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Estimating the contribution of microalgal taxa to chlorophyll *a* in the field—variations of pigment ratios under nutrient- and light-limited growth

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ABSTRACT: Cellular concentrations of chlorophylls and carotenoids were measured in nutrient- and light-limited cultures of marine microalgae to determine the utility of accessory pigments as proxies for the biomass of specific groups of microalgae in the ocean. In most species, concentrations of chlorophyll *a* (chl *a*) and photosynthetically active pigments varied linearly with growth rate in nitrate-limited continuous cultures or with the logarithm of the irradiance in light-limited and light-sufficient batch cultures, as has been observed before. Rates of pigment-concentration change as a function of irradiance or growth rate did not covary with rates of maximum growth. Concentrations of carotenoids covaried with chl *a* in most species analyzed; intraspecies variations of chl *a*-carotenoid ratios were usually smaller than variations of chl *a*:*b* or chl *a*:*c* ratios. These results were used to critically evaluate the assumptions underlying iterative methods used to determine the contribution of different algal taxa to chl *a* from ratios of chl *a* and accessory pigments. Estimates based on chl *a*:*b* or chl *a*:*c* ratios are prone to error because these ratios can vary by up to an order of magnitude among species and within species as a function of irradiance, thus violating an assumption of the iterative methods. Instead, such methods should rely on ratios of chl *a* and photosynthetically active carotenoids. Using simple models and field data from the Chesapeake Bay, USA, we showed that iterative methods are either prone to error when different populations of microalgae co-vary or do not give discrete solutions. As an alternative we suggest methods that rely more strongly on empirically determined pigment ratios.

KEY WORDS: Phytoplankton · Chlorophyll *a* · Carotenoids · HPLC · Regression analysis

INTRODUCTION

Concentrations of chlorophyll *a* (chl *a*) have been measured in the ocean for over 50 yr to estimate the biomass of phytoplankton (Harvey 1934, Richards & Thompson 1952, Holm-Hansen et al. 1965). Use of chl *a* as an indicator of phytoplankton biomass presupposes a consistent relationship between its cellular concentration and algal biomass. However, cellular concentrations of chl *a* (chl a_{cell}) in phytoplankton depend on a variety of environmental and physiological parameters, such as irradiance, growth rate, and nutritional state. In light-limited cultures of microalgae chl a_{cell} usually varies as a linear function of the logarithm of the irradiance (Falkowski 1980). In nutrient-limited algae grown in chemostats chl a_{cell} usually changes linearly as a function of growth rate (Laws & Bannister 1980). These results imply that phytoplankton biomass (µg C l^{-1}) cannot be determined from concentrations of chl *a* accurately without assuming *a priori* relationships between phytoplankton biomass and chl *a* (cf. Geider 1987).

Today, modern chromatographic methods are used to measure accessory chlorophylls and carotenoids to determine not only phytoplankton biomass, but also the biomass of specific groups of microalgae from concentrations of taxon-specific carotenoids such as fucoxanthin (diatoms), peridinin (dinoflagellates) and zeaxanthin (procaryotic photooxytrophs). Contributions of different groups of microalgae to biomass or total chl *a* can in principle be determined from concentrations of

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carotenoids, provided relationships between algal biomass and carotenoid concentrations are known. However, such relationships are currently lacking for most accessory pigments and are only now emerging for chl *a* (Geider et al. 1997). A simpler approach is to determine the contribution of different groups of microalgae to total chl *a*, thus avoiding the problem inherent in estimating the relationship between accessory pigments and phytoplankton carbon biomass. A variety of groups have used this approach (Gieskes et al. 1988, Everitt et al. 1990, Letelier et al. 1993, Mackey et al. 1996, Wright et al. 1996). The basic method relies on good first guesses for pigment ratios, refinement of these guesses iteratively using the minimization of calculated and measured chl *a* as a conversion criterion, and calculation of the contributions of different groups of microalgae to total chl *a* from the optimized pigment ratios. Implementations of this method differ primarily in the degree of mathematical sophistication, not in the basic approach.

A necessary assumption for these iterative methods is that ratios of taxon-specific pigments and chl *a* are constant. A corollary of this assumption is the requirement that 'all of the data samples in any given calculation are from the same phytoplankton community and physiological state' (Mackey et al. 1996). This requirement is impossible to meet as it is phytoplankton community structure—and ideally the physiological state of the populations—that we wish to determine. There is currently no obvious solution to the problem. Clearly, firm constraints on variations of pigment ratios in microalgae are required to determine which accessory pigments are best suited for such iterative methods. This information can then be used to determine the sensitivity of iterative methods to violations of their central assumptions. The sensitivity of a refined version of the iterative method to random errors associated with pigment ratios and measurements of pigment concentrations has been studied in detail by Mackey et al. (1996).

The objectives of this study were to quantify variations of cellular concentrations of chl *a* in eucaryotic microalgae as a function of growth conditions and to evaluate accessory pigments as indicators of phytoplankton biomass. We focus on cellular concentrations of pigments rather than pigment to carbon ratios to derive relationships that can be used to interpret pigment and flow-cytometry cell count data from the field. We present data for 7 species of microalgae grown in nutrient-limited chemostats and we analyze data from the literature for microalgae grown in light-limited batch cultures. These data are used to evaluate the use of chl *a* as a phytoplankton biomass indicator in the field and to evaluate methods currently used to calculate the contribution of different phytoplankton taxa to chl *a* (Gieskes et al. 1988, Everitt et al. 1990, Letelier et al. 1993, Mackey et al. 1996).

METHODS

Algal cultures. Methods used to maintain the microalgae in nitrate-limited continuous cultures have been described previously (Montoya & McCarthy 1995). To summarize, cultures were maintained under white continuous light (336 µEinst m^{-2} s^{-1}) at a temperature of 19°C in the enriched seawater medium f/2. The concentration of nitrate was reduced 4-fold to 220 µM. Cultures were harvested aseptically once steady state was attained. Samples were filtered on glass fiber filters (P < 25 cm Hg) and stored in a freezer at –22°C until analysis.

Chromatography. Glass fiber filters with field or culture samples were thawed and ground in 100% acetone. The extract was centrifuged (for 5 min) 30 min after grinding and decanted. Pigment extracts were analyzed on a reverse phase HPLC (high performance liquid chromatography) system as described by Goericke & Welschmeyer (1992a): column—Rainin Microsorb Short One, 10 cm, 3 µm C-18; gradient [time (min); % methanol with 0.15 parts aq. ammonium acetate, % methanol, % acetone]—[0; 100, 0, 0], [5; 0, 85, 15], [10; 0, 70, 30], [14; 0, 20, 80], [16; 0, 20, 80], [18; 100, 0, 0]. Pigments were detected by absorbance at 440 nm. The detector response was calibrated using

Table 1. Names, abbreviations, taxa and pigments of cultures used for the continuous culture experiments. Detailed information on clones and maximum growth rates can be found in Montoya & McCarthy (1995)

Species	Abbreviation	Taxon	Pigments
Thalassiosira weissflogii	*T. w.*	Diatom	Chl *a*, chl *c*, fucoxanthin
Skeletonema costatum	*S. cost.*	Diatom	Chl *a*, chl *c*, fucoxanthin
Phaeodactylum tricornutum	*P. tri.*	Diatom	Chl *a*, chl *c*, fucoxanthin
Isochrysis galbana	*I. gal.*	Prymnesiophyte	Chl *a*, chl *c*, fucoxanthin
Chroomonas salina	*C. sal.*	Cryptophyte	Chl *a*, chl *c*, alloxanthin
Dunaliella tertiolecta	*D. ter.*	Chlorophyte	Chl *a*, chl *b*, lutein
Pavlova lutheri	*P. luth.*	Prymnesiophyte	Chl *a*, chl *c*, fucoxanthin

standard procedures (Bidigare 1991). The HPLC system used does not separate the pigments chl c_1 - chl c_2 and lutein - zeaxanthin. We will refer to chl c_1 and chl c_2 as chl *c* and refer to lutein - zeaxanthin as lutein because zeaxanthin is a small fraction of the total pigments in the species examined (Hager & Stransky 1970).

Data analysis. Pigment data for continuous and batch cultures were also compiled from the literature. All data were analyzed using standard statistical procedures, all confidence intervals (CI) are 95% confidence intervals; standard deviations are denoted by the ± sign. Simple linear regressions were either model I or model II (Bartlett's 3-group method; Sokal & Rohlf 1981). Values of pigment ratios were optimized using the Solver module of Microsoft Excel 4.0. This module is based on a GRG2 nonlinear optimization code. We used quadratic optimization, forward differencing, the quasi-Newton gradient search method, and the minimization of least squares between measured and calculated chl *a*. Solutions were usually found in tens of seconds to minutes (33 MHz PC).

RESULTS

Continuous cultures

A total of 7 species (Table 1) were grown in nitrate-limited chemostats. Chl a_{cell} (pg cell^{-1}) varied linearly with the growth rate, μ (d^{-1}), in all species investigated except for the cryptophyte *Chroomonas salina* (Fig. 1). For this species 2 distinct linear regions, ranging from 0.05 to 0.5 and 0.5 to 1.4 d^{-1}, are discernible in its plot of chl a_{cell} versus μ. We characterized the relationship between pigment per cell or a pigment ratio, x (g g^{-1}), and growth rate, μ, by fitting the equation

$$x = x_{\mu=0} + a \cdot \mu \quad (1)$$

to the data and calculated the predicted value of x at a growth rate of 1.0 d^{-1}, $x_{\mu=1.0}$. We normalized the slope a by $x_{\mu=1}$, i.e. $\alpha = a \cdot (x_{\mu=1})^{-1}$, to correct for interspecies variability of the slope a due to varying cell size. The dimensions of α are time; the value of $1 - \alpha$ corresponds to the ratio of $x_{\mu=0}$ and $x_{\mu=1.0}$. Chl a_{cell} increased significantly as a function of μ in all species studied but *Isochrysis galbana* (Table 2). Values of α were calculated from our data (Fig. 1) and data from the literature (Fig. 2). The average value of $\alpha_{chl\ a}$ was 0.76 (±0.22, n = 17) for the combined data set, ranging from 0.31 to 1.3. Values of α did not vary significantly as a function of μ_{max}, the regression coefficient of α versus μ_{max} was −0.03 [model II regression, CI_{slope} = (0.65, −0.82)].

Chl *a*:*c* or chl *a*:*b* ratios varied significantly as a function of μ only in *Skeletonema costatum* and *Chroomonas salina* (Fig. 3, Table 2). The ratios did not vary significantly with μ in other species (Table 2); however, observed values varied in some species by a factor of 2. In *C. salina* the chl *a*:*c* ratio varied as a linear function of μ only for growth rates >0.3; the ratio increased from a value of 30 at μ = 0.3 to a value of 110 at μ = 0.09.

The cellular concentrations of all major xanthophylls (fucoxanthin in chromophytes, lutein in *Dunaliella*

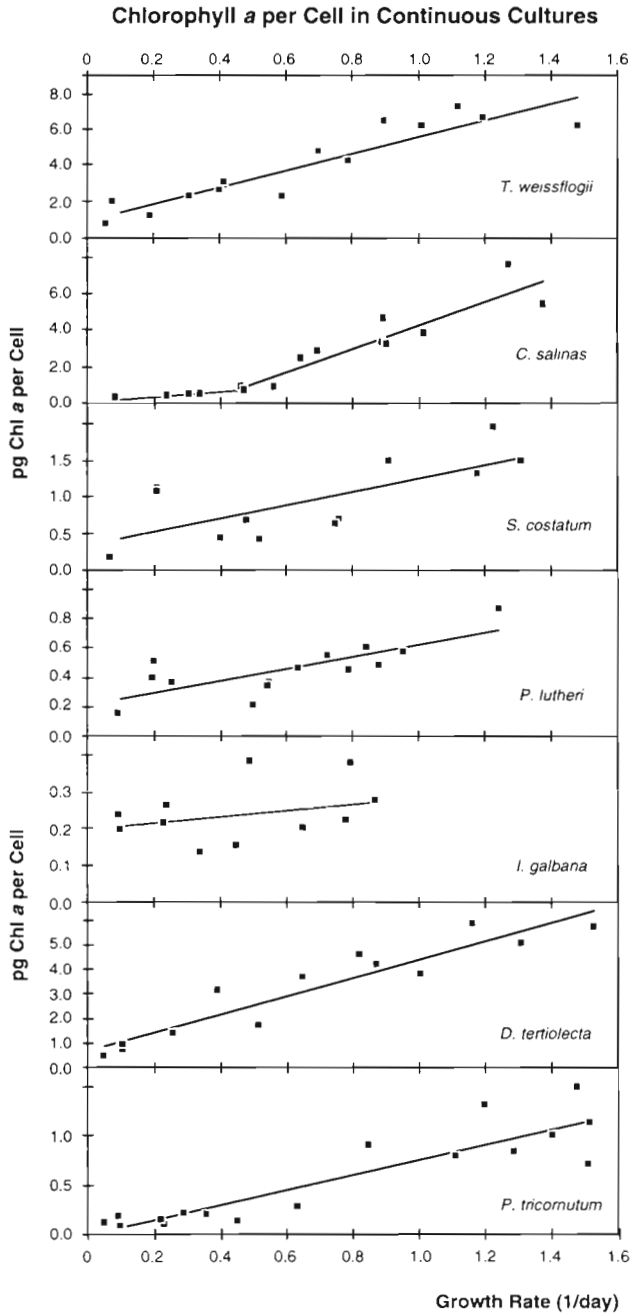


Fig. 1 Chlorophyll *a* per cell vs growth rate for 7 species of microalgae grown in nitrate-limited continuous cultures (Table 1)

Table 2. Summary of the regression statistics for continuous culture pigments. The equation $x = x_{\mu=0} + a\mu$ was fit to the data for any parameter x. Listed in the table for each parameter are its value for $\mu = 1$ d^{-1}, the slope a normalized by the value of the $x_{\mu=1}$, i.e. $\alpha = a/x_{\mu=1}$, and the probability that the value of a does not equal zero. We used a model I regression to fit the parameters because the growth rate was the controlled variable. Full names corresponding to the abbreviations are given in Table 1. Fucox.: fucoxanthin; allox.: alloxanthin. DD: diadinoxanthin; DT: diatoxanthin. ns: not significant

	D. ter.	*I. gal.*	*P. luth.*	*P. tri.*	*S. cost.*	*T w.*	*C. sal.* $\mu < 0.5$ d^{-1}	*C. sal* $\mu > 0.5$ d^{-1}
Chl *a*								
Value ($\mu = 1.0$)	4.388	0.285	0.620	0.759	1.252	5.533	1.473	4.315
Norm. slope	0.85	0.31	0.66	1.00	0.71	0.82	0.97	1.50
p ($a \neq 0$)	>99.9	ns	>99.9	>99.9	>99	>99.9	>99	>99.9
Chl *b* or chl *c*								
Value ($\mu = 1.0$)	0.822	0.023	0.019	0.053	0.112	0.349	0.180	0.727
Norm. slope	0.85	0.11	0.83	0.98	0.90	0.80	1.01	1.82
p ($a \neq 0$)	>99.9	ns	>99	>99.9	>99.9	>99.9	>99	>99.9
Carotenoid								
Name	Lutein	Fucox.	Fucox.	Fucox.	Fucox.	Fucox.	Allox.	Allox.
Value ($\mu = 1.0$)	1.387	0.044	0.107	0.196	0.272	1.237	0.180	0.727
Norm. slope	0.61	−0.07	1.14	0.91	0.95	0.69	1.01	1.82
p ($a \neq 0$)	>99.9	ns	>99	>99.9	>99	>99.9	>99	>99.9
DD+DT								
Value ($\mu = 1.0$)		0.010	0.104	0.030	0.040	0.182		
Norm. slope		−6.76	0.83	0.45	0.59	−1.13		
p ($a \neq 0$)		>95	ns	>95	ns	ns		
β- or α-carotene								
Value ($\mu = 1.0$)	0.179	0.001	0.023	0.010	0.018	0.068	0.022	0.096
Norm. slope	0.37	−8.75	−2.26	0.77	−0.92	0.91	1.06	1.89
p ($a \neq 0$)	ns	>99	ns	>99.9	ns	>99.9	>99	>99.9

	D. ter.	*I. gal.*	*P. luth.*	*P. tri.*	*S. cost.*	*T w.*	*C. sal.*
Chl *a*:*b* or chl *a*:*c*							
Value ($\mu = 1.0$)	5.36	12.7	35.5	15.5	11.5	15.7	20.0
Norm. slope	−0.03	0.17	−0.26	−0.01	−0.40	−0.02	−0.60
p ($a \neq 0$)	ns	ns	ns	ns	>95	ns	>95
Chl *a*:major xanthophyll vs μ							
Value ($\mu = 1.0$)	3.16	7.18	7.08	3.87	4.87	4.43	9.76
Norm. slope	0.52	0.43	−0.79	0.28	−0.90	0.25	−0.59
p ($a \neq 0$)	>99.9	ns	>99	>99.9	>95	>99.9	>99.9
Chl *a*:(DD+DT)							
Value ($\mu = 1.0$)		17.5	9.3	26.0	36.8	30.8	
Norm. slope		0.97	0.20	0.80	−0.15	0.71	
p ($a \neq 0$)		>95	ns	>99.9	ns	>99	
Chl *a*:$\sum$carotene vs μ							
Value ($\mu = 1.0$)	30.0	107.8	28.7	76.9	85.7	92.7	54.4
Norm. slope	0.56	0.95	0.45	0.50	0.20	−0.19	−0.64
p ($a \neq 0$)	ns	>99.9	ns	>99.9	ns	ns	>99

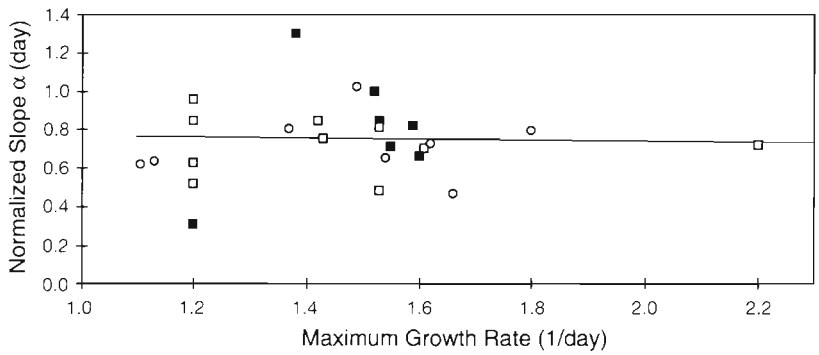


Fig. 2. Slopes of chl a_{cell} vs maximum growth rate normalized by chl a_{cell} ($\mu = 1$), i.e. α (see text), for microalgae grown in continuous cultures (■: this work; □: Laws & Wong 1978, Laws & Bannister 1980, Kolber et al. 1988) and for microalgae grown under light-saturated conditions at 10, 15, 20, and 25°C (○: Thompson et al. 1992; for these data α is plotted against growth rates at 20°C). Line represents the results of a linear regression of the chemostat data; the Thompson et al. (1992) data were not included in this regression

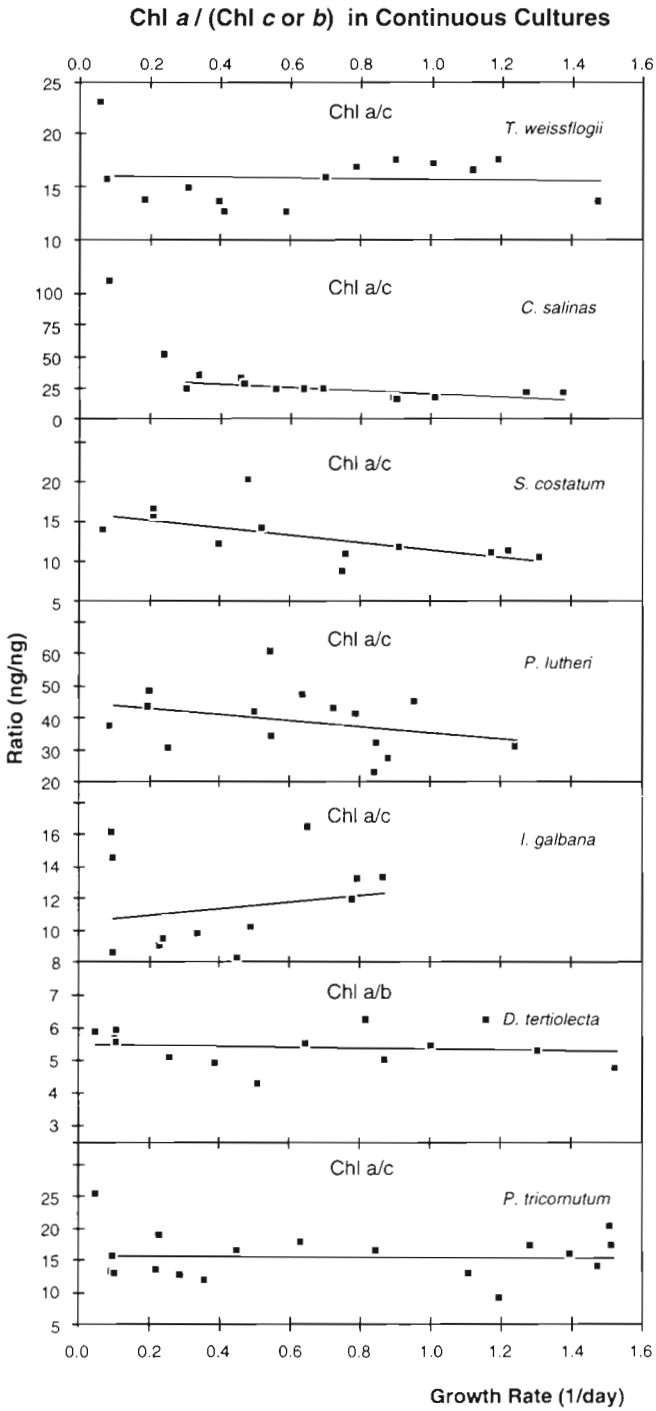


Fig. 3. Chl *a*:*c* or chl *a*:*b* ratio vs growth rate for 7 species of microalgae grown in nitrate-limited continuous cultures (Table 1)

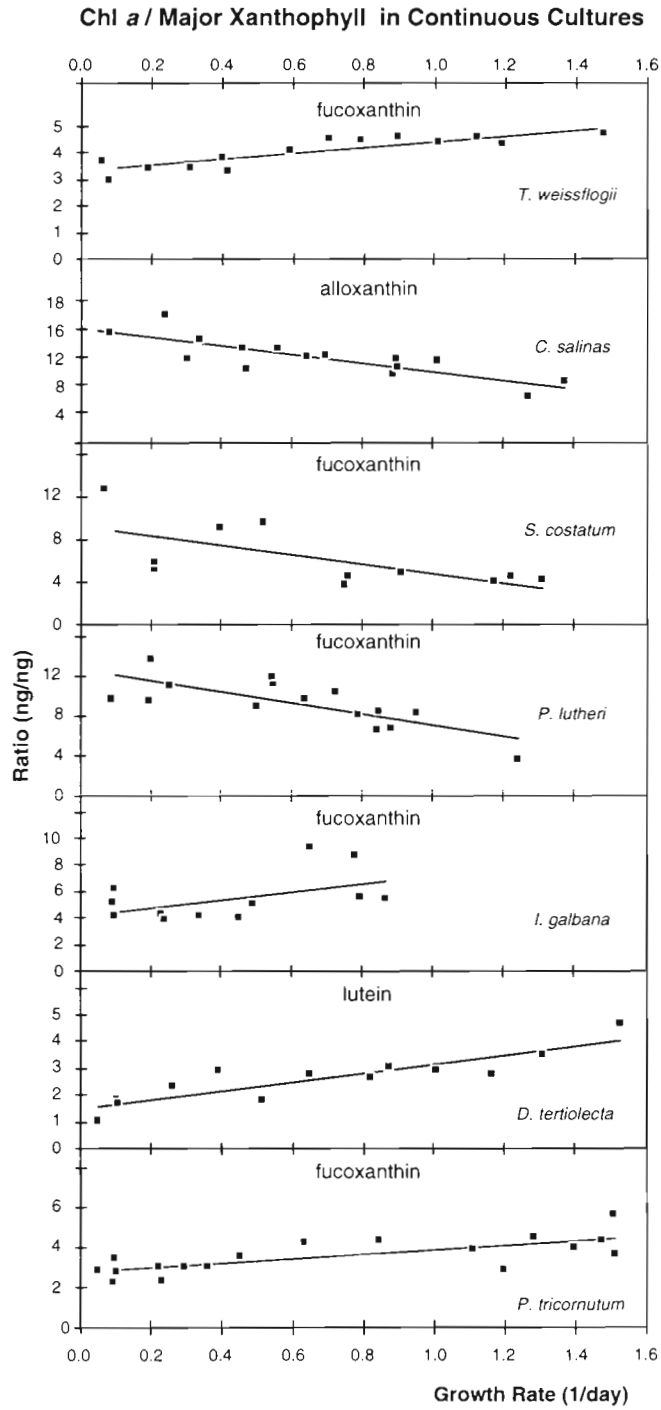


Fig. 4. Chl *a* to major xanthophyll ratio vs growth rate for 7 species of microalgae grown in nitrate-limited continuous cultures (Table 1)

tertiolecta and alloxanthin in *Chroomonas salina*) increased significantly as a function of μ in all algae studied except *Isochrysis galbana* (Table 2). The predicted ratio of chl *a* and fucoxanthin for $\mu = 1$ ranged from 3.9 to 7.2, decreased significantly as a function of μ in *Pavlova lutheri* and *Skeletonema costatum*, increased significantly with μ in *Thalassiosira weissflogii* and *Phaeodactylum tricornutum* (Fig. 4, Table 2), but did not vary significantly with μ in *I. galbana*. The ratio of chl *a* to alloxanthin in *C. salina* decreased signifi-

cantly as a linear function of μ. The ratio of chl *a* to lutein in *D. tertiolecta* increased significantly as a function of μ. However, absolute changes of the ratio chl *a* to major carotenoid in these species were small; values of the ratios for growth rates of 0.1 and 1.0 d^{-1} differed only by up to a factor of 3.

The pigments diadinoxanthin (DD) and diatoxanthin (DT) can rapidly be interconverted in microalgae in response to irradiance changes, a process called xanthophyll cycling (Hager 1980). It is possible that such interconversion occurred during the sampling of the cultures; thus we only consider the sum of the cellular concentrations of the 2 pigments ($DD+DT_{cell}$). The ratio if chl *a* and $DD+DT_{cell}$ did not vary consistently as a function of μ in most cultures studied (Fig. 5), even though observed variations were often large (Fig. 5). $DD+DT_{cell}$ decreased significantly with μ in *Isochrysis galbana* and increased significantly with μ in *Phaeodactylum tricornutum* (Table 2). The ratio of chl *a* and DD+DT increased significantly with μ in *Thalassiosira weissflogii*, *I. galbana*, and *P. tricornutum*, but may have varied nonlinearly with μ in *Skeletonema costatum* and *Pavlova lutheri* (Fig. 5).

Batch cultures

It has been observed previously (Falkowski 1980) that chl a_{cell} varies as a linear function of the logarithm of the irradiance in nutrient replete batch cultures of microalgae. We used the equation

$$x = x_0 + b \cdot \log(I) \quad (2)$$

to characterize the relationship between pigment concentrations or ratios, x, and the logarithm ($\log_{10}$) of the irradiance, $\log(I)$. x_0 is the predicted value of the pigment or ratio at an irradiance of 1 µEinst m^{-2} s^{-1} and b is the slope of the relationship. The slope b was normalized by the value of x predicted for an irradiance of 10 µEinst m^{-2} s^{-1} (x_{10}), i.e. $\beta = b/x_{10}$. β, which is dimensionless, designates the fraction by which the value of x changes when the irradiance is decreased from 10 to 1 µEinst m^{-2} s^{-1}. This normalization facilitates the comparison of species with differing cellular pigment concentrations or ratios. Chl a_{cell} varied as a function of $\log(I)$ in most of the species analyzed here with the exception of the dinoflagellates *Amphidinium carterae* (Sosik et al. 1989) and *Gyrodinium aureolum* (Nielsen 1992). For these latter species chl a_{cell} varied as a hyperbolic function of $\log(I)$. Nonetheless, for the sake of consistency, we also used Eq. (2) to describe the variation of pigment concentrations with irradiance in these species.

Values of β fell mostly in the range −0.2 to −0.7. Exceptions to this pattern were some cultures of

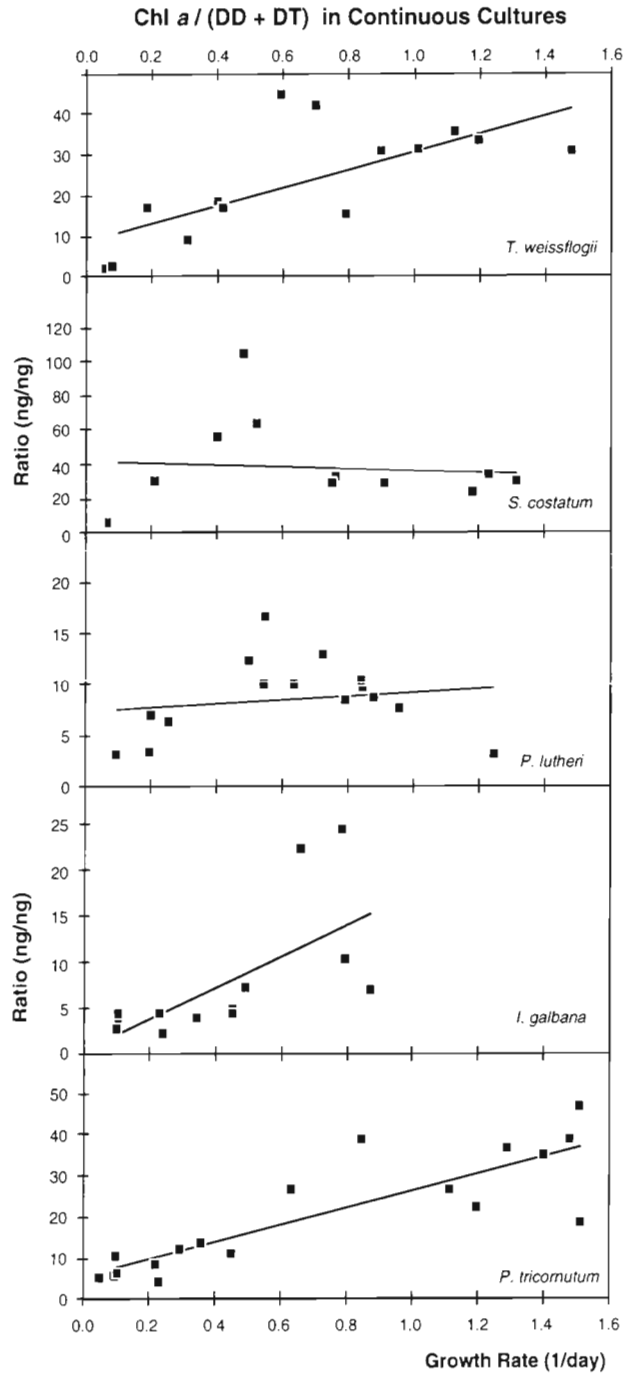


Fig. 5. The ratio of chl *a* and diadinoxanthin (DD) plus diatoxanthin (DT) vs growth rate for 7 species of microalgae grown in nitrate-limited continuous cultures (Table 1)

Olisthodiscus luteus (β = −0.01) and *Skeletonema costatum* (β = 0.02) and 2 cultures of a cryptophyte (β = −1.03) and a prasinophyte (β = −1.00) grown under continuous light at a temperature of 1°C (Buma et al. 1993). The high value of β for *S. costatum* contrasts with results from 6 other studies for the same species

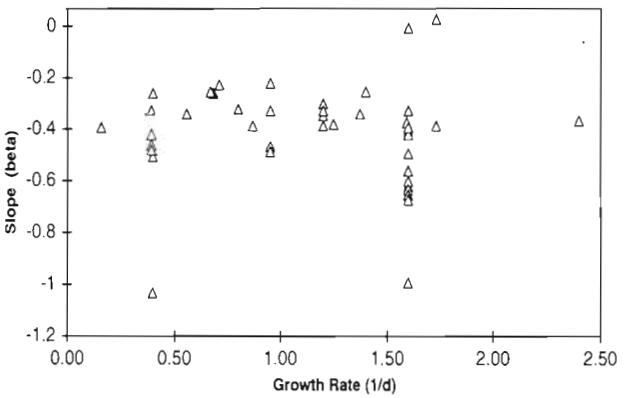


Fig. 6. Slopes of chl a_{cell} vs maximum growth rate normalized by chl a_{cell} (μ = 1), i.e. β (see text), for microalgae grown in batch cultures. Data are from Table 3 and from an analysis of data from the literature (Falkowski & Owens 1980, Falkowski et al. 1981, 1985, Raps et al. 1983, Post et al. 1985, Sakshaug & Andresen 1986, Langdon 1987, Kana et al. 1988, Kolber et al. 1988, Sosik et al. 1989, Buma et al. 1993, Garcia & Purdie 1992, Goericke & Welschmeyer 1992a, Nielsen 1992, Moore et al. 1995). Maximum growth rates were taken from the literature

(β = −0.29 ± 0.06), suggesting that the single value of 0.02 for *S. costatum* is an experimental artifact. The values of −1.0 observed for Antarctic microalgae grown under continuous light contrast with values of −0.51 and −0.62 determined for the same species grown under a 12:12 h light:dark cycle. The average value of β for all data is −0.39 ± 0.20, n = 34. β varied slightly as a function of μ_{max} (Fig. 6), the regression coefficient for β versus μ_{max} was 0.16 [model II regression; CI_{slope} was (0.30, 0.04)].

The effects of photoperiod length and irradiance on chl a_{cell} in *Skeletonema costatum* were investigated by Sakshaug & Andresen (1986). Chl a_{cell} varied linearly with log(I) for all photoperiods (Fig. 5 of Sakshaug & Andresen 1986), with the exception of short-daylength, low-irradiance cultures, in which chl a_{cell} did not increase with decreasing irradiance. Sakshaug & Andresen (1986) suggested that these cultures, grown under 'marginally low light', represented partially dormant states. We did not use these data points, 4 of 42 (see Fig 7), for the following statistical analysis. Values of β for these experiments, averaging −0.36, did not vary significantly as a function of daylength (test of equality of slopes: df = 5, F_s = 1.25 < $F_{5\%[5,25]}$ = 2.02). A plot of chl *a* per cell against daily light dose (Fig. 7) shows that chl a_{cell} also varied linearly with the logarithm of the daily light dose (Einst m^{-2} d^{-1}). This is expected because the change in units from irradiance to daily light dose represents an additive transformation in a log domain. We observe, however, that all lines collapse into one once the data are transformed to the daily-light-dose domain.

In nutrient replete batch cultures accessory chlorophylls usually co-vary with chl *a* (Table 3). Variations of chl *a*:*b* or chl *a*:*c* ratios with irradiance were at times large. Ratios always increased with irradiance, i.e. β was always positive. This increase was in most cases small with the exception for some nonlinear variations of chl *a*:*c* ratios, e.g. in *Thalassiosira weissflogii* (Goericke & Welschmeyer 1993). Rates of change of chl *a* to xanthophyll ratios as a function of log(I) were small in all except 1 case (Table 3). Values of β were close to zero, with an average value of 0.011 ± 0.100. Concentrations of β-carotene decreased as a function of irradiance relative to chl *a* in 4 chromophytes (Table 3).

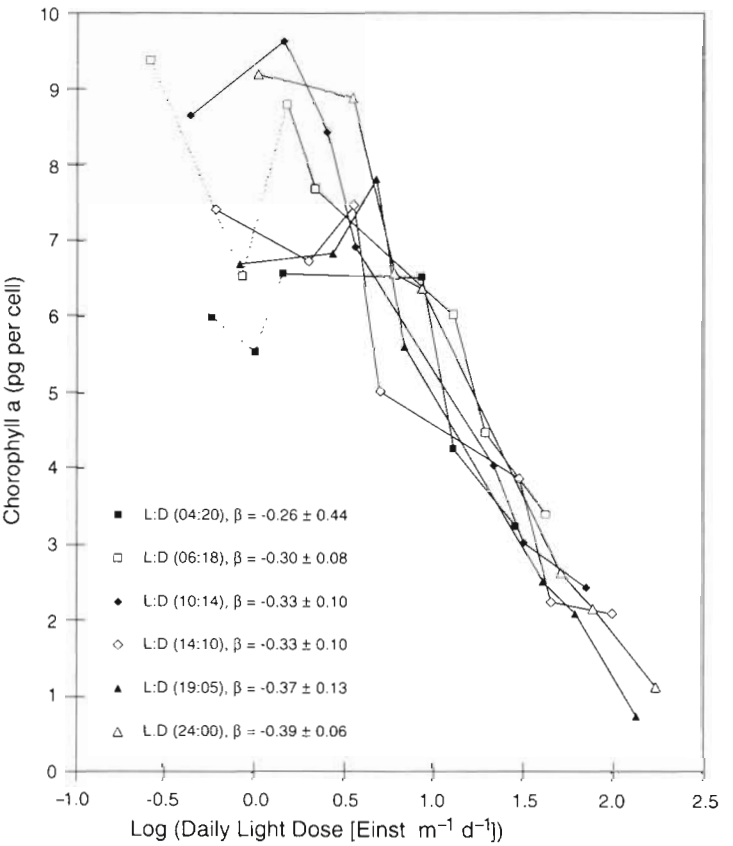


Fig. 7. Chl a_{cell} for *Skeletonema costatum* grown under varying irradiance and light:dark (L:D) cycles plotted against daily light dose (from Fig. 5 of Sakshaug & Andresen 1986). We excluded the data points denoted in the figure by dotted lines (low irradiance, short daylength) from our statistical analysis

Table 3. Literature data for the ratios of accessory chlorophylls or photosynthetically active carotenoids and chl *a* in light-limited and light- sufficient batch cultures. Data were fit to the equation $X = X_0 + b \cdot \log(I)$ as described in the text. Given in the table are the photocycle (P-cycle; light:dark), temperature (Temp., °C), the maximum growth rate of the species (μ_{max}, d^{-1}), the pigment ratio (g g^{-1}), the predicted value of the ratio for an irradiance of 100 µEinst m^{-2} s^{-1} (X_{100}), the normalized slope of the line (β), the probability that *b* is different from zero, and the source of the data. Full species names are either given in Table 1 or are *Amphidinium carteri* or *Hymenomonas carterae*. Allox.. alloxanthin; fucox.: fucoxanthin; perid.. peridinin; β-caro.: β-carotene. Sources: 1: Buma et al. (1993); 2: Falkowski et al. (1981); 3: Kolber et al. (1988); 4: Sosik et al. (1989), 5: Goericke & Welschmeyer (1992a), Goericke & Welschmeyer (1992b). ns: not significant, n: number of data points

Species	P-cycle	Temp.	μ_{max}	Pigment ratio	X_{100}	β	p ($b \neq 0$)	Source
Pyramimonas sp.	24:0	1	0.40	Chl *a*:chl *b*	1.44	0.112	95	1
Pyramimonas sp.	12:12	1	0.40	Chl *a*:chl *b*	1.42	0.081	ns	1
D. tertiolecta	24:0	15	1.25	Chl *a*:chl *b*	3.73	1.802	(n = 2)	2
S. costatum	24:0	15	0.95	Chl *a*:chl *c*	3.61	0.100	ns	2
S. costatum	24:0	18	0.95	Chl *a*:chl *c*	4.92	0.299	95	3
T. weissflogii	24:0	18	1.73	Chl *a*:chl *c*	8.32	0.049	ns	3
D. tertiolecta	24:0	18	1.37	Chl *a*:chl *c*	6.55	0.390	ns	3
I. galbana	24:0	18	1.20	Chl *a*:chl *c*	3.43	2.146	95	3
A. carteri	24:0	20	0.71	Chl *a*:chl *c*	3.83	0.268	99.9	4
T. weissflogii	24:0	20	2.40	Chl *a*:chl *c*	14.80	0.296	99	4
H. carterae	24:0	20	1.40	Chl *a*:chl *c*	16.50	0.920	ns	4
T. weissflogii	24:0	18	1.20	Chl *a*:chl *c*	28.14	3.530	99.9	5
Cryptomonas sp.143	24:0	1	0.40	Chl *a*:allox.	5.51	0.063	ns	1
Cryptomonas sp.169	24:0	1	0.40	Chl *a*:allox.	5.80	0.147	ns	1
Cryptomonas sp.143	12:12	1	0.40	Chl *a*:allox.	2.12	−0.395	ns	1
Cryptomonas sp.169	12:12	1	0.40	Chl *a*:allox.	3.26	−0.021	ns	1
T. weissflogii	24:0	20	2.40	Chl *a*:fucox.	2.98	0.040	ns	4
T. weissflogii	24:0	18	1.20	Chl *a*:fucox.	3.07	−0.095	99	5
H. carterae	24:0	20	1.40	Chl *a*:fucox.	3.33	0.138	99	4
A. carteri	24:0	20	0.71	Chl *a*:perid.	2.00	−0.039	99	4
A. carteri	24:0	20	0.71	Chl *a*:β-caro.	43.73	−0.213	99.9	4
H. carterae	24:0	20	1.40	Chl *a*:β-caro.	30.20	−0.188	99.9	4
T. weissflogii	24:0	20	2.40	Chl *a*:β-caro.	64.44	−0.005	ns	4
T. weissflogii	24:0	18	1.20	Chl *a*:β-caro.	28.88	−0.299	99	5

DISCUSSION

Cellular chl *a*

Chl a_{cell} varied significantly as a function of log(*I*) and growth rate in most species of eucaryotic microalgae studied. In order to use these data to predict variations of cellular chl *a* in field populations we quantified variations of pigment concentrations as a function of growth rate in continuous cultures or irradiance in batch cultures using simple linear models. Our analysis of continuous culture studies gave a mean value for the slope of chl a_{cell} versus μ normalized by chl $a_{cell}(\mu = 1)$, i.e. $\alpha_{chl\,a}$ of 0.76. This result implies that chl a_{cell} will increase on average by a factor of 3.2 when growth rate increases from 0.1 to 1.0 d^{-1}, values that are encountered when natural phytoplankton communities grow under nutrient-depleted and nutrient-replete conditions (Furnas 1990).

The type of nutrient limiting growth may have an effect on the value of α. For example, values of α as calculated from the data of Laws & Bannister (1980) for nitrate-, ammonia-, and phosphate-limited continuous cultures of *Thalassiosira weissflogii* are 0.95, 0.75, and 0.62, respectively. Differences between these values are small but significant (df = 2, 9; $F_s = 10.8 > F_{0.5\%[2,9]} = 10.1$). Thus we cannot rule out a small effect of the type of limiting nutrient on α. However, it is quite likely that the primary physiological force driving changes in cellular chl *a* is the variations of a cell's growth rate; the type of nutrient limiting growth may exert a secondary effect on cellular chl *a*. We can attempt to corroborate this hypothesis by using data on microalgae grown at varying temperatures under light- and nutrient-replete conditions. In this case variations of growth rates are entirely due to varying temperatures. The work by Thompson et al. (1992), who grew 8 species of microalgae at 10, 15, 20, and 25°C and determined their cellular composition, provides the data to test this hypothesis. The average value of α for the 8 species grown by Thompson et al. (Fig. 2) is 0.72, a value not significantly different (*t*-test, $t = 0.60 < t_{95\%,2\text{-tail}} = 2.1$) from the average of the α values derived from the chemostat experiments, 0.76. This result suggests that to understand or model variations of cellular chl *a* in the ocean we only need to consider the effects of growth rates and can neglect, at least to a first approximation, the factor limiting growth, as long as the irradiance is constant.

Our analysis of batch culture studies gave a mean value for the slope of chl a_{cell} versus log(I) normalized by chl a_{cell} (I = 10), i.e., $\beta_{chl\ a}$, of −0.39. This value implies that chl a_{cell} will decrease by a factor of 5 when nutrient-replete cultures are shifted from an irradiance of 10 to 1000 µEinst m^{-2} s^{-1}, irradiance values commonly encountered in the ocean at the bottom of the euphotic zone and at the surface, respectively.

Our analysis of the data of Sakshaug & Andresen (1986) showed that in *Skeletonema costatum* rates of change of chl a_{cell} as a function of irradiance ($\beta_{chl\ a}$) do not vary significantly with photoperiod length. This result, if generally true, implies that the effects of irradiance on chl a_{cell} can be considered to be independent of photoperiod as long as the photoperiod is constant, i.e. we can assume that a single value of $\beta_{chl\ a}$ will describe variations of chl a_{cell} as a function of irradiance independent of photoperiod length as long as the photoperiod is constant. However, if photoperiod varies, as it does over the seasons, its effects have to be taken into account. The plot (Fig. 7) of chl a_{cell} against log(daily light dose) shows that variations of chl a_{cell} in *S. costatum* only depend on the daily light dose and are independent of the photoperiod (Fig. 7). This implies, if the result is indeed generally valid, that variations of chl a_{cell} over the seasons can easily be modeled using daily light dose (Einst m^{-2} d^{-1}) instead of irradiance (µEinst m^{-2} s^{-1}).

A priori, temperature should not have an effect on $\beta_{chl\ a}$ since photochemistry is temperature independent. Indeed, the average value of $\beta_{chl\ a}$ for Antarctic microalgae grown in batch cultures at a temperature of 1°C, −0.53, was not significantly different from the average value of $\beta_{chl\ a}$ for microalgae grown at temperatures ranging from 15 to 29°C, −0.36.

Our analysis of photoacclimation in terms of chl *a* per cell is to some degree limited because cell size has been shown to vary in some species as a function of the factors that also affect cellular pigment concentrations. Thompson et al. (1992) have recently summarized the effects of varying irradiance on cell size and carbon quotas. No clear patterns emerged from this review of almost 40 species. Cell size and carbon quotas were observed to increase and decrease in response to increasing irradiance. Maximal increases of cell size were on the order of 60%, with extreme values of 95%, but on the average only small increases in cell size were observed. Patterns for carbon quotas were similar; some species responded to increasing irradiance with no response or decreasing carbon quotas but most responded with a moderate increase in carbon quotas. These changes in cell size and carbon quotas are significant but small compared to the factor of 3 to 5 changes in cellular chl *a* concentrations. We conclude that the cell size and carbon quotas do not affect the patterns that we have described.

Accessory chlorophylls

The ratio of chl *a* and accessory chlorophylls varies among species and within a species as a function of physiological state. Chl *a*:*b* ratios range from 1 to 10 in chlorophytes and from 0.5 to 3 in prasinophytes (Wood 1979). The ratio of chl *a* to chl *c* ranges from 1.5 to 7.5 in chromophytes (Jeffrey 1972, Stauber & Jeffrey 1988). Average values of the chl *a*:*c* ratio (predicted value for μ = 1, Table 2; predicted value for I = 100 µEinst m^{-2} s^{-1}, Table 3) calculated here ranged from 3.5 to 35. Within any one species changes of chl *a*:*b* and chl *a*:*c* ratios as a function of growth rate in nutrient-limited cultures and as a function of irradiance in light-limited and light-sufficient batch cultures were often small but mostly significant. Ratios varied usually by less than a factor of 2 (Tables 2 & 3). Large changes of chl *a*:*b* and chl *a*:*c* ratios were only observed in 3 out of 21 experiments analyzed: in continuous cultures of *Chroomonas salinas*, batch cultures of *Thalassiosira weissflogii* and batch cultures of *Isochrysis galbana* (Tables 2 & 3).

The chl *a*:*c* ratio was virtually constant in nutrient-limited and light-limited cultures of *Thalassiosira weissflogii*, but changed from a value of 12 to 68 in light-saturated batch cultures with increasing irradiance (Goericke & Welschmeyer 1992a). Such large variations of chl *a*:*c* ratios may indicate changing ratios of light-harvesting pigment protein complexes and reaction centers (cf. Prézelin 1981). Brown (1988) reported that chl *c* is only associated with the light-harvesting complexes in diatoms, whereas chl *a* is found in the reaction centers I and II and the light-harvesting complexes. The constancy of the chl *a*:*c* ratio under growth-limiting light intensities then suggests that the number of reaction centers did not change relative to the number of light-harvesting complexes. The increase of the chl *a*:*c* ratio at growth-saturating light intensities suggests that either the number or the size of light-harvesting complexes was reduced relative to the number of reaction centers. These dynamics of the intracellular chl *c* pools may offer a simple method to study photoadaptive processes in chromophytes.

Carotenoids

The carotenoids fucoxanthin, 19'-hexanoyloxyfucoxanthin, 19'-butanoyloxyfucoxanthin, peridinin, and alloxanthin function as light-harvesting pigments in microalgae (Siefermann-Harms 1987). The carotenoids diadinoxanthin and diatoxanthin in chromophytes and the carotenoids violaxanthin and zeaxanthin in chlorophytes have photoprotective roles (Siefermann-Harms 1987). Other carotenoids such as lutein in chlorophytes

and zeaxanthin in cyanobacteria are also present at high concentrations in the chloroplast envelope, though their function is largely unknown. These different functions will affect concentrations of these pigments in microalgae. For example, concentrations of photosynthetically active carotenoids should covary with chl *a*; however, cellular concentration changes of other carotenoids are more difficult to predict.

Ratios of chl *a* and photosynthetically active carotenoids ranged from 2 to 7 (Tables 2 & 3; Hooks et al. 1989). Cellular concentrations of fucoxanthin, peridinin, and alloxanthin co-varied with chl *a* under conditions of light-limited, light-saturated, and nutrient-limited growth. Although we often observed significant variations of the chl *a*:carotenoid ratio as a function of growth rate or irradiance, these variations were small when compared to variations of pigment concentrations. The ratio of chl *a* and fucoxanthin increased significantly as a function of growth rate in some continuous cultures but decreased significantly in others; no systematic pattern was evident. Our observations corroborate the results of similar studies with cryptophytes (Buma et al. 1993) and prasinophytes (Hooks & Bidigare 1988). To summarize, most available data indicate that concentrations of photosynthetically active pigments covary in microalgae under conditions of changing growth rates and irradiance.

Higher plants and algae have a photoprotective mechanism, commonly called the xanthophyll cycle, which involves the epoxidation and de-epoxidation of 2 xanthophylls, diadinoxanthin and diatoxanthin in chromophytes and violaxanthin, and zeaxanthin in chlorophytes (Hager 1980, Siefermann-Harms 1987, Demmig-Adams 1989). Considering the photoprotective function of these pigments, their concentrations might be expected to co-vary with concentrations of photosynthetically active pigments and possibly with irradiance. However, it is difficult to predict changes of $DD+DT_{cell}$ since the 2 factors are usually negatively correlated in the field. For example we observed large changes of the ratio of chl *a* and $DD+DT_{cell}$ in cultures grown in chemostats (Fig. 5) but these were not related to growth rate in these species. These large changes of $DD+DT_{cell}$ in any one species and differences among species that have also been observed in batch cultures (Sosik 1988, Goericke 1990) may preclude its use as an indicator of chromophyte biomass in the field.

Chl *a* and phytoplankton biomass changes in the field

The main factors that affect cellular concentrations of pigments in natural populations of microalgae are irradiance and growth rate for nutrient-limited populations and irradiance alone for light-limited populations. For example, C:chl *a* ratios (Geider 1987) and cellular concentrations of photosynthetically active pigments can vary by up to a factor of 10 in environments where growth might be limited by nutrients and light, e.g. the stratified water column in subtropical gyres (Goericke & Repeta 1993). This implies that phytoplankton biomass can only be predicted to within a factor of 10 from concentrations of pigments unless we make assumptions regarding the physiological state of the microalgae and their light environment. Although variations of photosynthetically active pigments as a function of growth rate and irradiance are predictable in most species of algae (see above), phytoplankton growth rates in the open ocean are virtually unconstrained *a priori*; estimates for the subtropical open ocean, for example, range from 0.2 to more than 3.0 d^{-1} (Goldman et al. 1979). Thus, it is difficult at the present time to use the derived relationships to calculate phytoplankton biomass unless growth rates are directly measured (cf. Goericke in press). However, the relationships derived here can be used to calculate relative biomass changes as a function of depth or time from changes of chl *a* concentrations.

We can apply this approach to the subarctic Pacific, where a major fraction of the phytoplankton community is believed to grow under nutrient-sufficient, light-limited conditions (Miller et al. 1988, 1991; but see Martin & Fitzwater 1988 for discussions on the effect of iron). In the subarctic Pacific mixed layer chl *a* does not vary systematically over the seasons and has an average value of about 0.4 µg chl *a* l^{-1} (Frost 1991). Based on this observation it has been concluded that there are virtually no seasonal variations of phytoplankton standing stocks (Miller et al. 1988, Frost 1991). This conclusion, however, does not take into consideration that irradiance and mixed layer depth both vary over the seasons in the subarctic Pacific (Fig. 8A) and that phytoplankton might photoacclimate. Based on our results we expect that chl a_{cell} varies in the subarctic Pacific over the seasons with high values in the winter and lower values in the summer This would imply that phytoplankton biomass changed over the seasons since mixed layer chl *a* remained constant. We can calculate relative phytoplankton biomass changes over the year by assigning the value of 1 unit of biomass to the winter solstice. We assume that the average irradiance experienced by the phytoplankton is equal to the higher value of either the irradiance at the depth corresponding to one half of the mixed layer depth or the irradiance at the 5% light level. The latter condition is imposed because phytoplankton in complete darkness, i.e. at the bottom of a deep mixed layer with a euphotic zone depth less than the mixed layer depth, will not photoadapt (authors' unpubl. data); changing this

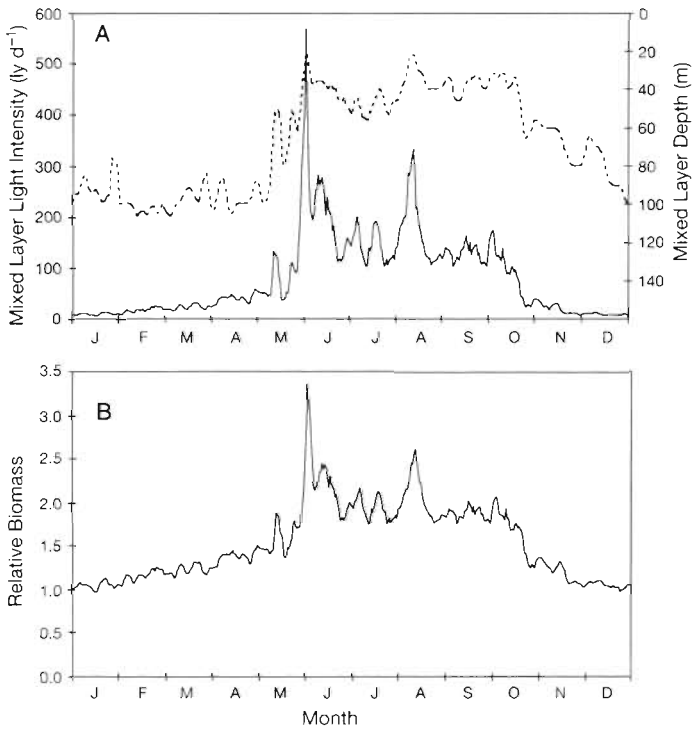


Fig. 8. (A) Mixed layer depth (dashed line) and average mixed layer irradiance (solid line) and (B) relative biomass units in the subarctic Pacific. Data in A represent 5-day running averages of data presented in Frost (1991) for 1970

value to 10 or 1% does not change the following conclusions qualitatively. Using available data for irradiance and mixed layer depth (Frost 1991) we can calculate variations of phytoplankton biomass over the year using Eq. (2) with a value of −0.39 for β (Fig. 8B) and assuming that chl *a* is constant over the year. Although relative phytoplankton biomass calculated as described above is highly variable, similar to observed short-term variations of chl *a* (Miller et al. 1991), phytoplankton biomass changes systematically by about a factor of 2 over the seasons. These calculations illustrate that irradiance-driven changes of chl *a* concentrations in the surface layer of the ocean are expected to be small under most conditions. The subarctic Pacific and other temperate or subarctic areas are probably extreme cases due to large seasonal variations of irradiance and mixed layer depths.

The regressions of cellular pigment concentrations against irradiance derived here cannot be used blindly as some of these predict negative pigment concentrations for high light levels (usually for values >1000 to 2000 µEinst m^{-2} s^{-1}). It is likely that the relationship between pigment concentrations and irradiance is nonlinear for species able to live at such high irradiances. However, we did not find such a nonlinear relationship at high irradiances in our data set.

Calculating contributions of different taxa to chl *a*

The strong covariation of chl *a* and photosynthetically active carotenoids suggests that these can be used as proxies for the biomass of specific taxa, just as chl *a* is used as a proxy for phytoplankton biomass. However, biomass estimates based on accessory carotenoids are subject to the same uncertainties that biomass estimates based on chl *a* are. A more conservative approach is to use accessory pigments and ratios of accessory pigments to chl *a* to calculate the contribution of different groups of algae to total chl *a* (Gieskes et al. 1988, Everitt et al. 1990, Letelier et al. 1993, Mackey et al. 1996). Estimates of these pigment ratios can be improved once large data sets are available. This is achieved by iteratively regressing variations of accessory pigment concentrations against variations of chl *a* (cf. Everitt et al. 1990, Letelier et al. 1993), akin to the estimation of phytoplankton carbon from a single linear regression of chl *a* against particular organic carbon (POC). However, estimating the contribution of different taxa to total chl *a* from multiple linear (or nonlinear) regressions may subject these estimates to bias similar to the bias inherent in estimates of phytoplankton carbon based on regressions of chl *a* and POC (Banse 1977). Critical to our understanding of the potential bias associated with these iterative methods are the explicit and implicit assumptions used. The 2 main assumptions are (cf. Mackey et al. 1996): (1) pigment ratios within any group are constant over the domain encompassed by the data set, and (2) variations in the abundances of different algal groups are not correlated.

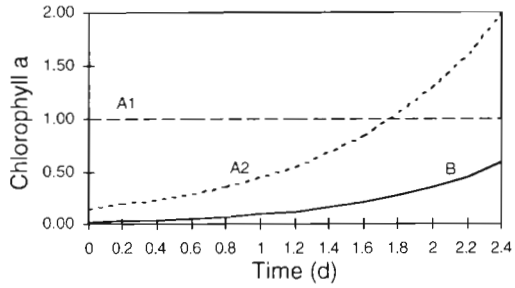
It is quite unlikely that the first assumption will ever hold in a strict sense. Attempts can only be made to minimize deviations from it. Thus, it is clearly advisable to use only those pigments for a regression analysis whose concentrations covary tightly with chl *a*. The large variations of chl *a*:*c* ratios observed in some species (Table 3) and the large differences of chl *a*:*b* ratios between closely related species, these ratios ranging from 0.3 (Moore et al. 1995) to 10 (Wood 1979), should caution us to use accessory chlorophylls in regression analyses. Most photosynthetically active carotenoids are well suited for this type of analysis. For example, fucoxanthin may be used to estimate the contribution of diatoms to total chl *a* because the ratio of chl *a* and fucoxanthin in diatoms only varies by up to a factor of 2 as a function of irradiance and growth rate within any species and has values of between 3 and 4 for different species (Tables 2 & 3). This result suggests

that the contribution of diatoms to total chl *a* can be calculated to within a factor of about 2.

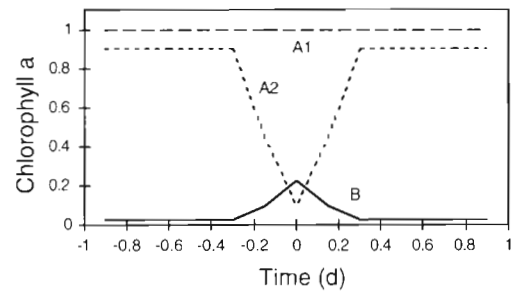
The second assumption, statistical independence among the dependent variables, i.e. pigment biomass of the individual taxa, is required since these independent variations are our only means of estimating ratios of chl *a* and carotenoids for the different taxa. However, it is unlikely that the second assumption will always hold since growth rates, and quite likely biomass as a consequence, may respond to environmental stimuli similarly in different taxa of microalgae.

Our personal experience with regression analyses suggests that at times they do not give consistent results or that they do not give realistic results, i.e. calculated values of pigment ratios are unrealistic. We believe that this may be due to violations of the above assumptions. To illustrate these problems we set up 2 model systems of phytoplankton communities, each consisting of 3 different populations (Box 1). These models, Case 1 and 2, consist of 2 populations, PA_1 and PA_2, with the accessory pigment A and a third population, PB, with the accessory pigment B. Using pigments as taxon-specific markers we are only able to differentiate between 2 populations in these 2 model systems. This is typical for pigment-based approaches to phytoplankton ecology because the taxonomic units delineated by pigments are rather coarse, i.e. usually at the class level. For both cases, abundances of populations PA_2 and PB are assumed to covary. For Case 1, PA_2 and PB are assumed to increase exponentially with time and for Case 2 the correlation between the 2 populations is assumed to be negative and transient, as might be observed as a response to short environmental stimuli. Ratios of chl *a* and the accessory pigments A and B were estimated using regression analyses. The estimated chl *a*:A ratio was in both cases realistic, 1.5 in Case 1 and 0.8 in Case 2, even though not correct since the true ratios (chl *a* weighted averages) were 1.36 and 1.35, respectively. The estimated Case 1 and Case 2 chl *a*:B ratios, −1.8 and 1.2, respectively, differed significantly from the true ratio, 0.33 for both cases. The error associated with the first case would have been easy to spot as negative pigment ratios are not possible. The error associated with the second case would have been difficult to detect without ancillary information. In that case the contribution of population PB to chl *a* would have been overestimated by a factor of more than 3. A 'fix' to the problem represented by

Box 1. Effects of covariations of populations on regression-based estimates of the contribution of different taxa to total chl *a* are explored using 2 simple models. Models were set up in MS Excel and solved numerically. Regressions on model results were performing using MS Excel Solver.



Case 1. Assume the following: (1) There are 3 populations of microalgae, PA_1, PA_2, and PB. (2) PA_1 and PA_2 have the accessory pigment A and PB has the accessory pigment B. (3) PA_1 and PA_2 have chl *a*:A ratios of 2 and 0.5, respectively, and PB has a chl *a*:B ratio of 0.33. (4) These ratios are constant. (5) PA_1 does not change its abundance and the abundances of PA_2 and PB increase exponentially at rates of 1.2 and 1.5 d^{-1}. Using pigments we can only differentiate between 2 populations with the accessory pigments A and B. The chl *a*:A and chl *a*:B ratios of these 2 populations are determined by regressing A and B against chl *a*. A chl *a*:A ratio of 1.5 and a chl *a*:B of −1.8 are derived from the regression analysis. The true value of the chl *a*:A ratio is 1.36 (chl *a* weighted average).



Case 2. A similar example is shown here for the same parameters as used above, except that the abundance of PA_2 and PB is assumed to covary inversely in response to a short environmental stimulus. Regression analysis gave a chl *a*:A ratio of 0.8 and a chl *a*:B ratio of 1.2. The latter ratio differs by almost a factor of 4 from the actual value of 0.33. The true value of the chl *a*:A ratio is 1.35 (chl *a* weighted average).

Case 1 is to add the constraint that pigment ratios fall into certain ranges. In that case the solution will be a boundary value and will no longer be the unbiased result of a statistical procedure but the result of our ability to select 'realistic' boundary values.

An additional problem is the discreteness of solutions for pigment ratios for a certain class of models, i.e. there is no finite number of solutions to the problem but instead an infinite number of solutions that are usually described by a simple function. For example, no discrete solutions exist for simple systems with pairs of populations, PA and PB under certain conditions, even if these are embedded in larger communities. Let us assume that PA has the carotenoids A and B, that PB has the carotenoid B only and that there are no other populations in the community that have these 2 carotenoids. The ratio of chl *a* to A in population PA is f_A(PA). f_B(PB) is similarly defined. The ratio of B to A in PA is given by f_{BA}(PA). The contribution of the 2 populations to the total chl *a* in both populations [Tchl *a* (PA, PB)] is calculated from

$$\text{Tchl } a\ (\text{PA, PB}) = \text{A} \cdot f_A(\text{PA}) + (\text{B} - f_{BA}(\text{PA}) \cdot \text{A}) \cdot f_B(\text{PB})$$

i.e. to calculate the contribution of PB to Tchl *a*, concentrations of B have to be corrected for the contributions to B by PA. In this case a discrete solution exists for f_B(PB) but not for f_A(PA) and f_{BA}(PA). To illustrate the mechanism, assume that the initial guess of f_A(PA) is an overestimate of the true value and that the other parameters are correct. In this case chl *a* associated with PA will be overestimated. This overestimation can be compensated for in 2 ways, either by choosing the correct value of f_A(PA) or by increasing f_{BA}(PA) such that chl *a* due to PB is decreased by exactly the amount by which chl *a* due to PA had been overestimated. In either case a perfect fit to the data is achieved, regardless of the number of samples as long as the samples are perfect representations of the underlying model. If both f_A(PA) and f_{BA}(PA) are allowed to vary, as is the case with regression programs, an infinite number of solutions can be generated, their values being driven by the initial conditions. As a consequence there are no constraints on the abundances of populations PA and PB, only on their sum. The problem manifests itself in a different form in real data. Real data never fit a perfect model, either due to analytical or sampling errors or due to violations of some assumptions, e.g. ratios of pigments in the different populations are not constant but variable. In this case discrete solutions exist for the problem but the solutions are driven by the random variability inherent in the data and not by the parameters we try to determine.

The above problem will arise whenever there exists one population that has one unique pigment and another pigment that is the primary taxon-specific, but not unique, pigment of another population. An example is zeaxanthin, the characteristic pigment of cyanobacteria, that is also found in chlorophytes (see below) in conjunction with lutein. The structure of models used for iterative regression analyses should be studied so that these trouble spots can be dealt with before regressions are performed. Results of regressions can be studied for the detection of such problems. A problem may exist whenever a parameter is not changed significantly from its initial value by the regression procedure. It is prudent in that case to study the sensitivity of the system to changes in these parameters and to test if changes in the initial value of the parameter will lead to a range of discrete solutions. The problems that arise in these cases are avoided by assuming an *a priori* value for factors like f_{BA}(PA), i.e. not using an iterative procedure to 'improve' values of these parameters.

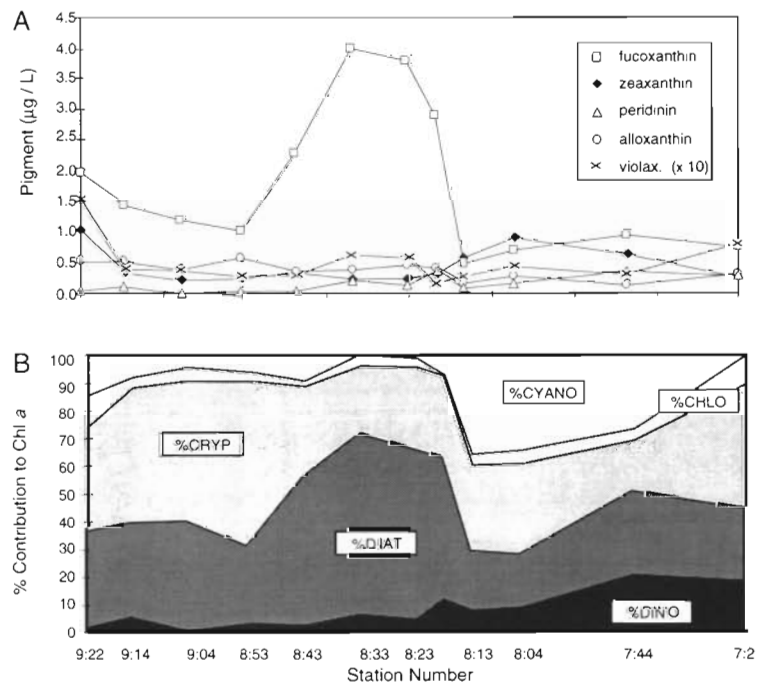


Fig. 9. (A) Concentrations of major photosynthetically active carotenoids and (B) the contribution of different taxa to chl *a* for a transect in the Chesapeake Bay, USA (27 to 29 September 1984). Data are plotted against station numbers which are based on the latitude of the station (e.g. Stn 8:33 is 8° 33' north of 30° N). Ancillary information on the system is given in Horrigan et al. (1990). CRYP: cryptophytes; DIAT: diatoms; CYANO: cyanobacteria; DINO: dinoflagellates; CHLO: chlorophytes

We first noticed the absence of discrete solutions in the case discussed above while analyzing a data set from the Chesapeake Bay, USA (Fig. 9A) that we will discuss in detail. We only considered the contributions of diatoms (fucoxanthin), dinoflagellates (peridinin), cryptophytes (alloxanthin), chlorophytes (violaxanthin, lutein/zeaxanthin which coelute on the system used), and cyanobacteria (zeaxanthin with a correction for the chlorophyte lutein/zeaxanthin contribution) to total chl *a*. Thus, we had to determine ratios of chl *a* to the above carotenoids for the individual groups and the ratio of lutein plus zeaxanthin to violaxanthin in green algae to correct total zeaxanthin for the contribution by green algae. A regression analysis returned realistic values for the ratios of chl *a* to peridinin (2.6 g g^{-1}), fucoxanthin (1.6 g g^{-1}), violaxanthin (20.8 g g^{-1}) and zeaxanthin in cyanobacteria (2.2 g g^{-1}). However, the calculated ratios of chl *a* and alloxanthin in cryptophytes and zeaxanthin and violaxanthin in green algae, 0.5 and −12, respectively, were unrealistic. Expected values are approximately 5 for both parameters. The consequence is a gross underestimation of the importance of cryptophytes in this system and an overestimation of the importance of cyanobacteria. Constraining the parameters to values larger than zero forced the value of the lutein plus zeaxanthin to violaxanthin ratio to zero. We studied the response of the system to varying values of the ratios chl *a*:violaxanthin and lutein plus zeaxanthin to violaxanthin in chlorophytes and chl *a*:zeaxanthin in cyanobacteria and found multiple solutions for different combinations of these parameters, an indication that the underlying model does not have discrete solutions for these parameters. A close inspection of the alloxanthin concentrations did not reveal any data points whose covariation with other pigments might have resulted in the suspect value of 0.5 for the cryptophyte chl *a*:alloxanthin ratio.

Instead of pure regression analysis that is prone to errors as discussed above we propose an alternative approach based primarily on values of pigment ratios derived from the literature or based on values determined from field samples under conditions that minimize the possibility of bias (see below). This approach is to some degree also practiced by others when parameter values are narrowly constrained or when certain parameters are fixed. It should be realized and be explicitly stated that the results of such an approach are no longer the results of an objective statistical analysis.

We will illustrate this approach using the Chesapeake Bay data. We started out using pigment ratios taken from our database or the literature (Tables 2 & 3; Hager & Stransky 1970, Kana et al. 1988, Moore et al. 1995: chl *a*:fucoxanthin = 3.5, chl *a*:alloxanthin = 4.4, chl *a*:peridinin = 2.0, chl *a*:violaxanthin = 5.0, chl *a*:zeaxanthin = 2.0 in cyanobacteria) to calculate the contribution of the different taxa to chl *a*. Zeaxanthin concentrations had to be first corrected for contributions by chlorophytes. To achieve this we used a value for 5.0 for the lutein plus zeaxanthin to violaxanthin ratio in chlorophytes (Hager & Stransky 1970). The set of values derived from the literature overestimated true chl *a* everywhere, but particularly at the location of the fucoxanthin maximum (Fig. 9A), a clear indication that the chl *a*:fucoxanthin ratio was too high. We applied a regression analysis to those data points and derived a value of 1.2 g g^{-1} for the chl *a*:fucoxanthin ratio. This is a robust estimation of the pigment ratio because diatoms dominated the community at these stations. An even better method would have been to use size-fractioned samples enriched with diatoms; however, such samples were not available. After this iteration, residuals (predicted minus true chl *a*) were acceptably low everywhere. However, predicted cyanobacterial biomass was at some stations negative, an indication that the assumed lutein plus zeaxanthin to violaxanthin ratio in chlorophytes was slightly too high at those stations. We changed the value of the ratio (from 5.0 to 3.6 g g^{-1}) such that all stations had positive or zero cyanobacterial biomass. As a consequence, squared residuals went up but our data set was now internally consistent. Residuals now ranged from −48 to 11% of the true chl *a*. On average, absolute residuals were 14% of true chl *a*. The resulting plot of the % contribution of the different taxa to chl *a* is shown in Fig. 9B. There is still no guarantee that our results are correct; for example, the chl *a*:fucoxanthin ratio may have been different between the fucoxanthin maximum and other areas of the bay, but the approach forced us to consider values of all parameters explicitly rather than blindly accepting the output of an automated regression analysis.

Conclusions

We observed strong covariations between photosynthetically active carotenoids and chl *a* in microalgae growing exponentially in batch cultures and in nutrient-limited chemostats. Ratios of chl *a* and photosynthetically active carotenoids varied only by up to a factor of 2 under these conditions, which is small compared to the observed variations of cellular pigment concentrations. Our most surprising result was that rates of change of pigment concentrations as a function of growth rate or irradiance were very similar for many microalgal species. These results suggest that it is as difficult to calculate the carbon biomass of different taxa in the field from concentrations of taxon-specific carotenoids as it is to calculate phytoplankton carbon

biomass from concentrations of chl *a*. Consequently, changes in the contribution of any group to chl *a* are a more robust data product than the absolute values of these contributions. Calculating the contribution of different algal taxa to chl *a* from chl *a*:carotenoid ratios (cf. Everitt et al. 1990, Letelier et al. 1993) is a more conservative approach. Regression analyses currently used to determine chl *a*:carotenoid ratios were shown to be susceptible to error under certain conditions. These errors primarily affect pigment ratios of populations that do not dominate the community. Consequently, these errors are acceptable if the objective is to explain variations of chl *a* in terms of accessory pigment variations. If, however, the objective is to determine variations of the abundance of different phytoplankton populations and study factors that control their abundance, such errors are not acceptable. Instead of regression analyses we proposed to rely more heavily on ratios directly derived from the literature and illustrated this approach using a data set from the Chesapeake Bay. In spite of these limitations pigment-based measurements of the contribution of different taxa to phytoplankton biomass in the field are a valuable tool for phytoplankton ecologists, as we illustrated using an example from the Chesapeake Bay where we were able to document dramatic variations of the biomass of dinoflagellates and cyanobacteria in this system. Future work has to be aimed at quantifying the uncertainties associated with this method, attempting to derive independent estimates of floristic composition from cell counts (cf. Anderson et al. 1996), and incorporating the effects of photoadaptation into models used to calculate the contribution of different taxa to total chl *a*.

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