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Size-scaling of macromolecules and chemical energy content in the eukaryotic microalgae

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The macromolecular composition and cell size of microalgae can influence their competitive interactions for nutrients and food quality for predators. Here we quantify the cell volume and dry weight based size-scaling of protein, lipid, carbohydrate and chemical energy content of eukaryotic microalgae from data extracted from the scientific literature. Across all the microalgae examined, cell size is an excellent predictor of macromolecular and chemical energy content with size-scaling exponents ranging from 0.8 to 0.93 for cell volume and 0.96 to 1.1 for dry weight. There are second-order taxonomic differences in the size scaling of macromolecular and chemical energy content. Relative to the green algae and dinoflagellates, the diatoms have lower cell volume size-scaling exponents for protein, lipid and chemical energy content due to their larger increase in vacuole volume with increasing cell volume. The dinoflagellates have a lower size-scaling exponent for carbohydrate relative to the diatoms and green algae and the green algae have a relatively high size-scaling exponent for protein as compared to the diatoms. Differences in the size-scaling of macromolecular and chemical energy content across the diatoms, green algae and dinoflagellates appear to reflect fundamental differences in cellular architecture and growth and storage allocation strategies across these microalgal phyla.

KEYWORDS: carbohydrate; cell size; lipid; phytoplankton; protein

INTRODUCTION

The macromolecular composition of the microalgae is of interest for understanding nutrient competition within microalgal communities (Follows and Dutkiewicz, 2011), food web interactions (Raubenheimer *et al.*, 2009), and for developing algal systems for the development of biofuels, nutraceuticals and for mariculture (Brown *et al.*, 1997; Spolaore *et al.*, 2006; Hu *et al.*, 2008). A recent analysis

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found the median macromolecular composition of microalgae under nutrient-sufficient, exponential growth conditions is 32.2% protein, 17.3% lipid, 15.0% carbohydrate and 6.8% nucleic acid on a percent dry weight basis (Finkel *et al.*, 2016). There is significant variability in the macromolecular composition of microalgae across species and phyla (Finkel *et al.*, 2016) and with environmental conditions (see below). An improved understanding of the macromolecular composition of microalgae is required to develop modeling frameworks for predicting macromolecular stoichiometry in microalgae.

Changes in growth rate and environmental conditions that alter physiological state are known to influence the macromolecular composition of microalgae (Fogg and Thake, 1987). Generally, nutrient starvation and decreases in growth rate are associated with a decline in RNA and protein content (Sterner and Elser, 2002; Flynn *et al.*, 2010; Loladze and Elser, 2011) and an increase in carbohydrate and lipid storage. Cellular protein content can decline more than 2-fold with large decreases in nitrate concentration or supply rate (Utting, 1985; Sukenik and Wahnon, 1991), but is variable across species and experimental conditions (Palmucci *et al.*, 2011). Protein content is generally less sensitive to phosphorus relative to nitrogen starvation (Kamalanathan *et al.*, 2016) and can even increase with phosphate supply rate (Kilham *et al.*, 1997). Cellular lipid can increase more than 2-fold under nutrient-sufficient growth conditions to more than 50% of dry weight under nutrient starvation and other stress conditions (Shifrin and Chisholm, 1981; Lynn *et al.*, 2000; Hu *et al.*, 2008). Light limitation tends to have a smaller influence on cellular protein and lipid content than nutrient limitation but can cause an approximate 3-fold decline in cellular carbohydrate content relative to light-saturating conditions (Renaud *et al.*, 1991; Sukenik and Wahnon, 1991).

Cell size may influence macromolecular composition independent of environmental conditions (Chan, 1978; Hitchcock, 1982; Moal *et al.*, 1987) through its influence on cell physiology and growth rate, sinking rate and investment in energy stores. Maximum growth rate (Y, often determined as carbon produced per organism per unit time) can be predicted from organism size: $Y = aM^b$, where a is growth rate at a reference size, M is an estimate of organism size, often cell volume for microalgae, and b is the metabolic size-scaling exponent. There is evidence that the size-scaling exponent b for growth rate (mass of C produced time^{-1}) for unicellular eukaryotes is ¾ if M is cell volume and approximately 1 if M is cell carbon (Delong *et al.*, 2010; Finkel *et al.*, 2010b). The growth rate hypothesis predicts that increases in growth rate, regardless of the environmental conditions, within and across species, requires an increase in RNA relative to protein content (Elser *et al.*, 2000; Sterner and Elser, 2002; Loladze and Elser, 2011; Daines *et al.*, 2014), although this is not always observed (Flynn *et al.*, 2010). If smaller microalgal cells have higher biomass-normalized growth rates than larger cells (Y/M, time^{-1}) then the growth rate hypothesis predicts that smaller cells will have higher protein and RNA content and a lower protein to RNA ratio relative to larger cells. Cell size also impacts sinking rate and storage capacity. Stokes' rule predicts a quadratic increase in sinking rate with increasing cell radius and a linear increase with density relative to the fluid medium. As a consequence larger microalgal cells may have higher cellular lipid concentrations than smaller cells to reduce their density. Alternatively it has been hypothesized that storage may be more advantageous for larger relative to smaller cells (Grover, 1991; Talmy *et al.*, 2014). If this is the case we may expect relatively more carbohydrate and/or lipid stores in larger relative to smaller microalgae. The size-scaling of protein, lipid and carbohydrate content will determine how total chemical energy content varies with cell size.

To address these hypotheses we analyze a database of microalgae macromolecular content gathered from the literature and quantify the size-dependence of protein, lipid, carbohydrate and chemical energy content of microalgae. In addition we determine if there are any differences in the protein, lipid, carbohydrate and chemical energy content predicted for three different phyla, diatoms, green algae and dinoflagellates, at three different cell volumes.

METHOD

Macromolecular data for eukaryotic microalgae was extracted from 53 studies from the literature. Data from figures was collected using ImageJ software. Macromolecular composition (protein, carbohydrate and lipid) as mass per cell was recorded along with physical and chemical measures of cell size (cell volume, dry weight per cell and carbon per cell), taxonomic information (phylum, genus, species and strain information), culture conditions (semi-continuous culture, turbidostat, batch culture) and growth phase (lag, exponential or stationary phase of the batch culture). Synonyms and phyla were identified using AlgaeBase during 2014–2016, an online database of terrestrial, marine and freshwater algae (Guiry and Guiry, 2016). Species identified to the genus but not to the species level are assumed to be different species unless identified as the same strain within or across studies. Our analyses focus on macromolecular observations from microalgae grown in nutrient-sufficient conditions in batch, turbidostat and semi-continuous cultures with an associated measure of cell volume and/or dry weight. Light and other environmental conditions not explicitly considered in this analysis can influence macromolecular content. Estimates of protein derived from total cellular nitrogen content were corrected for non-protein nitrogen as described in

Finkel *et al.* (2016). If linear cell dimensions were provided then cell volume was calculated following Hillebrand *et al.* (1999). The database and list of data sources is publically available at figshare.com (Finkel and Irwin, 2016).

The full dataset analyzed here includes 136 species from 6 phyla: the Bacillariophyta (diatoms), Chlorophyta (green algae), Dinophyta (dinoflagellates), Cryptophyta, Haptophyta, and Ochrophyta. Cell volume is used as the primary estimate of microalgal size because in total there are more estimates of macromolecular content (protein + lipid + carbohydrate) as a function of cell volume than for dry weight. In total there are 327 (193) observations of cellular protein, 184 (220) observations of cellular lipid, and 227 (191) observations of cellular carbohydrate with an associated measure of cell volume (or dry weight). There are very few observations of RNA content together with cell size, so we did not attempt to analyze the allometry of RNA content. The diatoms followed by the Haptophytes and the green algae have the largest number of macromolecular observations associated with a measure of cell volume. The size-dependence of protein, lipid, and carbohydrate content was analyzed for the full dataset, the diatoms, the green algae and the dinoflagellates. There are > 6 orders of magnitude of variation in cell volume associated with cellular protein, lipid and carbohydrate for the full dataset, > 6 orders of magnitude in cell volume associated with the diatoms, > 2 orders of magnitude in cell volume associated with the green algae and > 3 orders of magnitude associated with the dinoflagellates. The Cryptophyta, Haptophyta and Ochrophyta data are included in the pan-microalgae estimates of the size-scaling of macromolecular pools and chemical energy content estimates but because there are less than 15 observations of protein, lipid or carbohydrate (the Cryptophyta and Ochrophyta) with an associated measure of cell volume or there is less than two-orders of magnitude range in cell volume (the Haptophyta) the size-dependence of these individual groups was not analyzed. Chemical energy content was computed from joint observations of protein, lipid and carbohydrate content assuming 4.19 kcal per g protein, 9.5 kcal per g lipid and 4.20 kcal per g carbohydrate and converting calories to Joules by multiplying by 4.184 (Prosser and Brown, 1961, Hitchcock, 1982).

Major axis (MA) regression (Legendre, 2014) is used to determine the size-scaling exponents of protein, lipid, carbohydrate (pg cell^{-1}), and chemical energy content (nJ cell^{-1}) as a function of cell size (cell volume and dry weight per cell) and the inter-relationship between cell volume, dry weight and carbon content. Since there is uncertainty in both cellular composition and the estimates of cell size and none are experimentally controlled, the assumptions underlying ordinary least squares regression are not met. Major axis regression treats the predictor and response variables symmetrically and does not result in a flattening (regression to the mean) of the relationships. This is particularly important when comparing size-scaling exponents to each other and to reference values such as 1. We used a reference size of 100 μm^3 or 100 pg dry weight for the regressions, meaning that the intercepts of the regression equation correspond to predicted values for cells with those sizes. For the purposes of predicting cellular composition or energy content from cell size, it is more appropriate to use parameters obtained from ordinary least squares (OLS) regression (Legendre, 2014). Since both intercepts and slopes vary across phyla and macromolecules, we predicted cellular composition from OLS regressions for small (V = 10 μm^3, $\log_{10} V$ = 1), medium (V = 320 μm^3, $\log_{10} V$ = 2.5), and large (V = 100 000 μm^3, $\log_{10} V$ = 5) cells, chosen to span most of the range of observed sizes and so that the ratio of the size of a medium to small cell is the same as the ratio for a large to medium cell. We used *t*-tests to check for differences between MA regression slopes within phyla (comparing protein, lipid, and carbohydrate) and across phyla (comparing diatoms to greens, diatoms to dinoflagellates, and greens to dinoflagellates). We used a Bonferroni correction to account for the fact that we were making three tests with each set of results. We also used *t*-tests to compare the macromolecular composition predicted for small, medium, and large cells across phyla, with a Bonferroni correction. All analyses were performed with R version 3.2.2.

RESULTS

Relationship between physical and chemical measures of cell size

We use linear (MA) regression to determine the relationship between the physical measure of cell size (cell volume, usually calculated from linear dimensions) with two different estimates of cell mass: dry weight and cell carbon (Fig. 1, Table I). There are fewer published carbon than volume or dry weight data. There are 149 estimates of cell volume with associated cell carbon data, 139 observations of cellular volume with dry weight data, and 27 observations of cell carbon with associated dry weight data. Cellular carbon (C) scales sub-linearly with cell volume (V) with a size-scaling exponent of 0.78 with a 95% confidence interval of 0.75–0.81. Dry weight (DW) scales sub-linearly with cell volume (V) with a size-scaling exponent of 0.82 with a 95% confidence interval of 0.76–0.89. The size-scaling exponent for carbon and dry weight as a function of cell volume are not significantly different from one another (Fig. 1). Cellular carbon content as a function of dry weight per cell has a size-scaling exponent of 0.96 with a 95% confidence interval of 0.91–1.01 (Table I).

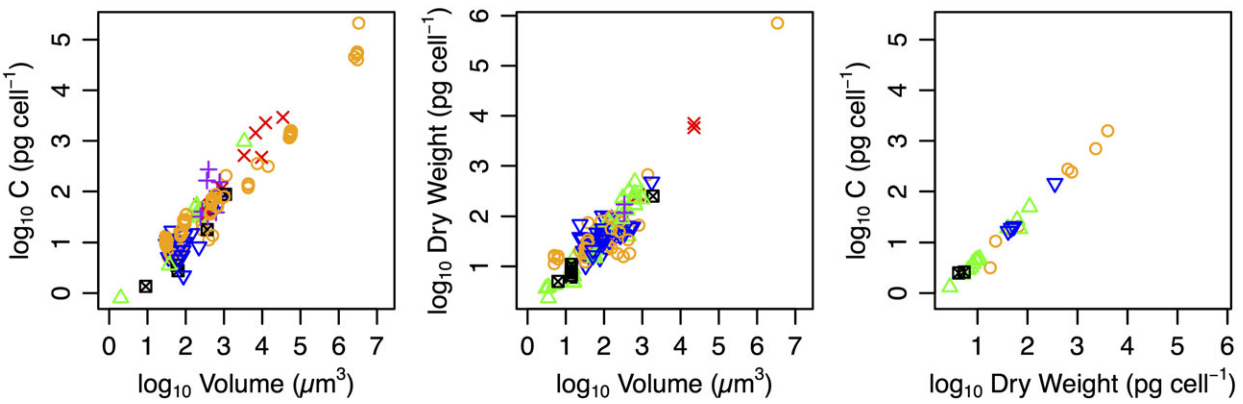

Fig. 1. Relationship between cell carbon ($\log_{10} C$, pg cell^{-1}), cell volume ($\log_{10} V$, μm^3), and dry weight ($\log_{10} DW$, pg cell^{-1}). Symbol legend: Bacillariophyta/diatoms (orange, open circle), Chlorophyta/green algae (green, upward triangle), Cryptophyta (purple, plus sign), Dinophyta/dinoflagellates (red, multiplication sign), Haptophyta (blue, downward triangle), Ochrophyta (black, box with multiplication sign), Rhodophyta (red, star).

Table I: Major axis (MA) linear regression relationship between cell carbon ($\log_{10}$ C, pg cell^{-1}), cell volume ($\log_{10}$ V, μm^3), and dry weight ($\log_{10}$ DW, pg cell^{-1})

Equation	Phytoplankton group	Intercept (*a*)	Slope (*b*)	*N*	R^2
$C = aV^b$	All	1.20 (1.18, 1.22)	0.78 (0.75, 0.81)	149	0.94
	Diatoms	1.21 (1.19, 1.23)	0.75 (0.72, 0.78)	95	0.97
	Green algae	1.31 (1.27, 1.34)	0.99 (0.80, 1.22)	9	0.95
	Dinoflagellates	1.20 (0.69, 1.58)	0.94 (0.68, 1.28)	8	0.91
$DW = aV^b$	All	1.64 (1.63, 1.64)	0.82 (0.76, 0.89)	139	0.83
	Diatoms	1.64 (1.63, 1.65)	0.78 (0.66, 0.91)	51	0.77
	Green algae	1.64 (1.64, 1.65)	0.89 (0.80, 0.99)	34	0.92
	Dinoflagellates	1.57 (1.36, 1.76)	0.95 (0.83, 1.08)	4	0.99
$C = aDW^b$	All	1.59 (1.57, 1.61)	0.96 (0.91, 1.01)	27	0.99

Intercepts correspond to $V = 100$ μm^3 or $DW = 100$ pg. Values in parentheses indicate 95% confidence intervals on estimates.

The size-scaling of carbon as a function of cell volume is significantly lower for diatoms (0.75, 95% CI: 0.72–0.78) than for the other microalgae, and in particular is smaller than the size-scaling exponent for carbon as a function of cell volume for the green algae (0.99, 95% CI: 0.80–1.2). The size-scaling exponent for dry weight as a function of cell volume is also lower for diatoms (0.78, 95% CI: 0.66–0.91) than for the green algae (0.89, 95% CI: 0.80–0.99).

Carbon as a percentage of dry weight

On average cell carbon is 35% of dry weight (95% CI: 27–43%) for diatoms and 44.6% (95% CI: 42–48%) of dry weight for the other microalgae. For comparison with these experimental observations we estimated carbon as percent dry weight based on the measured stoichiometry of elements in microalgal cells. Based on observations from the literature we assumed a C:N:P:Cl:K:Na:S:Mg:Ca:Fe:Sr:Zn:Cu:Cd:Co:Mo of microalgal biomass of 106:16:1:1.87:1.70:1.65:1.30:0.56:0.50:(7.5:5.0:0.8:0.38:0.21:0.19:0.03)$_{0.001}$. The stoichiometry of C:N:P follows the canonical Redfield ratio, Na and Cl (relative to P) was estimated from x-ray microanalysis of microalgae collected from the field (El-Bestawy *et al.*, 1996), and the trace metal stoichiometry was estimated from laboratory cultures (Ho *et al.*, 2003). The hydrogen and oxygen content associated with the major macromolecules (protein, lipid, carbohydrate and nucleic acids) was estimated using a theoretical approach (Anderson, 1995); 175 H and 42 O for every phosphorus atom. For diatoms $Si(OH)_4$ is added to the calculation assuming Si:N is 1 (Brzezinski, 1985). This approach estimates that carbon as percent dry weight is 31% for diatoms and 48.5% for other (not biomineralized) microalgae.

The size scaling of macromolecular and chemical energy content in microalgae

Across all the microalgae (full dataset), cellular protein, lipid and carbohydrate content, have cell volume based size-scaling exponents ranging from 0.80 to 0.93, with the lowest size-scaling exponent associated with lipid

Table II: Major axis (MA) regression results for $\log_{10}$ macromolecular and chemical energy content as a function of $\log_{10}$ cell volume

	Intercept	Slope	*n*	R^2
Protein (pg cell^{-1})				
All	1.09 (1.07, 1.10)	0.83 (0.80, 0.86)	327	0.90
Diatoms	1.10 (1.08, 1.13)	0.77 (0.74, 0.80)	172	0.93
Green algae	1.09 (1.09, 1.10)	1.03 (0.89, 1.18)	42	0.85
Dinoflagellates	1.01 (0.72, 1.25)	1.05 (0.89, 1.25)	31	0.83
Lipid (pg cell^{-1})				
All	0.80 (0.79, 0.81)	0.80 (0.76, 0.85)	184	0.88
Diatoms	0.73 (0.71, 0.75)	0.79 (0.74, 0.84)	80	0.91
Green algae	0.78 (0.76, 0.79)	0.87 (0.74, 1.03)	32	0.84
Dinoflagellates	0.54 (-0.49, 1.18)	1.04 (0.65, 1.7)	16	0.62
Carbohydrate (pg cell^{-1})				
All	0.72 (0.70, 0.73)	0.93 (0.87, 0.99)	227	0.80
Diatoms	0.62 (0.58, 0.65)	0.91 (0.83, 1.0)	103	0.82
Green algae	0.82 (0.80, 0.84)	1.05 (0.88, 1.2)	41	0.78
Dinoflagellates	1.05 (0.79, 1.3)	0.82 (0.67, 1.0)	18	0.88
Energy content (nJ cell^{-1})				
All	2.79 (2.78, 2.80)	0.83 (0.79, 0.87)	178	0.91
Diatoms	2.70 (2.69, 2.72)	0.80 (0.75, 0.85)	78	0.94
Green algae	2.83 (2.82, 2.85)	0.97 (0.84, 1.12)	32	0.87
Dinoflagellates	2.74 (2.24, 3.13)	0.99 (0.75, 1.3)	14	0.84

The regression equation was $\log_{10} y = a + b \log_{10} (x/100)$, where y is the macromolecular (pg cell^{-1}) or energy (nJ cell^{-1}) content, and x is cell volume (μm^3), so the intercept a corresponds to the value of $\log_{10} y$ for a cell with volume 100 μm^3. The slope b is the size-scaling exponent. Sample size is n and R^2 is the square of Pearson's correlation.

Table III: Major axis (MA) regression results for $\log_{10}$ macromolecular and chemical energy content as a function of $\log_{10}$ dry weight

	Intercept	Slope	*n*	R^2
Protein (pg cell^{-1})				
All	1.48 (1.47, 1.49)	0.96 (0.93, 0.99)	193	0.96
Diatoms	1.49 (1.46, 1.52)	0.98 (0.92, 1.04)	55	0.95
Green algae	1.57 (1.51, 1.63)	1.18 (1.07, 1.30)	37	0.93
Dinoflagellates	1.49 (1.34, 1.63)	0.87 (0.78, 0.96)	19	0.96
Lipid (pg cell^{-1})				
All	1.22 (1.21, 1.23)	0.97 (0.94, 1.01)	220	0.94
Diatoms	1.19 (1.16, 1.22)	0.92 (0.84, 1.00)	64	0.89
Green algae	1.13 (1.07, 1.19)	0.97 (0.87, 1.08)	50	0.89
Dinoflagellates	1.21 (1.03, 1.38)	1.01 (0.91, 1.11)	21	0.98
Carbohydrate (pg cell^{-1})				
All	1.03 (1.01, 1.04)	1.10 (1.05, 1.15)	191	0.90
Diatoms	0.93 (0.88, 1.0)	1.06 (0.92, 1.21)	55	0.81
Green algae	1.13(1.05, 1.22)	1.20 (1.05, 1.38)	37	0.87
Dinoflagellates	1.56 (1.31, 1.79)	0.75 (0.62, 0.90)	19	0.88
Energy content (nJ cell^{-1})				
All	3.18 (3.18, 3.19)	0.97 (0.95, 0.99)	191	0.98
Diatoms	3.14 (3.12, 3.16)	0.90 (0.86, 0.95)	55	0.97
Green algae	3.19 (3.16, 3.22)	1.05 (1.00, 1.11)	37	0.98
Dinoflagellates	3.31 (3.21, 3.41)	0.88 (0.82, 0.95)	19	0.98

The regression equation was $\log_{10} y = a + b \log_{10} (x/100)$, where y is the macromolecular (pg cell^{-1}) or energy (nJ cell^{-1}) content, and x is dry weight (pg cell^{-1}), so the intercept a corresponds to the value of $\log_{10} y$ for a cell with dry weight 100 pg. The slope b is the size-scaling exponent. Sample size is n and R^2 is the square of Pearson's correlation.

and then protein content and the highest size-scaling exponent associated with cellular carbohydrate content (Table II). Chemical energy content has a size-scaling exponent of 0.83 (95% CI: 0.79–0.87) with cell volume. The size-scaling exponents associated with the macromolecular pools increase when dry weight (0.96 to 1.1) is used as the estimate of cell size (Tables III and IV). Major axis regression analysis is used to compare size-scaling exponents across taxa but ordinary least squares regression analyses is used for predictions of macromolecular and chemical energy content based on cell volume (Tables V and VI).

The size scaling of the macromolecular pools differs across some of the phyla of microalgae (Fig. 2, Tables II, III and IV). For the diatoms, the cell volume based size-scaling exponent for carbohydrate is significantly higher than for protein and lipid. For the dinoflagellates the volume and dry weight based size-scaling exponent for carbohydrate and protein are lower than for lipid content. For the green algae the dry weight based size-scaling exponent for carbohydrate and for protein is significantly higher than for lipid. Both the green algae and dinoflagellates have significantly larger cell volume based size-scaling exponents for protein than the diatoms. A similar pattern is observed when dry weight is used as the estimate of cell size but the size-scaling exponent for protein for dinoflagellates is not significantly different from the size-scaling exponent for the diatoms.

Across all the microalgae, chemical energy content has a size-scaling exponent less than 1; 0.83 for cell volume and 0.97 for dry weight (Tables II and III). Across the taxa the diatoms have lower cell volume based size-scaling exponents than the green algae and dinoflagellates for protein, lipid and chemical energy content. On a dry weight basis the diatoms and dinoflagellates have lower size-scaling exponents for chemical energy content than the green algae that have a size-scaling exponent ≥ 1.

Taxonomic differences in the macromolecular and chemical energy content in small, medium and large cells

There are differences in cellular protein, lipid, carbohydrate and chemical energy content across different groups of microalgae at small (10 μm^3), medium (320 μm^3) and large (100 000 μm^3) cell volume (Table VI). Diatoms have 1.6-fold higher protein content than green algae microalgae at small size. In contrast, at medium and large cell size ranges, diatoms have lower protein content than other microalgae. Cellular carbohydrate content is larger for dinoflagellates and green algae relative to diatoms at medium size. There are no significant differences in lipid content across the groups of microalgae at small size but dinoflagellates have the highest and diatoms the lowest

Table IV: Results of t-tests comparing size-scaling exponents (slope b) reported in Tables II *and* III, reported as * for significant tests with $P < 0.05$, a blank for non-significant test results, and – where tests are not appropriate

(a) Comparisons between phytoplankton groups

	Diatoms	Green algae	Dinoflagellates
Protein			
Diatoms	–	*	*
Green algae	*	–	
Dinoflagellates		*	–
Lipid			
Diatoms	–		
Green algae		–	
Dinoflagellates			–
Carbohydrate			
Diatoms	–		
Green algae		–	
Dinoflagellates	*	*	–
Energy Content			
Diatoms	–	*	
Green algae	*	–	
Dinoflagellates		*	–

(b) Comparisons between macromolecular pools

	Protein – Lipid		Lipid – Carbo		Carbo – Protein	
	V	DW	V	DW	V	DW
All			*	*	*	*
Diatoms			*		*	
Green algae		*		*		
Dinoflagellates		*		*		

Sub-table (a) compares the size-scaling exponents for a macromolecule or energy content between the phytoplankton groups. Results above the diagonal refer to comparisons between exponents on a volume basis (Table II) and results below the diagonal refer to comparisons on a dry weight basis (Table III). Sub-table (b) compares size-scaling exponents within a phytoplankton group and between two macromolecular pools (indicated by column headings) on both a volume (V, Table II) and dry weight (DW, Table III) basis. Carbo = carbohydrates.

Table V: Ordinary least squares (OLS) regression results for $\log_{10}$ macromolecular and chemical energy content as a function of $\log_{10}$ cell volume

	Intercept	Slope	*n*	R^2
Protein (pg cell^{-1})				
All	1.11 (1.07, 1.15)	0.80 (0.77, 0.83)	327	0.90
Diatoms	1.12 (1.07, 1.17)	0.75 (0.72, 0.78)	172	0.93
Green algae	1.09 (0.99, 1.19)	0.94 (0.81, 1.07)	42	0.85
Dinoflagellates	1.15 (0.89, 1.4)	0.95 (0.79, 1.12)	31	0.83
Lipid (pg cell^{-1})				
All	0.80 (0.76, 0.85)	0.76 (0.71, 0.81)	184	0.88
Diatoms	0.74 (0.66, 0.81)	0.76 (0.71, 0.82)	80	0.91
Green algae	0.77 (0.67, 0.87)	0.81 (0.68, 0.94)	32	0.84
Dinoflagellates	0.92 (0.28, 1.56)	0.81 (0.45, 1.17)	16	0.62
Carbohydrate (pg cell^{-1})				
All	0.73 (0.68, 0.80)	0.84 (0.78, 0.89)	227	0.80
Diatoms	0.65 (0.54, 0.75)	0.83 (0.75, 0.90)	103	0.82
Green algae	0.80 (0.68, 0.92)	0.92 (0.77, 1.08)	41	0.78
Dinoflagellates	1.11 (0.86, 1.36)	0.78 (0.63, 0.93)	18	0.88
Energy content (nJ cell^{-1})				
All	2.80 (2.75, 2.84)	0.80 (0.76, 0.83)	178	0.91
Diatoms	2.71 (2.65, 2.78)	0.78 (0.73, 0.82)	78	0.94
Green algae	2.82 (2.73, 2.92)	0.91 (0.78, 1.04)	32	0.87
Dinoflagellates	2.88 (2.45, 3.3)	0.91 (0.65, 1.16)	14	0.83

The regression equation was $\log_{10} y = a + b \log_{10} (x/100)$, where y is the macromolecular (pg cell^{-1}) or energy (nJ cell^{-1}) content, and x is cell volume (μm^3), so the intercept a corresponds to the value of $\log_{10} y$ for a cell with volume 100 μm^3. Symbol legend as in Table II.

lipid content at medium and large size. At small size there are no significant differences in chemical energy content across the phyla but at medium size the green algae have higher chemical energy content than the diatoms and at large size the dinoflagaellates have significantly higher chemical energy content (3.6-fold) than the diatoms.

DISCUSSION

Microalgae cover an enormous size range from <1 µm in diameter for the smallest picoeukaryotes (for example: *Ostreococcus* spp.) to >1000 µm for some of the largest diatom and dinoflagellate species (for example: *Ethmodiscus rex* and some of the *Ceratium* spp.). This size range corresponds to >9 orders of magnitude in cell volume (Finkel, 2007; Beardall *et al.*, 2009). Cell size influences how organisms interact with their physical, chemical and biological environment (Haldane, 1926). For the microalgae, cell size is known to influence grazing susceptibility (Kiorbøe, 1993; Hansen *et al.*, 1997), sinking rate (Smayda, 1970; Waite *et al.*, 1997), and the acquisition of resources, including light absorption (Finkel, 2001; Mei *et al.*, 2009), CO_2 uptake (Wirtz, 2011; Wu *et al.*, 2014), nutrient uptake (Munk and Riley, 1952; Armstrong, 2008), as well as maintenance metabolic rate and maximum growth rate (Peters, 1983; López-Urrutia *et al.*, 2006). These cell size-linked eco-physiological traits can be used to interpret and predict changes in the size-structure of microalgal communities under different environmental and climatic conditions (Finkel *et al.*, 2007, 2010b; Clark *et al.*, 2013).

Here we find the macromolecular and chemical energy content of microalgae is influenced by cell size (Fig. 2). Across the microalgae, the size-scaling exponents for protein, lipid and carbohydrate content range from 0.80 to 0.93 for the full set of observations with an r^2-value ≥0.8 for cell volume and from 0.96 to 1.1 with an r^2-value ≥0.9 for dry weight (Tables II and III). There are some differences in size-scaling exponents for protein, lipid and carbohydrate content on both a cell

Table VI: Predictions of cellular macromolecular composition and energy content by taxon for a small (V = 10 μm^3), medium (V = 320 μm^3), and large (V=100 000 μm^3) cell from OLS regression models (Table V)

Protein	Small	*t*	Medium	*t*	Large	*t*
All	2.0 ± 7%		32 ± 4%		3080 ± 10%	
Diatoms	2.3 ± 8%		31 ± 5%		2340 ± 10%	
Green algae	1.4 ± 20%	D	36 ± 15%			
Dinoflagellates			42 ± 23%		10 300 ± 36%	D
Lipid						
All	1.1 ± 8%		15 ± 6%		1260 ± 15%	
Diatoms	0.94 ±12%		13 ± 8%		1060 ± 20%	
Green algae	0.91 ± 19%		15 ± 15%			
Dinoflagellates			21 ± 66%		2270 ± 77%	
Carbohydrate						
All	0.80 ± 11%		14 ± 7%		1750 ± 21%	
Diatoms	0.66 ± 19%		11 ± 12%		1360 ± 29%	
Green algae	0.76 ± 23		18 ± 19%	D		
Dinoflagellates			32 ± 21%	D	2810 ± 30%	
Energy content						
All	100 ± 8%		1570 ± 5%		153 000 ± 14%	
Diatoms	85 ± 11%		1260 ± 8%		111 000 ± 17%	
Green algae	83 ± 19%		1890 ± 15%	D		
Dinoflagellates			2140 ± 38%		399 000 ± 51%	D

Predicted macromolecular composition at three sizes between diatoms, green algae, and dinoflagellates were compared using *t*-tests. A *D* in the *t*-test column indicates a statistically significant difference ($P < 0.05$) between that group and the mean for diatoms. Entries are missing for unobserved sizes (small dinoflagellates, large green algae).
Composition is reported as a mean (pg cell^{-1} or nJ cell^{-1}, not on the log scale) and the standard error is given as a percent of the mean.

volume and dry weight basis. Generally, the microalgae have a significantly larger size-scaling exponent associated with carbohydrate as compared to lipid content (all microalgae, diatoms, but not the dinoflagellates). This result is consistent with an earlier study on 11 diatom and 8 dinoflagellate taxa (Hitchcock, 1982). Moal *et al.* (1987) in a study of 11 taxa, including 4 diatoms, 3 dinoflagellates, 1 green alga, 1 Haptophyta and 2 Crytophyta also found the cell volume based size-scaling exponent for cellular carbohydrate content was larger than the size-scaling exponent for protein content (Moal *et al.*, 1987). In this study we also find that the cell volume and dry weight based size-scaling exponent associated with cellular protein content is lower for diatoms relative to the green algae, indicating a larger increase in protein content with increasing cell size in the green algae relative to the diatoms.

The high size-scaling exponent associated with carbohydrate content is consistent with the hypothesis that larger microalgal cells may invest more heavily in storage materials on a volume basis than smaller cells. Microalgae use a variety of carbohydrate and lipid molecules for energy storage. Carbohydrate storage molecules such as starch, glucan, and chrysolaminarin are metabolically more energy efficient than triacylglyceride (TAG) lipid stores but TAG droplets are more energy dense (Subramanian *et al.*, 2013). Small microalgae can become space-limited (Raven, 1986) and starch-like granules can take up more than 20% of cellular area of eukaryotic picoplankton (< 2 μm in diameter) (Joint and Pipe, 1984). As a consequence it may be advantageous for small cells to accumulate more lipid than starch stores. Under nutrient-sufficient active growth, the conditions examined here, most microalgae have minimal energy stores. We currently lack the data to evaluate how cell size influences carbohydrate and lipid storage under nutrient starvation and other stress conditions that are known to stimulate hyper-accumulation of energy stores.

In contrast to the other microalgae, the dinoflagellates have relatively low volume and dry weight based size-scaling exponents for carbohydrate content (Tables II and III). Many dinoflagellates are characterized by carbohydrate-rich microfibrillar plates ($(C_6H_{10}O_5)_n$) (Dodge, 1973) and higher carbon content relative to other microalgae on a volume basis (Menden-Deuer and Lessard, 2000). This is because dinoflagellate walls are predominately carbohydrate and can account for a large proportion of total cellular carbohydrate, for example the wall (theca) of *Peridinium westii* is 95% glucose polymer (Nevo and Sharon, 1969). By comparison, carbohydrate is only a very minor component (few percent of dry weight) of the diatom cell wall (Hecky *et al.*, 1973). Here we find medium and large sized dinoflagellates have >2-fold higher carbohydrate content than equivalently sized diatoms (Table VI). Since the mass of dinoflagellate cell walls is an important component of the total carbohydrate pool, and expected to be roughly proportional to cell surface area, the cell wall component of total carbohydrate should reduce the size-scaling exponent for carbohydrate content away from 1 towards 2/3 on a whole cell basis, as observed here (Table III).

Diatoms tend to have lower volume-scaling exponents for protein, lipid and carbohydrate content relative to other microalgae (with the exception of carbohydrate in dinoflagellates). This is because with increasing cell volume diatoms become increasingly vacuolated relative to other groups of microalgae (Sicko-Goad *et al.*, 1984). As a result, the size-scaling exponent for cellular carbon as a function of cell volume is lower for diatoms than other microalgae (Strathmann, 1967; Menden-Deuer and Lessard, 2000) (Table I). A comparison of size-scaling exponents for macromolecular content on a dry weight basis eliminates much of variability across groups of microalgae due to differences in the size-scaling of vacuolation, accounting for the tighter relationship between macromolecular content and dry weight relative to macromolecular content and cell volume in Figure 2. But even on a dry weight basis diatoms have slightly lower size-scaling exponents for protein,

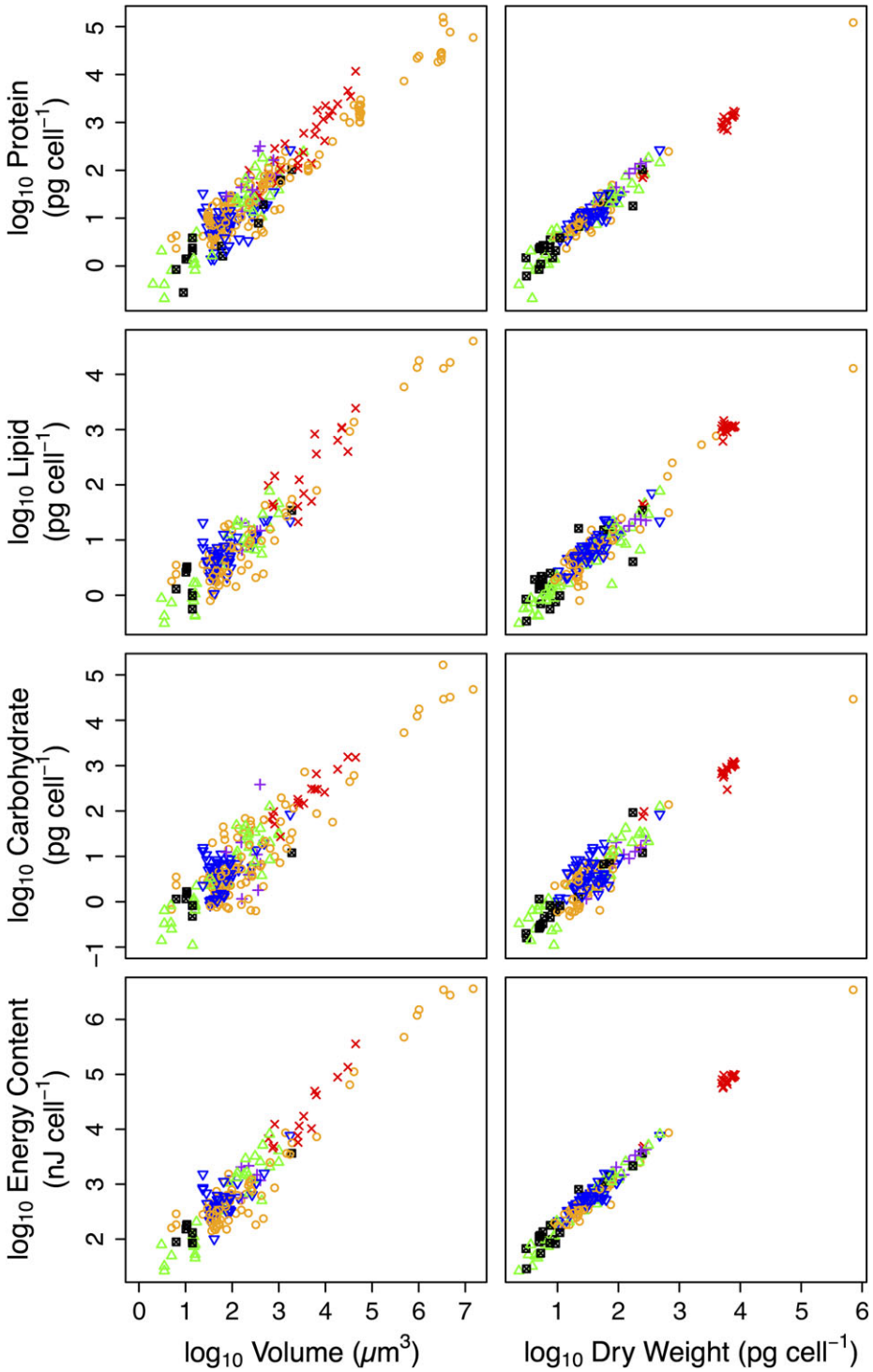

Fig. 2. Cellular protein, lipid, carbohydrate and chemical energy content ($\log_{10}$ pg or pJ cell^{-1}) as a function of cell volume ($\log_{10} V$, μm^3) and dry weight ($\log_{10} DW$, pg cell^{-1}). Symbol legend as in Fig. 1.

lipid and carbohydrate relative to the other microalgae (an exception being carbohydrate content in dinoflagellates). This is likely due to an increase in silicon-to-carbon in diatoms with increasing cell volume under standardized growth conditions (Brzezinski, 1985). As a general rule it is often assumed that organic carbon is 40–50% of the dry weight of a microalgal cell (Strickland, 1960; Shuter *et al.*, 1983). Based on relatively few observations of

carbon as a percent of dry weight determined by a chemical measure of carbon and oven-dried microalgal biomass ($n = 27$) we find carbon is 44.6% of dry weight for microalgae without biomineralized structures and 35% for diatoms. These estimates closely match the estimate of carbon as percent dry weight based on an average elemental composition of non-biomineralized microalgae and silicified diatoms (see Results). These estimates indicate the diatom frustule is a significant contributor to dry weight, reducing diatom macromolecular and chemical energy content on a dry weight basis relative to other microalgae. The contribution of Si to dry weight will vary across species, with silicon concentration, and other environmental conditions (Boyle, 1998; Martin-Jézéquel *et al.*, 2000; Finkel *et al.*, 2010a). The siliceous frustule and large vacuole allows diatoms to achieve relatively large physical sizes and lower carbon and macromolecular density than other microalgae.

The size-scaling exponent of protein may reflect the size-scaling of growth rate since biosynthetic machinery is rich in protein. Although we do not have growth rate data associated with the macromolecular observations presented here, on a volume basis larger microalgae tend to have relatively slower specific growth rates ($time^{-1}$) as compared to smaller cells (Flynn *et al.*, 2010; Finkel *et al.*, 2010b). The growth rate hypothesis predicts that larger, slower growing cells would have lower protein per unit cell volume than smaller, faster growing ($time^{-1}$) cells. Although the diatoms have an average size-scaling exponent for protein that is less than 1, surprisingly, we find the average volume- and dry weight size-scaling exponent for cellular protein content is larger than 1 for the green algae, indicating that green algae may (the 95% CI overlaps 1) invest more heavily in protein with increasing cell size (Tables II and III). This result may not be representative of green algae generally. Investigators may have disproportionally selected to study faster-growing large green algal taxa that tend to have higher protein content. There is not enough data in the literature to quantify the size-scaling of RNA content, so we did not attempt to look for changes in the ratio of RNA to protein. More work on the size-scaling of growth rate and macromolecular content across diverse phyla, such as the green algae, is needed to directly test these hypotheses.

Since lipid can increase buoyancy and could oppose increases in sinking rate resulting from increases in cell diameter we might expect a higher volume-scaling exponent for lipid relative to the other macromolecules. Contrary to this expectation, the volume based size-scaling exponent for lipid is lower than for carbohydrate in diatoms, and for the green algae and dinoflagellates the volume based size-scaling exponent for lipid is not significantly different from 1. In diatoms, the amount of Si per cell has a volume-scaling exponent less than 1 (Brzezinski, 1985), so that small cells have greater mass density attributed to Si than do large cells (Villareal, 1988). While the volume size-scaling exponent for lipid in diatoms is less than 1 (relatively more lipid per unit volume in smaller cells compared with larger cells), the dry weight based size-scaling exponent for lipid content is not different from 1, indicating that the lipid allometry is consistent with increased vacuolation with increasing cell volume in diatoms and not size-linked variation in silicification. It is possible that the size-scaling of lipid content is affected in opposing directions by changes in vacuolation, buoyancy, silicification, and energy storage strategies. An alternative interpretation is that the accumulation of lipid is not an effective way to alter cellular density and sinking rate in living phytoplankton (Smayda, 1970; Waite *et al.*, 1997). Smayda (1970) argues that although the accumulation of lipid stores is often assumed to regulate sinking rate, observations indicate it is, at best, only partially effective. To illustrate this point Smayda (1970) calculates that large increases in cellular lipid, up to 40% of dry weight, would result in only minor decreases in the density of a typical diatom cell; from 1.19 to 1.15 g cm^{-3}.

The size-scaling of chemical energy content of microalgae is a function of the size-scaling of the three major macromolecular pools: protein, lipid and carbohydrate. At small cell sizes, 10 μm^3, there are no significant differences in caloric content across diatoms, green algae or dinoflagellates (Table VI). But at intermediate cell volume, 320 μm^3, diatoms are lower in chemical energy content than the green algae and at large cell volumes, 100 000 μm^3, dinoflagellates are ~3-fold higher in caloric content than the diatoms, consistent with Hitchcock (1982). With increasing cell size diatoms have lower chemical energy content per unit volume than other microalgae because they become increasingly vacuolated relative to the other microalgae (Sicko-Goad *et al.*, 1984). As a consequence, on a cell volume basis the size-scaling exponents associated with chemical energy content are significantly less than 1 for diatoms and not significantly different from 1 for the green algae and dinoflagellates (Table II). This means that with increasing cell volume there is no significant change in chemical energy content per μm^3 in green algae and dinoflagellates but that larger-sized diatoms have fewer nJ μm^{-3} than smaller diatoms. Theoretically, chemical energy content has a size-scaling exponent of 1 when cellular carbon is used as an estimate of cell size because protein, lipid and carbohydrate have very similar chemical energy content on a carbon basis (Platt and Irwin, 1973; Gnaiger and Bitterlich, 1984). Here we find the dry weight based size-scaling exponent associated with chemical energy content is significantly less than 1 for the diatoms and dinoflagellates and not significantly different from 1 for

the green algae (Table III). While there is considerable uncertainty associated with the size-scaling exponent for macromolecular and chemical energy content for the dinoflagellates, the dry weight based size-scaling exponent for chemical energy content in the diatoms is likely less than 1 due to increases in silicon as a proportion of total dry weight with increasing size in diatoms. Changing macromolecular stoichiometry with cell size can affect the energy content of cells, but since lipid has only about twice the energy content per gram compared to protein and carbohydrate, the relatively small changes in lipid:carbohydrate:protein observed here will have a very small effect on energy content compared to changes in cell size, vacuolation and mineralization.

CONCLUSIONS

Macromolecular composition and chemical energy content of eukaryotic microalgae increase with cell size with size-scaling exponents varying from 0.8 to 0.93 with cell volume and 0.96 to 1.1 with dry weight. Although cell size explains much more of the variability in macromolecular and energy content than phylogenetic differences at a given cell size, there are phylum-level differences in the size scaling exponents associated with macromolecular and energy content that appear to be related to differences in cell biology, growth allocation and storage strategies across the phyla.

For example, the diatoms tend to have lower cell volume based size-scaling exponents because they are increasingly vacuolated with increasing cell volume and are lower in macromolecular and energy density (pg macromolecule or $\mathrm{nJ\,\mu m^{-3}}$) at large size relative to other microalgae. The size-scaling exponent for carbohydrate content is high relative to lipid in the diatoms and green algae but not in the dinoflagellates. We hypothesize that the high size-scaling exponent associated with carbohydrate versus lipid content in the diatoms and green algae reflect an advantage of increased carbohydrate relative to lipid storage in larger diatom and green algal cells. The smaller size-scaling exponent for carbohydrate in the dinoflagellates relative to the diatoms and green algae may be due to the high carbohydrate content of the dinoflagellate cell wall and the decrease in the surface area to cell volume ratio with increasing cell volume. The size-scaling exponent for protein is high ($\geq$1) in the green algae relative to the diatoms on both a cell volume and dry weight basis. We hypothesize that the green algae may invest more heavily in protein with increasing cell size or that there has been a selective bias towards studying faster growing large green algal taxa. Differences in the size-scaling of protein, lipid and carbohydrate content across diatoms, green algae and dinoflagellates may influence the nutritional quality and size-structure of phytoplankton communities and the biogeochemical cycling of nitrogen and carbon (Finkel *et al.*, 2010b). More experimental work is needed on the inter-relationship between cell biology, growth rate and macromolecular and chemical energy content to explicitly test these hypotheses.

DATA ARCHIVING

The macromolecular database analyzed in this manuscript is available at figshare.com. doi: 10.6084/m9.figshare.1600986.

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