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Estimation of intracellular carbon and silica content of diatoms from natural assemblages using morphometric techniques[1]

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Abstract

Electron microscopic morphometric techniques were used to measure accurately the cytoplasm and frustule volumes of 12 naturally occurring planktonic diatoms in the Laurentian Great Lakes. In addition, cytoplasm volumes were measured in 6 other algal species. Estimates of carbon content were then made by converting measured cytoplasm volumes to carbon biomass. From these measurements and conversions, the average percent carbon by dry weight was found to be 19% (range 10–30%) for diatoms and 35% (range 29–43%) for the nondiatom species. These values are lower than those previously reported. This is probably due to two facts: our estimates do not include extracellular carbon that may be associated with the cells, and vacuole relative volume (% of total cell volume) ranged from 22 to 70% in diatoms and 2 to 29% for nondiatoms. The carbon content of cells with lower vacuole volumes more closely approximate those previously reported. Our estimations of percent silica by dry weight of diatoms averaged 60% (range 40–78%). Our values for silica content are closer to those reported for naturally occurring populations than to those for cultured populations.

Estimation of biomass in individual phytoplankton populations in a mixed assemblage remains a complex problem. Traditionally, biovolume estimates have been used to assess the importance of certain species through conversion to organic carbon content, ash-free dry weight, or chlorophyll (Paasche 1960; Mullin et al. 1966; Strathmann 1967). The return to biovolume estimates is an attempt to escape the limitations of secondary measurement techniques such as Chl *a* or ATP since there is generally a poor correlation between estimates of biovolume and other independent measurements of biomass (Vollenweider et al. 1974). Sicko-Goad et al. (1977) have summarized three inherent problems associated with biovolume to biomass conversion: a general lack of accuracy and precision in measuring complex, irregularly shaped microscopic algae; differing amounts of "inert" space between different divisions of algae as well as differences between species in the same division; and physiological condition of the cells may drastically affect cell size as well as certain "volatile" compartments such as storage products.

Indirect estimates of cytoplasm volume have been made by extrapolation from cell carbon (Strathmann 1967) or Chl *a* and protein (Hitchcock 1983). Although these extrapolations provide estimates of cytoplasm volume, the numbers are not as accurate as those made by morphometric techniques. Although estimates of carbon content made by conversion of plasma volume may be inaccurate, there is no direct way to determine biomass from historical phytoplankton data.

The most direct method to determine if a relationship exists between cytoplasm volume and cell components would be to compare morphometric studies with accurate chemical analyses. While this approach could work in cultures, it still has several drawbacks. It assumes that the cells would have a constant amount of a component per unit volume. This relationship has been demonstrated for the chloroplast, where pigment content varies directly with chloroplast size (Brown and Richardson 1968; Messer and Ben-Shaul 1972). However, a component such as carbon would not lend itself directly to calculation per unit volume for two reasons. The amount of storage products in a given algal cell can change quite drastically in culture during growth

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Table 1. Collection information and sampling scheme used for morphometric analysis.

	No. photos examined	Standard magnification	Grid spacing (cm)	Points counted	Total area examined (μm^3)	Fixation procedure	Collection		Species code for Fig. 1
							Date	Site	
Stephanodiscus alpinus	25	13,200×	1.25	4,979	4,206	A	28 Apr 78	Saginaw Bay, sta. 25*	1
S. binderanus	46	13,600×	0.5	30,745	4,550	A	10 Apr 75	Saginaw Bay, sta. 56†	2
S. minutus	50	25,500×	1.25	3,929	944	A	28 Apr 78	Saginaw Bay, sta. 25*	3
S. niagarae	50	5,000×	1.25	5,329	33,306	A	28 Apr 78	Saginaw Bay, sta. 25*	4
S. subtilis	11	15,000×	1.0	1,668	741	B	20 Sep 78	Grand River	5
S. tenuis	50	13,400×	1.0	10,285	5,728	B	25 Sep 79	Grand River	6
Fragilaria capucina (A)	75	25,100×	0.5	33,263	1,320	A	10 Apr 75	Saginaw Bay, sta. 56†	8
F. capucina (B)	50	24,000×	1.0	4,390	750	A	7 Aug 77	Saginaw Bay, Caseville Pier	9
F. intermedia var. *fallax*	25	28,700×	1.0	4,327	525	A	17 May 77	L. Michigan, sta. 06‡	7
Melosira granulata	30	11,100×	1.0	8,799	7,272	A	7 Aug 77	Saginaw Bay, Caseville Pier	11
M. islandica	75	13,100×	1.0	8,034	4,682	A	17 May 77	L. Michigan, sta. 06‡	10
Cyclotella No. 6	30	39,600×	1.0	3,239	207	B	11 Sep 81	L. Michigan, 1.6 km offshore Grand Haven	13
C. meneghiniana	50	15,000×	1.0	12,158	5,404	B	25 Sep 79	Grand River	12

* Robbins 1984.
† Sicko-Goad et al. 1977.
‡ Schelske et al. 1983.

(Fogg 1966; Sicko-Goad unpubl. obs.). The amount of extracellular carbonaceous materials also changes. Due to the complex nature of carbohydrates and lipids, the amount of carbon per unit volume of cytoplasm will be higher in those cells having larger volumes occupied by storage products and proportionately smaller volumes occupied by "ground cytoplasm" consisting of proteinaceous materials.

We have used morphometric techniques to measure precisely the volume density of important cellular compartments in 12 planktonic diatoms and 6 other algae from natural assemblages in the Laurentian Great Lakes. We have used conversion factors to estimate the carbon content of all algae examined and the silica content of the 12 diatoms. The results indicate that previous estimates of carbon content based on conversion of plasma volumes may be high due to overestimates of the plasma volume. Our lower estimates of carbon content do not account for extracellular material.

Materials and methods

Samples were collected from three locations: Saginaw Bay (Lake Huron), the Grand River mouth (inshore Lake Michigan), and Lake Michigan (Table 1).

Samples were taken as 1.0-liter splits of Niskin bottles. The raw water was fixed by one of two methods. In method A sufficient glutaraldehyde (50%, biological grade) and sodium cacodylate were added immediately to give a final concentration of 3% glutaraldehyde and 0.1 M cacodylate at pH 7.2; the osmolality of this fixative was 490 mosmol. In method B paraformaldehyde, glutaraldehyde, and sodium cacodylate were added to the whole-water samples to give final concentrations of 1% paraformaldehyde, 1% glutaraldehyde, and 0.05 M sodium cacodylate; the pH of this solution was

7.2 and its osmolality was 515 mosmol. We prefer method B since it preserves all major algal groups well and is especially convenient for field use (Lazinsky and Sicko-Goad 1979).

Fixed samples were stored on ice and returned to the laboratory for further processing, usually within 24 h. Samples were concentrated by gentle centrifugation, rinsed four times in 0.1 M cacodylate buffer, pH 7.2, then post-fixed with 1% OsO_4 in cacodylate buffer for 1 h at 4°C. Fixed and concentrated cells were then dehydrated in a graded ethanol and propylene oxide series and embedded in Epon (Luft 1961).

Thin sections were cut with a diamond knife, collected on cleaned, Formvar-coated 200-mesh copper grids, and stained with aqueous uranyl acetate (Watson 1958).

The sampling scheme for quantitative stereology is also outlined in Table 1. Organisms were examined and electron micrographs taken at a standard magnification, dependent on the cell size, with either a Zeiss EM 9S-2 or a JEOL JEM 100B transmission electron microscope. Magnification calibrations were made periodically with a diffraction grating replica to check stability. In most instances variations were $<1\%$.

Micrographs were taken of the first identifiable organisms of interest in each section examined, including small grazing sections through cells. In cases where whole cells were larger than the photographic field, a photographic montage was made and used for analysis. With filamentous organisms, only one cell from each filament was sampled, since multiple samples from a single filament do not improve volume estimates and may bias the population estimate (Sicko-Goad et al. 1977). Only one section per grid was used for analysis to avoid bias by sampling adjacent cell sections. The minimum distance between series of sections was 10 μm.

Estimates of volume density, the fractional volume of a cellular component relative to its containing volume, were obtained with the grid-counting technique (Glagoleff 1933; Chalkley 1943). This method is an extension of the Delesse principle (*cited in* Weibel and Bolender 1973) that the areal density of profiles in sections of negligible thickness (of the order of 50 nm in this case) provides an unbiased estimate of the true volume of any identifiable structure relative to the containing volume. Thus, by counting points of a standard sampling grid superimposed over a particular component (i), one can obtain an estimate of the volume fraction of (i); i.e.

$$\frac{V_i}{V_t} = \frac{A_i}{A_t} = \frac{P_i}{P_t}$$

where V_i is the volume of a given component, V_t the total containing volume, A the area, and P the points counted.

Transparent square sampling lattices of dimensions appropriate to photographic magnifications were used for point counts. Between 30 and 75 micrographs of each taxon were measured. Estimation of coefficients of variation and plots of cumulative means and variances indicated adequate sampling of the material. Results are presented as the mean.

Estimates of total cell volume for each species were obtained from light microscope measurements made of the same populations measured by stereology. Ten independent measurements of each species were made from epoxy whole-cell slide mounts of the fixed and embedded material. Volume calculations are based on the best regular geometric approximation of cell shape for each taxon.

Morphometric data were consolidated into three categories to calculate carbon and silica content: relative cytoplasm volume, relative vacuole volume, and relative frustule volume. These were converted to estimates of absolute volume per cell by multiplying them by estimates of average cell volume. The vacuole was assumed to contain negligible dry matter, and this fraction was neglected in further calculations. Carbon weight per cell was estimated by multiplying cytoplasm volume by 0.11 (Strathmann 1967), which appears to be a reasonable estimate of carbon content. Conversion of frustule volume to weight is considerably less straightforward. The specific gravity of amorphous silica is about 2.2. However, silica in diatom frustules may be

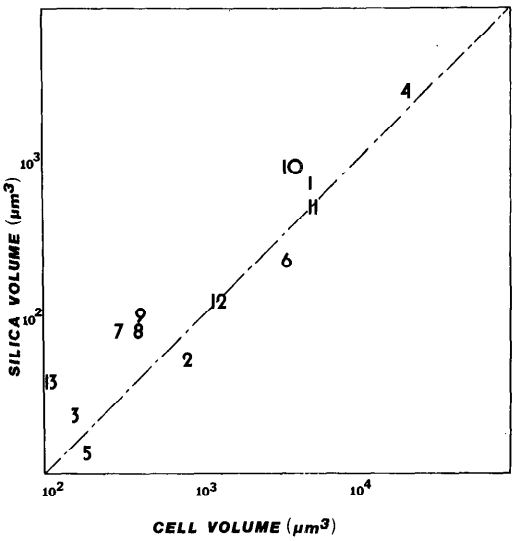

Fig. 1. Logarithmic plot of silica volume vs. cell volume for diatom species examined. (Key to species code given in Table 1.)

hydrated to varying degrees (Kamatani 1971) and may contain varying amounts of "micropore" space (Hurd et al. 1981) which would not be resolved at the magnifications we used. The frustule may also contain organic matter. Unfortunately, most recent measurements (discussed by Schmid et al. 1981) have been made on species which have atypical thin wall structure. For this reason we adopted the uncertain but probably conservative approach that 50% of the frustule is SiO_2 and multiplied frustule volume measurements by 1.1 to obtain silica weight estimates. Calculated dry weights were assumed to be the sum of the frustule weight and twice the carbon content (Shuter 1978) to correct for other organic material and ash.

Results

Our results show a significant correlation between cell volume and silica content (Fig. 1). This would be expected over a cell size range that varies by more than two orders of magnitude. Within this general trend there is considerable interpopulation variation. Relative silica volumes varied fivefold, from a high of 40% in *Cyclotella* sp. No. 6 to a low of 7–8% for three species of *Stephanodiscus*: *S. binderanus, S. subtilis,* and *S. tenuis.* Within the genus *Stephanodiscus* there is also considerable variability, with *S. alpinus, S. minutus,* and *S. niagarae* having silica volumes about twice as large as the three *Stephanodiscus* populations with the lowest frustule volume (Fig. 2). Silica

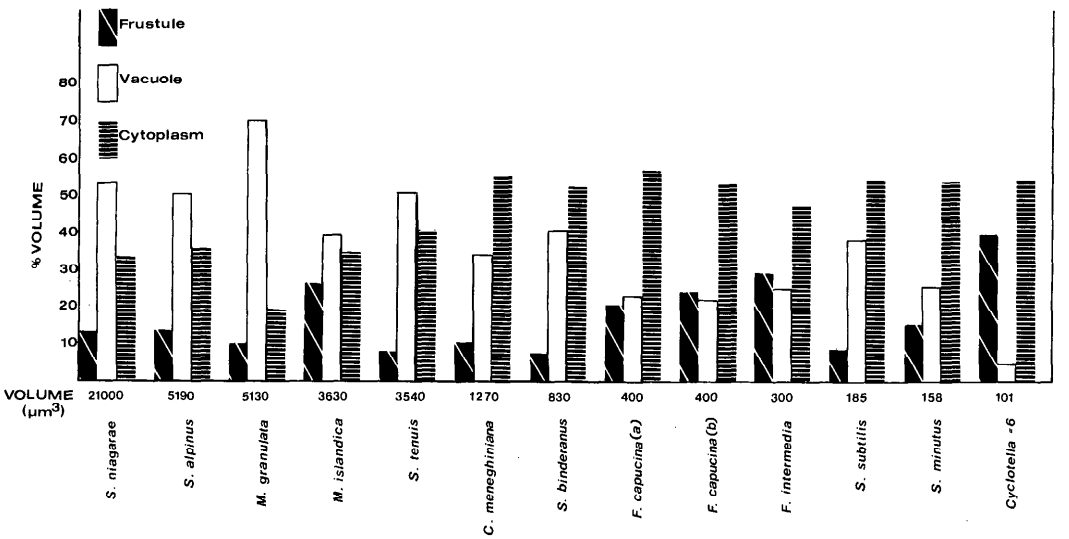

Fig. 2. Relative volumes (% total cell volume) of frustule, vacuole, and cytoplasm for diatom species examined. Diatoms are arranged in order of decreasing size. Cytoplasm is a composite category that includes all organelles other than the siliceous frustule and vacuole.

Table 2. SiO_2 and C dry weight composition of diatoms. Percentages are based on morphometric data converted to biomass by Strathmann's conversion.

	% C	% SiO_2	SiO_2:C	TP*
S. binderanus	30	39	1.30	EI
S. subtilis	29	41	1.41	EI
S. tenuis	27	45	1.67	EI
C. meneghiniana	26	49	1.89	EI
S. minutus	21	59	2.87	E
S. niagarae	17	66	3.86	E
S. alpinus	17	66	3.89	M_2
F. capucina (A)	18	64	3.57	E
F. capucina (B)	15	69	4.47	E
M. granulata	14	72	5.23	E
F. intermedia	12	76	6.32	E
Cyclotella No. 6	11	78	7.25	M_2
M. islandica	10	79	7.62	M_1

* Trophic preference (Tarapchak and Stoermer 1976): EI—recently introduced eutrophic species; M_2—mesotrophic, tolerant of nutrient enrichment; E—eutrophic; M_1—mesotrophic, intolerant of nutrient enrichment.

volumes were <15% for 8 of the 12 populations studied. *Fragilaria capucina, Fragilaria intermedia* var. *fallax,* and *Melosira islandica* had relatively large silica volumes, ranging from 23 to 30%.

Cytoplasm volumes were fairly constant and averaged about 50% for the seven populations with total cell volumes <1,270 μm^3 (Fig. 2). Cytoplasm volumes for the larger-celled species were more variable and generally smaller, ranging from 20 to 41%. Except for *M. islandica,* vacuole volumes for these populations ranged from 50 to 70% compared to 20 to 40% for the smaller species.

Because cytoplasm constituted 50% or less of the total cell volumes, our estimates of carbon in most populations investigated were relatively low. Carbon was estimated to be as little as 10–12% of the dry weight in *M. islandica, F. intermedia,* and *Cyclotella* sp. No. 6. At the other extreme, *Cyclotella meneghiniana, S. binderanus, S. subtilis,* and *S. tenuis* were estimated to contain 26–30% carbon. These and *S. minutus* were the only diatoms that contained >18% carbon. The average for all populations studied was 19±2%.

It is also clear from Table 2 that silica and carbon contents can be separated into three groups. Silica : carbon ratios for these groups are 1.3–1.9, 2.9–4.0, and 5.2–7.6. The silica:carbon ratio, of course, varies more than the silica or carbon contents, since it is the ratio of the product of the range in these contents.

Table 3. Carbon content by dry weight of nondiatom phytoplankton species (after Sicko-Goad et al. 1977). The specific gravity of the cells was assumed to be 1.00, and dry weight 25% of wet weight.

	Measured plasma vol (μm^3)	Total cell vol (μm^3)	% C
Anabaena flos-aquae	52	80	29
Euglena viridis	2,400	3,100	34
Cryptomonas erosa	1,200	1,300	41
Peridinium lindemanni	8,600	11,000	34
Prymnesiophycean No. 1	66	100	29
Prymnesiophycean No. 2	68	70	43

We have also estimated by conversion the carbon content for the phytoplankton species other than diatoms we previously studied (Sicko-Goad et al. 1977). These data are presented in Table 3.

Discussion

The results presented here clearly demonstrate two facts. Morphometric techniques may easily be applied to natural phytoplankton assemblages to measure accurately cellular compartments that may be both physiologically and ecologically significant. The relative volumes of the measured compartments then may be converted mathematically to provide first-order estimates of components such as carbon and silica if desired. Although we have no data on cultured phytoplankton to correlate accurately with the concentration of a particular component per unit volume, our data are in relatively close agreement with values in the literature for carbon or silica on a dry weight basis.

The morphometric techniques we have applied to the natural phytoplankton populations provide a statistical representation of the "average" cell in that population. This average cell represents various stages of development and physiological conditions because natural populations are usually asynchronous. We also believe that our estimates of cellular composition of the phytoplankton populations may differ from estimates

derived from laboratory cultures grown under uniform conditions. We base this statement on several facts. It has been shown that morphometric data from two populations of *F. capucina* were not statistically different although the populations sampled were collected at different locations and during different seasons (Sicko-Goad 1982). It has also been shown (Sicko-Goad and Stoermer 1979; Sicko-Goad 1982) that short term laboratory perturbations with heavy metals produced greater variations and alterations in subcellular structure than were found in the same natural populations collected at different times and locations. Finally, studies in our laboratory have indicated that there is a tendency toward storage product accumulation in short term incubations of natural phytoplankton populations (Sicko-Goad 1982 and unpubl. results). Such accumulation may be accompanied by a decrease in vacuole volume. The product of these two cellular changes in culture could reasonably account for the higher carbon : dry weight ratios previously reported for cultured species (Parsons et al. 1961; Goldman and Graham 1981).

It should also be recognized that our estimates of carbon-containing biomass include only cytoplasm; we have not tried to measure the variable and sometimes large fractions of carbon associated with extracellular products. Oksiyuk and Yurchenko (1971) and other investigators (e.g. Bakken and Olsen 1983) have suggested that it is difficult to compare biomass estimates based on cell volumes with chemical measurements of cell carbon on cultured species due to the variability of extracellular products. With appropriate staining techniques (Reimann 1961), it is possible to visualize and measure extracellular products morphometrically, but we have not done so here. Although visual comparisons with many other observations would indicate that the amount of extracellular products associated with the populations we measured is relatively small, it is a possible source of difference between our estimates and those made by chemical methods.

Our results show that there are problems associated with the direct conversion of

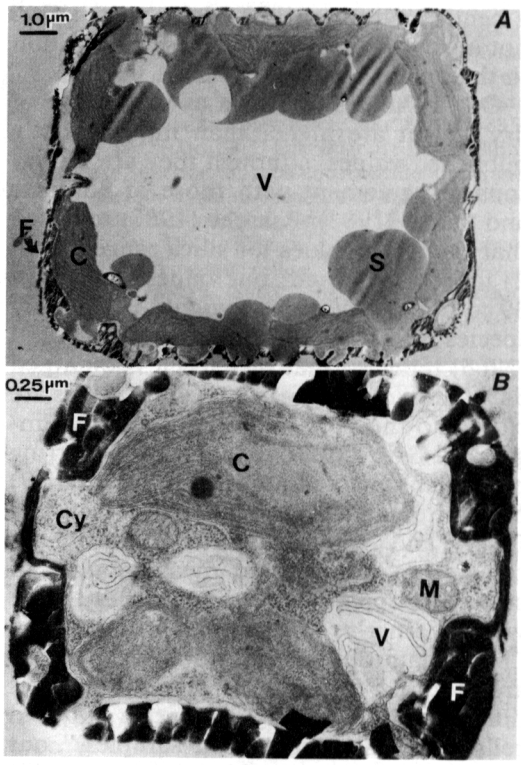

Fig. 3. Transmission electron micrographs of *Cyclotella*. Near median girdle view sections showing chloroplast (C), mitochondria (M), vacuole (V), other cytoplasm (Cy), storage (S), and siliceous frustule (F). Note the difference in frustule thickness and structure. A. *Cyclotella meneghiniana*. B. *Cyclotella* sp. No. 6.

biovolume to estimates of cellular carbon or other constituents. The greatest of these is the inability to estimate plasma volume accurately due to the variability of vacuole volume. Vacuole volume for the species we studied ranges from 2 to 70% of the total cell volume. It ranges from 2 to 34% for nondiatom species (Table 3). The difference for diatoms is even greater, ranging from 5% in *Cyclotella* sp. No. 6 to 70% in *Melosira granulata* (Fig. 2). The problem lies in the estimation of plasma volume on the basis of the assumption that the cytoplasm may be of constant thickness (Lohmann 1908; Strathmann 1967) or of variable thickness based on a cell area : volume ratio (as discussed by Hitchcock 1983). Either model is insufficient to describe a complex diatom ultrastructure (Fig. 3) consisting of

a central cytoplasmic bridge and smaller, not always coalesced vacuoles, in addition to the polar vacuoles.

Our estimates for silica as percentage of dry weight are higher than most recently published values, although they are in reasonable agreement with those of Sommer and Stabel (1983). Paasche (1980) reported that literature values for silica ranged from 21 to 64% dry weight; our values range from 39 to 78%. *Melosira granulata* is the only species common to both data sets. Lund (1965) reported that this species contained 54% silica; our value is 72%. Our values are closest to those originally reported by Einsele and Grim (1938) for naturally occurring phytoplankton communities. Werner (1977) has commented on the differences between Einsele and Grim's estimates and those derived from more recent measurements of cultured populations. While we fully agree with Werner's conclusion (p. 121) that "More data obtained by modern methods are required to decide if there is any real difference in the SiO_2 content in cultured cells and cells in plankton samples," our results do not support his concern (p. 121) that Einsele and Grim's "estimation from plankton samples might be much too high." If populations with similar morphology are compared, our estimates are in reasonable agreement with those of both Werner and Einsele and Grim. There may well have been an unintentional, but systematic, bias in results from laboratory cultures toward diatom species that have relatively thin frustules and low silica content. In fact, most recent measurements (Schmid et al. 1981) have been of species which have thin, atypical wall structure. Such populations are generally more easily maintained in culture because they undergo less rapid size diminution; there also seems to be a general, although certainly not universal, trend for them to tolerate elevated nutrient conditions and to have high growth rates.

The latter point is illustrated by the fact that calculated silica : carbon ratios in the diatom populations we studied were related to the trophic preference classification of Tarapchak and Stoermer (1976) based on range of occurrence in Lake Michigan. The species with the lowest silica : carbon ratios, 1.3–1.9 from our data, were classified by Tarapchak and Stoermer as "introduced eutrophic" species. These populations were not indigenous to the Great Lakes but now are abundant in the most enriched and disturbed parts of the system. None of the populations we studied was classified as "oligotrophic," but among the four populations with the highest silica : carbon ratios, three are classified as "mesotrophic" and two of these are indicated to be intolerant of nutrient pollution. Average silica : carbon ratios might be expected to decrease in diatom assemblages affected by increased phosphorus loadings because of silica limitation (Schelske and Stoermer 1971, 1972; Schelske 1975).

Many of the diatom species that we studied are polymorphic and hence able to adjust the amount of silica accumulated in their frustules to some degree. *Melosira granulata* and *M. islandica* (Stoermer et al. 1981), some varieties of *S. binderanus* (Stoermer et al. 1979), populations reported as *S. subtilis* (Stoermer and Håkansson in press), and *S. tenuis* (Stoermer et al. 1979; Håkansson and Stoermer in press *a*) are all able to form frustules of grossly different structure and thickness. *Cyclotella meneghiniana* (Tuchman et al. in press), *S. alpinus* (Theriot and Stoermer 1982; Håkansson and Stoermer in press *b*), and *S. niagarae* (Theriot 1983) all have lesser, but significant modifications in frustule structure. The case of *S. niagarae* is particularly interesting because modifications in frustule structure can be clearly related to nutrient conditions, specifically Si:P ratios, both in the Great Lakes and in smaller lakes (Theriot and Stoermer in press). It thus appears that silica limitation may operate to select either different species or polymorphic growth forms of certain species with reduced silica requirements. The latter is certainly a factor in our study. Our samples of *S. binderanus, S. subtilis,* and *S. tenuis* are certainly the more lightly silicified growth form of these species. Our results for less strikingly polymorphic species may also be biased toward the low end of the possible range of silica content for those species, but more extended sampling and analysis would be necessary to verify this.

Our results show the importance of ob-

taining morphometric data in certain types of ecological studies where accurate (precise) estimates of carbon content at the population level are necessary or are implied. Eppley (1980) pointed out that it is necessary to make estimates of biomass and growth rate if one is to calculate primary production unambiguously. Accurate estimates of biomass (carbon content) are fundamental in determining species-specific growth rates such as those estimated with autoradiography. Autoradiography may prove to be valuable in improving estimates of production rates for oligotrophic oceans (Peterson 1980). In the Great Lakes literature, rates of diatom production have been calculated from estimates of primary production and an average Si:C ratio (Johnson and Eisenreich 1979). Our results show that community averages are not appropriate, especially if there is no information on diatom species composition.

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