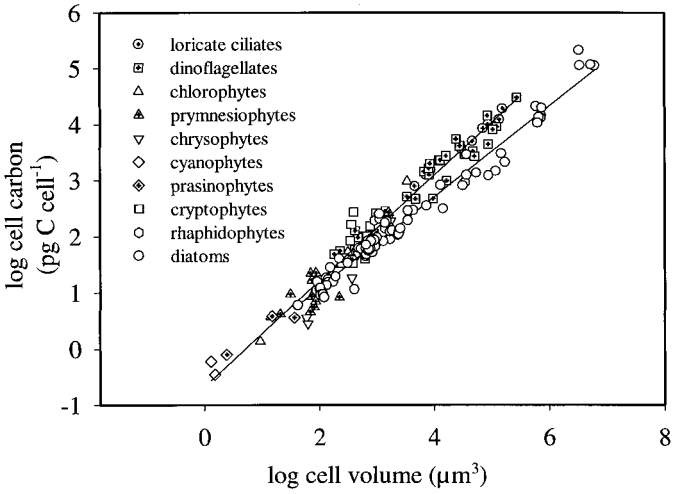

Fig. 5. C : vol relationship for diatoms compared to the one obtained for other protist plankton, determined using data from Parsons et al. 1961; Mullin et al (1966); Strathmann (1967); Verity and Langdon (1984); Moal et al. (1987); Verity et al. (1992); Montagnes et al. (1994), and this study. Data for aloricate ciliates (Putt and Stoecker 1989) were excluded from regression analysis. Regression statistics are given in Tables 3 and 4.

larger than 3,000 μm^3; smaller dinoflagellates and diatoms did not differ significantly in their carbon content.

Ciliates: Verity and Langdon (1984) measured carbon and lorica volume in nine species of loricate ciliates. We estimated cell volume using a conversion factor of 0.5 cell : lorica volume ratio (Beers and Stewart 1967) and determined a C : vol regression for the log-transformed data (Fig. 4, Table 4). Putt and Stoecker (1989) measured carbon and volume in five species of aloricate oligotrichous ciliates. As individual data values were not available, we predicted carbon values using the regression equation determined by Putt and Stoecker (1989, fig. 2b) for Lugol's fixed cells over the volume range of their studied species. We converted fixed to live volumes using their average shrinkage of 22% and log transformed the predicted values. Aloricate and loricate ciliates differed in their C : vol relationships (Fig. 4, Table 4). Whether this represents a true difference is not known. The discrepancy between the two data sets may be due to differences in methodology between the two studies, or our method of data analysis (e.g., using a constant cell : lorica volume ratio for the Verity and Langdon 1984 data, predicting data for Putt and Stoecker's 1989 cell volume). Therefore, we compared our results for dinoflagellates to aloricate and loricate ciliates separately. Neither the slope nor *y*-intercept of the regression for loricate ciliates is significantly different than that for dinoflagellates, suggesting that loricate ciliates and dinoflagellates have similar carbon densities. On the other hand, aloricate ciliates appear to have an average 43% greater biomass (range 34–50%) than a similar sized dinoflagellate over the size range of 1.8×10^4 to 9.2×10^4 μm^3. Comparisons of growth and grazing rates, as well as nutritional value, are dependent upon accurate carbon density estimates. Consequently, the discrepancy between loricate and aloricate ciliate C : vol and our inability to resolve whether dinoflagellates and ciliates have similar or different carbon biomass emphasizes the need to empirically determine the C : vol relationship(s) for more species of ciliates over a wider size range.

One C : vol relationship for protist plankton other than diatoms—Strathmann (1967) first attempted to determine a single C : vol relationship for phytoplankton but concluded that diatoms were significantly less carbon dense than a mix of 14 phytoflagellates measured by Parsons et al. (1961) and Mullin et al. (1966). Strathmann (1967) cautioned that the observed differences may be investigator-based and might not reflect true biological differences. Available C : vol data from regression studies for marine protist plankton (excluding Putt and Stoecker 1989) were combined to reexamine this result and to determine if a single regression could reasonably well describe one C : vol relationship for all non-diatom protist plankton. Combining C : vol data for protist plankton (excluding diatoms) from these studies with our dinoflagellate data yields a significant C : vol relationship expressed by the equation log pgC cell^{-1} = -0.665 + log vol $\times$ 0.939 r^2 = 0.96, (Fig. 5, Table 4). It is noteworthy that the limited data reported for small heterotrophic flagellates fell well within the range of this relationship (Børsheim and Bratbak 1987; Geider and Leadbeater 1988; Nakano 1994; Pelegri et al. 1999). The two values for cyanobacteria from Verity et al. (1992) also appear to agree well with the general trend (Fig. 5). Bearing in mind the limitations of combining data from many studies, we suggest that by using a large data set that covers a broad size range, particular concerns (fixation, differences in methodology) will have only a minimal effect on the magnitude of the regression coefficients. For instance, predictions for cell carbon in non-diatom protists based on regressions that include or exclude fixed data were on average less than 5% different. Thus, the C : vol conversion equation can be used to predict carbon content based on live as well as fixed cell volume data.

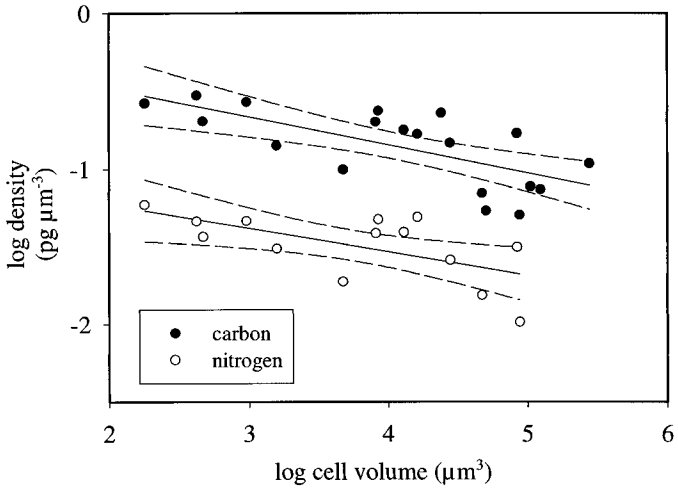

Fig. 6. C and N density (pg μm^{-3}) decreased significantly ($p \leq 0.01$) with increasing cell volume. Solid line represents line of best fit and dashed lines are 95% confidence intervals.

The regression for protist plankton is significantly different from the one for diatoms ($p < 0.0001$, Fig. 5), which supports Strathmann's (1967) suggestion that diatoms are significantly less carbon dense than other plankton and should be treated separately. However, this difference is only significant for species with volumes greater than 3,000 μm^3 (ESD = 17 μm); there is no significant difference between the C : vol relationships for diatom and non-diatom species smaller than 3,000 μm^3. We can only suggest possible reasons for the divergence between diatom and other plankton (e.g., vacuole size, silica vs. organic cell covering), but the fact that it does not seem to apply to smaller cells should be investigated further.

The result that plankton biomass can be estimated using only two C : vol relationships for mixed plankton, one for diatoms and one for taxonomically diverse protist plankton, could benefit plankton studies and ecosystem modeling. Depending on the aim of the investigation, taxonomic analysis of plankton samples could be minimized to distinguishing between diatom and non-diatom species, therefore decreasing labor-intensive microscopic analysis. If the focus of the study is on the <3,000 μm^3 size range, one C : vol equation could be used to determine plankton biomass (Table 4). Furthermore, these conversion equations could simplify and improve model applications, reducing the parameters necessary to accurately simulate development and fluxes of carbon biomass.

Density is size dependent—Our analysis showed that neither C nor N was a constant fraction of cell volume in dinoflagellates (Fig. 6), but rather decreased with increasing cell volume (i.e., the slopes of the C : vol and N : vol relationships are significantly <1, $p = 0.0009$ and $p = 0.018$, respectively). Size dependency of carbon density was first reported by Mullin et al. (1966) and can be shown for many C : vol analyses for plankton (Parsons et al. 1961; Strathmann 1967; Verity and Langdon 1984; Moal et al. 1987; Verity et al. 1992.) and even echinoderm eggs (Strathmann and Vedder 1977). However, some studies report constant carbon density irrespective of cell size (Rocha and Duncan 1985; Putt and Stoecker 1989; Montagnes et al. 1994; Pelegri et al. 1999). Rocha and Duncan (1985) collected data for freshwater plankton, which may have a different density relationship than marine plankton; the authors also report a weak dependence of density on size, after adding new data points to their original data. Putt and Stoecker (1989) analyzed ciliates spanning only 1 order of magnitude in size, and it may be that this range is insufficient to observe size-dependent effects of density. Montagnes et al. (1994) obtained a C : vol regression with a slope not different from one using microscopically measured live volumes. However, the slope of the regression using live volumes measured with a Coulter Counter is significantly less than one ($p = 0.007$). Since both methods of volume measurement have inaccuracies, this data set appears inconclusive with respect to size dependence of carbon density. Pelegri et al. (1999) report slopes both equal to and significantly different from one for different subsets of their data. The analysis of Pelegri et al. (1999) is largely based on bacteria, however, which may have different C : vol relationships than eukaryotic organisms.

When all C : vol data for protist plankton were combined, carbon density decreased significantly with increasing volume ($p < 0.003$) (Fig. 5, Table 4). Moal et al. (1987) observed a similar trend for a wide range of other cellular components such as chlorophyll *a* (Chl *a*), protein, and carbohydrates. It is likely that carbon density decreases due to changes in the relative contribution of cellular components such as membranes, nuclear material, organelles, and vacuole space. The latter has been suggested by Verity et al. (1992), who proposed that smaller cells contain less cellular water. The contribution of membrane material to the total volume should decrease significantly with increasing cell size, using the scaling argument by Raven (1994). The volume occupied by the surface membrane of 5-nm thickness (Alberts et al. 1989) is over 2% of the total volume in the smallest cell (1.3 μm^3), but only 0.01% for the largest cell (6×10^6 μm^3) for data shown in Fig. 5. Therefore, the proportion of a dense cellular component such as the surface membrane, which contains over 70% C (Reuter and Perdue 1984), decreases by 2 orders of magnitude over this size range. In order to make quantitative estimates of the significance of this observation, the relative contribution of other dense cellular components, such as DNA, plastids, and pigments, to total cellular content needs to be examined. Although we can only speculate on the biological function of size-dependence in density (i.e., metabolic constraints, buoyancy), the data and theoretical consideration are a clear demonstration that the application of a constant C : vol conversion factor over a large size range will significantly affect the predicted biomass. For example, Hansen et al. (1997) analyzed growth rates of heterotrophic dinoflagellates with respect to volume, implicitly assuming constant density. Hansen et al.'s volume to growth rate relationship has a slope of -0.26; reanalyzing their data by converting volume to C biomass using our regression for dinoflagellates results in a much greater decline in expected growth rate with increasing size (slope of -0.31).

Conclusions

Our results show that dinoflagellate carbon density was higher in photosynthetic and thecate dinoflagellates than in heterotrophic and athecate dinoflagellates, but that these differences were relatively small and dinoflagellate cell carbon can be determined from a single regression equation. The reexamination of available data for other phylogenetic protist groups indicated that most groups, except diatoms, have similar carbon to volume relationships. Therefore, we established two C : vol relationships for protist plankton, one for taxonomically diverse protist plankton and one for diatoms. These comprehensive relationships can serve to simplify methodology and analysis of experimental and model simulations of ecosystem biomass rates and fluxes. The underlying reasons explaining why the C : vol relationship for diatoms is distinct from other phylogenetic groups deserve further study. The available data for some groups (chlorophytes, chrysophytes, ciliates, and heterotrophic nanoflagellates) is sparse, and there is need to examine more species over a larger size range in each of these groups to confirm whether or not they are different. Our results show that carbon (and nitrogen) density change with cell size and emphasize that an inappropriate use of a constant C : vol conversion will underestimate the biomass of small cells and overestimate the biomass of large cells.

References

ALBERTS, B., D. BRAY, J. LEWIS, M. RAFF, K. ROBERTS, AND J. D. WATSON. 1989. Molecular biology of the cell. Garland.

ATCHLEY, W. R., C. T. GASKINS, AND D. ANDERSON. 1976. Statistical properties of ratios. I. Empirical results. Syst. Zool. **25:** 137–148.

BEERS, J. R., AND G. L. STEWART. 1967. Microzooplankton in the euphotic zone at five locations across the California Current. J. Fish. Res. Board Can. **24:** 2053–2068.

BELL, R. T. 1993. Estimating production of heterotrophic bacterioplankton via incorporation of tritiated thymidine. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], Handbook of methods in aquatic microbial ecology. Lewis.

BERGES, J. A. 1997. Ratios, regression statistics and 'spurious' correlations. Limnol. Oceanogr. **42:** 1006–1007.

BØRSHEIM, K. Y., AND G. BRATBAK. 1987. Cell volume and cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. Mar. Ecol. Prog. Ser. **71:** 185–194.

DEYOE, H. R., AND C. A. SUTTLE. 1994. The inability of the Texas ''brown tide'' alga to use nitrate and the role of nitrogen in the initiation of a persistent bloom of this organism. J. Phycol. **30:** 800–806.

DODGE, J. D. 1985. Atlas of dinoflagellates. Farrand.

EDLER, L. 1979. Recommendations on methods for marine biological studies in the Baltic Sea, Phytoplankton and Chlorophyll. The Baltic Mar. Biol. Publ. **5:** 1–38.

FLYNN, K. J., K. DAVIDSON, AND J. W. LEFTLEY. 1994. Carbon-nitrogen relations at whole-cell and free-amino-acid levels during batch growth of *Isochrysis galbana* (Prymnesiophyceae) under conditions of alternating light and dark. Mar. Biol. **118:** 229–237.

———, K. FLYNN, E. H. JOHN, B. REGUERA, M. I. REYERO, AND J. M. FRANCO. 1996*a*. Changes in toxins, intracellular and dissolved free amino acids of the toxic dinoflagellate *Gymnodinium catenatum* in response to changes in inorganic nutrients and salinity. J. Plankton Res. **18:** 2093–2111.

FLYNN, K., K. J. JONES, AND K. J. FLYNN. 1996*b*. Comparisons among species of Alexandrium (Dinophyceae) grown in nitrogen- or phosphorus-limiting batch culture. Mar. Biol. **126:** 9–18.

GEIDER, R. J., AND B. S. C. LEADBEATER. 1988. Kinetics and energetics of the marine choanoflagellate *Stephanoeca diplocostata.* Mar. Ecol. Prog. Ser. **47:** 169–177.

GUILLARD, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates. *In* W. L. Smith and M. H. Chanley. [eds.], Culture of marine invertebrate animals. Plenum.

HANSEN, P. J. 1991. Quantitative importance and trophic role of heterotrophic dinoflagellates in a coastal pelagic food web. Mar. Ecol. Prog. Ser. **73:** 253–261.

———, P. K. BJOERNSEN, AND B. W. HANSEN. 1997. Zooplankton grazing and growth: Scaling within the 2–2000 μm body size range. Limnol. Oceanogr. **42:** 687–704.

HILLEBRAND, H., C. D. DUERSELEN, D. KIRSCHTEL, U. POLLINGHER, AND T. ZOHARY. 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol. **35:** 403–424.

KROOPNICK, P. M. 1985. The distribution of $^{13}CO_2$ in the world oceans. Deep-Sea Res. **32:** 57–84.

LAWS, E. A., AND J. W. ARCHIE. 1981. Appropriate use of regression analysis in marine biology. Mar. Biol. **65:** 13–16.

LEAKEY, R. J. G., P. K. BURKHILL, AND M. A. SLEIGH. 1994. A comparison of fixatives for the estimation of abundance and biovolume of marine planktonic ciliate populations. J. Plankton Res. **16:** 375–389.

LESSARD, E. J. 1991. The trophic role of heterotrophic dinoflagellates in diverse marine environments. Mar. Microb. Food Webs **5:** 49–58.

MICHAELS, A. F., D. A. CARON, N. R. SWANBERG, F. A. HOWSE, AND C. M. MICHAELS. 1995. Planktonic sarcodines (Acantharia, Radiolaria, Foraminifera) in surface waters near Bermuda: Abundance, biomass and vertical flux. J. Plankton Res. **17:** 131–163.

MOAL, J., V. MARTIN-JEZEQUEL, R. P. HARRIS, J. F. SAMAIN, AND S. A. POULET. 1987. Interspecific and intraspecific variability of the chemical composition of marine phytoplankton. Oceanol. Acta **10:** 339–346.

MONTAGNES, D. J. S., J. A. BERGES, P. J. HARRISON, AND F. J. R. TAYLOR. 1994. Estimating carbon, nitrogen, protein and chlorophyll *a* from volume in marine phytoplankton. Limnol. Oceanogr. **39:** 1044–1060.

MULLIN, M. M., P. R. SLOAN, AND R. W. EPPLEY. 1966. Relationship between carbon content, cell volume and area in phytoplankton. Limnol. Oceanogr. **11:** 307–311.

NAGATA, T., AND Y. WATANABE. 1990. Carbon and nitrogen to volume ratios of bacterioplankton grown under different nutritional conditions. Appl. Environ. Microbiol. **56:** 1303–309.

NAKAMURA, Y. 1998. Growth and grazing of a large heterotrophic dinoflagellate *Noctiluca scintillans*, in laboratory culture. J. Plankton Res. **9:** 1711–1720.

NAKANO, S. I. 1994. Carbon : nitrogen : phosphorous ratios and nutrient regeneration of a heterotrophic flagellate fed on bacteria with different elemental ratios. Arch. Hydrobiol. **129:** 257–271.

PARSONS, T. R., K. STEPHENS, AND J. D. H. STRICKLAND. 1961. On the chemical composition of eleven species of marine phytoplankters. J. Fish. Res. Board Can. **18:** 1001–1016.

PELEGRI, S. P., J. DOLAN, AND F. RASSOULZADEGAN. 1999. Use of high temperature catalytic oxidation (HTOC) to measure carbon content of microorganisms. Aquat. Microb. Ecol. **16:** 273–280.

PUTT, M., AND D. K. STOECKER. 1989. An experimentally deter-

mined carbon : volume ratio for marine ''oligotrichous'' ciliates from estuarine and coastal waters. Limnol. Oceanogr. **34:** 1097–1103.

RAVEN, J. A. 1994. Why are there no picoplanktonic O_2 evolvers with volumes less than $10^{-19}m^3$? J. Plankton Res. **16:** 565–580.

REUTER, J. H., AND E. M. PERDUE. 1984. A chemical structural model of early diagenesis of sedimentary humous/proto-kerogens. Mitt. Geol.-Paleontol. Inst. Univ. Hamburg. **58:** 249–262.

ROCHA, O., AND A. DUNCAN. 1985. The relationship between cell carbon and cell volume in freshwater algal species used in zooplankton studies. J. Plankton Res. **7:** 279–294.

ROFF, J. C., AND R. R. HOPCROFT. 1986. High precision microcomputer based measuring system for ecological research. Can. J. Fish. Aquat. Sci. **43:** 2044–2048.

SIERACKI, M. E., C. L. VILES, AND K. L. WEBB. 1989. Algorithm to estimate cell biovolume using image analyzed microscopy. Cytometry **10:** 551–557.

SMETACEK, V. 1975. Die Sukzession des Phytoplanktons in der westlichen Kieler Bucht. Ph.D. thesis, University of Kiel.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. Freeman.

STOECKER, D. K., D. J. GIFFORD, AND M. PUTT. 1994. Preservation of marine planktonic ciliates: Losses and cell shrinkage during fixation. Mar. Ecol. Prog. Ser. **110:** 293–299.

STRATHMANN, R. R. 1966. Estimating the organic carbon content of phytoplankton from cell volume, cell area or plasma volume. Master's Thesis. Univ. Washington.

———. 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol. Oceanogr. **12:** 411–418.

———, AND K. VEDDER. 1977. Size and organic content of eggs of echinoderms and other invertebrates as related to developmental strategies and egg eating. Mar. Biol. **39:** 305–309.

UYE, S. I. 1982. Length-Weight relationships of important zooplankton from the Inland Sea of Japan. J. Oceanogr. Soc. Jpn. **38:** 149–158.

VERITY, P. G., AND C. LANGDON. 1984. Relationships between lorica volume, carbon, nitrogen, and ATP content of tintinnids in Narragansett Bay. J. Plankton Res. **66:** 859–868.

———, C. Y. ROBERTSON, C. R. TRONZO, M. G. ANDREWS, J. R. NELSON, AND M. E. SIERACKI. 1992. Relationship between cell volume and carbon and nitrogen content of marine photosynthetic nanoplankton. Limnol. Oceanogr. **37:** 1434–1446.

WELSCHMEYER, N. A., AND C. J. LORENZEN. 1984. Carbon 14-labeling of phytoplankton carbon and Chl *a*: Determination of specific growth rates. Limnol. Oceanogr. **29:** 135–145.

ZAR, J. H. 1996. Biostatistical Analysis. Simon and Schuster.

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