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A New Method for Estimating Phytoplankton Growth Rates and Carbon Biomass

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Abstract

A new method is described for the determination of phytoplankton growth rates and carbon biomass. This procedure is easy to apply and utilizes the labeling of chlorophyll *a* (chl *a*) with ^{14}C. Pure chl *a* is isolated using two-way thin-layer chromatography, and the specific activity of chl *a* carbon is determined. Data from laboratory cultures indicate that the specific activity of chl *a* carbon becomes nearly equal to that of total phytoplankton carbon in incubations lasting 6 to 12 h and can be used to calculate phytoplankton growth rates and carbon biomass. Application of the method to the phytoplankton community in an eutrophic estuary in Hawaii indicates that the cells are growing with a doubling time of about 2 d and that about 85% of the particulate carbon consists of phytoplankton carbon.

Introduction

At present, there is no reliable or widely accepted method for determining phytoplankton carbon biomass in the oceans or for estimating phytoplankton growth rates (Goldman *et al.*, 1979). The two problems are linked, because growth rate (μ, in units of inverse time) is generally calculated by the equation

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

where dC_p/dt is the photosynthetic rate and C_p is the concentration of phytoplankton carbon. The ^{14}C-method of Steeman Nielsen (1952) measures dC_p/dt with acceptable accuracy (e.g. ± 10%), although quantification of C_p, and hence μ, poses a more difficult problem. Measurements of particulate organic carbon (POC) in the ocean provide an upper bound to C_p. However, POC usually has a large component of non-phytoplankton carbon (detritus, zooplankton, etc.), and is thus greater than the true phytoplankton POC, especially in oligotrophic areas (Banse, 1977). An indirect estimate of C_p may be derived from either chlorophyll *a* (chl *a*) or adenosine triphosphate (ATP) concentrations if one makes assumptions about the chl *a*:C_p or ATP:C_p ratios (Steele and Baird, 1961; Holm-Hansen and Booth, 1966; Hunter, 1979; Sinclair *et al.*, 1979). However, such ratios can vary widely, depending upon mean growth rate, time of day, species composition, and the factor or factors limiting growth (Eppley *et al.*, 1971; Eppley and Renger, 1974; Perry, 1976; Laws and Wong, 1978; Hunter, 1979). C_p can also be determined from microscopic cell counts and particle volumes, but this method is tedious and inaccurate because photosynthetic and non-photosynthetic particles are easily confused and because the relationship between cell volume and carbon content varies (Strathmann, 1967; Caperon and Meyer, 1972; Beers *et al.*, 1975). Eppley *et al.* (1977) compared 7 indirect methods of estimating C_p in the field, but the relative accuracy of the methods was difficult to assess since the calculated values could only be compared to the total POC, which as noted provided an upper boundary to the true C_p.

Numerous researchers have attempted to evaluate μ in the ocean (Eppley, 1968; Sutcliffe *et al.*, 1970; Eppley *et al.*, 1972, Swift and Durbin, 1972; Eppley *et al.*, 1973, Weiler and Chisholm, 1976; Saino and Hattori, 1977; Sheldon and Sutcliffe, 1978; Weiler, 1980). Methods used in the above studies include the use of C and N stable isotope ratios, and the use of Eq. (1) combined with values of C_p based on ATP, chl *a*, cell counts or ^{14}C assmilation over long-term incubations, the frequency of cell division, and changes in ATP during short-tem incubations. Growth rates obtained by these

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methods have varied widely, from 0.07 to 8 cell divisions per day (Goldman *et al.*, 1979). Although some of the variation may reflect true differences, much of it is probably due to inaccuracies in the experimental methods which have been used. We report here the development of a new method for estimating both μ and C_p which should prove easy to apply and more accurate than existing techniques.

Our method utilizes ^{14}C-labeling of the chl *a* pool of phytoplankton exposed to ^{14}C-bicarbonate. chl *a* is uniquely associated with photosynthetic organisms, assuring the quantification of solely phytoplankton biomass, and is relatively easy to isolate and measure using thin-layer chromatographic methods (Jeffrey, 1974). In addition, the chl *a* pool appears to turn over much more rapidly that the generation time of the phytoplankton, thus permitting the chl *a* to be labeled uniformly in a matter of hours. For example, Hitchcock (1977) found that phytoplankton can alter cell quotas of photosynthetic pigments within a few hours in response to change in the light environment. In *Chlorella pyrenoidosa* chl *a* has a half-life of only 0.5 h in rapidly growing batch cultures, as indicated by the kinetics of $^{14}CO_2$ incorporation into chl *a* (Grumbach *et al.*, 1978). Riper *et al.* (1979) reported chl *a* turnover times of about 3 h for exponentially growing batch cultures of *Skeletonema costatum* (as compared to about 10 h for stationary-phase cells) after inoculation with ^{14}C-labeled δ-aminolevulinic acid, a precursor of chl *a*. There was also an indication of a direct recycling of chl *a* breakdown products, with a turnover rate of about 100 h (Riper *et al.*, 1979).

When an alga is incubated in the presence of $H^{14}CO_3^-$, the first stable intermediate to be labeled is 3-phosphoglyceric acid (Calvin *et al.*, 1951), which passes through the Calvin cycle and eventually is fixed into carbohydrates. Labeled carbon enters the chl *a* biosynthetic pathway via the condensation of succinyl CoA and glycine to form δ-aminolevulinic acid (Bogorad, 1976), the immediate precursor to the porphyrin ring of chl *a*. Grumbach *et al.* (1978) reported that ^{14}C activity appeared to flow directly from intermediates in the Calvin cycle to the isoprenoid side chains of the chl *a* molecules, with subsequent labeling of the porphyrin ring. Thus, there is good evidence that the labeling of chl *a* carbon occurs rapidly and early in the pathway of carbon metabolism, and that essentially uniform labeling of chl *a* can be achieved using incubations of no more than 6 to 12 h.

The ^{14}C activity in the protein, polysaccharide and lipid pools of *Thalassiosira fluviatilis* in batch culture was relatively constant as a percent of the total particulate ^{14}C activity (same populations as in Experiments 10-12, Table 1) after incubations of only 6 h (Redalje, 1980). Li *et al.* (1980), using similar methods with natural samples of the cyanophyte *Oscillatoria thiebautii* from the Caribbean Sea, found that populations achieved steady-state labeling after only 2 h, at the higher photosynthetic rates studied. Thus, there is also evidence that the ^{14}C activity in macromolecular pools other than chl *a* may become labeled in a matter of hours.

Materials and Methods

In the method described here, we will assume that, after a sufficiently long incubation, the specific activity of the chl *a* carbon ($R^*_{\text{chl }a}$, dis/min μg C^{-1}) is identical to the specific activity of the total phytoplankton carbon pool ($R^*_{C_p}$, dis/ min μg C^{-1}). $R^*_{\text{chl }a}$ and $R^*_{C_p}$ need not be constant, but they must become and remain equal after a sufficient period of time. Based on the foregoing discussion and the data to be presented, incubations of 6 to 12 h appear to be adequate to achieve this equality.

One must measure the ^{14}C activity of a phytoplankton sample at the end of an incubation (A*, dis/min l^{-1}) and determine $R^*_{\text{chl }a}$ (using methods to be described below). Once these quantities are known, one can calculate the C_p at the end of the incubation from the following equation:

$$C_p\ (\mu\text{g l}^{-1}) = \frac{A^*}{R^*_{\text{chl }a}}\ , \tag{2}$$

where $R^*_{\text{chl }a} = R^*_{C_p}$. One can relate the phytoplankton carbon concentrations at the beginning (C_p°) and end (C_p) of the incubation if one assumes exponential growth at a rate, μ, by the equation:

$$C_p = C_p^\circ\, e^{\mu t}\ , \tag{3}$$

where t is the time of incubation (h). The photosynthetic rate (P, μg C l^{-1} h^{-1}) for the incubation is then related to C_p through the equation:

$$Pt = C_p - C_p^\circ = C_p\,(1 - e^{-\mu t}), \tag{4}$$

so that

$$\mu = -\,t^{-1}\,\ln\,(1 - Pt \cdot C_p^{-1})\ . \tag{5}$$

In order to test the feasibility of using Eqs. (2) and (5) to estimate C_p and μ, we studied both continuous and batch cultures of marine phytoplankton in the laboratory and later applied the method in the field.

For the laboratory sites, continuous cultures of the marine diatom *Thalassiosira fluviatilis* Hustedt were established as described by Laws and Bannister (1980), with either nitrate or ammonium as the limiting nutrient. In addition, batch cultures of *T. fluviatilis* were grown in sterile 3-l flasks containing a sterile filtered (0.22 μm) seawater medium enriched with nutrients to the concentrations employed in the medium for the continuous culture studies. In some experiments, the cells were uniformly labeled with ^{14}C by adding $H^{14}CO_3^-$ to the reservoir medium of the continuous cultures or to the initial batch culture medium prior to inoculation with cells. Other cultures were labeled only after steady-state had been achieved in the continuous cultures or after

log phase had been reached in the batch cultures; the medium was inoculated with $H^{14}CO_3^-$, and the ^{14}C uptake was monitored over a set time interval. In conjunction with all experiments, samples were filtered onto Whatman glass-fiber filters (GF/C) for total particulate ^{14}C activity (triplicate 5 ml samples), POC (quadruplicate 25 ml samples), chl *a* (triplicate 25 ml samples) and for the isolation of pure chl *a* by two-way thin-layer chromatography (TLC) methods ($\geqslant$ 100 ml). Particulate ^{14}C activities were measured in a liquid scintillation counter (Searle Analytic Delta 300) in Aquascint fluor (ICN) according to the recommendations of Lean and Burnison (1979) to eliminate residual (^{14}C) bicarbonate activity. Liquid-scintillation counting efficiencies were determined by the external-standard ratio method. POC concentrations were determined on a Hewlett-Packard model 185B CHN analyzer following the recommendations of Sharp (1974). Chl *a* concentrations were measured on a Beckman Acta II spectrophotometer by the procedures of Jeffrey (1974) and Jeffrey and Humphrey (1975). Cellulose plates (MN 300, Macherey-Nagel), as described by Jeffrey (1974), were used for the TLC separations. A modification of the chromatography solvent system recommended by Jeffrey (1974) to a more polar solvent system isolated the chl *a* completely. The solvent system used was 0.5 to 1.0% (v/v) n-propanol in ligroine (63° to 75°C boiling range, Eastman) for the first dimension and 40% (v/v) dichloromethane in ligroine for the second dimension. We found that adjustment of the relative concentrations of the solvents is necessary to achieve adequate isolation of the chl *a* for samples from different cultures or natural environments. After the plate had been developed in both dimensions, the chl *a* spot was easily identified from its red fluorescence when exposed to long wavelength UV light (Mineralight ^{R}UVSL-13).

After the pigment had been isolated, the chl *a* spot was scraped from the plate and allowed to extract in 0.5 ml of 90% acetone at −20°C under N_2 for at least 1 h. The extracted pigment was then separated from the cellulose by filtration on a GF/C filter followed by 2 rinses with 90% acetone to bring the final filtrate to 3 ml. A 0.5 ml aliquot of the filtrate was then taken, diluted to 5 to 10 ml, and the chl *a* concentration measured on a Turner model 111 fluorometer which had been calibrated with pure chl *a*. The remaining 2.5 ml of filtrate was mixed with 10 ml of liquid scintillation cocktail (LSC) and the ^{14}C activity was measured on a liquid scintillation counter. The 2.5 ml of acetone was sometimes evaporated *in vacuo* prior to addition of the LS cocktail to reduce quenching. $R^*_{\text{chl }a}$ was then determined from the equation:

$$R^*_{\text{chl }a} = \frac{^{14}\text{C activity in chl } a \text{ (dis/min l}^{-1})}{\text{chl } a \ (\mu\text{g} \times \text{l}^{-1}) \ (0.7399)}, \qquad (6)$$

where 0.7399 is the fraction of the molecular weight of chl *a* contributed by carbon.

In the field experiments, water samples were collected just prior to sunrise in 20-l plastic carboys from a depth of 0.5 m in Kaneohe Bay, Hawaii. A 4-l subsample was inoculated with 500 μCi of $H^{14}CO_3^-$ and incubated at a depth of 1 m in Pyrex bottles from sunrise to sunset. From the remaining water, triplicate subsamples were filtered onto GF/C filters for the determination of initial concentrations of POC (250 ml) and chl *a* (100 ml, measured by fluorometry). At sunset the incubation was terminated, and triplicate subsamples were collected onto GF/C filters for measurement of total particulate ^{14}C activity (50 ml), final chl *a* concentration (100 ml) and final POC (250 ml). The remaining volume of the sample was filtered onto a GF/C filter, placed in 100% acetone under N_2, and stored in a freezer for subsequent TLC analyses, which were performed in triplicate or quadruplicate.

Because incubations lasting more than a few hours may sometimes result in erroneously low primary production measurements (Gieskes *et al.*, 1979; Smith and Barber, 1979), we performed an additional experiment in Kaneohe Bay to determine whether incubations as long as 12 h yielded results consistent with a series of short-term incubations. Twelve 1-h ^{14}C incubations were performed at a fixed location in the bay, and the results were compared to the total primary production measured during a 12 h incubation. The initial water samples were collected just at sunrise, dispersed into 300 ml glass biological-oxygen-demand bottles, inoculated with $H^{14}CO_3^-$ (5 μCi) and incubated at 1 m. One set (each set consisted of two light and one dark bottle) was incubated for the entire 12 h period; the second set was stopped and replaced each hour with bottles filled with water taken from the same location just prior to the start of each hourly incubation. At the end of each incubation triplicate 5 ml aliquots were withdrawn from each bottle and injected into LSC vials containing 0.5 ml of 1.2 N HCl to stop biological activity. ^{14}C activity in these samples was determined by the technique of Schindler *et al.* (1972).

Results

Table 1 summarizes the results of applying Eq. (2) to estimate C_p in continuous and batch cultures using both uniform labeling and shorter-term incubations lasting 6 to 24 h. When the mean of the ratios of estimated to experimentally measured C_p are calculated, that for the uniformly labeled cultures is 0.97 ± 0.2 (± SD) and that for the cultures incubated for 10 to 12 h is 1.07 ± 0.11 (± SD). The overall mean ratio (± SD) for cultures labeled for 10 h or longer is 1.03 ± 0.16.

A plot (Fig. 1) of the experimentally measured values of $R^*_{\text{chl }a}$ and $R^*_{C_p}$ shows the close correspondence between the two parameters. The slope (1.02) of the Model II regression line fit to the data is not significantly different from 1 ($P > 0.1$), and the intercept (− 1.45) is not significantly different from zero ($P > 0.1$).

In the 12 h time-series study in Kaneohe Bay, the mean hourly rate of primary production calculated from the 1 h incubations was 13.2 ± 1.2 μg C l^{-1} h^{-1}, whereas

Table 1. *Thalassiosira fluviatilis*. Estimated and actual values of C_p under a variety of NH_4^+-limited culture conditions (Experiment No. 4 was a NO_3^--limited culture)

Culture conditions and Experiment No.	C_p (μg-at C l^{-1})		% accuracy of estimate (estimate/actual × 100)
	Estimated	Actual	
Continuous culture, constant light, finite incubations			
(1) $\mu = 0.624\ d^{-1}$, 24 h incubation	534.61	524.02	102.0
(2) $\mu = 0.960\ d^{-1}$, 12 h incubation	584.38	463.49	126.1
(3) $\mu = 1.170\ d^{-1}$, 12 h incubation	404.74	402.00	100.7
(4) $\mu = 0.392\ d^{-1}$, 12 h incubation	526.75	472.58	111.5
Continuous culture, uniform labeling, light/dark cycle			
(5) $\mu = 0.314\ d^{-1}$, dark period (07.30 hrs)	309.06	294.23	105.0
(6) $\mu = 0.314\ d^{-1}$, light period (14.00 hrs)	556.14	428.42	129.8
(7) $\mu = 0.515\ d^{-1}$, dark period (07.30 hrs)	353.60	416.74	84.8
Batch culture			
(8) uniform labeling	478.66	557.55	85.9
(9) uniform labeling	502.69	616.13	81.6
(10) $\mu = 0.760\ d^{-1}$, 6 h incubation	946.95	801.91	118.1
(11) $\mu = 0.640\ d^{-1}$, 10 h incubation	946.92	919.83	103.0
(12) $\mu = 0.720\ d^{-1}$, 12 h incubation	935.46	958.54	97.6

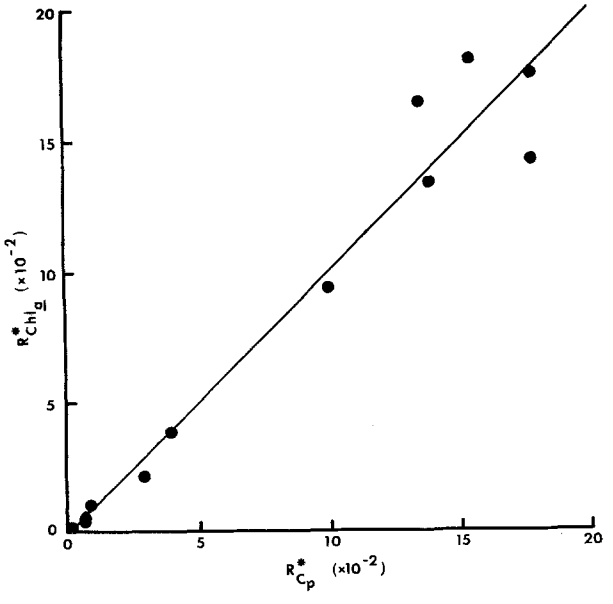

Fig. 1. *Thalassiosira fluviatilis*. Relationship between $R^*_{chl\,a}$ and $R^*_{C_p}$ for the 12 experiments listed in Table 1. The Model II, geometric mean, least-squares, linear regression equation $R^*_{chl\ a} = -1.45 + 1.02\ (R^*_{C_p})$ is significant at the 1% level ($r = 0.974$, DF = 10)

the hourly rate calculated from the single 12 h incubations was 14.1 μg C l^{-1} h^{-1}. Dark-bottle counts in the 12 h incubation were 5% of light-bottle counts.

Table 2 summarizes the growth rate and C_p estimates made in Kaneohe Bay on 13 November and 6 December 1979. The mean 24 h growth rates were estimated from the dawn-to-dusk growth rates by multiplying the dawn-to-dusk growth rate by $t/24$, where t is the photoperiod in hours, and by 0.85 to correct for dark respiration losses, which were assumed to be approximately 15% of daytime production rates (Steemann Nielsen and Hansen, 1959; Laws and Wong, 1978).

Table 2. Estimates of *in situ* specific growth rates (μ) and phytoplankton carbon concentrations (C_p) in Kaneohe Bay, Hawaii, on indicated dates. Samples were incubated from dawn to dusk. Standard errors indicated in partheneses

Date (1979)	Incubation time (h)	μ (d^{-1}) for:		C_p ($\mu g C\ l^{-1}$)	POC ($\mu g C\ l^{-1}$)	Pt ($\mu g C\ l^{-1}$)	$\frac{Pt}{\Delta POC}$	Chl a ($\mu g\ l^{-1}$)	C_p:Chl a ($\mu g\ \mu g^{-1}$)
		photoperiod	24 h						
13 November									
	0	–	–	–	207 (7)	–	–	0.76 (0.01)	–
	11.70	1.00 (0.04)	0.41 (0.01)	288 (9)	328 (2)	111	0.92	1.19 (0.06)	242
6 December	0	–	–	–	199 (5)	–	–	0.49 (0.03)	–
	11.25	0.86 (0.04)	0.34 (0.01)	261 (9)	311 (2)	86	0.77	0.96 (0.05)	272

Discussion

The results of the continuous and batch-culture studies strongly support the assumption that for practical purposes $R^*_{\text{chl } a}$ and $R\hat{C}^*_p$ are identical after incubations of no more than 12 h. The slowest growth rate studied, 0.392 d^{-1}, corresponds to a doubling time of 1.8 d, and is well within the range of estimated phytoplankton growth rates in oligotrophic parts of the ocean (Goldman *et al.*, 1979). The results are consistent with the earlier work of Grumbach *et al.* (1978) and Riper *et al.* (1979) in showing that incubation times of no more than 12 h are needed to achieve equality of $R^*_{\text{chl } a}$ and $R\hat{C}^*_p$, even for slowly growing populations (see Experiments 10–12: Table 1; the dense populations probably experienced some degree of light-limited growth).

The work of Li *et al.*, (1980) and Redalje (1980) indicates that under certain conditions, the carbon pools of protein, polysaccharides and lipids achieve steady-state labeling. However, specific activities of the carbon pools of these macromolecules were not determined in either of the above studies, and comparisons with $R^*_{\text{chl } a}$ and $R\hat{C}^*_p$ cannot be made.

The results of the 12 h time-series incubation in Kaneohe Bay indicate that at least in this system incubations of up to 12 h can be used to study phytoplankton photosynthesis. However, this condition will certainly not be found in all parts of the ocean. For example, Smith and Barber (1979) found that incubations of no more than 1 h were required to study photosynthesis by the ciliate *Mesodinium rubrum* in the Peru current because of the organism's sensitivity to confinement and rapid nutrient exhaustion. Gieskes *et al.* (1979), in a study of primary production in the North Atlantic Equatorial Current, reported clear evidence of algal mortality when cells were confined in 30 or 300 ml bottles from sunrise to sunset, and at one station reported a serious "bottle enclosure effect" even in 4-l bottles incubated over a similar time. In Kaneohe Bay, ^{14}C production rates derived from incubations in 60 ml bottles (5.83 $\mu g\ C\ l^{-1}\ h^{-1}$) were similar to those in 4-l bottles (6.52 $\mu g\ C\ l^{-1}\ h^{-1}$; Redalje, 1980). However, Sheldon *et al.* (1973) presented results indicating higher rates of production in 50-ml bottles than in 4-l bottles, the higher rates being attributed to the absence of grazing. We conclude that in applying our method, use of 4-l bottles will be essential not only to provide an adequate amount of chl a for TLC analysis, but also to minimize bottle-enclosure effects. Moreover, time-series incubations similar to those reported here should be performed to insure that production rates are not being affected by bottle confinement.

Whether incubations of as long as 12 h are required for the application of our method remains an open question. Estimates of phytoplankton doubling times as short as 3 to 3.5 h have been reported in some oligotrophic marine areas (Goldman *et al.*, 1979), although such doubling times are less than half the minimum doubling times of 8 to 12 h reported for laboratory cultures in the temperature range 20° to 25 °C by Goldman and Carpenter (1974). However, it is possible that phytoplankton may often be growing with doubling times of less than 12 to 24 h, and from our own results as well as those of Grumbach *et al.* (1978) and Riper *et al.* (1979), it is likely that in such cases incubations of no more than 3 to 6 h will be required to achieve equality of $R^*_{\text{chl } a}$ and $R\hat{C}^*_p$. However, if incubations less than one-half the daily photoperiod are used, either some correction for diel periodicity in photosynthetic rates will be required, or a time-series of incubations will be needed to obtain an average growth rate over the course of the photoperiod.

The two experiments in Kaneohe Bay, although not extensive, provide some interesting insights. In both experiments the total rate of primary production during the photoperiod agreed well with the observed change in POC, suggesting that phytoplankton carbon made a major contribution to the total POC. This speculation is borne out by the calculated C_p estimates, which were both about 85% of the total POC values. It is generally assumed that C_p contributes a higher percentage of POC in eutrophic than oligotrophic systems, and the high percentage of C_p in Kaneohe Bay, which is highly eutrophic compared to the adjacent open ocean, is in accord with this assumption.

The C_p:chl a ratios calculated for the Kaneohe Bay phytoplankton seem high compared to commonly assumed ratios of 50 to 100 (Strickland and Parsons, 1972), but are not surprising when one examines the estimated 24 h growth rates of 0.41 and 0.34 d^{-1}. These rates are considerably lower than the maximum growth rates of 1.8 d^{-1} estimated by Goldman and Carpenter

(1974) for laboratory cultures at 24 °C (Kaneohe Bay temperature was 24° to 25 °C at the time of the experiments); thus, although maximum growth rates are species-specific, it seems likely that most of the Kaneohe Bay phytoplankton species, which consist largely of diatoms (Redalje, unpublished data), are capable of growing at rates well in excess of 0.34 to 0.41 d^{-1} under ideal conditions at 24° to 25 °C. The C_p :chl *a* ratio in phytoplankton is negatively correlated with growth rate under nutrient limitation, and for diatoms the ratio can easily exceed 200 to 300 μgC μg chl a^{-1} at growth rates less than half the maximum growth rate (Laws and Bannister, 1980). We conclude that at the time of the present studies the Kaneohe Bay phytoplankton population was largely nutrient-limited and was growing at a rate less than half of its potential maximum. This conclusion tends to contradict the often-held notion that phytoplankton in nutrient-rich environments are growing rapidly. The standing crop of phytoplankton in Kaneohe Bay appears to be large, but its turnover rate is evidently low compared to the maximum rate sustainable under ideal conditions.

Although the method for estimating μ and C_p presented here appears to have worked well in its first field applications and may be widely applicable, several limitations to the method deserve consideration. First, it is imperative that physiological damage to cells does not occur during confinement in the incubation bottles. Such damage will be minimized if large bottles (e.g. 4-l) are used and incubations are confined to $\leqslant$ 12 h. Based on our work and that of Grumbach *et al.* (1978) and Riper *et al.* (1979), an incubation time of 12 h is more than adequate to assure that $R^*_{\text{chl } a} = R^*_{C_p}$. Where populations are growing with doubling times of 12 to 24 h or less, 6 h incubations will likely be adequate. If shorter incubations are used, some account must be taken of diel periodicity in the estimation of μ.

It is important that the concentration of chl *a* in the water be sufficiently high so that the chl *a* spot on the TLC plate is easily discernible. In highly oligotrophic areas this condition may pose a problem and necessitate the use of larger bottles or several 4-l bottles.

In addition, the effects of zooplankton grazing during the incubation are ignored in Eqs. (2) and (5). Although it would be difficult to quantify carbon losses due to grazing, such losses may result in a reduced estimate of C_p and an overestimate of μ. In the field experiments, the good agreement between production and observed increases in POC (Table 2) may indicate that grazing was negligible on these occasions. However, it is probable that some of the increase in POC is the result of bacterial conversion of excreted dissolved organic carbon to POC (Derenbach and Williams, 1974). The POC produced in this way would offset grazing losses to some degree. Since the observed increase in POC was greater than production in both cases (Table 2), one must conclude that heterotrophic production was significant and that combined losses to grazing and respiration cannot be evaluated.

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