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Phytoplankton carbon biomass and specific growth rates determined with the labeled chlorophyll *a* technique

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ABSTRACT: The labeled chlorophyll *a* technique, based on the incorporation of $H^{14}CO_3^-$ into chlorophyll *a* (chl *a*) gives accurate determinations of both phytoplankton carbon biomass (C_p) and specific growth rate (μ). C_p and μ, derived from the labeled chl *a* method, exhibited a diel periodicity and the mean μ over 24 h compared well with the dilution rate for an oceanic microflagellate in NH_4^+-limited continuous culture. The specific activity of carbon in C_p ($R^*_{C_p}$) and in chl *a* ($R^*_{chl\ a}$) were equal after only 2 h and remained the same through 24 h. This supports the main assumption of the technique that within a useful time scale, the specific activities are equal. Values of C_p and phytoplankton POC measured with a CHN analyser were the same for the microflagellate in continuous culture. Estimates of phytoplankton carbon derived from several indirect methods were either greater than (methods based on measurement of adenosine triphosphate and microscopic examination of cell counts and volumes) or less than (methods based on measurement of particulate organic carbon and chlorophyll *a* concentrations) values determined with the labeled chl *a* technique. Time course ^{14}C incubations indicate that both C_p and μ exhibit diel variability through 24 h in the fluorescence maximum layer and overlying water column in the Southern California Bight. Phytoplankton populations in the fluorescence maximum layer grew with generation times of 2 to 3 d, while generation times in the water column above averaged less than 1 d.

INTRODUCTION

Accurate determination of phytoplankton carbon biomass (C_p) and specific growth rate (μ) is essential in order to characterize the flow of organic matter through the pelagic ecosystem. The incorporation of ^{14}C by phytoplankton is a measure of the change in C_p over a period of time. However, an accurate determination of C_p is necessary before μ can be calculated, usually by the equation:

$$\mu = \frac{1}{C_p} \frac{dC_p}{dt} \quad (1)$$

Various indirect methods have been used to determine C_p with a variable degree of success. Some of these methods have been discussed elsewhere (Eppley et al., 1977; Redalje and Laws, 1981). Since particulate organic carbon (POC) contains a variable amount of non-phytoplankton carbon (e.g. detritus, zooplankton) and the ratios of chlorophyll *a* (chl *a*) or adenosine triphosphate (ATP) to C_p are variable, estimates of C_p based on these ratios are subject to an uncertain error.

Redalje and Laws (1981) presented a method which gives accurate determinations of both C_p and μ, based on the incorporation of $H^{14}CO_3^-$ into chl *a* (see 'Methods' for details). Initial experiments with diatoms in batch and continuous culture in the laboratory and with natural populations in a subtropical estuary gave reasonable estimates for μ and C_p (Redalje and Laws, 1981). However, there are several questions that must be answered before the method could be routinely applied. It is assumed in the method that by the end of the incubation the specific activity of carbon in chl *a* ($R^*_{chl\ a}$), which can be measured in the field, is equal to the specific activity of C_p ($R^*_{C_p}$), which cannot be measured in the field (see 'Methods'; Redalje and Laws, 1981). In this study, I asked: how do $R^*_{C_p}$ and $R^*_{chl\ a}$ vary

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for an oceanic microflagellate in continuous culture and what is the minimum amount of time required before $R^{*}_{chl\ a}$ and $R^{*}_{C_p}$ become equal?

Previously used methods for measuring C_p in the field all include a variable amount of contamination from non-phytoplankton carbon (Banse, 1977; Eppley et al., 1977). How do C_p values derived from the labeled chl *a* technique compare with those estimated by other indirect techniques?

Phytoplankton in laboratory culture frequently exhibit diel periodicity in growth rates and in photosynthetic parameters (Chisholm 1981; Harding et al., 1981). Cyclic periodicity is also important for phytoplankton in the field (Heath, 1982; Gargas et al., 1979; Maccaul and Platt, 1977). Do C_p and μ estimated by the labeled chl *a* technique exhibit diel variation?

The study reported here was designed to answer the above questions by investigating the time course of ^{14}C incorporation into phytoplankton carbon and into chl *a* using an oceanic microflagellate in continuous culture and natural populations in the waters of the Southern California Bight.

MATERIALS AND METHODS

The marine prasinophyte (clone MC-1), isolated from the North Pacific Central Gyre and tentatively identified as *Mantoniella* sp., was maintained in continuous culture. Cultures were established as described by Laws and Bannister (1980) with ammonium (NH_4^+) as the nutrient limiting growth. The culture was maintained at a dilution rate (= μ, mean over 24 h) of 0.0167 h^{-1} at 20 °C and illuminated with a light intensity of 290 $\mu E\ m^{-2}\ s^{-1}$ supplied by a bank of 40 watt cool white fluorescent bulbs on a 12 h : 12 h light : dark (L : D) cycle.

The continuous culture growth chamber was inoculated with 100 μ Ci of $H^{14}CO_3^-$ at the beginning of the photoperiod in this experiment. Samples were taken at the beginning of the experiment and after 2, 4, 6, 9, 12 and 24 h. Each experimental sample consisted of subsamples for POC and particulate nitrogen (PN) (15 ml), chl *a* (5 ml), total ^{14}C incorporated (triplicate, 5 ml) and for the isolation of pure chl *a* (150 ml). POC and PN samples were filtered onto precombusted Whatman glass fiber filters (GF/C, at 450 °C for 24 h) and analysed with a Hewlett-Packard model 185B CHN analyser according to the recommendations of Sharp (1974). Chl *a* samples were filtered onto GF/C filters and measured with a Turner Model 111 fluorometer (Holm-Hansen et al., 1965). Total particulate ^{14}C activity samples were filtered onto GF/C filters, treated as recommended by Lean and Burnison (1979) to eliminate residual $H^{14}CO_3^-$, and activities measured with a Beckman LS 100 C Liquid scintillation counter (LSC) using Betaphase (Westchem) liquid scintillation cocktail. LSC efficiencies were determined using internal standard (^{14}C-toluene, New England Nuclear) additions and the external standard ratio method. Samples for the isolation of pure chl *a* and for the determination of $R^{*}_{chl\ a}$, C_p and μ were treated as described by Redalje and Laws (1981). C_p and μ at the end of the incubation are calculated using Eq. 2 and 3 respectively, where A^* is the total ^{14}C incorporated by the phytoplankton (dpm l^{-1}), t is the length of incubation, and $P \cdot t$ is the total amount of carbon produced during the incubation.

$$C_p = \frac{A^*}{R_{chl\ a}} \tag{2}$$

$$\mu = -t^{-1} \ln \left(1 - \frac{P \cdot t}{C_p}\right) \tag{3}$$

It is assumed that, at time t, $R^{*}_{C_p} = R^{*}_{chl\ a}$.

Field experiments were carried out on 3 cruises to the Southern California Bight (SCBS-18), May, 1981; SCBS-19, September, 1981; SCBS-20, March, 1982) and 1 cruise off Baja California (SF3, July, 1981) (Fig. 1a, b). Samples were collected at sunrise in all cases. Incubations were performed at ambient surface water temperatures in on-deck incubators with neutral density screens to reduce incident irradiance to the appropriate level. For SCBS-18 samples were collected from the fluorescence maximum layer (about the 7 % light depth) and from near the surface (2 to 3 m) and incubated from sunrise to sunset in 4 l pyrex bottles. On SF3, samples were taken from the 6 % light depth and 50 % light depth and incubated from about 1 h after sunrise to sunset in 4 l pyrex bottles. The bottles were subsampled after 4, 8 and 12 h. On SCBS-19, 9 l samples from the fluorescence maximum layer were incubated for 24 h from sunrise, with subsamples taken after 6, 12 and 24 h. On SCBS-20, 9 l samples from the 37 %, 29 % and 12 % light depths and from the fluorescence maximum layer (about the 7 % light depth) were incubated for 24 h, from sunrise through the following sunrise with time course samples taken after 3, 6, 12 and 24 h.

In all field experiments, the water from 2 or more 5 l Niskin bottles were combined in a 20 l polyethylene carboy and distributed to the incubation bottles after pre-screening through 183 μm nitex mesh. The initial water was sampled for chl *a* (single or duplicate 50 ml or 100 ml) and POC and PN (single or duplicate 0.5, 0.75 or 1.0 l). Samples were inoculated with either 250 or 500 μCi of $H^{14}CO_3^-$. All subsequent samples (on each cruise) consisted of subsamples for chl *a* (single or

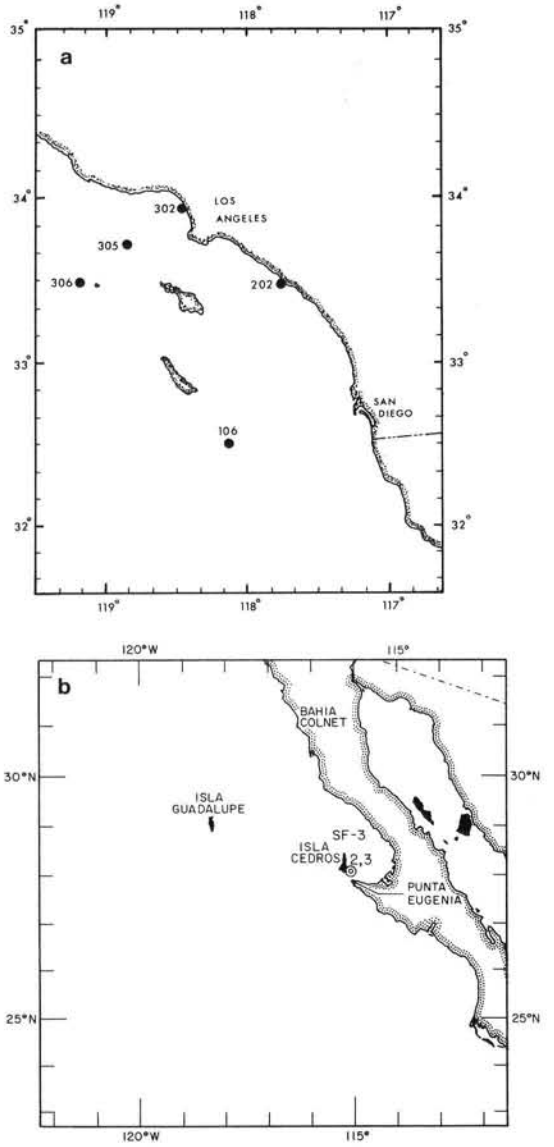

Fig. 1. Station locations. (a) Stations occupied on SCBS–18, SCBS–19 and SCBS–20; (b) stations occupied on SF3

duplicate 50 ml or 100 ml), POC and PN (single or duplicate 0.5, 0.75 or 1.0 l), total incorporation of ^{14}C (triplicate, 50 ml) and for the isolation of pure chl *a* (single, 1.5 or 2.0 l). All samples were treated and analysed as previously described for the laboratory studies.

RESULTS

Laboratory experiments

The specific activity of the total phytoplankton carbon, $R^*_{C_p}$, and the specific activity of the phytoplankton chlorophyll *a*-carbon pool, $R^*_{chl\ a}$, varied with time of incubation in a similar manner (Fig. 2) for *Mantoniella*

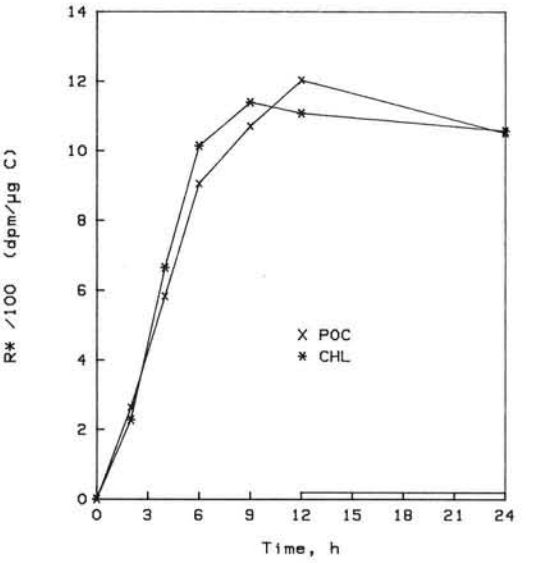

Fig. 2. *Mantoniella* sp. Variation in $R^*_{C_p}$ (dpm l^{-1}) and $R^*_{chl\ a}$ (dpm l^{-1}) with time (h) for NH_4^+-limited continuous culture under 12 h:12 h light: dark photocycle

sp. A least squares (Model II, geometric mean) linear regression of $R^*_{C_p}$ vs. $R^*_{chl\ a}$ (Eq. 4) is significant ($r^2 = 0.953$, $p < 0.001$); the slope is not significantly different from 1.0 and the intercept is not significantly different from 0 ($p > 0.05$).

$$R^*_{chl\ a} = 17.4 + 1.01\ (R^*_{C_p}) \quad (4)$$

The significant relationship between $R^*_{chl\ a}$ and $R^*_{C_p}$ (Fig. 2; Eq. 4) indicates that $R^*_{chl\ a}$ and $R^*_{C_p}$ were the

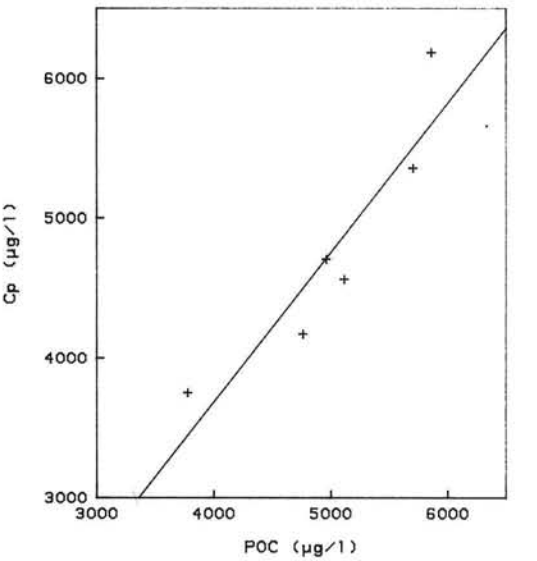

Fig. 3. *Mantoniella* sp. Comparison of C_p (µg Cl^{-1}) derived from labeled chl *a* method and phytoplankton POC (µg Cl^{-1}) measured with CHN analyzer. Least squares (Model II, geometric mean) linear regression: $C_p = -595 + 1.07$ (POC), $r^2 = .85$ is significant. Slope is not significantly different from 1 and intercept is not significantly different from 0 ($p > 0.05$)

same after only 2 h under these experimental conditions (e.g. moderately low μ, NH_4^+-limited continuous culture).

The calculated value of C_p at each sample time was highly correlated ($r^2 = .846$) to the phytoplankton POC, as measured by CHN analysis (Fig. 3). The least squares (Model II, geometric mean) linear regression (Eq. 5) is significant ($p < 0.01$); the slope of the regression line is not significantly different from 1.0 and the intercept is not significantly different from 0 ($p > 0.05$).

$$C_p = -595 + 1.07\ (POC),\ \mu g\ Cl^{-1} \qquad (5)$$

C_p averaged 95.1 ± 2.7 % of the phytoplankton POC.

One can calculate μ for each time interval sampled during the course of the experiment using Eq. 3 (Fig. 4). In this case, t is the interval between 2 sample time points, P·t is the ^{14}C production over this time interval and C_p is the phytoplankton biomass at the end of the time interval. Calculated values of μ were greater over the first 6 h of the photoperiod than over the second 6 h, with the maximum μ observed in the 4 to 6 h interval (Fig. 4). A mean μ over 24 h ($\bar{\mu}_{24}h$) can be calculated using the geometric mean value of μ over the photoperiod (= 0.0433 h^{-1}; dashed line in Fig. 4) and accounting for the specific dark ^{14}C loss rate and dilution. The resulting $\bar{\mu}_{24}h$ is nearly identical to the dilution rate of the continuous culture (Fig. 4).

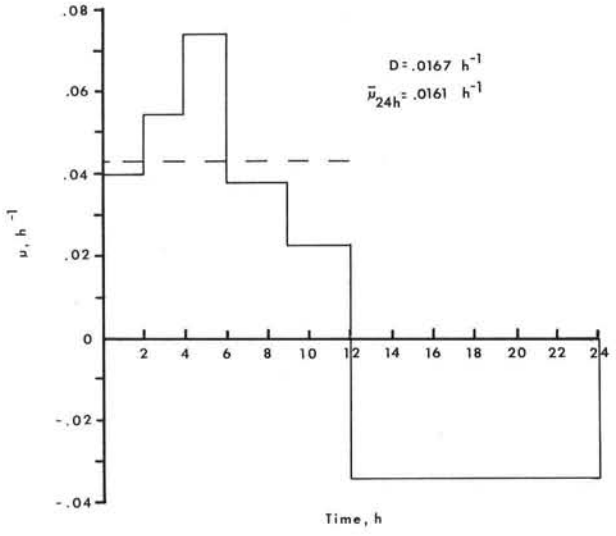

Fig. 4. *Mantoniella* sp. Variation in μ (h^{-1}) with time for NH_4^+-limited continuous culture under a 12 h:12 h light: dark photocycle

Comparison of biomass estimates for natural populations

The calculated C_p (Column A, Table 1) from 3 cruises off the coast of Southern California and Baja California (SCBS–18, 19 and SF3) have been compared to the phytoplankton C biomass estimates derived by the POC equation suggested by Eppley et al. (1977; phytoplankton C = 0.158 [POC] + 0.0007 $[POC]^2$), listed in Column B (Table 1). Biomass was also estimated using microscopic determinations of phytoplankton cell volumes and C/volume ratios (Column C, Table 1; Strathmann, 1967; Beers et al., 1975) and the use of an assumed C/ATP ratio of 250 (Column D, Table 1; Holm-Hansen and Booth, 1966) for samples taken at the beginning of the incubation on SCBS–18 (values shown in Table 1 have been corrected to biomass at the end of the incubation by including the

Table 1. Estimated phytoplankton biomass, μg C l^{-1}

Station/depth (Length of incubation)	A	B	C	D	E
I. SCBS-18	(May, 1981)				
106/22 m (12 h)	87	98	168	150	26
202/19 m (12 h)	167	90	149	213	38
302/ 1 m (12 h)	390	260	420	551	211*
302/ 9 m (12 h)	120	103	178	228	51
306/ 2 m (12 h)	165	152	304	367	210*
306/24 m (12 h)	101	92	155	245	49
II. SF3	(July, 1981)				
Sta. 2/ 2 m (8 h)	161	116			87*
Sta. 2/ 2 m (12 h)	135	127			121*
Sta. 2/22 m (8 h)	151	104			38
Sta. 2/22 m (12 h)	254	127			36
Sta. 3/ 4 m (12 h)	275	152			102*
Sta. 3/20 m (4 h)	72	26			25
III. SCBS-19	(September, 1981)				
106/50 m (t_o)	18	24			16
106/50 m (6 h)	21	15			17
106/50 m (12 h)	20	14			16
106/50 m (24 h)	12	14			16
305/40 m (t_o)	20	21			12
305/40 m (6 h)	25	16			12
305/40 m (12 h)	44	22			12
305/40 m (24 h)	34	24			12

Column A – C_p derived from labeled chl *a* method.

Column B – Phytoplankton C derived from POC equation of Eppley *et al.* (1977).

Column C – Phytoplankton C derived from microscopic cell volume determination, C/volume ratios of initial water sample plus the ^{14}C production during the incubation.

Column D – Phytoplankton C derived from the ATP concentration in the initial water sample plus the ^{14}C production during the incubation.

Column E – Phytoplankton C derived from the concentration of chl *a* multiplied by a C/chl *a* of 25 for deep samples and of 100 for near surface samples (*).

All values represent carbon biomass within the container at the end of the incubation.

^{14}C production over the duration of the incubation for these samples). Assumed C/chl *a* ratios of 25 for fluorescence maximum layer samples and 100 for near surface samples combined with ambient chl *a* concentrations yielded the phytoplankton C values listed in Column E (Table 1). These comparisons are also shown in Fig. 5.

Time Course Variation in C_p and μ

Both C_p and μ varied over 24 h for samples from the fluorescence maximum depth at 2 stations off of Southern California in September, 1981 (Figs. 6 and 7). C_p increased from sunrise through sunset and decreased during the night due to the combined C loss of respiration and excretion (Fig. 6). At both stations μ was greater during the first half of the day than during the second half (Fig. 7). One can calculate μ in 2 ways. In the first, the mean μ for the photoperiod was determined and corrected for the 24 h day using the specific dark C loss rate (rate [a] in Fig. 7). The second value of μ (rate [b] in Fig. 7) was calculated using the single 24 h endpoint C_p and ^{14}C production (Eq. 3). Both

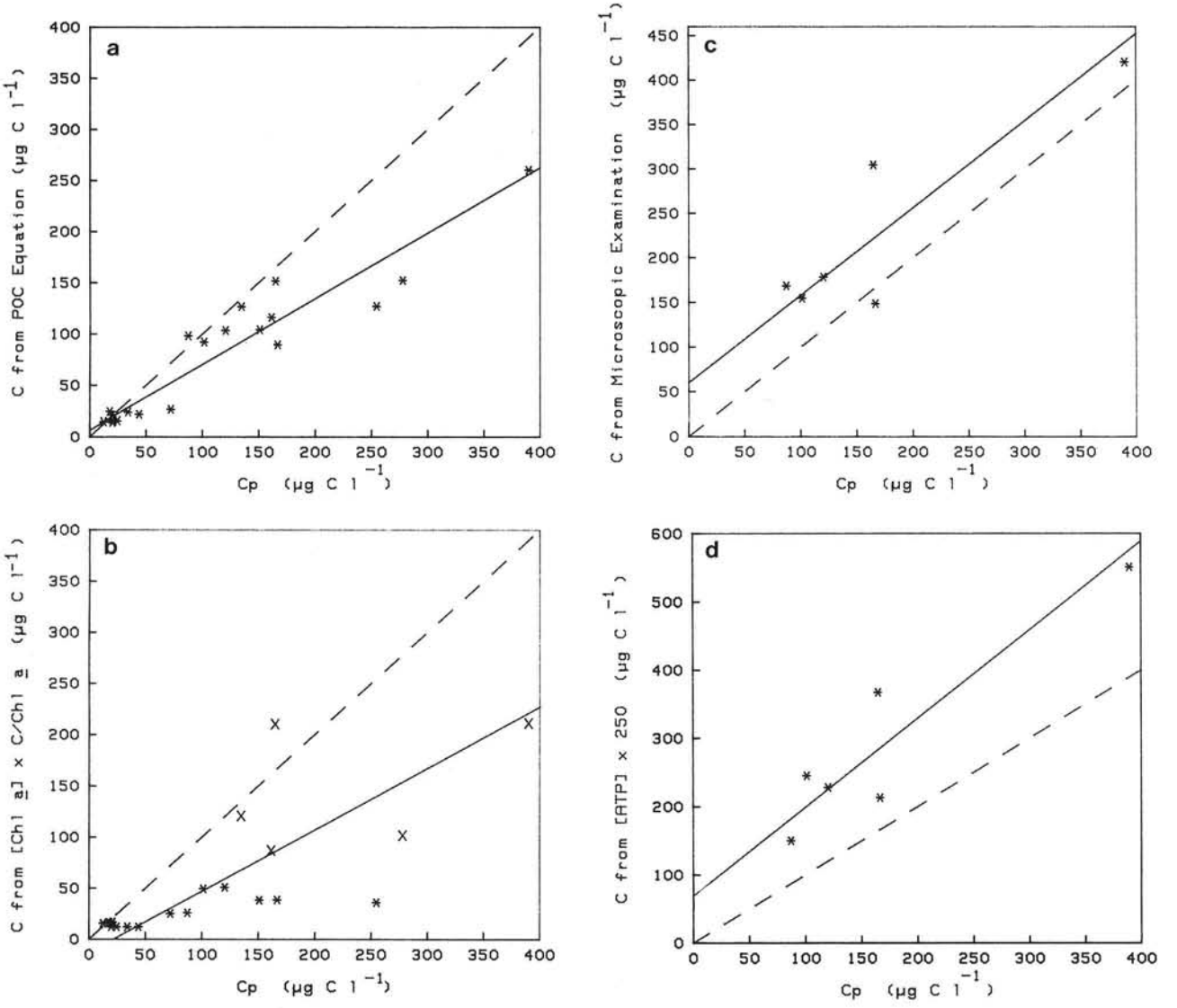

Fig. 5. Comparison of C_p determined by labeled chl *a* method with phytoplankton carbon determined by various standard techniques for natural populations listed in Table 1. In all cases the dashed line has a slope of 1 and passes through the origin. Solid line: particular comparison. (a) C_p vs. POC equation, (C_{POCeq}): $C_{POCeq} = 6.4 + 0.64\ C_p$, $r^2 = 0.891$. Intercept not significantly different from 0 ($p > 0.05$) and slope significantly different from 1.0 ($p < 0.05$). (b) C_p vs [chl *a*] · 25 or [chl *a*] · 100, ($C_{[chl\ a]}$): $C_{[chl\ a]} = -12.9 + 0.6\ C_p$, $r^2 = 0.546$. Intercept not significantly different from 0 ($p > 0.05$) and slope significantly different from 1.0 ($p < 0.05$). (c) C_p vs. C from microscopic examination, (C_{mic}): $C_{mic} = 69.1 + 0.98\ C_p$, $r^2 = 0.799$. Slope not significantly different from 1 and intercept not significantly different from 0 ($p > 0.05$). (d) C_p vs. [ATP] · 250, (C_{ATP}): $C_{ATP} = 69.2 + 1.3\ C_p$, $r^2 = 0.855$. Slope not significantly different from 1 and intercept not significantly different from 0 ($p > 0.05$)

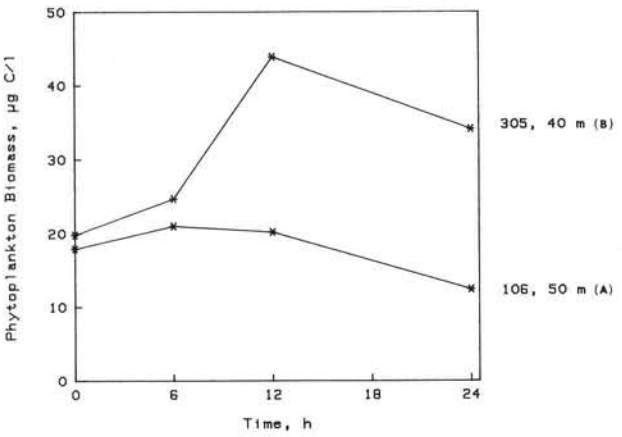

Fig. 6. Variation in C_p with time for samples from fluorescence maximum layer in September, 1981 (SCBS–19)

methods yield comparable values of µ which roughly correspond to cell generation times of 2 to 3 d.

In March, 1982 (SCBS–20), C_p and µ varied both during the day and with depth at Station 305 (Figs. 1, 8 and 9). There was a general increase in C_p through the day followed by a decrease at night at all depths (Fig. 8). As previously seen, µ was greater during the first half of the day and was markedly decreased during the second half (Fig. 9a, b, c). The variation in chl *a* and the POC/PN ratio within the bottles during the incubation are shown in Fig. 10a and b, respectively. The depth integrated µ for Station 305, calculated from the 24 h single endpoint results and extrapolated to the surface and 1 % light penetration depth, was 0.024 h^{-1}, corresponding to a cell generation time of 1.2 d (Fig. 11).

DISCUSSION

Laboratory studies

The results presented by Redalje and Laws (1981) strongly supported the assumption that after incubations of no more than 12 h, $R^*_{C_p}$ and $R^*_{chl\ a}$ were equal for diatoms in both continuous and batch culture. However, at that time, the minimum length of incubation required before $R^*_{C_p} = R^*_{chl\ a}$ was unknown. A 6 h incubation was sufficient for a rapidly growing batch culture of *Thalassiosira (fluviatilis) weissflogii* (Redalje and Laws, 1981). Welschmeyer and Lorenzen (manuscript in review) have presented results from time course batch culture experiments with the diatom *Skeletonema costatum* which indicate that under log phase growth in continuous light, $R^*_{C_p} = R^*_{chl\ a}$ after 12 h. A least squares linear regression of $R^*_{chl\ a}$ vs. $R^*_{C_p}$ had a slope of 1 and passed through the origin, indicating that $R^*_{C_p} = R^*_{chl\ a}$ from the beginning of the incubation (Welschmeyer and Lorenzen, manuscript in review).

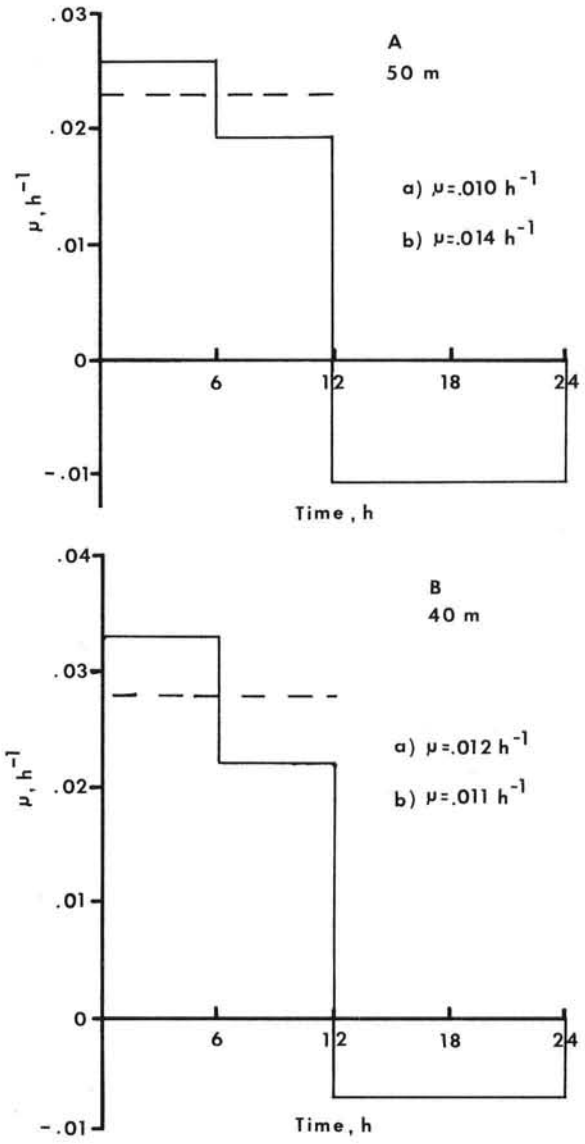

Fig. 7. Variation in µ (h^{-1}) with time for samples from fluorescence maximum layer in September, 1981 (SCBS–19). (A) Station 106; (B) Station 305

This is in contrast to the results of Riper et al. (1979), for the incorporation of ^{14}C labeled δ-aminolevulinic acid into chl *a* in *S. costatum*; they suggested that carbon growth and chl *a* synthesis were uncoupled allowing turnover times of chl *a* substantially more rapid than cell division rates. If chl *a* turnover was substantially greater than cellular C turnover, then $R^*_{chl\ a}$ could be the same as $R^*_{C_p}$ only if the chl *a* precursor pool had the same specific activity as cellular C and if there existed a dynamic equilibrium between the C in the precursor pool and the chl *a*–C pool. This is evidently the case when the cells are labeled with $H^{14}CO_3^-$, as evidenced by the results of this study and those of Welschmeyer and Lorenzen (manuscript in review).

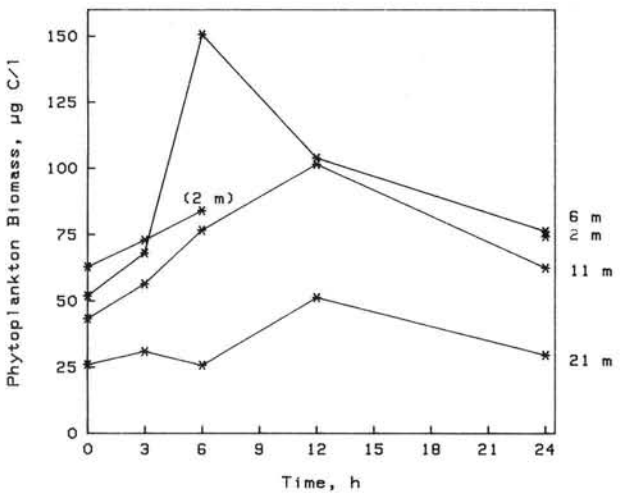

Fig. 8. Variation in C_p with time for samples taken at Station 305 from 2, 6, 11 and 21 m in March, 1982 (SCBS–20)

Comparison of phytoplankton biomass determination

In growing laboratory cultures, lacking detritus and other non-phytoplankton carbon, one can compare any indirect estimate of the phytoplankton carbon biomass to that measured by direct analysis (e.g. with a CHN analyser). However, in the field, one cannot determine the actual concentration of phytoplankton carbon present by comparable chemical analysis. C_p, determined by the labeled chl *a* method has provided an accurate estimate of the phytoplankton carbon in the laboratory, and as such, was used as the standard of comparison for C biomass in the field. The POC equation was thought to give the most reliable estimate of phytoplankton carbon in the Southern California Bight (Eppley et al., 1977). In this study, the POC equation also gave the best agreement to C_p (Table 1, Column B; Fig. 5a, $r^2 = 0.891$); however, the POC estimate was

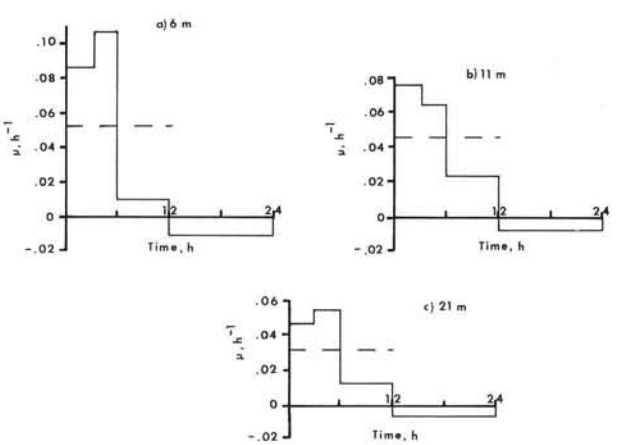

Fig. 9. Variation in µ (h^{-1}) with time for samples taken at Station 305 from (a) 6 m, (b) 11 m, and (c) 21 m in March, 1982 (SCBS–20)

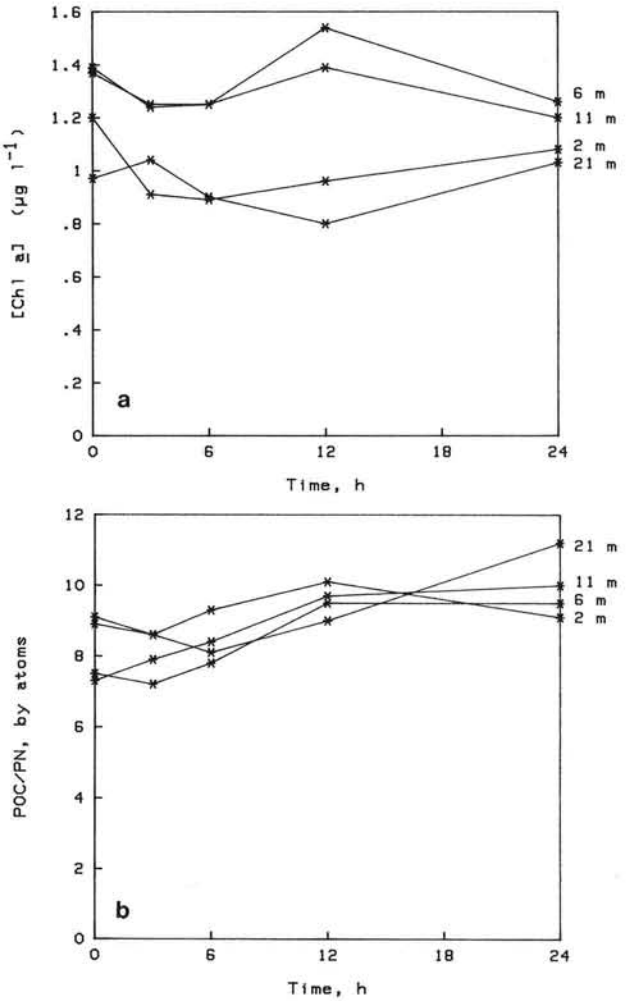

Fig. 10. Variation in chl *a* (a) and POC/PN (b) with time for samples taken at Station 305 from 2, 6, 11 and 21 m in March, 1982 (SCBS–20)

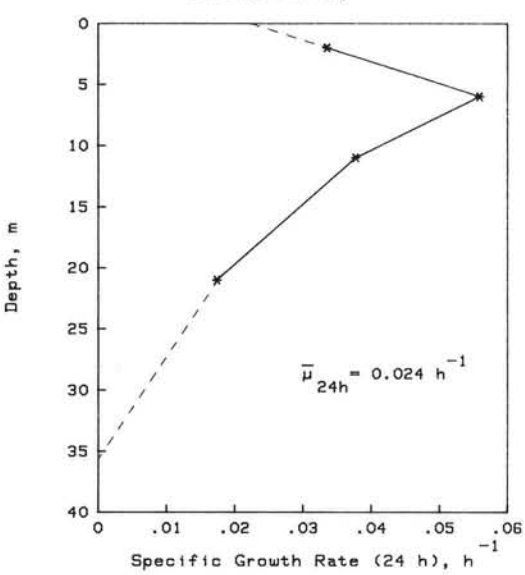

Fig. 11. Variation in µ, based on single 24 h endpoint data for Station 305 in March, 1982 (SCBS–20)

low by about 35 % for phytoplankton C levels up to 400 $\mu g\ Cl^{-1}$. The POC method may give low values because smaller cells (e.g. microflagellates, cyanobacteria, and very small diatoms) may have been neglected in the microscopic analysis upon which the POC equation was based (F.M.H. Reid, pers. comm.).

If some knowledge is assumed about the C/chl *a* ratio of the phytoplankton (e.g. about 100 for near surface samples and about 25 for deeper samples (Cullen, 1982), the phytoplankton C biomass determined from these ratios and measured chl *a* concentrations could underestimate C_p by about 40 % for stations occupied off of Southern California and Baja California in this study (Fig. 5b). The scatter in the regression of phytoplankton C by C/chl *a* ratios vs. C_p also makes use of the ratios unattractive (Fig. 5b, $r^2 = 0.546$). A least squares linear regression (Model II, geometric mean) of POC vs. chl *a* for the stations represented in Fig. 5b yields a slope (= C/chl *a*) of 238 and is significant ($p < 0.01$) although most of the variance remains unexplained ($r^2 = 0.39$). If a C/chl *a* of 238 is used to derive phytoplankton C, then C_p is overestimated by 40 %.

Phytoplankton C based on ^{14}C incorporation over the incubation period, combined with C from either direct microscopic determination of cell counts and cell volumes in the initial water sample and C/volume relationships (Strathmann, 1967), or ATP concentrations in the initial water sample and a C/ATP ratio of 250 (Holm-Hansen and Booth, 1966) systematically overestimate C_p (Fig. 5c, d). These comparisons are based on only 6 data points and as such, the 95 % confidence limits for both the slopes and intercepts were large (Fig. 5c, d); however, the tendency for C_p to be overestimated is clear. The presence of non-phytoplankton ATP and non-photosynthetic organisms may result in an unknown amount of non-phytoplankton C being included in each of these two methods. The POC method, originally based on the relationship between POC and phytoplankton cell counts and volumes, suffers from an unknown amount of contamination as well.

Field studies

The chlorophyll maximum layer off Southern California has been characterized as being dominated by dense aggregations of dinoflagellates with a C/chl *a* ratio of about 70 or by microflagellates or mixtures of flagellates and diatoms with a C/chl *a* ratio of about 25 (Cullen and Eppley, 1981; Cullen, 1982; Cullen et al., 1982). In these layers, the populations appear to grow with generation times of 2 to 2.5 d, which is roughly twice the generation time of the overlying populations (Cullen and Eppley, 1981). Chlorophyll maximum populations sampled in this study grew with generation times of about 2.3 d (Fig. 7 and 9c) and were characterized by C/chl *a* ratios generally between 20 and 40. Near-surface populations grew about 3 times as fast as those in the chlorophyll maximum layer, with generation times of about 0.7 d (Fig. 9) and had C/chl *a* ratios of about 50 to 70. Although some amount of disagreement is expected from the use of different methods, we see a similarity in the generation times and C biomass values derived from the labeled chl *a* method and previous estimates for Southern California Bight populations based on 24 h ^{14}C incubations and phytoplankton C from the POC equation of Eppley et al. (1977).

At Station 305 (SCBS–20, Fig. 1a), the mean growth rate integrated from the surface to the 1 % light level was 0.024 h^{-1} (Fig. 11). This value is roughly twice the average integrated growth rate from previous studies at the same station and time of year derived from 24 h ^{14}C incubations and biomass from the POC equation ($\mu = 0.011 \pm 0.005\ h^{-1}$, n = 3; Eppley, unpubl.)

Time-course ^{14}C incubations carried out on water taken from the fluorescence maximum layer and the overlying water column exhibit diurnal variability in both μ and C_p in a manner similar to that observed in the continuous culture study with *Mantoniella* sp. (c.f. Figs. 4, 6, 7, 8 and 9). There are several consequences of this periodicity which would have a significant bearing on oceanic productivity studies. Incubations conducted for a few hours near midday could give erroneous estimates of phytoplankton production and μ if they are extrapolated to 24 h rates (Fig. 9). However, long-term incubations may lead to complications due to containment effects (Gieskes et al., 1979). The marked decrease in μ observed on SCBS–20 (Fig. 9a, b, c) during the second half of the day may be an artifact due to containment or nutrient depletion within the bottles. Neither chl *a* or C_p exhibited a large decrease during this time period (Fig. 8) suggesting that mortality within the bottles was not a large factor. The POC/PN ratio within the bottles did not exhibit a marked increase in the second half of the day as one would expect had the population become N depleted leading to the reduced values of μ. Periodicity in C_p and μ would have proceeded without detection had not time course samples been taken.

In conclusion, results derived from the labeled chl *a* technique for estimating μ and C_p in the waters off Southern California and Baja California suggest: (1) Previous, indirect methods of estimating phytoplankton biomass tend to give either low (POC and chl *a* methods) or high (ATP and cell counts and volumes) values and that these values, along with measured rates of ^{14}C incorporation, lead to correspondingly high or low estimates of μ. (2) Errors due to use of indirect

biomass estimates are generally less than two-fold and are frequently better.

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