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Intra-class variability in the carbon, pigment and biomineral content of prymnesiophytes and diatoms

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ABSTRACT: Chlorophyll (chl) and carotenoid pigment data has significantly advanced our understanding of the distribution and class composition of phytoplankton biomass. However, the conversion of this data into quantitative and reliable estimates of biomass necessitates empirical carbon and pigment measurements on individual species. We have studied the carbon, pigment (chl and carotenoid) and biomineral (silicate and calcite) content of 20 prymnesiophytes and diatoms as key representatives of ecologically important phytoplankton in marine temperate waters. Batch cultures were sampled for each analysis in triplicate during early and late growth periods. To enable intra-class comparisons, pigment/chl *a* ratios are presented as are cellular constituent densities derived by normalising concentrations with cellular counts and volumes. For both prymnesiophytes and diatoms in early growth, chl *a* and carbon density were found to decrease from 8 to <2 fg μm^{-3} and from 0.5 to <0.1 pg μm^{-3} respectively as cell volume increased from 20 to >1000 μm^3. Pigment densities often decreased for the late growth period whereas carbon concentrations increased rapidly (up to 6-fold), resulting in a decrease in chl *a*/carbon ratio from an average 0.02 to 0.01. Regressions of POC and biovolume indicated that between 40 and 80% of the total POC for cells harvested during late growth was due to non-viable material. In addition, we found that chemotaxonomic marker pigments did not correlate with biominerals for either diatoms (fucoxanthin with silicate), or prymnesiophytes (19'hexanoyloxyfucoxanthin with calcite). Our empirical data, which are presented for a wider range of species than previously available, strengthen the basis upon which quantitative estimations of phytoplankton biomass in aquatic ecosystems are reliant.

KEY WORDS: Prymnesiophytes · Diatoms · Carbon · Chlorophylls · Carotenoids · Calcite · Silicate

'Only by studying individual biologies will we improve our understanding of the forces shaping ecosystem structure and driving biogeochemical fluxes'

Verity & Smetacek (1996)

INTRODUCTION

Prymnesiophytes and diatoms are 2 phytoplankton classes that often dominate the base of the food chain in temperate marine waters and may have a profound influence on global biogeochemistry. Diatoms, characterised by silica frustules, are ubiquitous in temperate oceans and dominate nutrient-enriched waters such as those found in upwelling regions and at the onset of the spring bloom. Following the spring bloom, nitrate and silicate become depleted and prymnesiophytes succeed in dominating the upper water column biomass while diatomaceous material dominates the flux of material sinking out of the water column (Sieracki et al. 1993, Weeks et al. 1993, Llewellyn & Mantoura 1996). Coccolithophorids, an important component of the prymnesiophyte biomass, play an important role in oceanic carbon cycling by transporting calcium carbonate from the upper oceanic layers to the abyssal sediments (Takahashi 1994).

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Table 1 Taxonomic designation, cell counts and volumes for early (1st harvest) and late (2nd harvest) cell harvests (1 and 2 respectively) as described in text. Standard deviations (%) are given in parentheses

Class / Order[a]	Culture code	Species	Cell count[b] (cells ml^{-1})		Growth phase[c]		Cellular volume[d] (µm^3)	
			1	2	1	2	1	2
Prymnesiophyceae[e]								
Prymnesiales	PLY-143[f]	*Chrysochromulina brevifilum*	1.48E+05 (11)	5.74E+03 (73)	L	D	63	63
	PLY-43[a]	*Chrysochromulina strobilus*	1.18E+05 (14)	5.38E+04 (26)	L	D	105	76
	PLY-559	*Chrysochromulina* sp.	1.41E+05 (14)	4.04E+05 (20)	L	S	44	40
	PLY-64	*Phaeocystis pouchetii*	2.28E+05 (15)	2.82E+05 (20)	L	D	19	24
Coccolithophorales	PLY-182[g]	*Coccolithus pelagicus*	1.12E+04 (39)	6.33E+03 (100)	L	S	1295[h]	617[h]
	PLY-61/7/3	*Emiliania huxleyi*	3.78E+05 (15)	4.75E+05 (9)	L	S	39[h]	36[h]
Pavlovales	PLY-244	*Diacronema vklianum*	4.18E+05 (17)	3.86E+06 (8)	L	S	32	25
	PLY-471	*Pavlova pinguis*	3.69E+05 (14)	1.25E+06 (14)	L	S	41	60
Isochryidales	PLY-B	*Dicrateria inornata*	5.14E+05 (14)	5.90E+05 (12)	L	S	16	12
	PLY-133	*Imantonia rotunda*	4.08E+05 (16)	3.26E+06 (13)	L	S	23	17
Bacillariophyceae[e]								
Centrales	CCAP-1000/1[g]	*Actinocyclus subtilis*	2.69E+02 (33)	5.03E+02 (42)	A	L	20056[i]	20056[i]
	CCAP-1009/2	*Asterionellopsis kariana*	7.99E+04 (25)	5.11E+05 (8)	L	S	185	137
	CCAP-1010/11	*Chaetoceros calcitrans*	2.36E+05 (17)	4.66E+05 (9)	L	D	29	17
	CCAP-1010/6	*Chaetoceros debilis*	2.26E+05 (19)	4.85E+05 (31)	L	D	125	47
	CCAP-1077/5	*Skeletonema costatum*	2.59E+05 (20)	1.17E+06 (17)	L	D	43	48
	CCAP-1084/1	*Thalassiosira weissflogii*	4.52E+04 (31)	2.68E+05 (20)	L	S	779	745
	CCAP-1029/18	*Fragilaria striatula*	1.37E+04 (100)	1.11E+05 (31)	A	L	98	162
Pennales	CCAP-1050/8	*Navicula hansenii*	1.08E+04 (39)	1.03E+05 (29)	L	S	33	61
	SMBA-302	Pennate diatom	1.13E+04 (31)	5.55E+04 (27)	A	L	92	120
	CCAP-1085/1	*Thalassionema nitzschioides*	3.42E+05 (17)	5.65E+05 (7)	L	S	120	120

[a]Taxonomic scheme for Prymnesiophyceae according to Chrétiennot-Dinet et al. (1993)
[b]Determined from microscopic analysis
[c]A, L, S and D denote acceleration, logarithmic, stationary and death phase respectively
[d]Determined from multisizer data using 1% Lugol's fixation. Shrinkage (1.33×) due to fixation taken into account (Montagnes et al. 1994). Standard deviation <10%; see text
[e]Commonly referred to as prymnesiophytes and diatoms
[f]PLY: Plymouth Culture Collection, CCMS-PML, Plymouth PL1 3DH, United Kingdom
[g]CCAP: Culture Collection of Algae and Protozoa, CCMS-DML, Oban PA34 4AD, United Kingdom. SMBA: Scottish Marine Biological Association, Oban DA34 4AD, United Kingdom
[h]Volume for naked cell: Lugol's fixation dissolves coccoliths
[i]Calculated from spherical diameter of 80 µm

Our understanding of these 2 important classes in terms of distribution of and contribution to phytoplankton biomass has increased significantly in recent years (Williams & Claustre 1991, Barlow et al. 1993, Llewellyn & Mantoura 1996). This is in part due to the introduction of high performance liquid chromatography (HPLC) techniques which have been applied with great success to the analysis of phytoplankton chlorophylls and carotenoids (Mantoura & Llewellyn 1983, Wright et al. 1991, Barlow et al. 1997). Chlorophyll *a* (chl *a*), ubiquitous in phytoplankton, is used to provide biomass estimates, whereas carotenoids are often associated with specific classes and their 'fingerprints' enable qualitative distribution patterns to be investigated. However, the conversion of these measurements into quantitative and reliable estimates of phytoplankton carbon biomass and class composition necessitates empirical carbon and pigment measurements on individual species. To date, there are few such published quantitative measurements.

The aim of this paper is to present and compare empirical measurements of carbon, chlorophylls, carotenoids, calcite and silicate in 20 prymnesiophyte and diatom species chosen as key representatives of temperate marine phytoplankton. Analyses were carried out on triplicate samples from batch culture harvestings from early growth and late growth. Concentrations are normalised using cellular volumes to provide cell constituent densities to facilitate comparisons between species and between the 2 phases of growth. Our results expand and update available pigment/chl *a* ratios for the accurate use of recent factorial approaches (Mackey et al. 1996) to determine the contribution of individual classes of microalgae to total chl *a*. The possibility that biomarker pigments could be used as proxy markers for biomineral fluxes was also investigated. We thus provide empirical data strengthening the basis upon which quantitative estimations of phytoplankton in aquatic ecosystems are reliant.

MATERIALS AND METHODS

Algal cultures. Inoculates from 10 prymnesiophytes and 10 diatoms (Table 1) were transferred aseptically (although the cultures were not kept axenic) into 2.5 l Erlenmeyer flasks and grown in 2.0 l of f/2 culture medium with sodium metasilicate (0.003% w/v). Cultures were illuminated at an irradiance of 98 to 146 µmol m^{-2} s^{-1} with 'daylight' fluorescent tubes (Philips) on a 12:12 h light:dark cycle, maintained at 15°C and mixed by swirling the flasks daily. Biomass and growth were monitored daily by measuring chl *a* using a Turner fluorometer (Yentsch & Menzel 1963) and cell counts using a Coulter multisizer. Samples were taken for each parameter described below by filtering triplicate aliquots of culture on 2 occasions: the first during early growth (1st harvest) where cells were predominantly in logarithmic growth phase (Table 1), and the second during late growth (2nd harvest) where cells were predominantly in the stationary growth phase (Table 1).

Particulate carbon and nitrogen. Aliquots of culture (10 to 40 ml) were vacuum filtered (< 50 mm Hg) onto ashed (12 h at 450°C) GF/F filters (25 mm, Whatman) and stored at –20°C. Prior to analysis, samples for total particulate carbon (TPC) and nitrogen were dried (12 h at 40°C), transferred onto ashed aluminium discs (30 mm), crimped to produce pellets (2 mm diameter) and kept desiccated. For particulate organic carbon (POC), samples were dried, transferred to ashed aluminium discs, and then acidified with 400 µl sulphurous acid to remove inorganic carbon (Verado et al. 1990). Samples were again dried and crimped into pellets. Pellets were analysed using an elemental analyser (Carlo Erba NA 1500) calibrated with acetinalide. Blanks, which were prepared in the same way as samples using un-inoculated culture media, were subtracted from samples. The contribution of heterotrophic bacterial carbon biomass was determined by using flow cytometric analysis of culture samples fixed with 0.1% glutaraldehyde and stained with the fluorescent dye SYBR green (Zubkov et al. in press, Glen Tarran, CCMS-PML, pers. comm.).

Calcite. Aliquots of culture (20 to 40 ml) were filtered onto GF/F filters (25 mm, Whatman) and stored at –20°C until analysis. Calcium was extracted with 1 ml concentrated HCl at 40°C for 5 min in acid-washed borosilicate vials and then made up to 10 ml with distilled water and 1 ml 10% lanthanum chloride solution. Calcium concentration was determined by flame atomic absorption spectrometry (Varian AA20) using an air-acetylene flame at 422.7 nm (Fernandez et al. 1993).

Silicate. Aliquots of culture (10 ml) were filtered onto Cyclopore polycarbonate membranes (25 mm, Whatman) and analysed using the method of Parsons et al. (1984). In summary: membranes were placed in 60 ml acid-washed polypropylene bottles with 25 ml of 85°C 0.1 M sodium hydroxide. Samples were shaken for 2 h at 85°C followed by the addition of 25 ml 1% sulphuric acid and 1 ml 0.16 M ammonium heptamolybdate. After 10 min, 1 ml 0.8 M oxalic acid and 1 ml ascorbic acid were added and mixed vigorously. Samples were left to colour stabilise for 10 min and then absorption was measured at 810 nm using a UV/vis absorbance spectrophotometer (Perkin Elmer Lamba 2). Sodium hexafluorosilicate was used in the calibration at 6 concentrations (0.5 to 5 mg Si l^{-1}), using the same protocol. All reagents were analytical grade (Aldrich) and prepared using Milli-Q water.

Table 2. Cellular pigment density (fg μm^{-3}) derived by dividing cellular concentrations by cellular volume for the 1st harvest and 2nd harvest (1 and 2 respectively). Chl c_3 = chlorophyll c_3; Chl c_1&c_2 = chlorophyll c_1&c_2; But-fuco = 19'butanoyloxyfucoxanthin; Hex-fuco = 19'hexanoyloxyfucoxanthin; Diadino = diadinoxanthin; Diato = diatoxanthin; Chl *a* = chlorophyll *a*; Phytol *c* = phytolated chlorophyll c_2

	Chl c_3		Chl c_1&c_2		But-fuco		Fuco		Hex-fuco		Diadino		Diato		Chl *a*[a]		Phytol *c*		Carotene	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Prymnesiophytes																				
Chrysochromulina brevifilum	0.40	0.11	0.91	0.25	0.05	0.08	0.11	0.07	3.86	1.32	1.37	0.44	0.08	1.20	5.46	5.38	0.28	–	0.12	–
Chrysochromulina strobilus	0.28	0.56	0.24	0.45	0.03	0.05	0.12	0.50	1.40	2.67	0.30	1.01	0.01	0.04	2.17	5.44	0.07	0.12	0.04	0.08
Chrysochromulina sp.	0.66	0.50	0.83	0.65	0.06	0.01	0.07	0.03	4.79	4.04	0.82	1.21	0.12	0.22	5.59	4.10	0.08	0.15	0.09	0.08
Phaeocystis pouchetii	0.79	0.45	1.33	0.61	0.14	0.12	3.62	2.02	0.54	0.61	1.37	1.63	0.41	0.62	7.38	3.93	0.30	0.17	0.13	0.05
Coccolithus pelagicus	0.08	–	0.25	0.23	0.01	–	0.72	0.56	0.08	0.02	0.22	0.13	0.05	0.28	1.41	1.39	0.04	–	0.02	0.02
Emiliania huxleyi	0.33	0.24	0.89	0.62	0.04	0.01	0.35	0.16	2.24	2.00	0.90	0.98	0.19	0.33	3.69	3.62	0.13	0.09	0.05	0.07
Diacronema vlkianum	–	–	0.12	0.12	–	–	0.58	0.47	–	–	1.20	1.48	0.27	0.45	4.13	2.83	–	–	1.35	2.44
Pavlova pinguis	0.01	–	0.30	0.17	–	–	0.97	0.58	–	–	0.79	1.14	0.20	0.58	3.77	2.98	0.01	0.02	0.36	0.66
Dicrateria inornata	0.88	0.70	1.22	0.79	0.72	0.31	0.36	0.17	4.94	3.92	0.96	0.02	0.21	0.75	6.69	5.38	0.19	0.13	0.14	0.10
Imantonia rotunda	1.56	1.17	1.96	0.83	1.07	0.44	1.44	0.24	6.18	4.53	2.28	1.77	0.84	1.04	6.35	5.87	0.20	0.15	0.20	0.14
Average	0.55	0.53	0.81	0.47	0.27	0.15	0.83	0.48	3.00	2.39	1.02	0.98	0.24	0.55	4.66	4.09	0.14	0.12	0.25	0.40
SD	0.48	0.34	0.59	0.26	0.40	0.17	1.07	0.58	2.25	1.68	0.59	0.61	0.24	0.37	1.96	1.44	0.10	0.05	0.40	0.79
Diatoms																				
Actinocyclus subtilis	–	–	0.02	0.04	–	–	0.08	0.12	–	–	0.02	0.03	0.01	0.02	0.16	0.26	–	–	0.01	0.01
Asterionellopsis kariana	0.02	0.02	0.38	0.27	–	–	0.76	0.68	–	–	0.10	0.28	0.04	0.11	2.23	1.32	–	–	0.04	0.02
Chaetoceros calcitrans	–	–	0.64	0.06	–	–	1.78	0.25	–	–	0.94	0.19	0.37	0.43	6.44	0.92	–	–	0.17	0.03
Chaetoceros debilis	–	–	1.14	0.11	–	–	1.43	0.45	–	–	0.21	0.17	0.02	0.26	3.62	1.34	–	–	0.06	0.05
Skeletonema costatum	–	–	1.68	0.29	–	–	1.18	0.02	–	–	0.17	–	0.05	–	8.23	0.33	–	–	0.08	–
Thalassiosira weissflogii	–	–	0.25	0.23	–	–	0.88	0.71	–	–	0.71	0.68	0.26	0.42	3.60	2.65	–	–	0.09	0.08
Fragilaria striatula	–	–	0.20	0.93	–	–	0.33	1.84	–	–	0.05	0.16	–	0.02	1.53	10.13	–	–	–	0.09
Navicula hansenii	–	–	0.43	0.20	–	–	1.57	0.89	–	–	0.44	0.43	–	0.17	3.74	2.83	–	–	0.05	0.06
Pennate diatom	–	–	0.46	0.47	–	–	1.89	1.50	–	–	0.54	0.82	0.02	0.33	4.71	3.50	–	–	0.09	0.05
Thalassionema nitzschioides	–	–	0.56	1.20	–	–	0.42	1.85	–	–	0.11	0.76	0.12	0.52	2.08	6.40	–	–	0.03	0.08
Average	–	–	0.64	0.42	–	–	1.14	0.91	–	–	0.36	0.44	0.13	0.28	3.63	2.97	–	–	0.08	0.06
SD	–	–	0.48	0.39	–	–	0.57	0.67	–	–	0.31	0.28	0.14	0.17	2.38	3.11	–	–	0.04	0.02

[a]Chl *a* includes chlorophyllide *a* and chl *a* allomer

Chlorophylls and carotenoids. Aliquots of culture (10 to 40 ml) were filtered on to GF/F filters and stored in liquid nitrogen. Pigments were extracted from the filter by ultrasonication and centrifugation using 2 ml methanol and analysed using the method described by Barlow et al. (1997) with a reverse-phase gradient HPLC system (Thermo Separation Products). Absorbance was monitored continuously between 300 and 700 nm and the pigments were quantified using canthaxanthin as an internal standard (Gibb et al. in press).

Cell counts and volumes. Aliquots of culture (100 ml) were preserved in 1% acid Lugol's iodine and stored in the dark at room temperature. Cells were counted using an inverted microscope (Olympus IMT-2) and counts averaged for 20 fields of view (average of 50 cells per field of view). In addition, cell counts and cell volumes were determined using a multisizer counter (Coulter II).

Microscopy data provided a more accurate estimate of cell counts than multisizer data and therefore we used these to calculate cell concentrations (Table 1). It is more difficult to ascertain the errors associated with cellular volume estimates. The standard deviation of cellular volumes calculated from multisizer data was less than 10% (Table 1). However, the multisizer assumes that cells have a spherical shape, and although this is true for most prymnesiophytes it is not true for some of the diatoms. Therefore, for non-spherical, chain-forming and clumping cells we obtained estimates of volume from microscopic analysis according to the basic geometry of the cell, e.g. sphere, cuboid and oblate ellipsoid. There is little published on cellular volumes; however, our calculated volumes were in the same range as those obtained by Montagnes et al. (1994), D. Harbour (unpubl. data) and C. Saunders (unpubl. data).

RESULTS

Pigments

Prymnesiophytes

All prymnesiophytes studied, except those in the order Pavlovales (i.e. *Diacronema vklianum* and *Pavlova pinguis*), contained chlorophyll *a* (chl *a*), chlorophyll c_3 (chl c_3), chlorophyll c_1 and c_2 (chl c_1&c_2), 19'butanoyloxyfucoxanthin (but-fuco), 19'hexanoyloxyfucoxanthin (hex-fuco), fucoxanthin (fuco), diadinoxanthin (diadino), diatoxanthin (diato) and β,β-carotene (Table 2). The Pavlovales had a pigment distribution more characteristic of diatoms, i.e. they were deficient in chl c_3, but-fuco and hex-fuco, and they contained β,ε-carotene in addition to β,β-carotene.

The cellular concentration of pigments per unit volume (cellular density; Table 2) was highest for chl *a*. This was followed by, in decreasing order, hex-fuco (average 65% [g/g] of chl *a*), diadino (average 22% of chl *a*), fuco (average 18% of chl *a*), chl c_1 and c_2 (average 17% of chl *a*), chl c_3 (average 12% of chl *a*) and finally by but-fuco, diato and carotene, which had similar densities (average 5% of chl *a*). Exceptions were for: *Diacronema vklianum* and *Pavlova pinguis*, which were absent in hex-fuco, and had a carotene density higher than that of fuco; *Phaeocystis pouchetii* and *Coccolithus pelagicus*, in which fuco dominated over hex-fuco; *Dicrateria inornata* and *Imantonia rotunda* (both in the order Isochryidales), in which but-fuco density was similar to that of fuco.

Pigment densities in cells from the 2nd harvest were generally less (by up to 80%) than for cells at 1st harvest, with chl *a* showing the least change (Table 2). Exceptions were diadino and carotene, which increased in density by up to 2.4-fold, and diato, which increased by up to 16-fold.

Diatoms

All diatoms contained chl c_1&c_2, fuco, diadino, diato, chl *a* and β,β-carotene (Table 2). In addition *Asterionellopsis subtilus* contained low amounts of chl c_3 (0.02 fg μm^{-3}). Pigment densities were generally lower, although more variable, in the diatoms than in the prymnesiophytes. Chl *a* had the highest density, followed by fuco (average 30% of chl *a*), chl c_1&c_2 (average 17% of chl *a*), diadino (average 10% of chl *a*), diato (average 3% of chl a) and carotene (average 2% of chl *a*) (Table 2). Chl *a* allomer and chlorophyllide *a*, whose formation is most likely triggered as an extraction artefact in ageing or stressed cultures, were present in some diatoms (Table 2). Interestingly fuco and chl c_1&c_2, frequently used as key markers of diatoms, were often present at a lower density in the diatoms than in the prymnesiophytes.

Pigment densities in diatom cells from the 2nd harvest were generally lower than those from the 1st harvest (except for diadino and diato, and chl *a* allomer and chlorophyllide *a*) and showed larger decreases (up to 98%) compared to the prymnesiophytes. Exceptions were for *Fragilaria striatula* and *Actinocyclus subtilus*, whose densities increased, likely as a result of the 2nd harvest being in log phase (Table 1), and for *Thalassionema nitzschioides*. Diadino and diato densities were lower overall in diatoms than in prymnesiophytes but showed a similar pattern of change at the 2nd harvest to that found for prymnesiophytes.

Table 3. Total particulate carbon (TPC), C/N, chlorophyll *a* (Chl)/C and mineral content for 1st harvest and 2nd harvest (1 and 2 respectively). Value is the mean for triplicate analysis with standard deviation in parentheses

	TPC ($\mu g\ l^{-1}$) 1	TPC ($\mu g\ l^{-1}$) 2	TPC ($pg\ cell^{-1}$) 1	TPC ($pg\ \mu m^{-3}$) 1	C/N 1	C/N 2	Chl/C 1	Chl/C 2	SiO_2 ($\mu g\ l^{-1}$) 1	SiO_2 ($\mu g\ l^{-1}$) 2	SiO_2 ($pg\ cell^{-1}$) 1	SiO_2 ($pg\ \mu m^{-3}$) 1	C in $CaCO_3$ ($\mu g\ l^{-1}$) 1	C in $CaCO_3$ ($\mu g\ l^{-1}$) 2	C in $CaCO_3$ ($pg\ cell^{-1}$) 1	C in $CaCO_3$ ($pg\ \mu m^{-3}$) 1
Prymnesiophytes																
Chrysochromulina brevifilum	2928 (7.6)	2431 (9.2)	19.8 (19)	0.32	5.2	3.8	0.0173	0.0003	333	725	2.3	0.036	–	–	–	–
Chrysochromulina strobilus	2225 (6.3)	2655 (14)	18.9 (20)	0.18	5.5	5.2	0.0121	0.0084	403	497	3.4	0.032	–	–	–	–
Chrysochromulina sp.	1334 (22)	5709 (8)	9.5 (36)	0.22	5	5.6	0.0259	0.0116	627	693	4.5	0.102	214		1.5	0.03
Phaeocystis pouchetii	1607 (23)	3427 (5)	7 (38)	0.38	4	2.3	0.0191	0.0077	497	743	2.2	0.117	–	–	–	–
Coccolithus pelagicus	2578	5735 (26)	230.2 (>39)	0.18		7.9	0.034[a]	0.0019[a]	301	844	26.9	0.021	1975	2827	176.3	0.14
Emiliania huxleyi	6493 (8)	16280 (2)	17.2 (23)	0.44	12.3	8.8	0.0135[a]	0.0080[a]	413	1073	1.1	0.029	2530	8556	6.7	0.17
Diacronema vklianum	2657 (6)	27263 (1)	6.4 (23)	0.20	3.9	5.6	0.0189	0.0189	249	904	0.6	0.019	–	–	–	–
Pavlova pinguis	3009 (9)	18017 (10)	8.2 (23)	0.20	8	7.6	0.0173	0.0117	631	10643	1.7	0.041	–	882	–	–
Dicrateria inornata	2262 (12)	5290 (5)	4.4 (26)	0.28	4.6	3.6	0.0243	0.0072	314	578	0.6	0.038	–	–	–	–
Imantonia rotunda	3644	23070 (9)	8.9 (>16)	0.39	5.7	5.6	0.0161	0.0143	470	603	1.2	0.051	–	474	–	–
Average	2874	10988		0.28	6.0	5.6	0.0189	0.0100	424	1730	4.4	0.049				
SD	1438	9286		0.10	2.6	2.0	0.0044	0.0055	133	3136	8.0	0.034				
Diatoms																
Actinocyclus subtilis	1166 (27)	2474 (12)	4334.6 (50)	0.02	7.3	5.6	0.0128	0.0186	2001	5078	7438.7	0.021	–	–	–	–
Asterionellopsis kariana	4293	7745 (13)	53.7 (>25)	0.29	6.7	6.8	0.0077	0.0114	1405	6199	17.6	0.095	–	–	–	–
Chaetoceros calcitrans	3061 (1)	7138 (2)	13 (18)	0.44	5.4	4.9	0.0264	0.0010	2004	3246	8.5	0.290	–	–	–	–
Chaetoceros debilis	2990 (15)	7794 (1)	13.2 (34)	0.11	4.9	4.2	0.0320	0.0017	2170	13952	9.6	0.077	–	–	–	–
Skeletonema costatum	2340 (1)	15648 (8)	9 (21)	0.21	5	6	0.0342	0.0009	3406	12231	13.2	0.309	–	–	–	–
Thalassiosira weissflogii	3905 (1)	20067 (18)	86.4 (32)	0.11	6.9	5.4	0.0296	0.0230	2625	15068	58.1	0.074	–	–	–	–
Fragilaria striatula	123	9061 (3)	9 (100)	0.09		6.7	0.0167	0.0122	423	6638	30.9	0.314	–	–	–	–
Navicula hansenii	204 (55)	5049 (39)	18.9 (94)	0.57	5.2	4.2	0.0070	0.0030	451	1953	41.8	1.256	–	–	–	–
Pennate diatom	559	2879 (19)	49.5 (>31)	0.54	8.6	8.9	0.0090	0.0079	1745	2035	154.4	1.683	–	–	–	–
Thalassionema nitzschioides	3332 (1)	14128 (2)	9.7 (18)	0.08	4.7	5	0.0231	0.0274	8677	17401	25.4	0.212	–	–	–	–
Average	2197	9198		0.25	6.1	5.8	0.0198	0.0107	2491	8380	780	0.433				
SD	1564	5723		0.20	1.4	1.4	0.0105	0.0096	2354	5760	2340	0.566				

[a]Calcite-C subtracted

Biominerals

Calcite was observed in 5 of the prymnesiophytes and in none of the diatoms (Table 3). Only 2 prymnesiophytes, however, both in the order Coccolithophorales (i.e. *Coccolithus pelagicus* and *Emiliania huxleyi*), contained high calcite concentrations, with calcite-carbon accounting for between 39 and 76% of total particulate carbon (TPC) for both harvests. The calcite density was similar for both *C. pelagicus* and *E. huxleyi* (0.14 and 0.17 pg μm^{-3} respectively). Calcite concentrations in the remaining 3 calcite-containing prymnesiophyte species (*Chrysochromulina* sp., *Pavlova pinguis* and *Imantonia rotunda*) were 10-fold less, with calcite-carbon contributing to <20% of TPC.

Silicate (Table 3) was present in both prymnesiophytes and diatoms. However, densities were on average 9 times lower in the prymnesiophytes (0.05 pg μm^{-3}) than in the diatoms (0.43 pg μm^{-3}). Prymnesiophyte silicate density was lowest for the 2 Coccolithophorales species and highest for *Phaeocystis pouchetti*, whose density was over twice the average value (0.12 pg μm^{-3}). Prymnesiophyte silicate concentrations for the 2nd harvest increased 4-fold. Diatom silicate density was particularly high for *Navicula hansenii* and the pennate diatom (1.3 and 1.7 pg μm^{-3} respectively) and low for the large-sized *Actinocyclus subtilus* (0.02 pg μm^{-3}). For the 2nd harvest, diatom silicate concentrations increased on average by 3-fold (i.e. similar to the increase for prymnesiophytes), with the largest increase in *Fragilaria striatula*, which had the lowest concentration for 1st harvest.

Particulate carbon

TPC concentrations are presented in Table 3. Bacteria contributed to <10% of TPC and blanks were less than 1% of TPC. Inorganic and thus organic carbon concentrations (POC) derived from elemental analysis are not tabulated because inorganic carbon results were erroneously high, suggesting that some organic material had been volatilised together with carbonate after the addition of sulphurous acid. Similar errors have been found in other studies using the same analytical method (King et al. 1998). Subsequently, where we refer to POC, it is derived by subtracting calcite-carbon from TPC.

Prymnesiophytes

Although TPC ranged from 1334 to 6493 µg l^{-1} (average 2874 ± 1438 µg l^{-1}) for the 1st harvest, carbon per unit cell volume, i.e. carbon densities, was more uniform (average 0.28 ± 0.10 pg μm^{-3}). Carbon density was highest for *Emiliania huxleyi* (0.44 pg μm^{-3}), possibly due to the 39% contribution from calcite-carbon. Carbon density was also high for *Phaeocystis pouchetti* (0.38 pg μm^{-3}), which contained high silicate density, and for *Imantonia rotunda*.

For the 2nd harvest, TPC concentrations increased in all cultures by an average of 4-fold (except for *Chrysochromulina brevifilum*, which declined). These higher TPC concentrations were, in part, caused by higher cell numbers and by the large numbers of lysed cells observed in the microscopic analysis (see Fig. 1).

The C/N ratio for the 1st harvest, excluding calcite carbon, was between 4 (*Phaeocystis pouchetti, Diacronema vklianum*) and 8 (*Emiliania huxleyi, Pavlova pinguis*), averaging close to the Redfield ratio (Takahashi et al. 1985). At the 2nd harvest there were slight changes in the C/N ratio, with an overall slight decrease, with *E. huxleyi* showing the largest decrease to a C/N of 4.

Diatoms

The range of TPC at the 1st harvest was larger for diatoms than for prymnesiophytes (123 to 3905 µg l^{-1}) as was the range in carbon density (0.02 to 0.57 pg μm^{-3}). However, average densities were similar for diatoms and prymnesiophytes (0.25 and 0.28 pg μm^{-3}). At the 2nd harvest diatom-TPC increased by the same amount as for the prymnesiophytes, i.e. 4-fold. C/N ratios for diatoms averaged at 6.0 for the 1st harvest and decreased by a similar amount to the prymnesiophytes at the 2nd harvest, i.e. to an average of 5.8.

DISCUSSION

Pigment ratios

Quantification of the abundance of various phytoplanktonic classes from marker pigment concentrations requires accurate pigment/chl *a* ratios (pigment ratios). However, there is little published information on pigment ratios: the most thorough study for quantifying pigments at the class level is by Jeffrey & Wright (1994), who tabulated the cellular concentration for 16 prymnesiophyte species. The few earlier attempts at quantifying prymnesiophyte biomass used less advanced chromatographic techniques (Berger et al. 1977), and the only survey of pigment diversity for diatoms (Stauber & Jeffrey 1983) used the cruder analytical technique of thin layer chromatography, where quantification was difficult although data were presented for chl *a* and *c*.

The limited set of ratios from the above citations, together with ratios for 1 or 2 other species, is summarised by Mackey et al. (1996) for use in a matrix fac-

Table 4. Pigment to chl *a* ratios for the 1st harvest and 2nd harvest (1 and 2 respectively). See Table 2 for pigment abbreviations. –: not detectable

	Chl c_1		Chl c_1&c_2		But-fuco		Fuco		Hex-fuco		Diadino		Diato		Phytol *c*		Carotene		Type	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2			a	b
Prymnesiophytes																				
Chrysochromulina brevifilum	0.074	0.021	0.167	0.047	0.010	0.014	0.019	0.013	0.713	0.247	0.252	0.083	0.014	0.226	0.051	–	0.022	–	A	4
Chrysochromulina strobilus	0.130	0.103	0.108	0.083	0.013	0.010	0.057	0.093	0.644	0.492	0.137	0.185	0.005	0.007	0.031	0.022	0.016	0.013	A	4
Chrysochromulina sp.	0.119	0.123	0.148	0.158	0.011	0.003	0.013	0.007	0.858	0.989	0.146	0.295	0.022	0.054	0.015	0.037	0.016	0.020	A	4
Phaeocystis pouchetii	0.109	0.114	0.184	0.157	0.019	0.030	0.500	0.515	0.075	0.156	0.190	0.415	0.056	0.158	0.041	0.042	0.018	0.012	C	4
Coccolithus pelagicus	0.060	0.000	0.177	0.164	0.004	0.000	0.506	0.400	0.057	0.016	0.152	0.091	0.038	0.204	0.027	–	0.016	0.015	C	4
Emiliania huxleyi	0.091	0.067	0.243	0.171	0.011	0.003	0.096	0.044	0.613	0.554	0.246	0.270	0.051	0.091	0.035	0.025	0.015	0.019	A	4
Diacronema vkhanum	–	–	0.028	0.083	–	–	0.141	0.333	–	–	0.291	1.028	0.065	0.306	–	–	0.326	0.833	D	1
Pavlova pinguis	0.002	0.000	0.078	0.057	–	–	0.255	0.196	–	–	0.208	0.385	0.052	0.196	0.002	0.005	0.097	0.225	D	2
Dicrateria inornata	0.132	0.130	0.183	0.147	0.108	0.059	0.054	0.031	0.740	0.730	0.144	0.003	0.032	0.139	0.028	0.024	0.022	0.019	B	4
Imantonia rotunda	0.249	0.196	0.313	0.139	0.171	0.074	0.231	0.041	0.989	0.758	0.364	0.297	0.135	0.174	0.031	0.026	0.032	0.022	B	4
Diatoms																				
Achnocyclus subtilis	–	–	0.135	0.157	–	–	0.468	0.468	–	–	0.137	0.128	0.070	0.055	–	–	0.027	0.032		
Asterionellopsis kariana	0.011	0.017	0.171	0.208	–	–	0.339	0.520	–	–	0.044	0.213	0.018	0.087	–	–	0.017	0.011		
Chaetoceros calcitrans	–	–	0.099	0.066	–	–	0.277	0.269	–	–	0.146	0.203	0.057	0.470	–	–	0.025	0.031		
Chaetoceros debilis	–	–	0.314	0.084	–	–	0.395	0.338	–	–	0.058	0.122	0.007	0.191	–	–	0.017	0.032		
Skeletonema costatum	–	–	0.206	0.835	–	–	0.143	0.050	–	–	0.020	0.015	0.005	0.000	–	–	0.010	0.009		
Thalassiosira weissflogii	–	–	0.069	0.085	–	–	0.245	0.270	–	–	0.197	0.255	0.073	0.159	–	–	0.024	0.030		
Fragilaria striatula	–	–	0.128	0.092	–	–	0.215	0.183	–	–	0.033	0.016	–	0.002	–	–	–	0.009		
Navicula hansenii	–	–	0.114	0.072	–	–	0.420	0.316	–	–	0.119	0.150	–	0.060	–	–	0.014	0.022		
Pennate diatom	–	–	0.097	0.136	–	–	0.401	0.430	–	–	0.115	0.234	0.005	0.095	–	–	0.019	0.015		
Thalassionema nitzschioides	–	–	0.265	0.187	–	–	0.202	0.290	–	–	0.050	0.119	0.056	0.082	–	–	0.014	0.012		

[a]Types according to Llewellyn & Gibb (this study); [b]Types according to Jeffrey & Wright (1994)

torization 'CHEMTAX' program for quantitative estimates of class abundances. Mackey et al. (1996) have developed a PC-based computer program ('CHEMTAX') based on factor analysis which requires an initial input of a pigment ratio matrix which is then used iteratively to obtain class abundances. The ratios used in this program are for log-phase cells only and therefore do not take into account changes in ratios that may occur in proceeding growth phases. Our results provide a more comprehensive assessment of the ratios for the prymnesiophytes and diatoms and highlight the extensive intra-class and intra-species variability (Table 4). Our pigment ratios generally fall within the ranges reported by Mackey et al. (1996) although we often found minimum values lower than those quoted for chl c_3, chl c_2, but-fuco and fuco and higher values for carotene, as shown in Table 5.

We found consistently higher diato and diadino ratios at 2nd harvest compared to the 1st harvest. The large changes in diadino and diato/chl *a* ratios between the 2 harvests are similar to changes observed in aging benthic diatoms by Klein (1988), who suggested that such changes may be related to nutrient limitation. We conclude that such large changes in diadino and diato/chl *a* ratios preclude their use in estimating biomass contributions.

Using our pigment ratios (Table 4) we have divided the prymnesiophytes studied into 4 pigment types and have tabulated the minimum and maximum ratios for these in Table 5. These types can be summarised as: Type A with low fuco ratios (0.013 to 0.096); Type B with high but-fuco ratios (0.059 to 0.171); Type C with high fuco ratios (0.4 to 0.515), i.e. comparable to that in diatoms; and Type D with no but-fuco and hex-fuco, with high carotene ratios (0.097 to 0.833) and with low phytol *c* ratios (<0.005). Our 4 types are identical to the morphological taxonomic order scheme proposed by Chrétiennot-Dinet et al. (1993) (Table 1) excepting *Emiliania huxleyi* and *Phaeocystis pouchetii*. However, this classification differs slightly to the chemotaxonomic types suggested by Jeffrey & Wright (1994) (Table 5) and subsequently used by Mackey et al. (1996) for estimating phytoplankton distribution.

Pigment ratios for the diatoms show no clear divisions within the 10 species studied (Table 5). Our ratios concur with those used by Mackey et al. (1996), with the exception that we found chl c_3 in the diatom *Asterionellopsis subtilus*, and that the chl c_1&c_2 ratio was high and the fuco ratio low for *Skeletonema costatum*.

Physiological and biochemical changes

Microalgae grown in batch culture rapidly become self shading and nutrient depleted. Such conditions mimic to some extent conditions during and proceeding a spring bloom. Our results show that microalgae change physiologically and biochemically according to growth conditions. Our cultures reached maximum chl *a* concentrations in about 10 d, after which there was a rapid decline, indicating that either senescence and/or bacterial or viral attack had occurred, resulting in destruction of cellular structure and the photosynthetic pigment system.

Pigment concentrations were found to decrease faster than cell numbers as growth progressed, resulting in a decrease in cellular pigment content, and indicating that the photosynthetic system is destroyed ahead of other cellular components. Few pigment transformation products of the chlorophylls (e.g. pheophorbides, pheophytins; Owens & Falkowski 1982) or the carotenoids (e.g. *cis*-carotenoids, ester-hydrolysed products; Repeta & Gagosian 1982) were detected at the 2nd harvest, indicating that the tetrapyrrolic structure of chlorophyll and the isoprenoid structure of carotenoid are rapidly cleaved into products that are undetectable during HPLC pigment analysis. These observations suggest that a similar process of mass chlorophyll and cell destruction could occur at the end of a spring bloom without zooplankton herbivory being involved.

Overall diatom pigments degraded more rapidly than those of prymnesiophytes from the 1st harvest to the 2nd harvest. This may be because silicate depletion occurred in the diatom cultures or that prymnesiophytes are better able to cope with nutrient limitation. The C/N ratio of prymnesiophyte and diatom cells was close to the Redfield ratio for the 1st harvest, and did not increase at the 2nd harvest, suggesting that the cultures were not nitrogen limited.

Table 5. Pigment to chl *a* ratios from the literature with revised ratios from this study. See Table 2 for pigment abbreviations. Types as assigned by Jeffrey & Wright (1994). Literature values taken from collation by Mackey et al. (1996) (Table 1). Revised values from this study: values in **bold** highlight ratios out of literature range. A, B, C, D: groupings from this study

		Chl c_3		Chl c_1&c_2		But-fuco		Fuco		Hex-fuco		Diadino		Diato		Phytol *c*		Carotene[a]	
		Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Prymnesiophytes																			
Type 1	Literature	0	0	0.08	0.306	0	0	0.36	0.948	0	0	0.107	0.254	0	0.014	–	–	0.02	0.123
	Revised	0	0	**0.028**	0.306	0	0	**0.141**	0.948	0	0	0	**1.028**	**0.065**	**0.306**	**0**	**0**	0.02	**0.833**
Type 2	Literature	0.062	0.141	0.175	0.304	0	0	0.465	1.115	0	0	0.055	0.105	0.001	0.006	–	–	0.016	0.026
	Revised	**0.002**	0.141	**0.057**	0.304	0	0	**0.196**	1.115	0	0	0.055	**0.385**	0.001	**0.196**	**0.002**	**0.005**	0.016	**0.225**
Type 3	Literature	0.054	0.17	0.104	0.33	0	0.023	0.038	1.205	0	1.36	0.034	0.28	0	0.035	–	–	0.009	0.03
		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Type 4	Literature	0	0.3	0.097	0.421	0.023	0.521	0.08	0.89	0.01	1.067	0.06	0.426	0	0.179	–	–	0	0.02
	Revised	**0.06**	0.3	0.097	0.421	**0.004**	0.521	**0.013**	0.89	0.01	1.067	**0.003**	**1.028**	**0.005**	**0.204**	**0.015**	**0.05**	0	**0.032**
A		0.021	0.13	0.047	0.243	0.003	0.014	0.013	0.096	0.492[b]	0.713	0.083	0.27	0.005	0.226	0.015	0.051	0	0.022
B		0.13	0.249	0.139	0.313	0.059	0.171	0.031	0.231	0.73	0.989	0.003	0.364	0.032	0.174	0.024	0.031	0.019	0.032
C		0	0.114	0.157	0.184	0	0.019	0.4	0.515	0.016	0.156	0.091	0.415	0.038	0.204	0.027	0.042	0.012	0.018
D		0	0.002	0.028	0.083	0	0	0.141	0.333	0	0	0.208	1.028	0.052	0.306	0	0.005	0.097	0.833
Diatoms																			
	Literature	0	0	0	0.183	0	0	0.159	0.755	0	0	0.03	0.448	0	0.269	–	–	0	0.154
	This study	0	**0.017**	**0.835**		0	0	**0.143**	0.755	0	0	**0.02**	0.448	0	**0.47**	–	–	0	0.154

[a]β,ε-carotene and β,β-carotene; [b]Excludes *Chrysochromulina brevifilum* at 2nd harvest

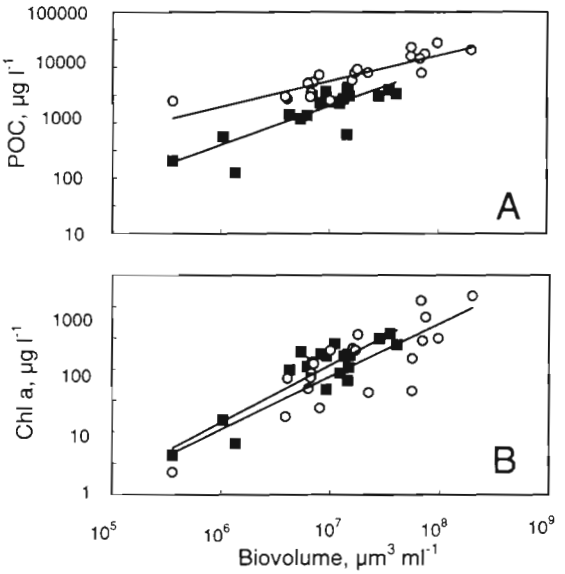

Fig. 1. (A) Particulate organic carbon (POC) and (B) chlorophyll *a* (chl *a*) versus biovolume (V), for early (1st harvest, ■) and late growth (2nd harvest, ○).

1st harvest: $\log_{10}POC = 0.699 \log_{10} V - 1.596$, $r^2 = 0.67$, $p < 0.0001$

2nd harvest: $\log_{10}POC = 0.462 \log_{10} V - 0.518$, $r^2 = 0.75$, $p < 0.0001$;

1st harvest: $\log_{10}chl\ a = 0.91 \log_{10} V - 4.813$, $r^2 = 0.78$, $p < 0.0001$;

2nd harvest: $\log_{10}chl\ a = 0.844 \log_{10} V - 4.531$, $r^2 = 0.67$, $p < 0.0001$

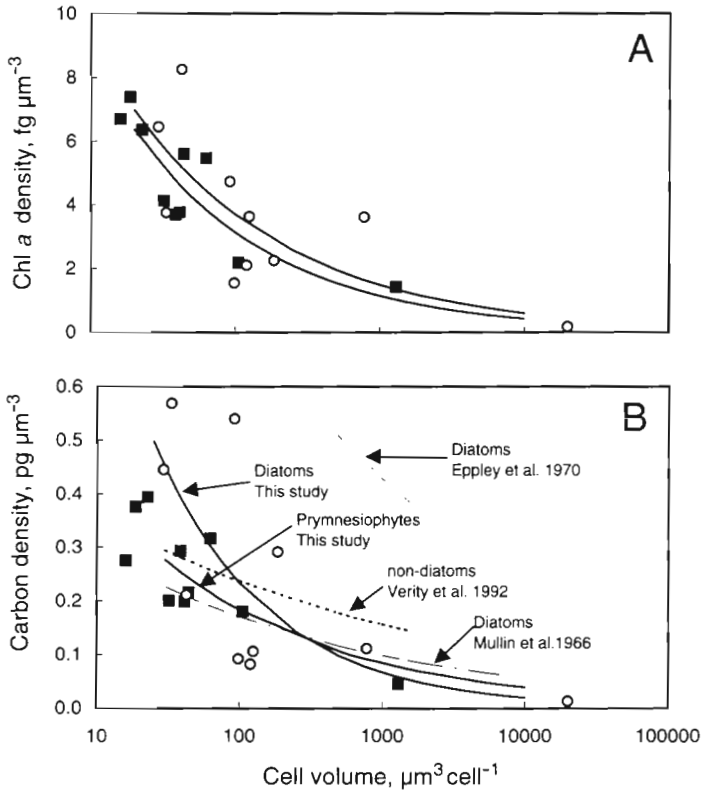

Fig. 2. (A) Chlorophyll *a* (chl *a*) and (B) carbon density (C) against cell volume for early growth (1st harvest) prymnesiophytes (■) and diatoms (○). Regressions using least squares method:

prymnesiophyte: $\log_{10}chl\ a = -0.441 \log_{10} V + 1.38$, $r^2 = 0.75$, $p = 0.001$;

diatom: $\log_{10}chl\ a = -0.397 \log_{10} V + 1.36$, $r^2 = 0.5$, $p = 0.02$;

prymnesiophyte: $\log_{10}C = -0.34 \log_{10} V - 0.06$, $r^2 = 0.63$, $p = 0.006$, 95% confidence limits, intercept −0.17 to 0.4, slope 0.61 to −0.29;

diatom: $\log_{10}C = -0.546 \log_{10} V + 0.46$, $r^2 = 0.47$, $p = 0.028$, 95% confidence limits, intercept −0.45 to 0.9, slope −0.72 to −0.17.

Verity et al. (1992) for non-diatomaceous cells: $\log_{10}C = -0.181 \log_{10} V - 0.26$, $r^2 = 0.64$.

Mullin et al. (1966) for predominantly diatoms: $\log_{10}C = -0.241 \log_{10} V - 0.29$.

Eppley et al. (1970) for diatoms: $\log_{10}C = -0.24 \log_{10} V + 0.35$

Chl *a*:carbon ratios for both prymnesiophytes and diatoms were on average 0.02 for the 1st harvest. However for the 2nd harvest carbon concentrations increased by typically 4-fold (Fig. 1), resulting in average chl *a*:carbon ratios of 0.01. The higher carbon levels at the 2nd harvest are likely to have been produced from the large amounts of dead cell material which were observed microscopically. Using regressions obtained from POC and biovolume data (Fig. 1) we predict that between 40 and 80% of the total POC in our 2nd harvest cultures was due to non-viable material. Low chl *a*:carbon ratios concurrent with an absence in pigment transformation products were also found in vertical depth profiles in the northeast Atlantic Ocean at the end of the spring bloom (Llewellyn & Mantoura 1996). This suggests that similar production of large amounts of non-viable carbon may occur in the open ocean without the involvement of protozoan or zooplankton grazing. The high TPC levels in our cultures were paralleled by high particulate nitrogen values, suggesting that the particulate organic material associated with non-viable cells had a similar nitrogen content to that of viable cells.

An alternative to using chl *a* as a proxy for microplankton carbon biomass is to determine the cell abundance and average cell volume and to estimate carbon from an empirically derived TPC per unit cell volume conversion factor (Booth et al. 1988, Sieracki at al. 1993). Regression models describing the relationship between carbon content and cell volume have been reported frequently (e.g. Mullin et al. 1966, Eppley et al. 1970, Moal et al. 1987, Verity et al. 1992).

However, the relationships have typically been determined for larger phytoplankton that contain significant vacuolar space, not present in smaller nanophytoplankton. For both prymnesiophytes and diatoms in early growth, chl *a* and carbon density decreased from 8 to <2 fg μm^{-3} and from 0.5 to <0.1 pg μm^{-3} respectively as cell volume increased from 20 to over 1000 μm^3 (Fig. 2). Slope and intercept regressions of carbon density versus cell volume produced 95% confidence limits that encompassed those of previous studies on prymnesiophytes (Verity et al. 1992) and diatoms (Mullin et al. 1966, Eppley 1970) (Fig. 2).

Biomineral correlations

We also investigated the hypothesis that fuco and hex-fuco can be used as proxies for diatom-silicate and prymnesiophyte-calcite abundances respectively. Fuco within diatoms did not correlate with silicate across the class or within species examined for the 2 harvests. This is likely to be because fuco is relatively labile with high turnover rates (Goericke & Welshmeyer 1992) whereas silicate decomposes slowly. For the prymnesiophytes, hex-fuco was found in 3 out of the 4 orders studied whereas calcite was found predominantly within the order Coccolithophorales. Therefore, in mixed communities of prymnesiophytes a correlation between calcite and hex-fuco is unlikely. In a situation where populations are dominated by 1 Coccolithophorales species, such as is known to occur during the North Atlantic spring bloom (Weeks et al. 1993), again, there is unlikely to be a correlation. This is because hex-fuco, like fuco, is a relatively labile pigment and its cellular concentration decreases as cells age (Table 3). In contrast, calcite coccoliths are cast off as cells age, increasing calcite concentrations rapidly (Table 5). Our results are consistent with findings in the marine environment, where 85% of the total carbon stored in the sedimentary sink is in the form of calcium carbonate, while only 15% is organic carbon (Westbroek et al. 1994). Thus, although carbonate transport out of the euphotic zone is dwarfed by the export of POC, the ultimate sink of carbonate is far greater than of organic carbon. We conclude that biomarker pigments cannot be used as proxies of biomineral abundance except for, perhaps, in an early bloom situation where Coccolithophorales dominate.

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