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The relationship between cell carbon and cell volume in freshwater algal species used in zooplanktonic studies

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Abstract. New records of cell carbon and cell volume are given for five species of freshwater algae cultured optimally for use in physiological studies on *Daphnia* spp. These are listed with other values for freshwater algae from the literature, giving a total of 41 determinations for 25 species, together with details of culture media employed, formulae used for calculation of volume and techniques for carbon determination. The following significant regression of cell carbon on cell volume was fitted to 37 points: $C = 0.1204V^{1.051}$ (pg C, μm^3) and the regression coefficient was found not to differ significantly from 1.0. Significant simple linear and polynomial regressions could also be fitted to the untransformed data. There was a significant reduction of residual mean square in the polynomial regressions when tested against the linear regressions and this demonstrated the curvilinearity of the relationship. The polynomial regressions predicted a more or less constant carbon-volume ratio of ~ 0.2 pg C μm^{-3} over the size range of freshwater algae available but the double logarithmic regression was better for comparison. The double logarithmic regression is compared with other regressions for freshwater and marine algal species and it appears that freshwater and marine species differ in the way carbon content per unit volume changes with cell size, as this relationship is inverse in marine algae. It is suggested that the relationship described here might be applied to estimate the availability of edible algae in nature to herbivorous zooplankton.

Introduction

Some knowledge of the physical and chemical characteristics of algal species is of special interest for studies on zooplanktonic filter-feeders since their main nutritive food in natural waters is phytoplankton. It is well known that food quantity and quality are important factors controlling development, growth and reproduction of zooplankton (Lefèvre, 1942; Edmondson, 1957; Hrbáčková, 1974; Weglenska, 1971; Pourriot, 1977). Although it is relatively easy to quantify the food level and to define the physical and chemical characteristics of monospecific algal food in laboratory experiments, it is quite difficult or almost impossible to determine the quantity of food available to planktonic animals in their food situation. This is because of the technically unsolved problem of how to separate the seston into living algal cells, detrital particles, bacteria and smaller zooplanktonic organisms in order to assess their relative abundance. Measurements of chlorophyll *a* or total particulate carbon in the seston do not provide an estimate of the concentration of particles available as food to filter feeders, since as much as 50% may be unsuitable because of size, shape, presence of projections or indigestible sheaths or because of toxic chemical properties (Lefèvre, 1950; Taub and Dollar, 1968; McNaught *et al.*, 1980; Porter and Orcutt, 1980; Lampert, 1981).

However, when something is known about the taxonomic composition and density of the phytoplankton, its size distribution and the size-shape characteristics of the algal cells ingested by the herbivores being studied, there is the possibility of applying a

cell carbon-cell volume relationship to cell densities in order to estimate the carbon biomass of the edible algal species present in the seston. For this reason, it is desirable to establish whether a predictable relationship between cell carbon and cell volume can be derived.

The application of such a relationship for the conversion of algal numbers and volumes to carbon biomass of available food is also a step towards expressing the grazer-grazed interaction in common units. This is desirable for the ecosystem models of planktonic communities of lakes and reservoirs in which photosynthesis, respiration and primary production have long been expressed in carbon per unit time. In more recent times, the feeding, respiration and growth of zooplanktonic species have been quantified as carbon rates (Lampert, 1977a, 1977b, 1977c, 1977d). What has been missing is a reliable estimate of what proportion of the net primary production is available to the main herbivorous populations, although attempts to assess this have been made using a crude conversion factor for carbon-volumes (Duncan, 1975; Nadin-Hurley, 1976).

A proportionality between the organic content and volume of algal cells has been reported by many investigators (Riley, 1941; Rodhe, 1948; Cushing, 1958; Strickland, 1960; Lund, 1964; Nalewajko, 1966). The first detailed study of the relationship between cell carbon and cell volume was made by Mullin *et al.* (1966) for a range of marine algal species from different taxonomic groups; this was followed by another for mainly marine diatoms by Strathmann (1967). Strathmann considered whether a carbon-plasma volume relationship provided a more precise prediction of cell carbon than a cell carbon-total cell volume one, since the cytoplasm of diatoms is confined to a thin film between the cell vacuole and the cell walls. More recently, Vidal (1978) presented a carbon-volume relationship for the marine diatoms he used in his growth experiments on marine copepods. For freshwater algal species, much less information is available and the first directly determined cell carbon-cell volume relationship was produced by Chalk (1981) for a series of algal species which she used during feeding and growth studies on *Daphnia magna*. The equations for the above four relationships are presented in Table V.

Methods

Algal cultures

The species *Scenedesmus acutus, S. quadricauda, S. brasiliensis* and *Monodus subterraneus* were grown in batch culture in Chu 12 medium (Stein, 1973) under continuous illumination from fluorescent lamps and at a constant temperature of $20 \pm 2°C$. The cultures were not free of bacteria. The species *Cryptomonas curvata* (Javornicky and Popovsky, 1971) from Dr R.S.Stemberger and a small chlorophycean (not identified) were grown under similar conditions in the Woods Hole MBL medium which contained vitamins. The cultures were kept in the exponential phase of growth by sub-culturing.

Table II lists the physical dimensions and carbon content of freshwater algal cells cultured in a variety of media. The following code identifies the media used: (1a) CHU 12 ($0.161\ g\ l^{-1}$) or a modified CHU 12 (Müller, 1972); (1b) CHU 10 EVT ($0.1041\ g\ l^{-1}$ with vitamins) (Stein, 1973); (1c) Blue-green algal medium, De's modification ($0.86\ g\ l^{-1}$) (Chalk, 1981); (1d) Kuhl's medium ($1.99\ g\ l^{-1}$) (Kuhl, 1962); (1e) Woods Hole MBL medium ($0.2163\ g\ l^{-1}$, with vitamins) (Stein, 1973);

Table I. The linear dimensions, calculated cell volumes and the carbon content of six species of freshwater algae determined in this study.

Species	(1)[a]	(2)[b]	n	Length ± S.D. (μm)	Width ± S.D. (μm)	Volume(3)[c] (μm³)	Volume ± S.D.(4)[d] (μm³)	n	Carbon ± S.D. (pg)
Scenedesmus acutus Meyer	1a	2a	50	10.34 ± 2.44	4.68 ± 1.36	109.39	106.58 ± 45.17	12	11.78 ± 0.30
Scenedesmus quadricauda Bréb.	1a	2b	50	14.55 ± 1.54	4.86 ± 0.64	269.69	276.21 ± 93.12	12	37.50 ± 4.26
Scenedesmus brasiliensis Bohl.	1a	2b	30	10.21 ± 1.75	3.96 ± 0.36	125.75	123.33 ± 41.23	12	10.64 ± 0.21
Monodus subterraneus Petersen	1a	2d	50	7.31 ± 1.47	3.79 ± 1.15	81.02	89.27 ± 69.74	12	6.55 ± 0.49
Cryptomonas curvata Ehr.	1e	2d	370	37.94 ± 2.38	19.91 ± 1.84	11 445.25	11 750.89 ± 1879.63	7	2197 ± 657.61
Unidentified small green alga	1e	2c		3.0		14.14		21	2.40 ± 0.223

[a]Culture media, see Methods.
[b]Formula for calculating cell volume, see Methods for key and formulae.
[c]Mean cell volume calculated from mean values of length and width.
[d]Mean cell volume calculated from individual volumes and accompanied by standard deviation.

Table II. Physical dimensions and carbon content of cultured freshwater algal species (used in zooplankton experiments) as obtained in this study and as available in the literature. (1), (2) and (3) refer to culture media, formula used for volume calculation and the carbon method. Explanation of the codes for the authors is given at the bottom of the table. L = length; W = width.

Species	Size of cell								
	Linear L(μm)	W(μm)	Volume (pg C)	Weight pg μm^{-3}	C/V	(1)	(2)	(3)	Author(s)
Chlorophyceae									
Scenedesmus acutus	10.34	4.68	102.39	11.78	0.115	1a	2a	3a	OR
S. acutus	15	4	125.66	15.63	0.124	1a	2a	3d	WL
S. acutus	14	4.5	111.00	21.3	0.192	1a	2b	3f	WG
S. obliquus			59.4	6.58	0.111	1g	2f	3e	K&R
S. obliquus			82.7	7.83	0.095	1g	2f	3e	K&R
S. basilensis			56.8	6.62	0.116	1g	2f	3e	K&R
S. brasiliensis Bohl.	10.21	3.99	125.33	10.64	0.085	1a	2a	3a	OR
S. brasiliensis Bohl.[a]			92.08	2.97	0.032	1d	–	3c	SP
S. quadricauda	14.55	4.86	269.69	37.5	0.139	1a	2b	3a	OR
S. quadricauda			255.25	46.00	0.180	1b	2c	3a	EC
S. quadricauda			296.25	53.45	0.180	1b	2e	3a	EC
Chlorella vulgaris	2.77		11.09	3.0	0.262	1b	2c	3a	EC
C. vulgaris			11.09	2.9	0.271	1b	2c	3a	EC
S. vulgaris			12.13	1.9	0.157	1a	2c	3c	SP
C. vulgaris			49.8	5.0	0.100	1g	2f	3e	K&R
C. pyrenoidosa			37.6	3.59	0.096	1g	2f	3e	K&R
'Small green'	3.0		14.14	2.40	0.170	1e	2c	3a	AD
Stichococcus minutissimus	3.0	1.0	3.14	0.83	0.264	1a	2b	3f	WG
S. bacillaris			24.9	3.32	0.133	1g	2f	3e	K&R
Pandorina morum	15.5		1206.0	384.0	0.320	1b	2c	3a	EC
Staurastrum planctonicum	50.0	40.0	40 000.0	11 500.0	0.288	1a	2c	3f	WG
Xanthophyceae									
Monodus subterraneus	7.31	3.79	81.02	6.55	0.081	1a	2d	3a	OR
Tribonema aequale[a]	13.3		1671.0	995.0	0.595	1b	2c	3a	EC
T. aequale[a]			2015.0	1029.4	0.511	1b	2c	3a	EC
T. spp	26.92	8.46	1513.24	305.0	0.202	1a	2b	3a	OR
T. spp			1513.24	310.0	0.205	1a	2b	3a	OR
Cryptophyceae									
Cryptomonas ovata	12.5		689.0	171.1	0.264	1b	2c	3a	EC
C. erosa	23.4	11.0	1478.0	125.3	0.085	1e	2d	3b	SA&WTE
C. curvata			10 300.0	2197.0	0.213	1e	2d	3a	AD
Rhodomonas minuta	5.9		60.0	9.8	0.163	1b	2c	3a	EC
Bacillariophyceae									
Stephanodiscus hantschii	7.3	6.0	250.0	27.8	0.111	1a		3f	WG
S. hantschii	10.7		390.0	84.7	0.217	1b	2c	3a	EC
S. hantzchii			394.0	64.0	0.162	1b	2c	3a	EC
S. astraea	28.7		12 382.0	2990.0	0.242	1b	2c	3a	EC
Nitzschia actinastroides	25.0	5.0	327.24	25.0	0.076	1a	2a	3f	WG
Asterionella formosa	27.0	3.0	190.85	21.9	0.115	1a	2b	3f	WG

Table II *Continued*.

Species	Size of cell								
	Linear L(μm)	W(μm)	Volume (pg C)	Weight pg μm^{-3}	C/V	(1)	(2)	(3)	Author(s)
Cyanophyceae									
Microcystis aeruginosa[a]	6.0		113.0	3.6	0.032	1f	2c	3f	WG
M. aeruginosa	5.0		65.45	7.14	0.109	1f	2c	3f	WL
Synechococcus elongatus	2.0	1.0	1.57	0.164	0.105	1f	2b	3d	WL
Anabaena flos-aquae	31.9	3.0	132.0	36.6	0.277	1c	2b	3a	EC
A. flos-aquae			207.0	53.4	0.258	1c	2c	2a	EC

[a]Omitted from regression 1 in Table III.
Authors: AD, A.Duncan; EC, E.A.Chalk (1981); K&R, B.H.Ketchum and A.C.Redfield (1949); OR, O. Rocha; SA&WTE, S.Abella and W.T.Edmondson (personal communication); SP, S.Piyasiri (personal communication); WG, W.Geller (1975); WL, W.Lampert (1981).

(1f) Blue-green algal medium of O'Flaherty and Phinney (1970) (0.26 g l^{-1}); (1g) medium of Ketchum *et al.* (1949) (1.76 g l^{-1}).

Algal cell size

Cell size was determined by the measurement of linear dimensions of a number of cells under high magnification using an ocular micrometer fitted into one eyepiece. A magnification of x1000 was used for *Scenedesmus*, *Monodus* and the small green alga and x400 for *C. curvata*. Living *Scenedesmus* and *Monodus* cells each from a single culture were measured whereas the cells of *Cryptomonas* and the small green alga were preserved in Lugol's iodine just prior to measurement and came from a series of batch cultures being grown as food for zooplankton. For cylindrical and oval shapes, two measurements were taken, one for the major and another for the minor axis. Volumes were calculated by applying formulae for the most appropriate geometrical shape which are given below. Mean volumes were derived by two calculation procedures: one from the mean linear dimensions and the other by averaging the individual volumes.

The following code identifies the geometric shapes and formulae adopted in Tables I and II for calculation of cell volume, where r refers to radius and h to height or length:

(2a) mean cylinder $\frac{1}{2}[(\Pi r^2 h) + \frac{(\Pi r^2 h)}{3}]$

(2b) cylinder $\Pi r^2 h$

(2c) sphere $\frac{4}{3} \Pi r^3$

(2d) elipsoid $\frac{4}{3} \Pi r_1 r_2^2$

(2e) four cylinders with a hemisphere at each end $4[(\Pi r^2 h) + \frac{1}{2} (\frac{4}{3} \Pi r^3)]$

(2f) centrifuged displaced volume.

Determination of organic carbon

Samples of *Scenedesmus* and *Monodus* from cultures kept in the exponential phase of growth were harvested, centrifuged and washed twice in distilled water before being

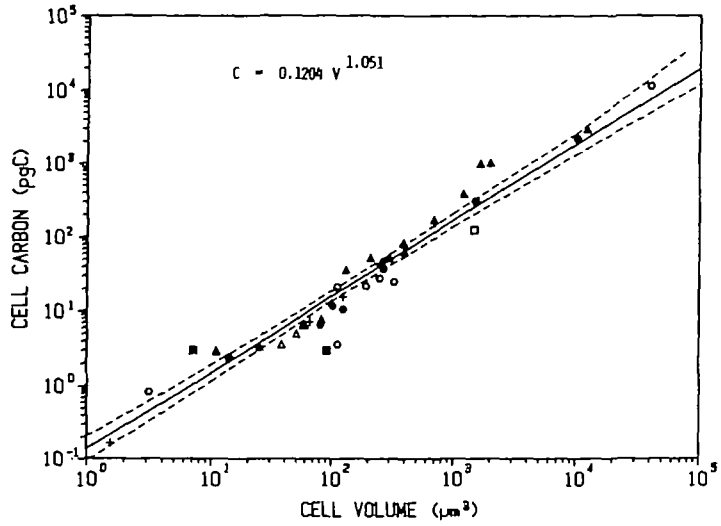

Fig. 1. The relationship between cell carbon and cell volume of freshwater species of algae. The dashed lines represent the 95% confidence limits. Authors of determinations (see Table II for code): □ SA&WTE; ▲ EC; ○ WG; △ K&R; + WL; ■ SP; ● OR&AD.

concentrated by filtration on pre-combusted glass fibre filter pads (GFF, 0.7 μm pore size); the same procedure was adopted for *Cryptomonas* and the small green alga, apart from the centrifugation. Organic carbon was determined by wet oxidation in dichromate and sulphuric acid at 100°C and the decrease in the oxidant was determined by titration with a ferrous salt. The end-point was detected amperometrically which increased the sensitivity of the technique and the lower limit of detection of the method was 30 μg carbon (Mackereth *et al.*, 1978).

The following code identifies the method of carbon determination for results cited in Tables I and II: (3a) wet combustion, as described above but with the titration end-point detected either amperometrically (OR&AD) or colorimetrically using the FERROIN indicator (EC) (Mackereth *et al.*, 1978); (3b) dry combustion of a filtered algal sample (silver pad) in a flow of oxygen and helium and the chromatographic detection of carbon dioxide by a Carlo Erba CHN Analyzer (Pella and Colombo, 1970); (3c) dry combustion of an algal sample in flowing oxygen and the detection of carbon dioxide by a Hartmann and Braun URAS2T nondispersive i.r. gas analyzer (Salonen, 1979); (3d) dry combustion in oxygen of an algal sample (GFF pad) and the detection of carbon dioxide by an UNOR i.r. gas analyzer (Krambeck *et al.*, 1981); (3e) dry combustion by Pregl's microcombustion method in which carbon dioxide is absorbed by ascarite which is weighed before and after (Fieser (1935) in Ketchum *et al.*, 1949); (3f) dry combustion in a Pregl-Roth combustion furnace and the carbon dioxide absorbed in a known volume of NaOH. The remaining NaOH is titrated against HCl using phenophthalein as an indicator (Müller, 1972).

Results

New data on the linear dimensions and carbon content of cultured freshwater algal species

are presented in Table I. All these algae have been cultivated for use as food in experiments on the growth of the planktonic animals under defined food conditions where it was advantageous to quantify food concentrations in carbon biomass. The algal species were cultured under similar conditions, according to the techniques described in Methods and in one of two media; they were harvested whilst growing expontentially and only one technique was used to determine carbon levels. Cell volumes were calculated from the measured linear dimensions, using the most appropriate shapes which fitted cell shapes. The particular formula adopted is given in Table I. Mean cell volumes were calculated either from the mean linear dimensions or from the individual volumes but the difference between these two was very small and the latter mean was used because there was a standard deviation which indicated the variability of the volume estimates. In general, Table I shows there to be a greater variability within the volume data compared with the carbon values.

The new values are included in Table II which lists other linear dimensions, cell volumes and cell carbon content of freshwater algal species which have been collected from the literature or from personal communications and includes the original data from Chalk's 1981 regression. The list contains 41 determinations by eight workers for 25 species which belong to five algal phyla and almost all come from long-term cultures maintained for zooplankton experiments, only *Tribonema* spp being recently isolated from the wild. The column with carbon weight per unit volume (as pg C μm^{-3}) provides values useful for comparing algae of different size. Information is provided in columns (1), (2) and (3) on the culture media used, the geometric formulae adopted for calculation of cell volume and the method of carbon determination, since there is some indication that these variables may affect the carbon-volume ratio. The data for *Scenedesmus brasiliensis*, which came from the same stock (the culture collection Bourrelly in Paris) provides an example of variation which may be due to technique. The algae had a smaller size and carbon-volume ratio when cultivated on Kuhl's medium (1.99 g l^{-1}) and when the carbon was determined by dry combustion (Salonen, 1979) but had a large size and carbon-volume ratio when cultured on Chu 12 (0.161 g l^{-1}) and with a wet combustion technique for carbon determination (Mackereth *et al.*, 1978). It may well be that the variation in cell size and carbon-volume ratios exhibited by *Chlorella vulgaris* in Table II is also associated with the different techniques employed.

Figure 1 is a plot of the data listed in Table II using logarithmic scales for convenience since the algal size covers several orders of magnitude. A curvilinear regression of the form

$$\ln C = \ln a + b \ln V$$

has been fitted by least squares, where C is cell carbon in pg C, V is cell volume in μm^3, ln a is the regression intercept and b is the regression coefficient. The resulting regression, which was fitted to 37 of the points listed in Table II and illustrated in Figure 1, is given as regression 1 in Table III. The regression was significant and the 95% confidence limits for the values of cell carbon predicted by it for given values of cell volume are plotted in Figure 1. These confidence limits were calculated by the formula given on page 422 of Sokal and Rohlf (1969). Figure 1 shows that certain determinations fall well outside these confidence limits but otherwise there is a reasonable fit to this heterogeneous data.

Table III. Various regressions relating cell carbon (pg C) to cell volume (μm^3) in freshwater algal species listed in Table II (n = 37).

	Regression equation	a ± 95% cl	b ± 95% cl	c	d	RMS	d.f.	F	*p*
1	$\ln C = \ln a + b \ln V$	-2.117 ± 0.346 $(0.1204)^a$	1.051 ± 0.065	–	–	0.17	1,35	1078	0.0001
2	$C = a + bV$	-51.6 ± 53.4	0.281 ± 0.008	–	–	23 574	1,35	5781	0.0001
3	$C = a + bV$	0	0.279 ± 0.008	–	–	25 441	1,36	5719	0.0001
4	$C = a + bV + cV^2$	-7.3 ± 18.8	0.208 ± 0.010	2.039×10^{-6}	–	2672	2,34	25 640	0.0001
5	$C = a + bV + cV^2$	0	0.205 ± 0.009	2.067×10^{-6}	–	2643	2,35	27 683	0.0001
6	$C = a + bV + cV^2 + dV^3$	4.2 ± 20.5	0.161 ± 0.042	7.196×10^{-6}	-1.007×10^{-10}	2382	3,33	19 176	0.0001
7	$C = a + bV + cV^2 + dV^3$	0	0.165 ± 0.034	6.708×10^{-6}	-9.140×10^{-11}	2342	3,34	20 990	0.0001

[a]Antilog; cl, confidence limits; RMS, residual mean square; d.f., degrees of freedom; F, variance ratio; *p*, probability

Table IV. Cell carbon (C) predicted from cell volumes (V) by the regressions in Table III.

Given values of cell volume (μm^3)	Regression 1 $\ln C = \ln a + b \ln V$	Regression 3 $C = 0 + bV$	Regression 5 $C = 0 + bV + cV^2$	Regression 7 $C = 0 + bV + cV^2 + dV^3$
Predicted cell carbon (pg C) ± S.E. (standard error of the predicted value)				
1.57	−1.643 ± 0.883 (0.193)[a]	0.438 ± 27.20	0.322 ± 0.067	0.350 ± 0.006
11.09	0.412 ± 0.511 (1.510)[a]	3.094 ± 27.19	2.273 ± 0.048	2.474 ± 0.041
102.39	2.748 ± 0.106 (15.6)[a]	28.6 ± 27.10	21.0 ± 0.446	22.8 ± 0.376
1206	5.340 ± 0.397 (208)[a]	336 ± 26.36	250 ± 5.099	269 ± 4.422
10 300	7.594 ± 0.805 (1987)[a]	2874 ± 40.40	2330 ± 33.17	2342 ± 35.28
Range of C/V (pg C μm^{-3}) from above predicted values of C ± S.E.				
1.57	0.051-0.298[a]	−17.04 -17.60	0.162-0.248	0.219-0.227
11.09	0.082-0.227[a]	−2.172 -2.730	0.201-0.209	0.219-0.227
102.39	0.137-0.170[a]	0.014 -0.544	0.200-0.210	0.219-0.227
1206	0.116-0.257[a]	0.257 -0.301	0.203-0.212	0.220-0.227
10 300	0.086-0.431[a]	0.275 -0.283	0.223-0.229	0.224-0.231

[a]Antilog of lnc.

A test using student's t showed that the coefficient of the above regression was not significantly different from 1.0 (d.f. 36; t = 1.581; $p = 0.15$) so that a linear regression of untransformed values of C and V might be more appropriate and simpler, particularly if the carbon content per unit volume can be shown to be constant over the range of algal sizes. However, although the simple linear regression 2 in Table III was significant, it predicted negative values for cell carbon for all cell volumes below a certain size due to the high negative intercept. When a zero origin is imposed, the result is regression 3 in Table III in which cell carbon is predicted from volume using a constant C/V ratio of 0.279, but when the standard error of the predicted carbon is included (Sokal and Rohlf, 1969, p. 422) Table IV shows that the sizes and ranges of predicted (C ± S.E.)/V ratios are unacceptable for the smaller sizes of algae.

A series of polynomial regressions fitted to the untransformed variables of C and V, some with zero origins, is given in Table III and all are statistically significant. A comparison of the residual mean squares shows a large decrease with the addition of a quadratic term and a further, though smaller, decrease with the addition of a cubic term. This suggests curvilinearity in the original relationship which is being satisfied by the relatively small values of the regression coefficient 'c' in the polynomial regressions. When Snedecor and Cochran's (1956, p. 455) test of significance of the departure from linear regression was applied to regressions 2 and 4, or to regressions 3 and 5, in Table III, the reduction in sums of squares, when tested against the mean squares remaining after curvilinear regression, proved to be significant in both cases (d.f. 1,34; F = 275; $p = 0.0001$ and d.f. 1,35; F = 312; $p = 0.0001$, respectively).

The next question is whether, unlike the linear regression, both types of curvilinear regressions (double logarithmic and polynomial) provide predictions which are useful practically. Table IV lists a series of predicted cell carbons ± the standard errors of the predicted values calculated for five cell volumes selected from Table II to cover four orders of magnitude in size. The range of carbon/volume ratios [(C−S.E.)/V to

Table V. The relationship between cell carbon and cell volume of cultured algae as obtained in this study and as available in the literature. Equation: $C = aV^b$, where C is pg C per cell and V is μm^3 per cell; d.f., degrees of freedom; F, variance ratio and p, probability.

Material		Equation: $C = aV^b$(pgC;μm^3) a	b ± 95% cl	Test of significance of the regression d.f.	F	p	Authors
Freshwater algae	1	0.1204	1.051 ± 6.2%	1,35	1078	0.001	This study (Table II)
Freshwater algae	2	0.210	1.041 ± 12.8%	1,12	291	0.001	Chalk (1981)
Marine algae	3	0.519	0.757 ± 6.6%	1,35	874	0.001	Mullin *et al.* (1966)
Marine diatoms	4	0.485	0.712 ± 4.9%	1,66	–	–	Strathmann (1967)
Two marine diatoms	5	0.357	0.814 –	–	–	–	Vidal (1978)

Comparison by covariance analysis (Steel and Torrie, 1980) between some of the regressions.

Regression 1 and 2:
Bartlett's test for homogeneity of residual variances: d.f. = 1; $X^2 = 0.27$; $p = 0.91$.
Similarity of regression coefficients (b): d.f. = 1,47; F = 0.014; $p = 0.60$.
Similarity of adjusted means: d.f. = 1,49; F = 12.72; $p = 0.0008$.

Regression 1 and 3:
Bartlett's test for homogeneity of residual variances: d.f. = 1; $X^2 = 0.012$; $p = 0.91$.
Similarity of regression coefficients (b): d.f. = 1,70; F = 40.39; $p = 0.0001$.
Similarity of adjusted means: cannot be tested since slopes are not parallel.

(C+S.E.)/V] for each selected cell volume is given in the lower half of the table. All three curvilinear regressions predict realistic values for these, but the polynomial ones provide a series of more constant ratios (~ 0.2 pg C μm^{-3}) over the whole algal size range. In the double logarithmic regression the range widens at each extreme of the algal size range and is narrowest about the mean tendency.

Although the polynomial regressions provide a more or less constant C/V ratio, they are much less amenable to regressional analysis and testing of significance when the range of variables covers so many orders of magnitude and comparison is difficult with other algal carbon/volume relationships in the literature. For this purpose, the double logarithmic regression 1 in Table III was used. Table V lists the regressions available and the intercept values have been back-transformed for comparability. The two freshwater regressions are very similar and covariance analysis (details in Table V) shows that, although the slopes are the same ($p = 0.91$), the elevations are statistically different ($p = 0.00008$), the Chalk regression lying higher than that of Rocha and Duncan. The consistently higher original values from Chalk (1981) can be seen in Figure 1. Table V shows that the three marine regressions also look rather similar to each other but are strikingly different from the freshwater ones in possessing slopes of < 1.0. This is illustrated in Figure 2 which also shows that the two sets of regressions overlap at cell sizes of $\sim 25 - 100$ μm^3. The marine and freshwater regressions are rather different and this was confirmed by covariance analysis of the Mullin *et al.* and the Rocha and Duncan regressions, only made possible because Mullin *et al.* published their data in full. Homogeneity of the residual variances ($p = 0.91$) permitted a comparison to be made between these two regressions but the regression coefficients were different ($p = 0.0001$) and when slopes are not the same there is no possibility for comparison of regression elevations.

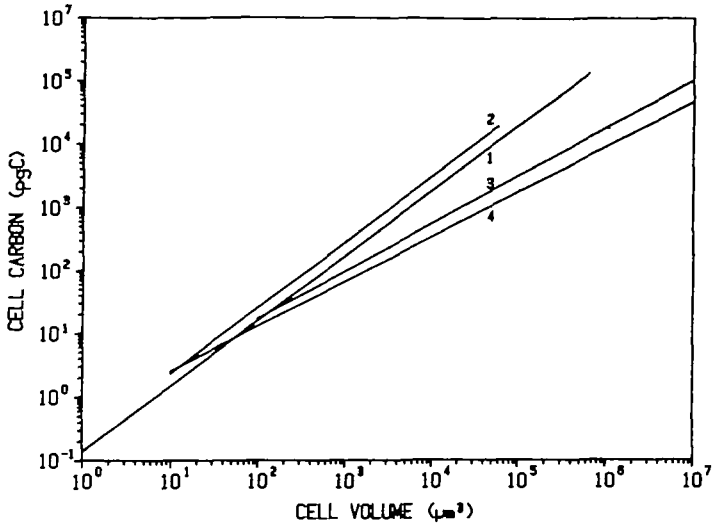

Fig. 2. Plots of the relationship between cell carbon and cell volume of freshwater and marine algae which are given in Table V. (1) This study and Table II; (2) Chalk (1981); (3) Mullin *et al.* (1966); (4) Strathmann (1967).

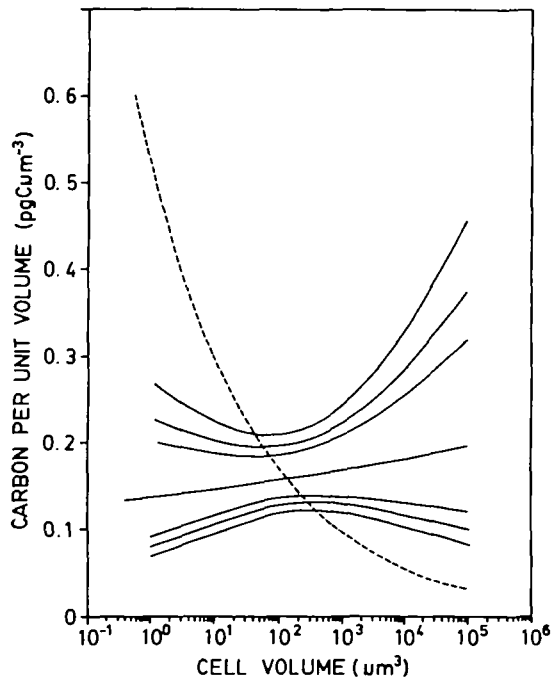

Fig. 3. A comparison between the carbon per unit volume against cell volume of marine (dashed line) and freshwater (solid line) algae, together with the 95%, 99% and 99.9% confidence limits about the freshwater line.

Table VI. Various regressions relating cell carbon (pg C to cell volume (μm^3) in freshwater algal species listed in Table II (n = 47). RMS, residual mean square.

	Regression equation	a ± 95% cl	b ± 95% cl	c	d	RMS	d.f.	F	p
1	$\ln C = \ln a + b \ln V$	−1.952 ± 0.376 (0.1420)[a]	1.996 ± 0.065	–	–	0.24	1,45	963	0.0001
2	$C = a + bV$	−104.8 ± 88.8	0.273 ± 0.014	–	–	82 560	1,45	1651	0.0001
3	$C = a + bV$	0	0.268 ± 0.014	–	–	90 908	1,46	1599	0.0001
4	$C = a + bV + cV^2$	−13.6 ± 50.5	0.160 ± 0.023	3.218×10^{-6}	–	23 588	2,44	2948	0.0001
5	$C = a + bV + cV^2$	0	0.157 ± 0.020	3.274×10^{-6}	–	23 218	2,45	3199	0.0001
6	$C = a + bV + cV^2 + dV^3$	36.6 ± 50.7	0.018 ± 0.075	1.974×10^{-5}	-3.250×10^{-10}	17 700	3,43	2624	0.0001
7	$C = a + bV + cV^2 + dV^3$	0	0.049 ± 0.062	1.655×10^{-5}	-2.650×10^{-10}	18 151	3,43	2733	0.0001

This difference between the marine and freshwater regression cofficients implies that the way cell size influences the carbon content per unit volume is different in marine and freshwater algae. As illustrated in Figure 3, in marine algae, it is the smaller cells which have the highest carbon per unit volume and there is a decrease in the ratio as the cell size increases whereas this ratio appears to be more or less constant in the freshwater algae over a similar size range. The expected values for pg carbon per μm^3 volume in freshwater algae, calculated from the Rocha and Duncan regression with b = 1.051, range from 0.120 for a 1 μm^3 cell to 0.216 for a 10^5 μm^3 cell but the confidence limits about these estimates are much wider, as illustrated in Figure 3. Kersting's (1983) value of 2.0 10^{-10} mg per C μm^{-3} for *Chlorella vulgaris* falls within the 99% confidence limits and agrees well with the prediction by the polynomial regressions.

Discussion

The relationship between cell carbon and volume for freshwater algae used in physiological zooplankton experiments and presented in this paper is not considered to be a definitive one. It is rather a development of Chalk's original freshwater regression, but with more points, which can serve as a base-line for assessing future determinations. It appears that cell size is not a variable affecting the carbon content per unit volume in freshwater algae. However, there are other influences on the C/V ratio which are technical in origin and which could be avoided with further investigation to clarify their effect. Of those examined in this paper, these are the nature and concentration of the culture medium and the method of carbon determination. For example, two workers obtained very different values of carbon per unit volume for the same stock of *S. brasiliensis* when it was cultured in media of different concentration and when carbon was determined by different methods. Sometimes a detectable and constant bias in the data may be caused by an inadequate sensitivity of the carbon technique; for example, the values for carbon per unit volume determined by a titration end-point detected colorimetrically by an indicator is significantly higher than the one presented in this paper. Further investigation on these technical matters may result in more replicable values for algae cultured optimally.

How well the geometric shape used to calculate volume from linear dimensions fit the actual cell shape is another consideration affecting the relationship between the carbon and volume and contributing to the overall variability, particularly between data sets of different workers. Rott (1981) reports on the findings of several workshops of algologists and this paper contains some valuable recommendations. Anyone who has calculated algal cell volume from linear dimensions knows how greatly the final value can vary according to the shape and equation adopted, so that it is clearly advantageous for published volumes to be accompanied by the linear dimensions from which they have been derived. The greater variability associated with the values of volumes compared with those of carbons in Table I suggests that carbon rather than volume should be the independent variable in the regression.

However, for obtaining a reliable estimate of what proportion of the algal biomass in a water body is available to the herbivorous population, volume has to remain the independent variable, since microscopic analysis is the best, and probably only reliable, method of distinguishing the edible sizes and species of algae in nature. More effort

is needed to increase the accuracy of cell volumes, for example, by more careful choice of geometrical shape for volume calculation and checking by other methods of volume measurement, such as density gradient, volume displacement or Coulter electronic techniques. In the authors' experience, it is probably more accurate to use for one algal species a combination of two or more formulae for different geometrical shapes [e.g., the mean of cylinder and cone for *S. acutus*; the mean of oblate and prolate spheroids (ellipsoids) for *C. ovata, C. curvata* and *M. subterraneus* and a cylinder with two terminal hemispheres for *S. quadricauda*; but see Rott (1981) for algologists' advice] than the spherical shape which is more generally used by some authors. It is also necessary to test whether the carbon-volume relationship presented here and mostly coming from laboratory algal cultures can predict well the carbon content of wild algal species from the phytoplankton. In some waters, this would be possible by determining the carbon content and volume size of the algal species at a time when they are producing abundant and almost monospecific crops in lakes and reservoirs. The presence of non-algal particulate carbon might obscure the relationship in water bodies with an organically loaded riverine input. In such situations, if the growing algal crop can be sampled at intervals prior to the maximum, a regression of total particulate carbon versus chlorophyll *a* can be obtained:

$$\text{Total particulate carbon} = a + b\ (\text{chlorophyll } a) \quad \text{(Steel } et\ al.\text{, 1972)}$$

in which the slope 'b' provides a carbon-chlorophyll *a* ratio for the crop species and the intercept 'a' gives some measure of the prevailing level of non-algal particulate carbon.

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Addendum

Recently, Infante and Litt (personal communication) have increased by ten the number of available records in the literature on cell carbon and cell volume of freshwater algal species and have generously sent us a list of their data. The following new regression of carbon on volume was calculated using the original data together with these new points:

$$C = 0.1420V^{0.996} \quad \text{pg C } \mu m^3 \quad (\text{d.f. } 1,45;\ F = 963;\ p = 0.0001)$$

This is presented in Table VI together with a series of simple linear and polynomial regressions fitted to untransformed variables and some with zero origins, as was done earlier in Table III. As before, the polynomial regressions provide evidence of curvilinearity in the relationship between cell carbon and cell volume in freshwater algae and, as before, the double logarithmic regression above has been used for comparison.

A comparison of regressions 1 in Tables III and VI shows that they are similar with respect to residual variance ($p = 0.45$), regression coefficients ($p = 0.31$) and ad-

justed mean carbons ($p = 0.28$). As in the regression with 37 points, this regression with 47 points has a slope which does not differ significantly from 1.0 (d.f. 46; t = 0.109; $p = 0.90$).

The technique for carbon determination used by Infante and Litt was dry combustion [(3b) in Methods]. A comparison of the influence which the technique of carbon determination (wet or dry) has on the carbon-volume relationship of freshwater algae showed that the adjusted mean carbon obtained by wet combustion was significantly higher (d.f. 1,82; F = 12; $p = 0.0001$; adjusted mean for wet combustion was 3.551 pg C and 3.109 pg C for dry combustion in their transformed forms). However, this comparison includes values determined by the wet combustion technique with the relatively insensitive colorimetric titration end-point. When these are deleted, the regression coefficients become significantly different (d.f. 1,56; F = 8.15; p = 0.16; b = 1.113 ± 0.086 95% cl for wet combustion and b = 0.956 ±0.061 for dry combustion) so that the adjusted mean carbon (now 3.183 and 3.019, respectively) can no longer be compared, but the difference between them is much less. Clearly we, as zoologists, must await a critical study by algologists (as in Rott, 1981) to resolve whether these differences are biological or technological. As there is a big difference in the cost of wet or dry combustion techniques, confirmation of the finding that both methods can provide reliable measurements would be valuable for limnologists with a tight budget.

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