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Source: *The New Phytologist*, May, 1987, Vol. 106, No. 1 (May, 1987), pp. 1-34

Published by: Wiley on behalf of the New Phytologist Trust

Stable URL: https://www.jstor.org/stable/2434683

LIGHT AND TEMPERATURE DEPENDENCE OF THE CARBON TO CHLOROPHYLL *a* RATIO IN MICROALGAE AND CYANOBACTERIA: IMPLICATIONS FOR PHYSIOLOGY AND GROWTH OF PHYTOPLANKTON

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(*Accepted 8 January 1987*)

CONTENTS

SUMMARY

The carbon:chlorophyll *a* ratio (C:chl *a* or θ) is a sensitive indicator of physiological state in microalgae. The dependence of θ on photon flux density (PFD or I) and temperature in exponentially growing nutrient-sufficient microalgae can be described by an empirical equation with four coefficients. C:chl *a* increases linearly with increased light level at constant temperature and decreases exponentially with increased temperature at constant light level. Both the slope (ϵ) and intercept (θ_0) of linear regressions of θ on photon flux density increase at low temperature. The intercept, θ_0, increases from 6 to 40 g of carbon per gram of chlorophyll *a* (g C g chl a^{-1}) between 30 and 0 °C and ϵ increases by over an order of magnitude from 0·04 to 1·9 g C g chl a^{-1} m^2 s μmol photon^{-1} over the same temperature range.

Low-temperature chlorosis can be interpreted as an adaptive response in the allocation of cell resources between temperature-independent biophysical reactions involved in light-harvesting and temperature-dependent biochemical reactions. This response also reduces the potential for photoinhibitory damage at high light levels which can be exacerbated by low temperatures.

The range of values for θ in nature has not been adequately determined because of difficulty in separating phytoplankton from detritus, bacteria and microzooplankton. Based on the

0028-646X/87/050001+34 $03.00/0

laboratory observations summarized in this paper, it would appear that use of a single value of θ for phytoplankton is inappropriate for ecological studies. For example, at a PFD of 50 μmol m^{-2} s^{-1}, θ increases from 10 to 130 g C g chl a^{-1} between 30 and 0 °C under nutrient-sufficient conditions.

These conclusions are based on observations for eight diatoms, two green algae, one euglenoid and two cyanobacteria for which the appropriate data are available. In contrast to these groups, the dinoflagellates contain substantially less chlorophyll *a*. The available data indicate that θ is about three times larger in dinoflagellates than in other algae under comparable PFDs at 20 °C. There are insufficient data available, however, to evaluate the light and temperature dependence of θ in dinoflagellates.

Key words: Chlorophyll, phytoplankton, photon flux density, temperature.

I. INTRODUCTION

The carbon:chlorophyll *a* ratio (θ) is a key, yet poorly studied, factor in phytoplankton growth and ecology. Photosynthesis rates are commonly expressed on a unit chlorophyll *a* basis (Platt, Denman & Jassby, 1977) because chlorophyll *a* is the most readily and unambiguously measured indicator of phytoplankton abundance (Cullen, 1982). The chl *a*-specific, light-saturated photosynthesis rate (P_m^{chl}) has been used to infer the physiological state (*sensu* Malek, 1976) of phytoplankton in nature (Thomas, 1970; Thomas & Dodson, 1972; Laws & Bannister, 1980), but P_m^{chl} cannot provide a direct estimate of phytoplankton growth rates without knowledge of θ. A value for θ is also needed in order to relate the dynamics of chlorophyll *a* and its degradation products (Welschmeyer & Lorenzen, 1985) to carbon cycling and to estimate phytoplankton biomass from satellite observations of chlorophyll *a*. It has often been assumed that θ is constant in ecological studies on phytoplankton. Strickland (1960) for example recommends $\theta = 30$ g C g chl a^{-1} for nutrient-rich waters and $\theta = 60$ g C g chl a^{-1} for nutrient-impoverished waters.

One of the most striking features of algal physiology is the marked phenotypic variation in chemical composition and rates of physiological processes. Variations in θ are indicative of the extreme range of phenotypic (physiological) plasticity of microalgae. In diatoms, for example, θ can vary from less than 10 to over 200 g C g chl a^{-1} depending on preconditioning light level, temperature or nutrient availability (Yoder, 1979; Laws & Bannister, 1980; Verity, 1982; Terry, Hirata & Laws, 1983, 1985; Geider, 1984; Geider, Osborne & Raven, 1985, 1986b; Osborne & Geider, 1986).

The C : chl *a* ratio is an important variable in recent models of microalgal growth (Kiefer & Mitchell, 1983; Geider, Platt & Raven, 1986a), but its fundamental role is also implicit in earlier physiological models (Steele, 1962; Eppley & Sloan, 1966; Bannister, 1979; Shuter, 1979). The importance of θ arises, in part, because cell carbon is a measure of cell energy content (Platt & Irwin, 1973) and cell chlorophyll *a* limits the energy supply rate through the chlorophyll *a*-specific light absorption coefficient (Shuter, 1979; Kiefer & Mitchell, 1983).

This paper provides an empirical account of the variability of θ as a function of temperature and light level in nutrient-sufficient cultures of microalgae and cyanobacteria. It is shown that a single function of temperature and photon flux density with four coefficients can account for much of the systematic variation of θ in nutrient-sufficient cultures. The discussion considers some of the ecophysiological implications of this relationship. Differences between θ in diatoms and

dinoflagellates, the two major groups of marine, autotrophic nanoplankton (Sieburth, 1979), are also considered. However, the temperature dependence of algal growth rate (Eppley, 1972) is not examined except insofar as it pertains to θ.

II. The Available Data

The analysis presented in this paper is based on data obtained from a review of published observations of θ and growth rate (μ) as a function of light level and temperature in nutrient-sufficient cultures of marine and freshwater microalgae. Table 1 summarizes the organisms under consideration and relevant information on culture conditions. Most studies were conducted with cells grown in batch cultures, although chemostat or turbidostat cultures were occasionally employed.

Table 1. *Summary of organisms used, environmental conditions and sources of information for the analysis of the dependence of the carbon : chlorophyll* a *ratio on photon flux density and temperature*

Species	T	I	L:D	n
Skeletonema costatum (1)	0	4–46	12:12	4
	5	2–92	12:12	7
	10	5–80	12:12	5
	16	5–94	12:12	4
	22	19–119	12:12	4
S. costatum (2)	20	15–650	12:12	4
Leptocylindrus danicus (3)	5	6–72	9:15, 12:12, 15:9	12
	10	6–79	9:15, 12:12, 15:9	12
	15	7–132	9:15, 12:12, 15:2	13
	20	6–127	9:15, 12:12, 15:9	13
Thalassiosira weisflogii (4)	18	30–600	24:0	5
T. weisflogii (5)	20	2–105	12:12	6
Thalassiosira pseudonana (6)	18	14–512	24:0	12
Phaeodactylum tricornutum (7)	23	1–230	24:0	11
P. tricornutum (8)	25	12–230	12:12	11
	25	52–277	12:12	6
Fragillaria crotonensis (9)	20	13–154	24:0	10
Scenedesmus sp. (9)	20	15–82	24:0	6
Nannochloris atomus (10)	23	1–200	24:0	6
Euglena gracilis (11)	25	9–483	24:0	6
Chlorella pyrenoidosa (12)	26	11–80	24:0	4
Dunaliella tertiolecta (13)	34	n.s.	24:0	8
Oscillatoria redekii (14)	15	15–250	24:0	7
Microcystis aeruginosa (15)	29	20–565	24:0	6

Temperature (T) is in °C; photon flux density (I) has units of μmol photon m^{-2} s^{-1}; L:D gives the durations of the light and dark periods of a light:dark cycle both expressed in hours; n, is the number of observations; n.s., not specified. Sources for information included in this table are: (1) Yoder (1979); (2) Cosper (1982); (3) Verity (1982); (4) Falkowski *et al.* (1985); (5) Laws & Bannister (1980); (6) Geider (1984); (7) Terry *et al.* (1983); (8) Geider *et al.* (1985, 1986b); (9) Rhee & Gotham (1981); (10) Geider & Osborne (1986); (11) Cook (1963); (12) Myers & Graham (1971); (13) Eppley & Dyer (1965); (14) Foy & Gibson (1982); (15) Raps *et al.* (1983).

In all cases, the cells were allowed to acclimate to the experimental conditions for a sufficient length of time so that balanced growth (*sensu* Shuter, 1979) could be assumed.

A variety of techniques were employed for determination of chlorophyll *a* and carbon concentrations in the different investigations and no attempt will be made to discriminate among the methods used in treating the data. Where carbon was not directly measured, θ was estimated from the contribution of chlorophyll *a* to dry weight by assuming that carbon was 50 % of ash-free dry weight (Strickland, 1960; Myers, 1980; Pirt, 1986). A variety of light sources were employed and measurements of light levels were not always reported in the same units. The following conversions were applied to obtain all results in units of μmol photons $m^{-2}\ s^{-1}$: 1 μmol $m^{-2}\ s^{-1} = 0{\cdot}2$ W $m^{-2} = 0{\cdot}41$ ly d^{-1} for photosynthetically active radiation (400 to 700 nm wavelength band). The observations of Cook (1963) for *Euglena gracilis* were reported in units of foot-candles. Cook's (1963) observations of light 'intensity' were converted to photon flux density by using the conversions 1 foot-candle = 10·76 lux, 1 lux = $8{\cdot}6 \times 10^{-5}$ ly min^{-1} and 1 ly min^{-1} = 3200 μmol $m^{-2}\ s^{-1}$ (after Parsons, Takahashi & Hargrave, 1977) which were assumed to be appropriate for the high-intensity incandescent light source used by Cook (1963). For cells cultured under a light : dark (L : D) cycle the mean light level for 24 h (i.e. light dose) was used in all calculations. The time of day at which samples were collected was not taken into consideration for studies employing L : D cycles.

Linear regression analysis was performed using Minitab (Ryan, Joiner & Ryan, 1976). The BMDP statistical package (Dixon, 1983) was used for non-linear least squares curve fitting.

Table 2 defines the symbols used in this paper.

III. DISCUSSION

1. *Light dependence of growth rate and the carbon : chlorophyll* a *ratio*

At constant temperature, θ increases linearly [Eqn (1)] with light level in all nutrient-sufficient, uni-algal cultures examined (Table 1) as illustrated for *Thalassiosira pseudonana* in Figure 1:

$$\theta = \theta_0 + \epsilon I, \tag{1}$$

where θ is the C : chl *a* ratio (g C g chl a^{-1}), θ_0 is the value of θ at $I = 0$, and I is the photon flux density (μmol photons $m^{-2}\ s^{-1}$). The empirical regression coefficient ϵ has units g C g chl a^{-1} m^2 s μmol $photon^{-1}$. The intercept, θ_0, varies from about 6 to 40 g C g chl a^{-1} and the slope, ϵ, varies by over an order of magnitude from 0·04 to 1·9 g C g chl a^{-1} m^2 s μmol $photon^{-1}$ (Table 3). An exceptional report of increased θ in *Skeletonema costatum* and *Dunaliella tertiolecta* at low light levels (Falkowski & Owens, 1980) has been excluded from this analysis. Also excluded is a report by Cosper (1982) of a decrease in θ between $I = 650$ and $I = 1500$ μmol $m^{-1}\ s^{-1}$ in *S. costatum* which may be related to the onset of photoinhibition of growth at these high light levels.

Growth rate (μ) is a saturating function of PFD in nutrient-sufficient cultures at constant temperature. The μ–I curve has been described by several functions including the rectangular hyperbola (Quraishi & Spencer, 1971), a modified rectangular hyperbola (Bannister, 1979) and a hyperbolic tangent function (Yoder,

Table 2. *List of commonly used symbols*

Symbol	Description	Typical units
a_c	Absorption cross-section of the light-harvesting catalysts	m^2 mg C^{-1}
a_{chl}	Absorption cross-section normalized to chlorophyll *a*	m^2 mg chl a^{-1}
c_T	Temperature coefficient	Dimensionless
q_l	Proportion of carbon devoted to light reactions of cell metabolism	Dimensionless
q_d	Proportion of carbon devoted to dark reactions of cell metabolism	Dimensionless
I	Photon flux density	μmol m^{-2} s^{-1}
I_0	Compensation I for growth	μmol m^{-2} s^{-1}
I_k	Light saturation parameter	μmol m^{-2} s^{-1}
P_m	Light-saturated rate of photosynthesis	d^{-1}
T	Temperature	°K or °C
Z	Dimensional constant	0·012 mg C μmol C^{-1}
Δ	Initial slope of the light curve for growth	m^2 μmol photon^{-1}
ϵ	Slope of linear regression of θ on I	(g C m^2 s) (g chl *a* μmol photon)$^{-1}$
ϕ	Photon efficiency of photosynthesis	mol C mol photon^{-1}
Φ	Photon efficiency of growth	mol C mol photon^{-1}
η	Parameter governing abruptness of the relation between μ and θ	g chl *a* g C^{-1}
μ	Growth rate	d^{-1} or s^{-1}
μ_0	Maintenance metabolic rate	d^{-1} or s^{-1}
μ_m	Maximum growth rate	d^{-1} or s^{-1}
ν	Energy efficiency of biosynthetic reactions	Dimensionless
θ	Carbon : chlorophyll *a* ratio	g C g chl a^{-1}
θ_0	θ at $I = 0$	g C g chl a^{-1}
ρ	Cell-specific reaction rate	g $cell^{-1}$ s^{-1}
$\rho_d{}^r$	Specific reaction rate of 'dark' reactions of cell metabolism	d^{-1} or s^{-1}

1979; Verity, 1982). Three parameters are usually required to describe the μ–I curve: these are the light-saturated growth rate, the hypothetical negative growth rate in darkness and a parameter that characterizes the light level at which growth rate becomes light-saturated. A three-parameter exponential function [Eqn (2)] will be used in this analysis:

$$\mu = \mu_m[1 - \exp(-I/I_k)] - \mu_0, \tag{2}$$

where μ is the growth rate (d^{-1}), μ_m is the light-saturated maximum growth rate, I is the photon flux density (μmol m^{-2} s^{-1}), I_k characterizes the high level at which growth becomes light-saturated (μmol m^{-1} s^{-1}), and μ_0 is the intercept of the μ–I curve at $I = 0$. The parameters of Eqn (2) can be used to obtain the initial slope of the μ–I curve ($\Delta = \mu_m/I_k$), which characterizes the dependence of μ on I under light-limiting conditions and the compensation light level for growth ($I_0 = \mu_0\Delta$).

Table 3. *Results of linear regression for the dependence of θ on* I *Eqn (1)*

Species	Temperature	θ_0 $\bar{x}$ (SD)	ϵ $\bar{x}$ (SD)
Skeletonema costatum	0	32·2 (12·1)	1·88 (0·471)
	5	42·4 (4·8)	0·513 (0·104)
	10	28·6 (4·6)	0·511 (0·103)
	16	27·5 (7·0)	0·246 (0·117)
	22	30·2 (4·7)	0·133 (0·066)
S. costatum	20	27·7 (2·1)	0·066 (0·003)
Leptocylindricus danicus	5	33·5 (4·0)	0·960 (0·109)
	10	34·6 (5·8)	0·774 (0·144)
	15	28·8 (3·2)	0·234 (0·046)
	20	28·0 (2·3)	0·155 (0·035)
Thalassiosira weisflogii	18	18·1 (1·6)	0·079 (0·005)
T. weisflogii	20	18·3 (1·2)	0·214 (0·026)
Thalassiosira pseudonana	18	19·7 (2·7)	0·0755 (0·001)
Phaeodactylum tricornutum	23	13·8 (0·85)	0·119 (0·008)
P. tricornutum	25	12·6 (1·8)	0·130 (0·019)
	25	8·6 (2·7)	0·140 (0·017)
Fragillaria crotonensis	20	17·7 (2·1)	0·102 (0·026)
Scenedesmus sp.	20	23·0 (2·7)	0·179 (0·083)
Nannochloris atomus	23	12·8 (1·9)	0·137 (0·016)
Euglena gracilis	25	10·9 (4·5)	0·074 (0·014)
Chlorella pyrenoidosa	26	9·0 (0·2)	0·078 (0·005)
Dunaliella tertiolecta	34	n.d.	n.d.
Oscillatora redekii	15	44·6 (8·0)	0·526 (0·061)
Microcystis aeruginosa	29	6·4 (0·58)	0·0388 (0·002)

Units for θ_0 are g C g chl a^{-1}; units for ϵ are g C g chl a^{-1} m^2 s μmol photon^{-1}; n.d., not determined because there were no data available for photon flux density; $\bar{x}$, estimated value; SD (in parentheses), standard deviation of the estimate.

The intercept at $I = 0$, i.e. μ_0, can be interpreted as the maintenance metabolic rate (Geider *et al.*, 1985) and I_k can be used in comparisons of genotypic sun–shade adaptation (Richardson, Beardall & Raven, 1983). If the carbon : chlorophyll *a* ratio did not depend on photon (flux density (I), then Δ would equal the initial slope of the PI curve. Specifically, it would characterize the efficiencies of light absorption and photosynthetic energy conversion where the end product is new cells. Because θ varies with I [Eqn (1)], such a simple proportionality does not apply.

Light-limited growth rates often extrapolate to $\mu = 0$ at $I = 0$ (Quraishi & Spencer, 1971; Beardall & Morris, 1976; Rhee & Gotham, 1981; Thomas & Carr, 1985; Geider *et al.*, 1985; Geider & Osborne, 1986) implying that $I_0 = 0$ and $\mu_0 = 0$. For many microalgae the maintenance metabolic costs appear to be very small and I_0 indistinguishable from zero! Pirt (1986) came to the same conclusion for two *Chlorella* strains grown in light-limited chemostat cultures. Two of the studies listed in Table 1 lead to estimates of $\mu_0 < 0$ when the observations are fitted to Eqn (3) (i.e. *T. pseudonana* at 18 °C and *Scenedesmus* sp. at 20 °C). In the majority of cases (19 out of 21) the three-parameter equation led to estimates of μ_0 which were less than one standard error removed from the origin. The

exceptional cases may be due to experimental errors in measured values of μ at low light levels or they may represent real genotypic differences. The observations of Schlesinger & Shuter (1981) using continuous cultures, for example, indicate variability of I_0 and μ_0 among four species of freshwater green algae.

For the remainder of this discussion it is assumed that $\mu_0 = 0$ and that a two-parameter function adequately describes the dependence of μ on I:

$$\mu = \mu_m[1 - \exp(-I/I_k)]. \tag{3}$$

This choice is not based on a comparison of alternative models using statistical criteria such as has been made for the photosynthesis–light curve (Jassby & Platt, 1976; Lederman & Tett, 1981). Often, the limited number of observations, especially in the light-limited initial slope region, precludes a rigorous comparison for the various two- and three-parameter models. Eqn (3) has been chosen, in part, because of ease of manipulation, but the general conclusions which follow do not depend on this particular description. The μ–I curve for *T. pseudonana* is illustrated in Figure 2 where the solid line represents the best fit of Eqn (3).

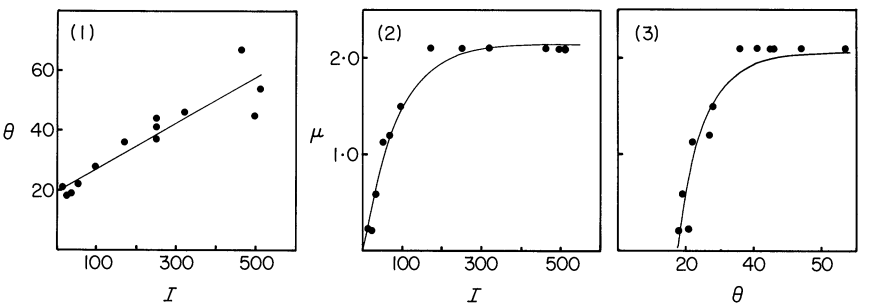

Fig. 1. Light dependence of θ for the diatom *Thalassiosira pseudonana* grown under continuous illumination at 18 °C (observations for Figs 1, 2 and 3 are from Geider, 1984). The line is the best fit of the data to Eqn (1) (see Table 3 for regression coefficients). Units for I are μmol m^{-2} s^{-1} and units for θ are g C g chl a^{-1}.

Fig. 2. Light dependence (I) of growth rate (μ) for *Thalassiosira pseudonana*. The line indicates best fit of the data to Eqn (3) (see Table 4 for regression coefficients). Units for I are μmol m^{-2} s^{-1} and units for μ are d^{-1}.

Fig. 3. Relationship between growth rate (μ) and carbon:chlorophyll *a* (θ) for *Thalassiosira pseudonana*. The line indicates the best fit to Eqn (4) (see Table 5 for regression coefficients). Units for μ are d^{-1} and units for θ are g C g chl a^{-1}.

Solving Eqn (1) for I and substituting into Eqn (3) leads to the following description of the relationship between μ and θ:

$$\mu = \mu_m\{1 - \exp[-\eta(\theta - \theta_0)]\}, \tag{4}$$

where

$$\eta = 1/(I_k \epsilon). \tag{5}$$

The parameter η has units of g chl *a* g C^{-1}. The relationship between μ and θ for *T. pseudonana* is illustrated in Figure 3 where the solid line represents the best fit to Eqn (4).

Eqns (1), (3) and (4) describe the interdependence of three variables (i.e. the

independent variable I and the dependent variables θ and μ) in terms of five parameters (θ_0 ϵ, μ_m, I_k and η). Only four of the parameters, however, are independent. This is a mathematical statement of the interdependence of the rates of light absorption and growth in microalgae. It is clear (Figs 2 and 3) that μ can be described as a saturating function of either I or θ [Eqns (3) and (4)] as is expected given the linear relationship between θ and I [Fig. 1, Eqn (1)].

Linear and non-linear regression analyses were used where appropriate to obtain estimates of the parameters of Eqns (1), (3) and (4) for all of the data sets listed in Table 1. These estimates are summarized in Tables 3, 4 and 5. Values are missing from the tables where the original observations were incomplete or where convergence was not attained [five out of 22 cases for Eqn (4)]. Even when convergence was attained, the errors associated with estimated parameter values for Eqn (4) were often high. This was especially so for η in which the standard deviation exceeded the estimate in six out of 15 cases.

Not surprisingly, Eqns (2) and (3) lead to similar estimates of μ_m and Eqns (1) and (3) lead to similar estimates of θ_0 when applied to the same data set (Figs 4 and 5). Eqn (4) occasionally leads to unreasonably high estimates of μ_m, but large errors are associated with these estimates. At low temperatures, Eqn (1) leads to larger values of θ_0 than Eqn (4), but again the errors associated with these estimates are often high. The three parameters I_k, ϵ and η are related through Eqn (5). The

Table 4. *Results of non-linear regression analysis to determine the parameters of the light curve of growth [Eqn (3)]*

Species	Temperature	μ_m $\bar{x}$ (SD)	I_k $\bar{x}$ (SD)
Skeletonema costatum	0	0·48 (0·039)	6·7 (1·8)
	5	0·95 (0·053)	12·3 (1·9)
	10	1·72 (0·15)	30·0 (6·4)
	16	2·88 (0·32)	35·3 (11)
	22	3·21 (0·085)	27·2 (2·2)
S. costatum	20	2·08 (0·059)	205 (15)
Leptocylindricus danicus	5	0·31 (0·019)	16·8 (2·9)
	10	1·11 (0·063)	30·1 (3·6)
	15	2·46 (0·14)	28·2 (4·7)
	20	2·59 (0·11)	27·2 (3·8)
Thalassiosira weisflogii	18	1·91 (0·089)	164 (20)
T. weisfloggi	20	1·15 (0·046)	22·8 (2·3)
Thalassiosira pseudonana	18	2·17 (0·073)	85·5 (9·2)
Phaeodactylum tricornutum	23	1·47 (0·030)	52·1 (3·3)
P. tricornutum	25	1·00 (0·031)	27·4 (3·2)
Fragillaria crotonensis	20	0·99 (0·11)	44·1 (11)
Scenedesmus sp.	20	1·59 (0·30)	31·8 (12)
Nannochloris atomus	23	1·29 (0·046)	49·5 (6·4)
Euglena gracilis	25	1·53 (0·044)	32·5 (3·1)
Chlorella pyrenoidosa	26	2·39 (0·038)	60·2 (2·5)
Dunaliella tertiolecta	34	n.d.	n.d.
Oscillatoria redekii	15	n.d.	n.d.
Microcystis aeruginosa	29	1·20 (0·084)	59·9 (13·6)

Units for μ_m are d^{-1}; units for I_k are μmol photon m^2 s^{-1}; n.d., not determined because data were not available for photon flux density; $\bar{x}$, estimated value; SD, standard deviation of the estimate.

Table 5. *Results of the non-linear regression analysis for determination of the parameters of Eqn (4)*

Species	Temperature	μ_m $\bar{x}$ (SD)	η $\bar{x}$ (SD)	θ_0 $\bar{x}$ (SD)
Skeletonema costatum	0	n.c.	n.c.	n.c.
	5	0·85 (0·062)	0·76 (0·46)	40·7 (0·38)
	10	1·85 (0·95)	0·043 (0·051)	25·1 (4·0)
	16	2·78 (0·93)	0·19 (0·252)	28·8 (2·9)
	22	n.c.	n.c.	n.c.
S. costatum	20	n.c.	n.c.	n.c.
Leptocylindricus danicus	5	0·33 (0·095)	0·032 (0·030)	20·7 (11)
	10	1·30 (0·82)	0·018 (0·024)	18·2 (15)
	15	4·19 (3·5)	0·022 (0·033)	15·4 (8·0)
	20	5·29 (10·8)	0·022 (0·068)	17·4 (11)
Thalassiosira weisflogii	18	1·84 (0·020)	0·093 (0·041)	18·5 (0·2)
T. weisfloggi	20	1·58 (1·86)	0·51 (0·12)	15·0 (5·5)
Thalassiosira pseudonana	18	2·06 (0·16)	0·131 (0·058)	17·4 (1·2)
Phaeodactylum tricornutum	23	1·56 (0·14)	0·098 (0·025)	12·9 (0·4)
P. tricornutum	25	0·94 (0·046)	0·31 (0·12)	9·8 (0·9)
Fragillaria crotonensis	20	n.c.	n.c.	n.c.
Scenedesmus sp.	20	n.c.	n.c.	n.c.
Nannochloris atomus	23	1·44 (0·37)	0·071 (0·005)	7·3 (2·7)
Euglena gracilis	25	1·53 (0·060)	0·31 (0·079)	7·1 (0·5)
Chlorella pyrenoidosa	26	2·37 (0·052)	0·22 (0·020)	9·0 (0·1)
Dunaliella tertiolecta	34	2·20 (0·21)	0·46 (0·29)	6·9 (0·9)
Oscillatoria redekii	15	n.d.	n.d.	n.d.
Microcystis aeruginosa	29	1·16 (0·10)	0·67 (0·37)	6·8 (0·4)

Units for μ_m are d^{-1}; units for η are g chl *a* g C^{-1}; units for θ_0 are g C g chl a^{-1}; n.d., not determined because data for μ were not available; n.c., the curve fitting was unsuccessful due to lack of convergence; $\bar{x}$, estimated value; SD, standard deviation of the estimate.

parameter η is plotted against $1/(I_k \epsilon)$ in Figure 6. The two parameters that are more precisely estimated were combined in the product for comparison with the less precise estimates of η. The independently estimated values of η and ϵ/I_k are correlated, although agreement is poor.

As noted previously only two of the parameters I_k, ϵ and η are independent. If it can be shown that there is limited interspecific variability in one of these parameters, then the variability in the other two will also be constrained. Goldman (1980) showed that both C : N and C : chl *a* scale with relative growth rate ($\mu' = \mu/\mu_m$) in *nutrient-limited* cultures, indicating that phenotypic variability has more effect on chemical composition than interspecific differences. Does the same conclusion hold for *nutrient-sufficient* but *light-limited* phytoplankton?

Figure 7 illustrates the relationship between θ and growth rate in *D. tertiolecta* (34 °C), *Microcystis aeruginosa* (29 °C), *Chlorella pyrenoidosa* (26 °C) and *E. gracilis* (25 °C). These results are replotted in Figure 8 after dividing the growth rates by species-specific maximum rates to obtain relative growth rates ($\mu' = \mu/\mu_m$). In all cases θ shows little phenotypic variation at $\mu' < 0{\cdot}5$, with a marked increase in θ as μ' approaches unity. Thus, the interspecific variability in the relationship

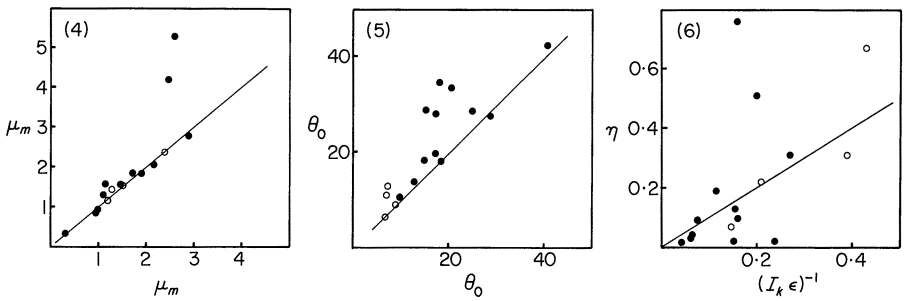

Fig. 4. Comparison of the values for μ_m estimated by Eqns (3) (x axis) and (4) (y axis). The line indicates equality. Solid symbols are for diatoms while open symbols are for all other groups. Errors of the estimated parameter values are given in Tables 3, 4 and 5. Units for both axes are d^{-1}.

Fig. 5. Comparison of values for θ_0 estimated by Eqns (1) (y axis) and (4) (x axis). The line indicates equality. Solid symbols are for diatoms, while open symbols are for all other groups. Errors of the estimated parameter values are given in Tables 3, 4 and 5. Units for both axes are g C g chl a^{-1}.

Fig. 6. Comparison of values for η and $1/I_k\epsilon$. The line indicates equality as is required by the identity in Eqn (5). Solid symbols are for diatoms while open symbols are for all other groups. Errors of the estimated parameter values can be calculated from Tables 3, 4 and 5. Units for both axes are g chl a g C^{-1}.

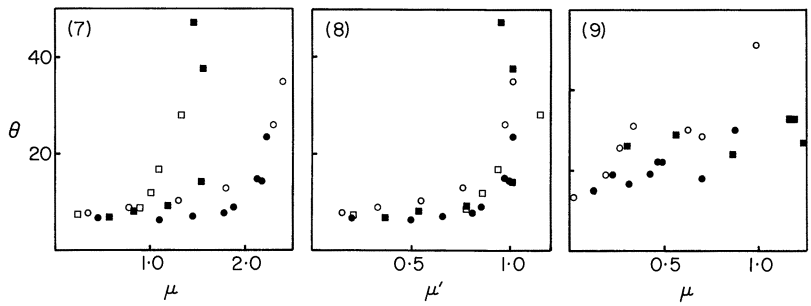

Fig. 7. Dependence of carbon:chlorophyll a (θ) on growth rate (μ) in *Dunaliella tertiolecta* at 34 °C (closed circles), *Microcystis aeruginosa* at 29 °C (open squares), *Euglena gracilis* at 25 °C (closed squares) and *Chlorella pyrenoidosa* at 26 °C (open circles). Sources for the observations illustrated here can be found in Table 1. Units for θ are g C g chl a^{-1} and units for μ are d^{-1}.

Fig. 8. Dependence of carbon:chlorophyll a (θ) on relative growth rate (μ') for the observations illustrated in Figure 7. Relative growth rate (μ') was obtained by dividing the observed growth rate (μ) by the species-specific maximum rate μ_m obtained from Eqn (3) (*Microcystis aeruginosa*, *Euglena gracilis*, *Chloretta pyrenoidosa*) or Eqn (4) (*Dunaliella tertiolecta*). Symbols are the same as in Figure 7. Units for μ are g C g chl a^{-1} and μ' is dimensionless.

Fig. 9. Relationship between carbon:chlorophyll a (θ) and growth rate (μ) in *Scenedesmus* species. Observations are for *Scenedesmus protuberans* at 20 °C (open circles), or 28 °C (closed circles) [1977 observations of Gons as reported in Shuter (1979)], and for *Scenedesmus* sp. at 20 °C (closed squares) (observations of Rhee & Gotham, 1981). Units for μ are d^{-1} and units for θ are g C g chl a^{-1}.

between θ and μ for these organisms grown at 25 to 35 °C can be largely accounted for in terms of changes in μ_m.

The observations for two *Scenedesmus* species (Fig. 9) at 20 and 28 °C do not appear to conform to the same generalization. The almost linear relationship of θ

and growth rate in *S. protuberans* may indicate that some factor other than light level is limiting at intermediate to high growth rates. In contrast, θ appears to be independent of μ' in the *Scenedesmus* sp. studied by Rhee & Gotham (1981).

2. *Temperature dependence of carbon : chlorophyll* a

The results summarized in Tables 3 and 5 indicate a general reduction in θ_0 at high temperatures. Studies conducted at temperatures between 15 and 30 °C indicate an almost linear decline of θ_0 with increasing temperature (Fig. 10), although the observations for *L. danicus* and *S. costatum* between 0 and 22 °C indicate only a slight change in θ_0 [estimated from Eqn (1)].

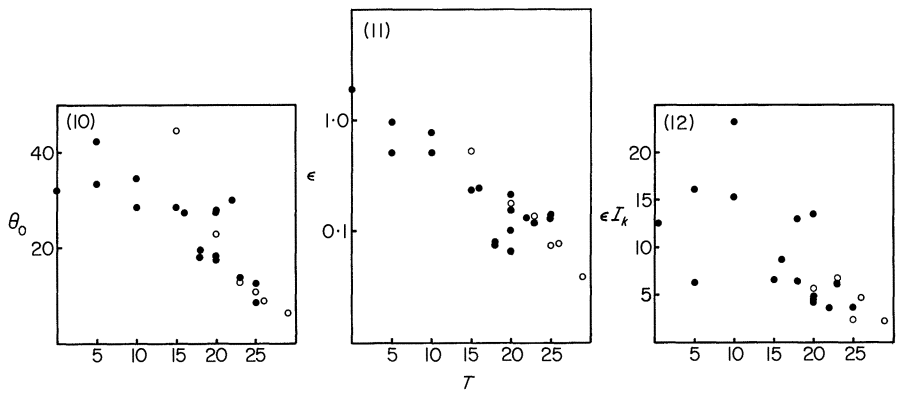

Fig. 10. Temperature (T in °C) dependence of θ_0. Solid symbols are for diatoms while open symbols are for all other groups. Values for θ_0 were obtained from Eqn (1) and errors on the estimated values are given in Table 3.

Fig. 11. Temperature dependence of ϵ. Solid symbols are for diatoms while open symbols are for all other groups. Values for ϵ were obtained using Eqn (1) and errors on the estimated values are given in Table 3.

Fig. 12. Temperature dependence of $\epsilon' = \epsilon I_k$. Solid symbols are for diatoms and open symbols are for other groups.

The slope (ϵ) of the regression of θ on I varied by over an order of magnitude. It was greatest at low temperatures, decreasing exponentially with increasing temperature (Fig. 11) as summarized by Eqn (6):

$$\epsilon = 1{\cdot}85 \exp(-0{\cdot}126\, T). \tag{6}$$

Thus, much of the variability in ϵ can be accounted for by its dependence on temperature. Can the relationship be improved if account is taken of interspecific variability in light requirements for growth?

As noted previously, the C:chl *a* ratio appears to vary with μ' in light-limited phytoplankton (Fig. 8). Given the interrelationship of θ and μ [Eqn (4)], it is expected that interspecific variability in θ at a given temperature will be reduced if θ is described as a function of non-dimensional light intensity ($I' = I/I_k$). In other words, the slope of a regression of θ on I' can be expected to account, in part, for interspecific variability in chemical composition. We obtain a new parameter $\epsilon' = \epsilon I_k$ with units g C g chl a^{-1} (note that $\epsilon' = 1/\eta$). Figure 12 illustrates that ϵ', like ϵ, decreases with increasing temperature. The range of ϵ' is

considerably less than that of ϵ, consistent with the observation that I_k increases as temperature is raised (Yoder, 1979; Verity, 1982), whereas ϵ decreases (Fig. 11). The parameter ϵ' varies by a factor of about four, but errors in estimated values are too large to allow the differences to be assigned to interspecific variability. Because ϵ, I_k and η are interdependent [Eqn (5)], a further examination of variability in these parameters is warranted (see Section III.3). At this point there does not appear to be any benefit to introducing a non-dimensional light intensity scale and therefore θ will be treated as a function of photon flux density and temperature for the remainder of this discussion.

Taken together, Figures 10 and 11 suggest that the dependence of θ on I and temperature can be represented by

$$\theta = (a - bT) + cI \exp(-dT). \tag{7}$$

From regressions of θ_0 and ϵ on temperature (Figs 10 and 11), the following numerical values for the coefficients are obtained: $a = 43{\cdot}4$, $b = 1{\cdot}14$, $c = 1{\cdot}85$, $d = 0{\cdot}126$.

The light-dependence of θ at 5, 15 and 25 °C is illustrated in Figure 13 together with the predictions obtained from Eqn 7. Clearly, θ increases at low temperatures and this increase is most pronounced at high light levels. The increase of θ with decreased temperature at a fixed light level has been noted previously (Eppley, 1972; Li, 1980). Eppley (1972) reports an increase in θ from 16 to 38 g C g chl a^{-1} for *D. tertiolecta* between 25 and 12 °C at a light level of 0·07 cal cm^{-2} min^{-1} (approximately 55 μmol photon m^{-2} s^{-1}), Li (1980) reports an increase in θ from 62 to 111 g C g chl a^{-1} for *Phaeodactylum tricornutum* between 25 and 5 °C at $I = 250$ μmol m^{-2} s^{-1} (Li & Morris, 1982), and Sakshaug (cited in Li, 1980) reports an increase in θ from 23 to 80 g C g chl a^{-1} for *S. costatum* between 15 and 4 °C.

Although Eqn (7) was derived from studies on temperate zone microalgae, limited observations for psychrophilic species are consistent with a general increase in θ at low temperatures (Bunt & Lee, 1972; Durbin, 1977; Van Baalen, 1985).

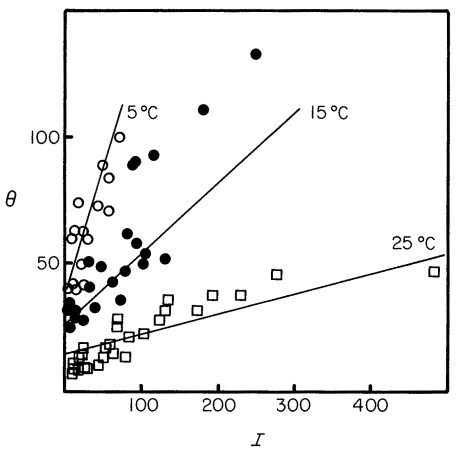

Fig. 13. Light (I) dependence of carbon:chlorophyll a (θ) for temperatures of 5, 15 and 25 °C:5 °C, open circles; 15 and 16 °C, closed circles; 25 and 26 °C, squares. Units for I are μmol m^{-2} s^{-1} and units for θ are g C g chl a^{-1}. This figure illustrates the typical extent of variation in observations used to derive Eqn (7). Unfortunately, it is difficult to determine the sources of error which may lead to a systematic variation from the predicted values.

Evidence from natural populations also supports the assertion that θ increases at low temperatures. Minimum C : chl *a* during the spring diatom bloom in Bedford Basin, Canada (temperature of 0·4 to 1·4 °C) was 180 g C g chl a^{-1} (Smith, Platt & Harrison, 1983). High ratios (> 200) have also been reported for particulate matter in arctic waters (Smith *et al.*, 1985). Steele (1962) suggested that high θ observed in winter was due to low-light chlorosis. This hypothesis seems untenable for cells in balanced growth, and an alternative explanation of the high θ as due to low-temperature chlorosis (Eppley, 1972) seems more in keeping with observations (Fig. 13).

In contrast to the results for Bedford Basin and the arctic, Bunt & Lee (1970) obtained C : chl *a* as low as 12, but more often between 25 to 60 for low-light environments of interstitial water and ice during an Antarctic sea ice bloom. The light levels under Antarctic sea ice are likely to be lower than those found in ice-free surface waters above the pycnocline. Bunt & Lee's observations may correspond to minimum θ (θ_0), whereas those from Smith *et al.* (1983, 1985) may characterize phytoplankton from higher light levels. With regard to Bunt & Lee's (1970) observations, also note that the evidence for an increase in θ_0 at temperatures below 15 °C is equivocal (Fig. 10). Finally, it is possible that Bunt & Lee (1970) underestimated organic carbon content due to inefficiencies in the wet-oxidation procedure which they employed, thus leading to low estimates of C : chl *a*.

3. *Limits of interspecific variability in* I_k

Richardson *et al.* (1983) summarize observations of the light dependence of growth in several classes of microalgae. The minimum light level at which growth rate becomes light-saturated does not vary greatly within each of four major subdivisions of algae and the cyanobacteria that were examined. Given the differences in light sources, culture vessel geometries and instrumentation used in the different investigations, and associated errors in estimates of light level, this similarity can be interpreted to indicate limited interspecific variability in I_k within major taxa, e.g. classes or divisions (Geider *et al.*, 1986a).

Few detailed observations of the μ–I curve for balanced growth of microalgae allow unambiguous interspecific comparisons of the saturation parameter I_k, but available observations indicate that the range of I_k is limited. Although the diatoms *P. tricornutum* and *T. pseudonana* differ in maximum growth rate, they have similar values of I_k (Nelson, D'Elia & Guillard, 1979). The same holds for the chlorophyte *Nannochloris atomus* and the diatom *P. tricornutum* (Geider & Osborne, 1986) and for the chlorophyte *Scenedesmus* sp. and the diatom *Fragillaria crotenesis* (Rhee & Gotham, 1981). The growth rates of five species of marine diatoms examined by Chan (1978) appear to saturate at similar light levels. Finally, the light levels at which growth rate becomes half saturated in five species of marine algae differed by only $\pm 50\%$ (Quraishi & Spencer, 1971).

The linear relationship between θ and light level has already been noted [Eqn (1), Fig. 1, Table 3], and similarly, light dependencies of the ratios of protein, carbohydrate and lipid to total carbon can be expected. The observation that cell chemical composition (Blasco, Packard & Garfield, 1982; Hitchcock, 1982) is independent of cell size in diatoms is consistent with size independence of I_k given the relationship between θ and μ' (Fig. 8). Despite the lack of a size dependence, there may be about 1·5 to two-fold variations in chemical composition at any given light level (Chan, 1978; Blasco *et al.*, 1982).

Thus, on the basis of direct comparisons of the μ–I curve between different

species (Quraishi & Spencer, 1971; Nelson *et al.*, 1979; Rhee & Gotham, 1981; Geider & Osborne, 1986), a review of independent observations (Richardson *et al.*, 1983) and indirect evidence obtained from interspecific variability of θ (Blasco *et al.*, 1982; Hitchcock, 1982), it appears that I_k shows only two-fold variations. This conclusion contrasts with the evidence for the existence of sun and shade flora in vertical distributions of phytoplankton in nature (Sournia, 1982). It may be that those species studied most frequently in axenic cultures have been inadvertently selected to have similar light requirements for growth. Alternatively, the vertical distributions may reflect the influences of environmental variables other than light level. Venrick (1982), for example, suggests that shallow (< 100 m depth) and deep (> 100 m depth) floral associations in the oligotrophic North Pacific Central Gyre correspond to 'nutrient-limited' and 'light-limited' physiological regimes.

4. Complicating effects of growth on a light : dark cycle

The use of 24 h mean light level (light dose) in the preceding analysis allowed observations obtained under continuous illumination and on a variety of light : dark (L : D) cycles to be combined. This approach may lead to errors if (1) the magnitude of the diel periodicities in θ are large, or (2) growth rate and chemical composition depend on the duration of the L : D cycle as well as light dose. These questions require further investigation in their own right. As discussed below, however, it is likely that the magnitude of these errors is small (a factor of 2) relative to the temperature effect (a factor of 10) that is under investigation (Figs 10 and 11). A detailed consideration of these potential problems is beyond the scope of this paper. However, the following points will be noted.

First, C : chl *a* will be lowest at the start of the light period if carbon-specific dark respiration rates are larger than chlorophyll-specific chlorophyll *a* degradation rates. This appears to be so for nutrient-sufficient *P. tricornutum* (Terry *et al.*, 1983) and *Oscillatoria redekei* (Foy & Smith, 1980). The magnitude of the diel variation of θ will depend on the relationship between photosynthesis rates during the light period, overall growth rate and the extent of mobilization of energy storage products during darkness to fuel continued cell synthesis. In *T. weisflogii* cultured on a 12 : 12 L : D cycle at 18 °C cell chlorophyll *a* content varied by 25 % at $I = 72$ μmol m^{-2} s^{-1} and by 50 % at $I = 593$ μmol m^{-2} s^{-1} (Post *et al.*, 1985). Cell chlorophyll *a* reached a minimum 8 h into the dark period and a maximum 8 h into the light period. The variation of θ will be less than that of chl *a* : cell since C : cell will increase during the light and decrease in darkness. Cosper (1982) found that θ varied by only 20 % over a 12 : 12 L : D cycle in *S. costatum* grown at light levels ranging from 15 to 1500 μmol m^{-2} s^{-1}. Similarly, in *O. redekei* the dry weight : chl *a* ratio increased by 25 % during a 6 h light period at $I =$ 27 μmol m^{-2} s^{-1} (Foy & Smith, 1980), but larger variations are expected at higher PFDs due to carbohydrate accumulation during the day and mobilization at night (Foy & Gibson, 1982; Foy, 1983).

Second, the assumption that growth rate can be expressed as a function of the 24 h light dose, independent of photoperiod duration, may be unrealistic. Although light-limited growth rates may scale with light dose (Yoder, 1979), maximum growth rates are often considered to depend on duration of photoperiod (Eppley, 1972; Foy & Gibson, 1976). In those species in which μ_m is greater under continuous illumination than under the L : D cycle, the increase in μ_m is usually less than proportional to the increase in percentage of the photoperiod illuminated (see entries for *S. costatum*, *T. weisflogii* and *P. tricornutum* in Tables 1 and 4; Foy

& Gibson, 1976; Yoder, 1979). However, some species will not grow, and others may show reduced growth rates, under continuous light (Brand & Guillard, 1981).

Finally, there is some evidence to suggest that different values of θ can be obtained at the same light dose under different photoperiods (Foy & Gibson, 1982; Post *et al.*, 1984). For example, Post *et al.* (1985) observed that the mean chl *a* : cell at a given incident PFD was the same whether cells were grown on a 12 : 12 L : D cycle or under continuous illumination despite a factor of two difference in light dose. If mean cell carbon content was the same under both culture conditions then θ at a given PFD, and as a consequence ϵ, would vary by a factor of two.

5. *Minimum carbon : chlorophyll* a *ratio in microalgae*

Most of the chlorophyll *a* in plant cells is contained within light-harvesting pigment–protein complexes (Prézelin, 1981; Barber, 1983) which are in turn embedded within or on lipid bilayers consisting largely of mono and digalactosyldiglycerides (Douce & Joyard, 1980). The light-harvesting antennae also contain accessory pigments (chlorophyll *c*, and carotenoids in the Chromophyta, chlorophyll *b* in the Chlorophyta, and phycoerythrin and phycocyanin in Cryptophyta and Cyanophyta) (Richardson *et al.*, 1983). Along with the antennae pigment–protein complexes, the thylakoid membrane contains reaction-centre pigment-protein complexes, various redox carriers and ATP synthetase (Prézelin, 1981). All of these pigments, catalysts and supporting lipid bilayers are necessary for photosynthetic production of ATP and reductant. Use of the products of the 'light' reactions of photosynthesis for CO_2 fixation requires the enzymes of the photosynthetic carbon reduction cycle of which the most abundant is the soluble enzyme ribulose bisphosphate carboxylase oxygenase (RUBISCO). Clearly, chlorophyll *a per se* accounts for only a fraction of the photosynthetic apparatus (*sensu* Shuter, 1979).

One measure of the intracellular allocation of resources is the relative size of various organelles. The chloroplast can comprise a variable fraction of the microalgal cell (Raven, 1984a) consistent with the observations of variable θ. Depending on preconditioning light level and nutrient availability, chloroplast volume ranged from 3 to 30 % of cell volume in *Cyclotella meneghiniana* (Rosen & Lowe, 1984). This range understimates the contribution of the chloroplast to cell dry weight or carbon content because of the large vacuole (30 to 50 % of cell volume) in this species (Rosen & Lowe, 1984). In contrast to the 10-fold range in chloroplast volume, the surface density of chlorophyll *a* on the thylakoid membrane varied by only a factor of two and cell chlorophyll content was well correlated with chloroplast volume (Rosen & Lowe, 1984).

Chlorophyll comprises about 4 to 5 % of the dry weight of terrestrial vascular plant (*Spinacia oleracea* and *Antirrhinum majus*) chloroplasts, protein divided equally between water-soluble and insoluble forms accounts for about 50 to 60 % of the dry weight, colourless lipid for 15 %, RNA for about 4 %, polysaccharides and amino acids for about 4 %, with inorganic ions contributing about 4 % (Kirk & Tilney-Bassett, 1978). If insoluble protein, lipid and chlorophyll *a* are the only components of the chloroplast membranes, then it follows that these membranes are composed of about 10 % chlorophyll *a*, 60 % protein and 30 % colourless lipid by weight. This calculation compares favourably with Murphy & Woodrow's (1983) analysis showing that chloroplast membranes from *S. oleracea* contained 7 % chlorophyll $a+b$, 68 % protein and 24 % lipid. Using a ratio of lipid : carbon of 0·7 and protein : carbon of 0·5 leads to an estimate for

θ of 7 to 8 for chloroplast membranes in vascular plants. This value exceeds the minimum ratio observed for microalgal cells. The chloroplast of vascular plants would have a C : chl *a* ratio of about 11 to 12.

Shuter (1979) indirectly derived a value for the C : chl *a* ratio of the photosynthetic apparatus from an analysis of growth rate-dependent biochemical composition under light-limiting conditions. He obtained a value of 6·0 for Cook's (1963) observations on *E. gracilis*. Laws *et al.* (1985) applied the same type of analysis to obtain values of 10·5 for *T. weissflogii* and 7·1 for *P. tricornutum*. These values apply to the sum of thylakoid membranes and the dark enzymatic components that together make up the photosynthetic apparatus. Shuter's (1979) analysis is based on the assumptions that the size of the photosynthetic apparatus is proportional to cell chlorophyll *a* content, the size of the synthetic apparatus is proportional to cell RNA content, the specific reaction rate constants for light harvesting and energy conversion by chlorophyll *a* and of protein synthesis by RNA are constant, and that storage and structural (including genetic) components of the cell can be independently estimated. These assumptions are open to criticism (Thomas & Carr, 1985) and more direct estimates of C : chl *a* in microalgal chloroplasts are desirable.

Raven's (1980) analysis for *Chlorella* leads to a ratio of thylakoid : chlorophyll *a* of 4·1 : 1 by weight. This is only 50 % of the ratio obtained for higher plant chloroplast membranes. More recently, Raven (1984b) suggested ratios of protein : total pigment and lipid : total pigment of 2·0 kg protein mol chromophore^{-1} and 0·38 kg lipid mol chromophore^{-1} for the Chlorophyta which leads to a thylakoid : chl *a* ratio of 4·5 : 1 by weight under the assumption that all of the chromophore is chlorophyll with a chlorophyll *a* : *b* weight ratio of 2 : 1 (Burkill *et al.*, 1987). In addition, assuming that protein is 50 % carbon and lipid is 70 % carbon, Raven's (1984b) figures lead to $\theta = 3$ g C g chl a^{-1} for the thylakoid membranes.

The minimum whole-cell C : chl *a* ratio observed at high temperature and low light levels is 6·4 g C g chl a^{-1} in the cyanophyte *Microcystis aeruginosa*. This ratio is lower than that calculated for higher plant chloroplasts, equal to that inferred for the photosynthetic apparatus of *Euglena* by Shuter (1979) and twice that for thylakoid membranes (based on Raven 1980, 1984b). The minimum ratio observed for a diatom is 10 for *P. tricornutum* at 25 °C, about the same as that for the greens at a similar temperature despite a difference in the accessory pigments between these groups : specifically, the carotenoid : chlorophyll *a* ratio is only about 1 : 3 in green algae and $> 1 : 1$ in diatoms (Kirk, 1983). The minimum C : chl *a* ratio (θ_0) rises rapidly with a drop in temperature and is equal to about 20 g C g chl a^{-1} at 20 °C, a value more in keeping with that observed for higher plant chloroplasts. Unfortunately, neither Kirk & Tilney-Basset (1978) nor Murphy & Woodrow (1983) give information about the growth temperature or light level.

6. *Mechanisms responsible for the temperature dependence of carbon : chlorophyll* a

(a) *Temperature dependence of carbon : chlorophyll* a

The low-temperature chlorosis exhibited by microalgae (Fig. 13) is typical of the response found in other plants. A loss of chlorophyll is observed in terrestrial vascular plants exposed to low temperatures (Berry & Björkman, 1980) and has been reported in the macroalga *Gracilaria tikvahiae* (Lapointe, Dawes & Tenore, 1984). As in the microalgae, low-temperature chlorosis in terrestrial vascular plants is accentuated by exposure to high light levels (Berry & Björkman, 1980).

It may be important to distinguish between the transient developmental response which has been studied in vascular plants (Berry & Björkman, 1980; Graham & Patterson, 1982) and the complete phenotypic adaptation which has been studied in the microalgae. In addition, the emphasis in studies on vascular plants has often concerned processes that occur at the tolerance limits to high and low temperatures, whereas the work of interest to algal ecologists is often directed at changes that occur within the normal physiological limits of a species. This is the distinction between 'resistance' and 'capacity' adaptations (Li, 1980). Similar explanations may pertain to low-temperature chlorosis in both algae and vascular plants, even though the context within which the question is approached may differ. In fact, the discussion which follows borrows heavily from work on vascular plants.

It is tempting to suggest that the temperature dependence of the C:chl *a* ratio is determined by the physical properties of the components of the photosynthetic apparatus. For example, an increase in the ratio of membrane lipid to protein may be required at low temperatures to maintain the required fluidity of the thylakoid membrane and/or to allow adjustment of intermolecular forces between membrane components (Raison *et al.*, 1980). Alternatively, the temperature dependence of enzyme reaction rates may place catalytic constraints on microalgal growth and chemical composition. Finally, protection from photo-oxidative damage at high PFDs should not be ignored (Raven, 1980).

(b) *Temperature effects on plant lipid content*

The proper functioning of the various catalysts and redox carriers contained on and in the thylakoid membrane depends on the physical state of the lipid matrix and in particular on its fluidity (Quinn & Williams, 1978; Raison *et al.*, 1980). Solidification of lipids in the thylakoid membrane may set the lower limit of the temperature range for a plant and other membrane properties may determine the upper limit (Berry & Björkman, 1980). Raison *et al.* (1980) suggest that the hydrophobic interactions between protein particles and the core of the lipid membrane increase and hydrophilic interactions between protein particles decrease at high temperatures leading to dissociation of membrane protein subunits. Temperature changes may effect the permeability of thylakoid membrane, influence the configuration and catalytic efficiency of membrane-bound proteins, or alter membrane-bound proteins in other ways (Berry & Björkman, 1980; Raison *et al.*, 1980). Changes in diffusivity of mobile electron carriers such as plastoquinone (Haehnel, 1984) within lipid-protein membranes may account in part for the observed temperature dependence of electron transfer reactions and ATP synthesis (Armond, Screiber & Björkman, 1978; Berry & Björkman, 1980; Öquist, 1983). Given the role of lipids in membrane structure and function, it is tempting to ask if increases in lipid content can account for an increase in C:chl *a* at low temperatures.

The lipid composition of plant membranes varies with temperature in such a manner that fluidity appears to be maintained within the tolerance range of a species (Quinn & Williams, 1978; Berry & Björkman, 1980; Lynch & Thompson, 1984a, b). Qualitative and quantitative changes in the polar lipids of various cell membranes (including the thylakoid membrane) have been measured but the ratio of chlorophyll *a*:lipid or protein:lipid in the membranes has not been determined. To the best of the author's knowledge there have not been any studies of the temperature dependence of the bulk chemical composition of either vascular plant or microalgal chloroplasts. Results of a structural study (Smillie *et al.*, 1978)

showed only slight differences in leaf chlorophyll *a*, chlorophyll *a*:*b* ratio, and number of thylakoids per granum for barley (*Hordeum vulgare*) grown at 11 and 27 °C. There were, however, slight increases (10 to 15%) in the ratio of stroma:grana and width of grana. These structural changes, if significant, are small in comparison with the observed change of θ in microalgae over the same temperature range.

Although plant lipid content increases in response to decreased temperature (Quinn & Williams, 1978; Graham & Patterson, 1982), the magnitude of this increase does not appear to be sufficient to account for the temperature dependence of C:chl *a*. For example, leaf lipid increased from 76 to 140 mg g^{-1} (weight lipid per gram dry weight) in winter rape (*Brassica napus*) between 25 and 5 °C (Smolenska & Kuiper, 1977). These values are, however, near the low end of the range of typical lipid contents for microalgae (Hitchcock, 1982; Terry *et al.*, 1983, 1985). A report of high rates of lipid synthesis (up to 80% of ^{14}C incorporation) in Antarctic phytoplankton under low light and temperature (Smith & Morris, 1980) has not been observed for Arctic water (Li & Platt, 1982). It also appears that the bulk macromolecular composition of microalgae is independent of temperature (Goldman, 1979, 1980; Van Baalen & O'Donnell, 1983).

(c) *Enzyme kinetic constraints on temperature acclimation of carbon:chlorophyll* a

An alternative explanation for the temperature dependence of θ may rest in the relationship between the 'light' and 'dark' reactions of cell metabolism. The specific reaction rate for the 'light' reactions of photosynthesis is often considered to be independent of temperature whereas the rates for enzymatic, 'dark' reactions are temperature-dependent.

If an enzyme-catalyzed reaction is not substrate-limited, then maintenance of the same cell-specific reaction rate at a lower temperature requires an increase of the intracellular concentration of the rate-limiting catalyst. Alternatively, if the intracellular concentration is independent of temperature, then the cell-specific reaction rate can be expected to decline as temperature is reduced. A quantitative analysis of these effects begins with the recognition that the rate of an enzymatic reaction will equal the product of enzyme concentration and specific reaction rate:

$$\rho = vq, \tag{8}$$

where, ρ is the cell-specific reaction rate (mass $cell^{-1}$ s^{-1}), v is the specific reaction rate of the catalyst (mass s^{-1}), and q is the intracellular concentration of catalyst ($cell^{-1}$). If ρ is the rate-limiting reaction for growth, then the temperature dependence of μ_m can be related to changes in the concentration (q) or specific activity (v) of the rate-limiting catalyst. For example, if v is the chlorophyll *a*-specific, light-saturated photosynthesis rate ($v = P_m^{chl}$ with units g C g chl a^{-1} s^{-1}) and q is the reciprocal of the carbon:chlorophyll *a* ratio ($q = 1/\theta$), then ρ has units of inverse time, which for light saturation of growth and photosynthesis with $\rho = \mu_m$ leads to

$$\mu_m = P_m^{chl}/\theta. \tag{9}$$

Eqn (9) can be considered as a useful summary of the relationship between three physiologically important variables. It also follows directly from the definition of specific growth rate (i.e. $\mu_m = (1/C)\,(dC/dt)_m = (1/B)\,(dC/dt)_m\,(B/C) = (dB/dt)_m\,(B/C)$; where C is cell carbon content, B is cell chlorophyll *a* content,

and the subscript m refers to maximum rates). Although it is tempting to treat one of the variables of Eqn (9) as dependent on the other two, this may not be mechanistically justified. It can, however, lead to useful ecological insights. For example, empirical relationships between μ_m and θ with temperature allowed prediction of the temperature dependence of P_m^{chl} (Eppley, 1972) for comparison with observations.

Overall, the maximum growth rate of microalgae (μ_m) is temperature-dependent with a Q_{10} of about 2 (Eppley, 1972), although higher values appear to apply to individual species (Eppley, 1972), although higher values appear to apply to individual species (Eppley, 1972; Yoder, 1979; Verity, 1982; Li & Morris, 1982). It is tempting to speculate that this temperature dependence results from a decrease in the specific reaction rate of an enzymatic process which controls growth rate at a fixed concentration of catalyst. Several candidates for this role can be identified. Ribulose bisphosphate carboxylase oxygenase (RUBISCO) is one of the most abundant enzymes in plants, and is a likely candidate for the rate-limiting enzyme for light-saturated rates of photosynthetic carbon reduction (Bassham, 1973). The activity of RuBP-carboxylase parallels changes of net photosynthesis in several C_4 species of vascular plants (Öquist, 1983) and in natural populations of marine phytoplankton (Li, Smith & Platt, 1984). In C_4-species, an increase in RuBP-carboxylase activity appears to result from an increase of enzyme concentration (Öquist, 1983). Correlations between light-saturated photosynthesis rate and RUBISCO activity notwithstanding, it would seem that RUBISCO rarely limits CO_2-saturated photosynthesis in C_3 land plants (Lilley & Walker, 1975; Caemmerer & Farquhar, 1981). Some aspect of RuBP regeneration is the more likely limiting step (Lilley & Walker, 1975; Caemmerer & Farquhar, 1981). Alternatively, the activity of one of the electron transfer chain intermediates may determine the maximum photosynthesis rate (Lilley & Walker, 1975; Fleischhacker & Senger, 1978; Caemmerer & Farquhar, 1981; Wilhelm & Wild, 1984). The growth rate need not necessarily be determined by the photosynthesis rate, especially at light saturation. Some aspect of cell growth, DNA replication or division (Tamlya *et al.*, 1953; Heath & Spencer, 1985; Olsen, Vaulot & Chisholm, 1986) may determine the maximum growth rate.

Control of flux through a complex reaction sequence is likely to be distributed among different steps (Kacser & Burns, 1973). It is possible that more than one metabolic pathway will be operating at near maximal capacity and the balanced growth rate will be co-limited by more than one catalyst. Specifically, if the cell resources are optimally divided amongst the catalysts of all essential biochemical pathways, an increase in the concentration of the rate-limiting catalyst for one pathway would require the reduction of concentration of the rate-limiting catalyst of another pathway and so reduce μ_m. For photo-autotrophic growth, however, it is possible to distinguish between catalysts of temperature-independent, biophysical 'light' reactions and temperature-dependent, biochemical 'dark' reactions. Raven (1987) recently considered the intracellular allocation of carbon between various components of cell metabolism. The components considered were: (1) catalysts for light harvesting, (2) catalysts for photosynthetic ATP and NADPH production, (3) catalysts for photosynthetic carbon fixation, (4) catalysts of the general cell metabolism (Raven's catalysts for chemo-organotrophic growth), and (5) cell structural, genetic and storage components. For this discussion it is assumed that the first component will comprise the catalysts for 'light' reactions and the second, third and fourth components comprise the

catalysts of the 'dark' reactions. This terminology differs from that often employed in discussions of photosynthesis by including enzymes of general cell metabolism and ATP/NADPH production together with those of CO_2 fixation in the 'dark' reactions and limiting the 'light' reactions to light-absorbing pigments and supporting lipid/protein.

For a cell with a maximum growth rate of 30×10^{-6} s^{-1} (2·6 d^{-1}) at 20 °C, Raven (1987) calculated that 10% of cell carbon was required for catalysts of photosynthetic ATP and NADPH production, 4% for enzymes of photosynthetic carbon fixation, 27% for the catalysts of chemo-organotrophic growth and 30% for structural material. This leaves a variable fraction (0 to 29% of cell carbon) for light-harvesting components. If 29% of cell carbon is used for light-harvesting pigment-protein membranes when the whole cell C : chl *a* is 20 (i.e. the minimum value at 20 °C), then C : chl *a* of the membranes would be 5·8 which is in reasonable agreement with observations (Section III.5).

A maximum growth rate of 30×10^{-6} s^{-1} (2·6 d^{-1}) can be attained at $I = 50$ μmol m^{-1} s^{-1} where $\theta = 27$ g C g chl a^{-1} if the light absorption cross-section (a_{chl}) equals 0·015 m^2 mg chl a^{-1} (Bannister, 1979) and the photon efficiency for growth (Φ) equals 0·09 mol C (mol photon)$^{-1}$ (Pirt, 1986; Osborne & Geider, unpublished data). These values for a_{chl} and Φ are likely to be the maximum attainable (Myers, 1980; Morel & Bricaud, 1981; Bannister & Weidemann, 1984). From Table 4 it is evident that most estimates of I_k are less than 50 μmol m^{-2} s^{-1} and that μ_m is usually less than 2·6 d^{-1} (at 20 °C). The low values for μ_m may be related to reduced values of a_{chl}. For example, a_{chl} can be as low as 0·004 m^2 mg chl a^{-1} (Falkowski, Dubinsky & Wyman, 1985; Geider *et al.*, 1985). For a whole cell with $\theta = 27$ and C : chl $a = 5{\cdot}8$ for the light-harvesting catalysts, one calculates that 21% of cell carbon is devoted to light reactions (as defined above).

For the remainder of this discussion we assume, for a cell growing at μ_m (20 °C and 50 μmol m^{-2} s^{-1}), that 38% of cell carbon is tied up in non-catalytic structural and storage material, 21% is tied up in catalysts of the 'light' reactions and 41% in catalysts of the 'dark' reactions. The maximum growth rate could be increased at the expense of the 'light'-reaction catalysts with a consequent increase in I_k. The microalgae apparently do not maximize μ_m (Raven, 1987), but instead sacrifice a significant increment in μ_m to maintain a higher μ under light-limiting conditions and/or to accumulate energy storage reserves at higher light levels. In this sense the microalgae as a group are shade-adapted.

With a decrease in temperature, cell resources can be mobilized from the catalysts for the 'light' reactions to compensate in part for the reduction in μ_m caused by a decrease in the specific activity of the catalysts of the 'dark' reactions. As a first approximation to the extent of re-allocation of resources, consider the following requirement for balanced rates of 'light' and 'dark' reactions:

$$a_c q_l I_k \Phi Z = \rho_d^r q_d c_T, \tag{10}$$

where q_l is the proportion of cell carbon in the catalysts of the light reactions (dimensionless), q_d is the proportion of cell carbon in the catalysts of the dark reactions (dimensionless), a_c is the light-harvesting efficiency associated with q_l (m^2 mg C^{-1}), Z is a dimensional constant equal to 0·012 mg C μmol C^{-1}, Φ is the photon efficiency of growth (mol C mol photons^{-1}), ρ_d^r is the catalytic efficiency (i.e. specific reaction rate with units of s^{-1}) of the catalysts for the dark reactions

at some reference temperature T_r (°K), and c_T is a temperature coefficient (dimensionless) defined from the Arrhenius equation (Raison, 1973):

$$c_T = \exp -(E_a/R)(1/T - 1/T_r), \tag{11}$$

where E_a is the temperature characteristic, R is the universal gas constant, T is absolute temperature (°K). This temperature coefficient [Eqn (11)] is of the same form as that used by Li *et al.* (1984) in an analysis of the temperature dependence of carboxylating enzyme activity in marine phytoplankton. Rearranging Eqn (10) leads to:

$$q_l/q_d = A\, c_T, \tag{12}$$

where $A = \rho_d{}^r/(a_c\, I_k\, \Phi Z)$. The whole cell C : chl *a* ratio can be calculated from q_l by using a C : chl *a* ratio of 5·8 for the light reaction pigment-protein complexes and associated lipid:

$$\theta = 5{\cdot}8/q_l. \tag{13}$$

At a reference temperature of 20 °C we take $I_k = 50$ μmol m^{-2} s^{-1}, $q_l = 0{\cdot}21$, $q_d = 0{\cdot}41$, $\rho_d = 9{\cdot}5 \times 10^{-5}$ s^{-1}, $a_c = 0{\cdot}0026$ m^2 mg C^{-1} (consistent with $a_{\text{chl}} = 0{\cdot}015$ m^2 mg chl a^{-1} and C : chl $a = 5{\cdot}8$ for the photosynthetic 'light' reactions), and $\Phi = 0{\cdot}09$ mol C (mol photon)$^{-1}$. Calculated values for θ at $I_k = 50$ μmol photons m^{-2} s^{-1} based on Eqn (13) with $E_a/R = 3{\cdot}0$ (Li *et al.*, 1984) are illustrated in Figure 14. There is good agreement between the predictions of this simple calculation and observations of θ over most of this temperature range. At both very low and very high temperatures the theoretical calculation overestimates θ, perhaps indicating that E_a/R should be less than 3·0.

The values for μ_m corresponding to these θ values (Fig. 14) are illustrated by the solid symbols in Figure 15, and values of μ_m which would be found in absence of compensation by the above scheme [Eqns (10) to (13)] are illustrated as the open symbols in Figure 15. The maximum growth rate is reduced by an additional 40 % between 20 and 0 °C in the absence of compensatory changes in θ. With any increase in temperature above 20 °C μ_m remains unchanged at 50 μmol m^{-2} s^{-1} if

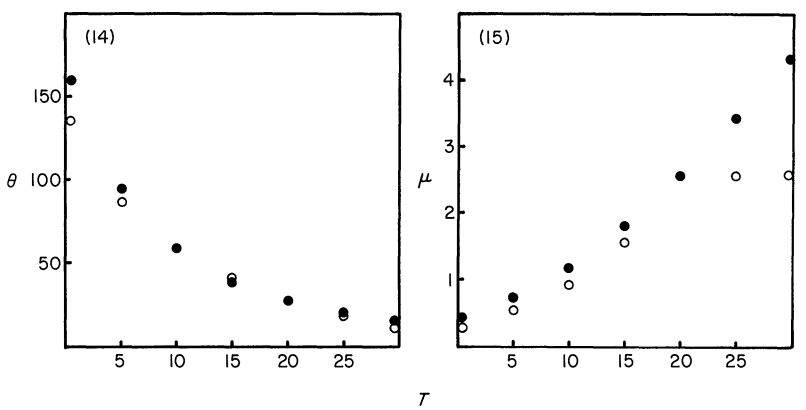

Fig. 14. Predicted (open symbols) and observed (closed symbols) dependence of θ on temperature (T) at $I = 50$ μmol m^{-2} s^{-1}. Observed values are based on Eqn (7). Predicted values are obtained from Eqns (10) to (13) as described in the text. T is in °C and θ has units of g C g chl a^{-1}.

Fig. 15. Predicted temperature (T) dependence of growth rate (μ) at $I = 50$ μmol m^{-2} s^{-1}. Closed symbols assume that growth rate is maximized through intracellular reallocation of resources described by Eqns (10) to (13). Open symbols are for fixed resource allocation. See the text for details. Units for μ are d^{-1}.

θ were held constant. Modifications to the response illustrated in Figures 14 and 15 would occur if I_k is temperature-dependent.

The maximum activity of an enzyme may not be the most appropriate measure of catalytic activity *in vivo* if the reactions are substrate-limited. For substrate-limited reaction rates the Michaelis half saturation constant (K_m) must be considered. Changes in the K_m of RUBISCO for CO_2 and O_2 with temperature (K_m-CO_2 increases more rapidly than K_m-O_2) leads to a reduction in the photon efficiency of the C_3 photosynthetic pathway as temperature increases (Berry & Björkman, 1980). This consequence of photorespiration has been observed in *Chlamydomonas reinhardii* (Coleman & Colman, 1980). It may be an important factor in the temperature dependence of microalgal growth given the evidence for photorespiration in microalgae (Burris, 1981; Kaplan & Berry, 1981; Birmingham, Coleman & Colman, 1982). Most microalgae, however, show some suppression of RUBISCO oxygenase activity related to operation of a CO_2-concentrating mechanism (Raven, 1984a). The Michaelis constant (K_m) has been shown to depend on temperature in ectothermic animals and vascular plants (Graham & Patterson, 1982). Qualitative and quantitative changes in isozymes present can apparently account for acclimation of K_m such that minimum values are found at ambient temperatures in vascular plants (Teeri, 1980).

A consideration of substrate-limited reaction rates leads to the possibility that the concentration of intermediates (S), as well as changes in v_m or K_m may be important in controlling cell metabolic and growth rates. Ehrenberg & Kurland (1984), for example, conclude that it is necessary to take the exponential increase of substrates into account as an explicit cost of growth. The effects of changes in v_m, K_m and S on metabolic rates can be described by $v = (v_m S)/(K_m + S)$. This equation illustrates that reduction of v_m will decrease the reaction rate at all substrate concentrations, but an increase in S or decrease in K_m will increase the reaction rate at low substrate concentrations.

(d) *Other constraints on the temperature dependence of carbon : chlorophyll* a

Raven (1980) considers three design criteria for the photosynthetic apparatus; these are (1) catalytic efficiency, (2) energy efficiency and (3) safety from photo-oxidative damage. The preceding analysis has considered the first of these criteria in terms of allocation of resources within the chloroplast and between the chloroplast and the remainder of the cell. The analysis focused on temperature dependence of v_m, but the possible complications due to variations of K_m were mentioned. Implicit in the analysis is the assumption of constant energy efficiency of photosynthesis and growth. Possible effects of temperature on these efficiencies are considered in the remainder of this section, as is the possibility of photo-oxidative damage.

The maximum photon efficiency of photosynthesis (ϕ), the energy efficiency of biosynthetic reactions (ν) and maintenance metabolic rates (μ_0) are often considered to be constant in organisms physiologically adapted to the ambient temperature within their normal tolerance limits (Shuter, 1979). The assumption of constant ϕ may not be universally valid if the oxygenase and carboxylase activities of RUBISCO show a marked temperature dependence at ambient O_2 and CO_2 concentrations. The increasing oxygenase activity of RUBISCO with increased temperature would lead to a continuous decrease of ϕ.

There is some indication that ν and μ_0 may also depend on temperature. In light-limited chemostat cultures, Lee, Tan & Hew (1985) report a maximum in

Φ for growth of *C. vulgaris* at 37 °C, and continuous increase in Φ for light-limited growth of *Chlorogonium* sp. over a temperature range of 15 to 40 °C. This was attributed to effects of temperature on chemical composition and ν, with ϕ presumably constant: low temperatures led to increases of both ν and cell protein content (Lee *et al.*, 1985). Unfortunately, photosynthetic pigments were not analyzed.

This leaves the remaining criterion of safety to be considered. Berry & Björkman (1980) suggest that, 'since a reduction in temperature causes a general decline in the rate of dark reactions of photosynthesis, the light required to saturate this capacity falls as temperature decreases and the threshold for sensitivity to photoinhibition increases'. High θ in microalgae at low temperatures may be considered as an adaptive response which ameliorates the potential damage due to photoinhibition. This benefit may arise as an indirect consequence of the reallocation of cell resources to the catalysts of the dark reactions of photosynthesis and growth at the expense of the light-harvesting pigments. Such a reallocation of resources would both reduce the absorption rate at potentially damaging photon flux densities and increase the rate of utilization of energy in absorbed photons. Alternatively, higher θ could indicate an increase in the components of the cell used for repair from photo-oxidative damage (Raven & Samuelsson, 1986).

7. *Implications of the temperature dependence of carbon : chlorophyll* a *for ecology of phytoplankton*

Estimating the growth rate of phytoplankton in nature is one of the major problems in studies of aquatic primary productivity (Eppley, 1981; Redalje & Laws, 1981). Direct estimates of productivity and biomass have been complimented by measurements of physiological state (Thomas, 1970; Thomas & Dodson, 1972; Goldman, 1980). Specifically, the carbon:nitrogen (C:N) ratio and C:chl *a* are useful indices of the physiological state of phytoplankton (Goldman, 1980). C:chl *a* may prove to be the more useful indicator in nature because (1) there is greater phenotypic variability of C:chl *a* than C:N and (2) a recently developed method is available to directly measure C:chl *a* of phytoplankton (Redalje & Laws, 1981). The empirical relationship between photon flux density, temperature and θ in phytoplankton (Section III.2) should have practical applications in studies of the distribution, productivity and physiological state of phytoplankton.

Several different approaches have been applied to the problem of estimating θ in nature (Eppley *et al.*, 1977a; Redalje & Laws, 1981). These are considered briefly before proceeding to a discussion of applicability of Eqn (7) to phytoplankton in the sea. The most direct approach compares chemical determinations of chlorophyll *a* with direct estimates of phytoplankton carbon from microscopy. Using this approach, Hobson, Menzel & Barber (1973) obtained phytoplankton C:chl *a* ratios ranging from 10 to 145. The method suffers from the difficulty of completely enumerating the phytoplankton within a sample and the inaccuracy of converting phytoplankton cell volume measurements into estimates of phytoplankton carbon. Nevertheless, Eppley *et al.* (1977a) regarded this method as the most reliable in a comparison of seven indirect methods for estimating θ in Californian coastal waters. An indirect approach to evaluating θ relies on linear regression between particulate organic carbon and chlorophyll *a* measurements. Values for θ ranging from 20 to 300 have been obtained from this method (Steele & Baird, 1962; Lorenzen, 1968; Eppley *et al.*, 1977a). These can underestimate or overestimate θ because of co-variation of the abundances of bacteria, micro-

zooplankton and detritus with phytoplankton (Banse, 1977). Specifically, phytoplankton can vary from less than 10% of particulate matter in oligotrophic waters (Hobson *et al.*, 1973) to almost 100% of particulate matter in phytoplankton blooms (Eppley *et al.*, 1977a). In a third method, Eppley (1968) compared estimates of phytoplankton carbon at the start of an incubation, as determined from the subsequent rate of ^{14}C-bicarbonate uptake, with the initial chlorophyll *a* concentration to determine the C:chl *a* ratio of phytoplankton in the original sample. He obtained values of $\theta = 33 \pm 18$ for nutrient-rich waters and $\theta = 91 \pm 19$ for nutrient-impoverished waters, consistent with expectations based on physiological responses of phytoplankton in laboratory cultures. Finally, a recently developed technique measures the ratio of ^{14}C activities in particulate carbon and chlorophyll *a* (Redalje & Laws, 1981). The technique overcomes the problem of estimating phytoplankton carbon and chlorophyll *a* contents by using ^{14}C as a tracer for both. It relies, however, on the assumption that growth during the sample incubation is balanced and indicative of natural conditions. Eppley's (1968) method relies on similar assumptions. These two approaches require observations on populations isolated in bottles and as such suffer from the consequences of containment (Venrick, Beers & Heinbokel, 1977). In particular, phenotypic plasticity of microalgae in response to alterations in light levels, temperature or nutrient concentrations may result in a difference between the ratio of C:chl *a* synthesis and the ratio of C:chl *a* in the original population (Eppley, 1968; Welschmeyer & Lorenzen, 1984).

The analysis of the C:chl *a* ratio presented in Section III.2 applies to nutrient-sufficient growth of phytoplankton. It is a truism that a necessary condition for development of a phytoplankton bloom is nutrient availability. Phytoplankton are likely to be nutrient-sufficient in coastal, temperate waters during the winter and spring prior to the peak of the spring bloom (Walsh *et al.*, 1981) within or below the deep chlorophyll *a* maximum layer which characterizes most ocean regions and accounts for most of the chlorophyll *a* in the open ocean (Venrick, McGowan & Mantyla, 1973; Herbland & Voituriez, 1979), and in arctic waters (Harrison, Platt & Irwin, 1982). The co-variation of total production with new production (Eppley & Petersen, 1979; Platt & Harrison, 1985) and of sedimenting particle flux with total production (Suess, 1980) suggests that nutrient-sufficient phytoplankton growth is one of the important links in the global carbon cycle (Walsh *et al.*, 1981).

In nature, phytoplankton growth may be limited by nutrient concentration rather than by light or temperature. The analysis of Section III.2 does not apply to these nutrient-limited conditions because θ can increase significantly with decreased nutrient availability at optimal light levels for growth (Laws & Bannister, 1980; Goldman, 1980; Osborne & Geider, 1986). Nutrient limitation of growth may occur in the surface waters of oligotrophic ocean gyres (Eppley *et al.*, 1973, 1977b) and stratified coastal waters (Eppley, Renger & Harrison, 1979). The open ocean which accounts for about 90% of the sea surface area and 75% of oceanic primary productivity (Walsh *et al.*, 1981) is often considered to be nutrient-limited for phytoplankton growth.

Some recent evidence (Goldman, McCarthy & Peavey, 1979; Goldman, 1980) suggests that phytoplankton growth rate is not nutrient-limited even in oligotrophic central gyres. If this is so, the analysis in Section III.2 should apply. Assume that the daily mean incident solar radiation at the water surface in subtropical gyres

is 200 W m^{-2}, 50 % of which is photosynthetically active radiation (PAR) (Miller, 1981) and that 1 W m^{-2} PAR = 5 μmol photons m^{-2} s^{-1} PAR (Richardson *et al.*, 1983). If the surface waters are mixed to the 10 % light level on a time-scale that is short relative to phytoplankton growth rate, phytoplankton in the mixed surface layer will be exposed to a mean light level of 195 μmol m^{-2} s^{-1}. Using this light level and a temperature of 28 °C which is appropriate for the North Pacific central gyre (Welschmeyer & Lorenzen, 1985) in Eqn (7) leads to $\theta = 22$ g C g chl a^{-1} for nutrient-sufficient cells. This is considerably lower than the measured $\theta = 114$ (Sharp *et al.*, 1980), leading to the conclusion that the oligotrophic ocean phytoplankton are nutrient-limited as is commonly (Eppley *et al.*, 1973, 1977b; Sharp *et al.*, 1980) but not universally (Goldman *et al.*, 1979; Goldman, 1980) accepted. The conclusion may be in error if Eqn (7) is inappropriate for the dominant phytoplankton in the oligotrophic oceans (see the discussion of θ in dinoflagellates in the following section). The potential complication of temporal variations in light level is discussed below.

Interestingly, Welschmeyer & Lorenzen (1985) recently calculated that $\theta = 11$ to 25 g C g chl a^{-1} for the euphotic zone (surface to 0·1 % light level) in the oligotrophic North Pacific central gyre. These low values for θ were obtained indirectly from observations of primary productivity and pigment production and degradation rates. Welschmeyer & Lorenzen (1985), however, rejected the low θ as being unrealistic given more direct estimates obtained by other investigators (Sharp *et al.*, 1980). The likely cause of the low θ was attributed to an underestimate of primary production (Welschmeyer & Lorenzen, 1985). Interestingly, the low θ value obtained by Welschmeyer & Lorenzen (1985) is consistent with the calculation made in the preceding paragraph for nutrient-sufficient phytoplankton growth. Such low C : chl *a* ratios, however, would imply that a very small percentage of the open ocean particulate matter is contained in the phytoplankton.

One aspect of the physiological ecology of phytoplankton that is poorly understood, but perhaps of great significance, is the response of growth, photosynthesis and chemical composition to fluctuations in environmental conditions (Harris, 1978; Falkowski & Wirick, 1981; Falkowski, 1980; Lewis *et al.*, 1984b). Conclusions derived from observations on cultures in balanced growth may not be directly applicable to phytoplankton in nature. However, given the poor quantitative understanding of the effects of environmental fluctuations on microalgal physiological responses, it is reasonable to proceed from inferences derived for the well-defined condition of balanced growth. One problem that must be faced in applying these results to nature is choosing the appropriate time and space scales for integrating environmental fluctuations (Lewis, Cullen & Platt, 1984a; Gallegoes & Platt, 1985). A recent theoretical analysis (Geider & Platt, 1986) suggests that changes of θ in fluctuating light can be interpreted in terms of photosynthesis and chlorophyll *a* synthesis rates. The appropriate time and space scales for comparing chemical composition and environmental variables would appear to be determined by the growth rate (Geider & Platt, 1986). A fuller discussion of the possible implications of fluctuations in environmental variables on phytoplankton physiological responses is beyond the scope of this paper.

8. Comparison of growth rate and carbon : chlorophyll a in dinoflagellates and diatoms

The results presented in Figures 10, 11 and 12 are derived primarily from observations on diatoms. Limited data for green algae and cyanobacteria appear to be in general agreement with those for diatoms. Schlesinger & Shuter (1981), however, report minimum $\theta = 10$ to 15 in four chlorophytes cultured under light-limiting conditions at 15 °C. They also have observations of $\theta = 8{\cdot}5$ to 12 for the same species at 25 °C. The results at 15 °C are somewhat lower than those derived for microalgae in this paper (III.2), while the results at 25 °C are in general agreement. The suggestion of variable temperature dependence of θ in different algal classes requires further attention.

The diatoms and dinoflagellates have been considered to be the most abundant oceanic phytoplankton (Sieburth, 1979), although the importance of picoplanktonic cyanobacteria and *Chlorella*-type green algae (Johnson & Sieburth, 1979, 1982) as a major component of the ocean flora has recently been recognized (Li *et al.*, 1983; Platt, Subba Rao & Irwin, 1983; Takahashi & Hori, 1984). In this regard, neglect of dinoflagellates can be considered a major limitation of the preceding analysis of temperature and light effects on θ. The following discussion seeks to rectify, in part, this omission. The available data limit a comparison of growth and pigment content in the diatoms and dinoflagellates to temperatures of about 20 °C. At this temperature, there are significant differences between these two classes.

Banse (1982) has recently reviewed the range of growth rates in diatoms and dinoflagellates, concluding that μ_m is about three times greater in diatoms than in dinoflagellates of comparable volume. Light saturation of growth in dinoflagellates has been reported to occur at much lower light levels than in diatoms consistent with the lower light-saturated growth rates (Richardson *et al.*, 1983). This observation might be interpreted to indicate that dinoflagellates are genotypically adapted to a shade environment. If adaptation implies a competitive advantage then this conclusion would be in error. Two attributes of a shade species in microalgae, as in vascular plants, are (1) a relatively high pigment content per unit mass which may contribute to (2) a higher growth rate at low light. By these criteria, dinoflagellates are less fit at low photon flux densities than diatoms as discussed below. A third attribute, which could contribute to enhanced μ at low light conditions and/or allow survival through prolonged adverse conditions (e.g. darkness) is reduced respiration rate. Respiration rates in dinoflagellates, however, account for the same fraction of light-saturated photosynthesis rates as in diatoms (Dunstan, 1973; Humphrey, 1975). Reduced respiration rates appear to parallel the reduced maximum growth rate of dinoflagellates relative to diatoms (Chan, 1978; Banse, 1982; Loeblich, 1984). A fourth criterion, facultative phagotrophy by an otherwise autotrophic organism (Loeblich, 1984), although potentially of ecological significance will not be considered here.

Observations on natural blooms of diatoms and dinoflagellates (Eppley *et al.*, 1977a) indicate lower pigment contents (i.e. higher θ) in dinoflagellates than in diatoms and studies on axenic cultures in the laboratory lead to similar conclusions. Chlorophyll *a* contributes from 0·75 to 1·5 % of cell mass in *Amphidinium carterii* at 20 °C corresponding to $\theta = 33$ to 66 (assuming that 50 % of dry weight is carbon) (Thomas & Carr, 1985). These are somewhat higher than in diatoms at similar temperature. Chan (1978) found that the average protein : chl *a* ratio was consis-

tently higher in dinoflagellates than in diatoms over a range of photon flux densities from 8 to 256 μmol photon m^{-2} s^{-1} (means for diatoms and dinoflagellates varied by a factor of 2 to 6). These protein : chl *a* ratios are roughly equal to θ (Chan, 1980). C : chl *a* averaged seven times greater, and growth rate 10 times lower, in the dinoflagellate *Prorocentrum micans* in a direct comparison with the diatom *T. weisflogii* at $I = 70$ to 600 μmol photons m^{-2} s^{-1} at 18 °C (Falkowski *et al.*, 1985). It would appear that the variations in μ_m between diatoms and dinoflagellates (Banse, 1982) are accompanied by a similar range of variation in θ.

The differences in growth rate at saturating PFDs appear to extend to subsaturating light levels as well. Chan (1978) observed that the growth rates of five dinoflagellate species are consistently lower than those of five diatom species at $I = 8$ to 256 μmol m^{-2} s^{-1}. The average growth rate for dinoflagellates was only one-third of that for diatoms cultured at the same light level (excluding those observations in which photoinhibition was observed).

Both light-limited and light-saturated growth rates, as well as the ratio of chl *a* : C ($1/\theta$) are lower in dinoflagellates than in diatoms. Dinoflagellates do not appear to be better adapted to low light than diatoms. As for the inferred 'preference' for low light (Richardson *et al.*, 1983), Chan's (1978) data have been interpreted (Loeblich, 1984) to indicate similar light requirements for growth in both groups.

Interestingly, when observations from both diatoms and dinoflagellates were considered together, Chan (1978) found that growth rate was proportional to chl *a* : protein ratio for both light-limited and light-saturated cultures. The slope of the regression of growth rate on protein : chl *a*, however, varied with photon flux density. Perhaps the two groups should be considered as possessing a continuum of physiological responses rather than as being distinctly different. Some dinoflagellates have very high growth rates approaching those for diatoms of similar size, and similarly, some diatoms have growth rates significantly less than expected based on the general size dependence of growth (Banse, 1982).

Chan's (1978) observations for diatoms alone are consistent with the conclusion of limited (i.e. a factor of 2) interspecific variability in θ and I_k. Inclusion of dinoflagellates in the analysis, however, indicates that this conclusion is too simple. Considering diatoms and dinoflagellates in the same analysis extends the range of both θ and μ at any given light level, thereby elucidating potentially unifying trends which would be obscure if the groups were considered separately. Thus, there appears to be some uniformity in the physiological processes responsible for the observed co-variation of μ and chl *a* : protein (or chl *a* : C) in both groups. As a consequence, the dependence of θ on photon flux density and temperature may not be as simple as is depicted by Eqn (7); however, this equation remains a useful summary of available observations of light and temperature effects on θ. Determining the range of interspecific variability of I_k and ϵ awaits further data.

IV. Conclusion

The preceding analysis has revealed some robust relationships amongst the carbon : chlorophyll *a* ratio (θ), growth rate, temperature and light level in microalgae. These observations indicate that phenotypic responses are more important than genotypic differences in accounting for variability of θ in diatoms and perhaps also chlorophytes and cyanobacteria. A single function of temperature and photon flux density appears to describe the variation in θ irrespective of the species under consideration. This optimistic appraisal needs to be tempered by the

fact that only a few species have been included in the analysis and that there are limited observations at low temperatures. Evidence from a number of independent sources does, however, support the conclusions reached in this analysis.

It is perhaps surprising and encouraging that these phenotypic responses to light and temperature are so clearly evident in a data set that has been drawn from a diversity of sources. The observations have been taken from 15 publications, by authors from 11 different laboratories using different analytical techniques for determining particulate carbon, chlorophyll *a* and photon flux density. In addition, organisms were grown under both continuous illumination and on a variety of light : dark cycles.

It is possible that the similarity of phenotypic responses and the limited genotypic variability arises in part through the selection of similar physiological types during isolation for laboratory studies. Observations from phytoplankton assemblages in nature, however, do not contradict the general conclusion that θ increases at low temperatures. Use of the chlorophyll *a*-labelling technique (Redalje & Laws, 1981) in conjunction with systematic experiments on phytoplankton in nature may provide the observations necessary to test the generality of responses described in this paper.

That both θ and relative growth rate (μ') depend on light level leads to the conclusion that θ and μ' are interdependent. Thus, the analysis of temperature dependence of θ leads immediately to a consideration of the temperature dependence of growth.

The mechanistic explanation for an increase in θ at low temperature is still largely speculative. Several hypotheses have been suggested in the discussion. These are based on the optimum allocation of resources between the functional and structural compartments of a microalgal cell under constraints imposed by physical–chemical properties of lipids and enzymes. Clearly, more experimental work is required in this much neglected aspect of algal physiology.

ACKNOWLEDGEMENTS

This work was initiated while the author was a Ph.D. student at Dalhousie University and supported by Dalhousie University and Isaac Walton Kilham Memorial Fellowships. Preliminary results were presented by the author and Dr T. Platt at the January 1984 Ocean Sciences Meeting, New Orleans. This work has benefited from discussions with Drs M. Lewis, R. E. H. Smith and B. A. Osborne, and Professor J. A. Raven. Suggested improvements from B. A. Osborne, T. Platt and J. A. Raven on a draft of the manuscript are gratefully acknowledged. The facilities of the University of Birmingham Computer Centre were used for statistical analyses. R. G.'s work at the University of Birmingham is supported by N.E.R.C. grant GR3/5900.

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