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An incubation procedure for estimating carbon-to-chlorophyll ratios and growth-irradiance relationships of estuarine phytoplankton

C. L. Gallegos[1,*], W. N. Vant[2]

[1]Smithsonian Environmental Research Center, PO Box 28, Edgewater, Maryland 21037, USA
[2]National Institute of Water and Atmospheric Research, PO Box 11–115, Hamilton, New Zealand

ABSTRACT: The carbon-to-chlorophyll ratio of phytoplankton, θ, is difficult to determine by direct chemical measurement because natural waters also contain particulate carbon due to heterotrophic organisms and detritus that cannot be separated from the phytoplankton. When growth is balanced phytoplankton produce new C and chlorophyll in proportion to θ, but growth will be unbalanced in the short-term when there is accumulation of C that has not had time to be proportionately allocated to chlorophyll, or when the phytoplankton are adjusting θ to a new light regime (i.e. photoadaptation). We conducted incubations in Manukau Harbour, New Zealand, to estimate θ from increments in ^{14}C and chlorophyll using highly diluted water (fraction of unfiltered seawater = 0.05 to 0.1) to greatly reduce grazing by microzooplankton. Estimated θ ranged from 21.5 to 46.6 mg C (mg chl *a*)$^{-1}$, typical of healthy, nutrient-sufficient diatoms. Maximal growth rates varied from about 1 to 2 d^{-1}, and C- and chlorophyll-based growth rates agreed well with one another. Growth rates predicted from separate, short-term measurements of photosynthesis-irradiance (*P-I*) curves agreed well with light-saturated rates measured in 24 h incubations, but were generally higher than the 24 h measurements at lower irradiances, possibly due to greater effect of respiration in the longer incubations. Dilution had contrasting effects on chlorophyll and ^{14}C increments because grazed chlorophyll was degraded, but grazed C appeared to be conserved in the particulate matter. Failure to use diluted water for the incubations would have resulted in large overestimates in θ. We constructed a model of ^{14}C tracer flux and chlorophyll production to explore the consequences of unbalanced growth, e.g. photoadaptation, on estimates of θ determined using incubations substantially free of grazing. Simulations indicated that accurate estimates of θ can be obtained by commencing 24 h incubations prior to sunrise before new C accumulates, and by avoiding major shifts in the range of light intensities to which the phytoplankton are adapted. The procedure should be applicable in other environments provided precautions about sunrise start and avoidance of light shifts and photoinhibiting irradiances are observed.

KEY WORDS: Phytoplankton · Carbon-to-chlorophyll ratio · Growth rate · Estuarine

INTRODUCTION

The important role of phytoplankton in the global carbon budget has given new impetus to the accurate determination of standing stocks, carbon fixation, and growth rates of phytoplankton. Much emphasis has been given to the validation of the ^{14}C technique for estimating phytoplankton carbon fixation, and to the development of remote sensing techniques for improving temporal and spatial coverage of phytoplankton pigment concentrations. In spite of advances in these areas, determination of the amount of carbon contained in living phytoplankton in a sample of seawater has remained problematic. Any sample of seawater contains, in addition to phytoplankton, particulate carbon as heterotrophic organisms and nonliving detrital and fecal particulate material

*E-mail: gallegos@serc.si.edu

(Banse 1977). This non-phytoplankton carbon ranges in size from bacteria (<1 µm) to large zooplankton (100s of µm) and overlaps completely with the size range of phytoplankton, making separation by filtration impossible. The chlorophyll in seawater is almost entirely associated with living phytoplankton, but the ratio of phytoplankton C to chlorophyll (chl *a*), θ, varies over a wide range with species and growth conditions (Chan 1980).

The ^{14}C pigment labeling method, introduced by Redalje & Laws (1981) and modified by Welschmeyer & Lorenzen (1984) and Goericke & Welschmeyer (1993), provides an estimate of specific growth rate and θ from the specific activity of ^{14}C-labeled chl *a* after a period of incubation in the light. The method relies on the assumption that phytoplankton growth is balanced so that ^{14}C fixation and ^{14}C incorporation into chl *a* proceed at the same specific rate (Gieskes et al. 1993). Serious bias in growth rate estimates can occur if growth is unbalanced due to photoadaptive changes in the compositional ratios of the phytoplankton (Goericke & Welschmeyer 1993). Another process that potentially confounds interpretation of ^{14}C tracer experiments is grazing, particularly by microzooplankton, that can occur inside incubation bottles (Eppley 1980). In order to circumvent uncertainties in the measurement and interpretation of ^{14}C uptake, more recent applications of the ^{14}C labeling of chl *a* have stressed labeling kinetics of the pigment pools to estimate specific growth rates irrespective of total carbon uptake (Welschmeyer & Lorenzen 1984, Goericke & Welschmeyer 1993). The chlorophyll-labeling technique is capable of estimating specific growth rate even in the presence of herbivory, because it relies on the specific activity of pigment, not on absolute concentrations. The chlorophyll-labeling technique, however, requires multiple applications of high performance liquid chromatography (HPLC) to isolate radiolabeled chl *a*, and care must be taken to assure that the pigments are not contaminated by unknown ^{14}C-labeled compounds (Goericke & Welschmeyer 1992, 1993). A simple alternative to chlorophyll labeling for estimating θ in natural phytoplankton remains a desirable goal for those without access to preparatory HPLC.

When phytoplankton growth is balanced θ may be estimated from the ratio of the increments of cellular particulate C and chl *a* (Eppley 1968), termed the C:chl *a* production ratio (Goericke & Welschmeyer 1993). In culture the C:chl *a* production ratio agreed well with direct determinations of θ on the particulate matter and with estimates made by the ^{14}C-labeling technique (Welschmeyer & Lorenzen 1984). With natural populations however, the increments in chlorophyll concentration were generally less than implied by the patterns of ^{14}C labeling (Welschmeyer & Lorenzen 1984, Welschmeyer et al. 1991, Goericke & Welschmeyer 1993). Grazing by microzooplankton was believed to be the cause of the lower increments in pigment concentrations relative to the rates of synthesis implied by the ^{14}C labeling of chlorophyll (Welschmeyer et al. 1991).

The dilution technique (Landry & Hassett 1982) for estimating rates of microzooplankton grazing also provides an estimate of phytoplankton growth rates. In a series of incubations in which whole seawater has been diluted with varying amounts of filtered water from the same site, the phytoplankton growth rate is given as the intercept of apparent growth rate regressed against fraction of whole (unfiltered) seawater. In applying the dilution technique in eutrophic waters in which the feeding kinetics of the microzooplankton might be expected to be nonlinear, Gallegos (1989) showed that the phytoplankton growth rate could still be resolved by employing highly diluted incubations of about 95% (i.e. X, fraction whole seawater, = 0.05). If highly diluted incubations can be considered to result in virtually complete elimination of grazing, then the potential exists to estimate the C:chl *a* production ratio by measuring simultaneously ^{14}C uptake and the chl *a* increment in highly diluted incubations. Provided growth is balanced, the ratio of ^{14}C uptake to chl *a* produced will give an estimate of θ.

Finally, the photosynthesis-irradiance (P-I) relationship for phytoplankton is easily measured and is an important tool in the assessment of regional and annual phytoplankton production (Platt & Sathyendranath 1988). If an estimate of θ is available, it then becomes possible to convert daily rates of C uptake to estimates of phytoplankton growth rate (μ). The growth-irradiance (μ-I) relationship is useful for modeling water column population dynamics and for assessing the factors limiting growth of natural phytoplankton.

Here we report results of experimental and modeling investigations to test the utility of C and chl *a* increments in highly diluted incubations for estimating the C:chl *a* production ratio and μ-I relationships of estuarine phytoplankton. We constructed a model of tracer flux through a precursor pool to pigment and cellular C to test the consequences of unbalanced growth and photoadaptation on estimated C:chl *a* production ratio. The model and experiments indicated that the C:chl *a* production ratio should give good estimates of θ provided that the plankton are sampled prior to sunrise when precursor pools are expected to be empty, and provided major shifts in growth irradiance are avoided. Growth-irradiance (μ-I) relationships for C and chlorophyll-specific rates agreed closely with one another.

THEORY

C:chl *a* production ratio. The differential equation for phytoplankton biomass in the presence of grazing may be written (Landry & Hassett 1982):

$$\frac{dB}{dt} = (\mu - g)B \quad (1)$$

where B is phytoplankton biomass (mg m^{-3}), μ (d^{-1}) is specific growth rate, and g (d^{-1}) is grazing loss rate (see Table 1). When considering microzooplankton as grazers, Landry & Hassett (1982) showed that estimates of μ and g could be made by incubating a water sample at a series of dilutions with filtered water from the same site. Dilution reduces the encounter rates between phytoplankton and their microzooplankton grazers so that the impact of grazers on phytoplankton growth becomes progressively reduced as the proportion of undiluted water declines. Under the assumption that microzooplankton clearance rate remains constant as the phytoplankton become more dilute (i.e. microzooplankton feeding kinetics are linear), and that microzooplankton biomass remains constant, the average net specific rate of biomass growth, μ_n, may be solved as a function of the proportion of undiluted water, X, after a time period, Δt

$$\mu_n(X) = \frac{1}{\Delta t} \cdot \ln\left(\frac{B_X(\Delta t)}{XB_{X=1}(0)}\right) = \mu - X\ g \quad (2)$$

where $B_X(\Delta t)$ and $XB_{X=1}(0)$ are, respectively, the final and initial phytoplankton biomasses at dilution X (Landry & Hassett 1982). In a series of incubations at a range of values of X, a plot of μ_n versus X should yield a line with slope = $-g$ and intercept = μ.

As noted above, μ may be estimated regardless of whether the feeding kinetics are linear, provided sufficiently dilute incubations are conducted so that the linear range of the feeding response curve is resolved (Gallegos 1989). In that case μ may be estimated by extrapolation of a few highly diluted incubations back to the origin, $X = 0$. That is, regardless of the microzooplankton feeding kinetics, $\mu_n(X) \rightarrow \mu$ as $X \rightarrow 0$.

As initially introduced, phytoplankton biomass was measured as the concentration of chl *a* (Landry & Hassett 1982), although Eq. (1) also holds for cell numbers (Landry et al. 1984) and taxon-specific accessory pigments (Burkhill et al. 1987). In principle, the technique can be applied to the change of carbon biomass, but as

Table 1. Definition of symbols and terminology used in measurement of carbon-to-chlorophyll ratio and modeling tracer flux in phytoplankton

Symbol	Definition
B	Phytoplankton biomass, mg m^{-3}
C_1	Concentration of 'new' (i.e. recently fixed) phytoplankton carbon, mg m^{-3}
C_2	Concentration of 'old' (i.e. producing biomass) phytoplankton carbon, mg m^{-3}
C_P	Phytoplankton carbon, mg m^{-3}
D	Photoperiod (d)
F_1, F_2	^{14}C fixed in pools C_1 and C_2, mg m^{-3}
g	Phytoplankton mortality rate due to microzooplankton grazing, d^{-1}
I	Incident irradiance, µmol quanta m^{-2} s^{-1}
I_{max}	Maximum incident irradiance at solar noon, µmol quanta m^{-2} s^{-1}
I_k	Light saturation parameter, µmol quanta m^{-2} s^{-1}
m	Rate constant for transfer of carbon from C_1 to C_2, d^{-1}
P	Specific photosynthesis rate, d^{-1}
P_{max}	Maximal specific photosynthesis rate, d^{-1}
P_m^B	Maximal photosynthesis rate normalized to chl *a*, mg C (mg chl *a*)$^{-1}$ h^{-1}
R_1, R_2	Specific respiration rates of the carbon pools C_1 and C_2, d^{-1}
R_3	Specific rate of chlorophyll degradation, d^{-1}
t	Time (d)
X	Fraction of unfiltered water in incubation
ΔC_P	Increment in phytoplankton carbon measured by ^{14}C uptake, mg m^{-3}
Δchl *a*	Increment in phytoplankton chl *a*, mg m^{-3}
Δt	Duration of incubation (d)
μ	Phytoplankton specific growth rate, d^{-1}
μ_n	Net specific growth rate, $\mu - g$, d^{-1}
μ^C, $\mu^{chl\,a}$	Carbon- and chl *a*-specific growth rates, d^{-1}
μ_m^C, $\mu_m^{chl\,a}$	Maximal carbon- and chl *a*-specific growth rates, d^{-1}
θ, θ_B	Ratio of carbon to chlorophyll in phytoplankton biomass, mg C (mg chl *a*)$^{-1}$
θ_A	Proportion of C_1 used to produce chl *a*, mg C (mg chl *a*)$^{-1}$
θ_P	Ratio of increments in carbon and chl *a*, $\Delta C_P : \Delta$chl *a*, mg C (mg chl *a*)$^{-1}$

noted above, the concentration of particulate carbon in living phytoplankton is difficult to estimate because of heterotrophic organisms and organic detritus. However, the ^{14}C technique measures the change, ΔC_P, in phytoplankton carbon (C_P), which is related to carbon-based growth by (Eppley 1972):

$$\Delta C_P = C_P(\Delta t) - C_P(0) = C_P(0)\cdot\left(e^{\mu^C \Delta t} - 1\right) \quad (3)$$

where μ^C (d^{-1}) is the carbon-based growth rate.

If grazing can be neglected by virtue of nearly complete dilution, then a similar relation holds for the increment in chlorophyll.

$$\Delta \text{chl}\,a = \text{chl}\,a(\Delta t) - \text{chl}\,a(0) = \text{chl}\,a(0)\left(e^{\mu^{\text{chl}\,a}\Delta t} - 1\right) \quad (4)$$

where $\mu^{\text{chl }a}$ is the chlorophyll-specific growth rate (d^{-1}). If growth is balanced then by definition all cellular components increase at the same specific rate, i.e. $\mu^C = \mu^{\text{chl }a}$. Because at any time $\theta = C_P/\text{chl } a$, it follows from Eqs. (3) and (4) that if growth is balanced, the C:chl *a* production ratio, $\Delta C_P/\Delta$chl *a*, gives an estimate of θ. We denote the ratio of increments in C and chl *a* as θ_P. Our intent here is to develop an incubation procedure to estimate θ in natural phytoplankton populations based on Eqs. (3) and (4), while employing dilution to minimize microzooplankton grazing.

STUDY SITE AND METHODS

Site description. Experiments to measure phytoplankton C:chl *a* production ratios and μ-*I* relationships were carried out in Manukau Harbour on 4 occasions during the austral summer of 1994-1995. Manukau Harbour (37° S, 174° E) is a turbid, shallow, macrotidal estuary west of Auckland on North Island of New Zealand. Samples for this study were collected from the region previously designated northeast (Vant & Budd 1993). Secchi depth at the site varies from <0.5 to ~1.4 m. Salinity ranges from 28 to 33 (practical salinity scale), with the water column generally vertically well mixed by strong tidal currents. Mean tidal range varies from 2 m neap to 3.4 m spring. Nutrient concentrations are high due to discharge from a waste treatment plant about 4 km from the sampling site. Concentrations of total inorganic N generally exceed 35 µM but can briefly drop to <5 µM when phytoplankton biomass is high in late summer due to an annually recurring bloom of the large diatom *Odontella sinensis*. Soluble reactive P varies from about 3 to 6 µM (Vant & Budd 1993). Chl *a* concentrations generally vary seasonally from 5 to 15 mg m^{-3}, except during the summer blooms of *O. sinensis* when concentrations as high as 66 mg m^{-3} have been measured (Vant & Budd 1993); but most years the bloom peaks at about 25 to 50 mg m^{-3} (W. N. Vant unpubl. data).

Experimental procedures. Incubations were carried out on samples highly diluted with filtered water to minimize grazing by microzooplankton. Water for the diluent was collected by bucket from an abandoned harbor bridge and, due to the time required to prepare the large volume of water, filtered the afternoon prior to the incubation. We filtered first through a 125 mm GF/C filter then through 47 mm GF/F filters because of the high concentrations of suspended solids (Vant 1991).

Water for plankton was collected prior to sunrise the morning of the incubation, except on 2 November when the sample was collected at midday. We added 2.4 l of unfiltered water containing phytoplankton and microzooplankton to about 45 l of diluent to make a suspension having a dilution factor (i.e. fraction of unfiltered water, X) of about 0.05. Initial chlorophyll analyses of diluted and undiluted plankton samples indicated that achieved dilution factors ranged from 0.052 to 0.062. Water was collected from the bridge using a slowly sinking bucket covered with a 200 µm Nitex net to exclude larger zooplankton. We modified this protocol slightly for the February experiment to avoid excluding the large diatom *Odontella sinensis* from the incubation. On that date we collected an unscreened sample and we used a target dilution factor of 0.1 to include a greater proportion of the larger cells.

The diluted sample was gently stirred for about 5 min with a plastic rod, then dispensed into twelve 2.4 l clear polycarbonate bottles for determination of chlorophyll growth. Replicate bottles were placed in 6 trays located in an open field and cooled with running tap water. Temperature of the tap water was within ±1°C of harbor temperatures and generally was stable to within ±1°C during the incubations. All trays were covered with a green shade cloth to broadly mimic the underwater light spectrum (see e.g. Fig. 4.21 in Davies-Colley et al. 1993) and 5 of the 6 were covered with 1 or more additional layers of black shade cloth to achieve a range of photon flux density (PFD, 400 to 700 nm) under the screens from ~1 to 24 mol quanta m^{-2} d^{-1}.

Water from the same diluted sample was dispensed into eighteen 300 ml borosilicate glass BOD bottles (12 light, 6 DCMU controls) for determination of ^{14}C uptake in parallel incubations following the procedure of Vant & Budd (1993). We also inoculated 6 bottles of undiluted water to determine the effect of dilution on ^{14}C uptake. Incubations for ^{14}C uptake were carried out in the same flowing-water trays used for determination of chlorophyll growth.

Incubations were terminated after 24 h. Initial and final chl *a* concentrations were measured with and without prefiltration through a 5 µm Nuclepore pre-

filter and a 22 µm Nitex screen to define operationally 3 size classes: <5, 5–22, and >22 µm. In all but the February experiment the >22 µm fraction was too erratic in the 24 h samples for reliable calculation of growth rates. Duplicate filters were frozen and stored for <1 to 4 wk before analysis. Filters were ground in 90% acetone and fluorescence measured on an Aminco fluorometer before and after acidification (Strickland & Parsons 1972). The fluorometer was calibrated with pure chl *a* (Sigma, St. Louis, MO, USA).

^{14}C uptake was also partitioned into the same 3 size-fractions (Vant & Safi 1996). At the end of the incubation, 30 to 50 ml subsamples from each bottle were filtered onto 0.8 µm Nuclepore membrane filters, with and without prefiltration through a 22 µm screen. A third subsample was filtered onto a 5 µm Nuclepore filter, following prefiltering through a 22 µm screen. Filters were rinsed with about 10 ml of filtered seawater and placed in vials to which 5 ml ACS II scintillation fluid (Amersham) was added; the quench-corrected ^{14}C activity was determined using a Wallace 1409 scintillation counter. The carbon uptake was partitioned into >22 µm (difference between whole and <22 µm results), 5–22 µm, and <5 µm (difference between <22 µm and 5–22 µm results).

Data analysis. θ_P was estimated as the slope of a regression of increments in carbon, ΔC_P, against increments in chlorophyll, Δchl *a*, in the 6 light treatments. Δchl *a* was calculated as the difference between final and initial concentrations. Type II regression (geometric mean method; Laws & Archie 1981) on means of the duplicates was used because light, not Δchl *a*, was the control variable. Thus chl *a*:C ratios can be obtained by inverting our reported values of θ_P.

Chlorophyll growth rates, $\mu^{chl\ a}$, were calculated as ln[(final chlorophyll + dark loss)/initial chlorophyll]. Addition of the dark loss of chlorophyll (when observed) was made because, even though ^{14}C uptake is widely believed to represent net particulate production (but see Williams 1993), negative values are impossible for ^{14}C uptake but not for chlorophyll changes. Due to the logarithmic transformation in calculating growth rate, the addition of dark chlorophyll loss was only important at low light intensities. Phytoplankton carbon at the start of the incubation, C_P, was estimated as the product of initial chl *a* concentration and estimated θ_P. Carbon based growth rates, μ^C, were estimated as $\ln(1 + \Delta C_P/C_P)$ (Eppley 1972).

Many investigators use measurements of the photosynthesis-irradiance (*P-I*) curve along with measurements of incident PFD and light attenuation to calculate daily depth-integrated production (Parsons et al. 1984). Conversion of production measurements to growth rates requires an estimate of C:chl *a*. To examine the relationship between conventionally measured *P-I* curves and the estimated growth-irradiance (μ-*I*) relationships, we conducted additional short-term incubations (i.e. 4 h centered about midday) of undiluted water on 2 dates, 7 February and 16 March 1995. Short-term measurements of ^{14}C uptake were used to estimate parameters in Eq. (6) (see below) using the procedure of Fee (1990). The fitted equation was used with incident PFD measured at 5 min intervals and measured screen transmittances to calculate the increment in C in the 6 light treatments for the size-fractions resolved. The resulting value of ΔC_P was used to determine μ^C from the 4 h incubations using the same θ_P estimated from the 24 h incubation.

RESULTS

Carbon and chlorophyll increments

In the first experiment (2 November 1994) we sampled and commenced incubation at midday. Increments in C were linear with Δchl *a* (Fig. 1a) but there

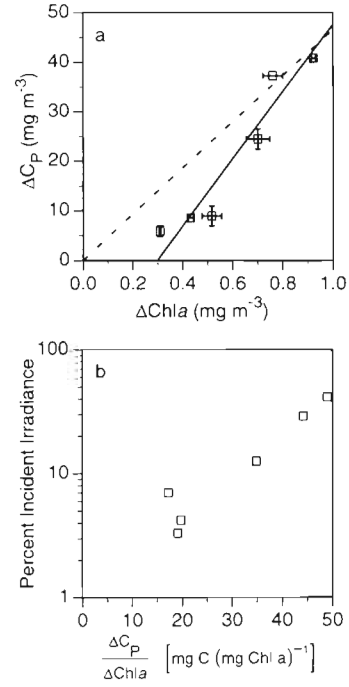

Fig. 1 (a) Plots of increment in C, ΔC_P, against increment in chlorophyll, Δchl *a*, on 2 November 1994. Plankton sample was collected midday and dilution and incubation commenced shortly thereafter. Solid line is type II regression with slope = 67.9 mg C (mg chl *a*)$^{-1}$. Dashed line is mean of 2 light-saturated incubations, slope = 46.6 mg C (mg chl *a*)$^{-1}$. (b) Discrete ratios of $\Delta C_P/\Delta$chl *a* in paired incubations as a function of percent incident PFD (i.e. screen transmittances in the simulated *in situ* trays). Percent incident PFD is on logarithmic vertical axis to imitate depth profile

was substantial incremental chlorophyll in the low-light incubations, resulting in a steeper slope than observed on subsequent dates. The slope estimated by type II regression was 67.9 mg C (mg chl *a*)$^{-1}$, compared with a mean of the 2 discrete $\Delta C_P/\Delta$chl *a* at saturating light of 46.6 mg C (mg chl *a*)$^{-1}$ A plot of paired ratios against PFD (log transformed on the vertical axis to represent depth profiles) decreased systematically with decreasing PFD (Fig 1b) as would be expected if there were unbalanced growth due either to photoadaptation or to synthesis of chlorophyll from C fixed prior to sampling ('Modeling tracer flux', see below). In subsequent experiments, however, sunrise sampling of plankton eliminated this pattern.

Our most complete data sets are for February and March. In each of these we obtained good linearity between ΔC_P and Δchl *a* in 2 size-fractions, 5–22 µm and >22 µm in February, and <5 µm and 5–22 µm in March. Δchl *a* was difficult to determine precisely in certain size-fractions when initial biomass in that size-fraction was low. Low precision was obtained in the <5 µm fraction in February and the >22 µm fraction in March. Complete results of calculated θ_P, and maximal C- and chl *a*-specific growth rates for all dates, are given in Table 2.

Estimated θ_P for the 5–22 µm size-fraction in February was 29.6 mg C (mg chl *a*)$^{-1}$ (Fig. 2a), and was somewhat higher, 38.5 mg C (mg chl *a*)$^{-1}$, in the >22 µm size-fraction (Fig. 2b). Carbon uptake was greater and Δchl *a* was more precise in the >22 µm size-fraction on this day. Maximal growth rates for both size-fractions were about 1.4 d^{-1} (Table 2). Estimated intercepts on the ΔC_P axes in Fig. 2a, b were very near 0 on this day, so no dark correction was employed in calculation of $\mu^{chl\ a}$. Estimates of μ^C (o) and $\mu^{chl\ a}$ (□) were in close agreement at all light intensities, especially for the >22 µm size-fraction (Fig. 2d). Such agreement is expected since both θ_P and growth rates were calculated from the same measured increments in carbon and chlorophyll.

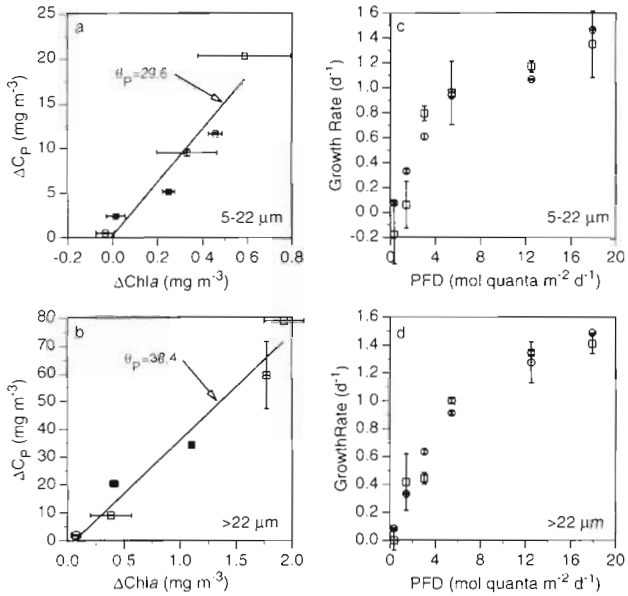

Fig. 2. (a) Plots of increment in carbon, ΔC_P, against increment in chlorophyll, Δchl *a*, for 5–22 µm size-fraction, 7 February 1995. θ_P are C:chl *a* production ratios estimated as the slope of a type II regression (plotted line). (b) As in (a) for >22 µm size-fraction. (c) Growth rates versus incident PFD calculated from increments in carbon (o) and chl *a* (□) plotted in (a). Initial phytoplankton carbon calculated from initial chlorophyll concentration and calculated θ_P. (d) As in (c), for >22 µm size-fraction calculated using incremental carbon and chl *a* and θ_P in (b). Error bars are range of duplicate measurements

In March we obtained good resolution of increments in C and chl *a* in the <5 µm and 5–22 µm size-fractions (Fig. 3a & b). θ_P was slightly higher in the <5 µm size-fraction (Table 2), but growth rate (Fig 3c) was about 40% lower than in the 5–22 µm size fraction (Fig. 3d). Discrepancies between μ^C and $\mu^{chl\ a}$ at low light intensities in Fig. 3d occurred because the calculated intercept on the abcissa in Fig. 3b was less than the observed dark loss. Consequently $\mu^{chl\ a}$ in Fig. 3d (□) are lower than the corresponding μ^C (o). At the highest light intensities the difference is only 0.1 d^{-1}, but at lower PFDs the difference is as high as 0.5 d^{-1}. The C- and chl *a*-based growth rates are virtually indistinguishable if we use the calculated intercept rather than the observed dark chlorophyll loss (data not shown), but the calculated chlorophyll-based growth rate in the dark becomes +0.5 d^{-1} The discrepancy between observed dark loss rate and the calculated intercept on

Table 2. Calculated C:chl *a* production ratios, θ_P, and maximal C- and chl *a*-specific growth rates (μ_m^C and $\mu_m^{chl\ a}$ respectively) of Manukau Harbour phytoplankton for 4 dates during the 1994–1995 growing season. Values in parentheses are approximate 95% confidence intervals (CI) for estimated θ_P, calculated as in Reckhow & Chapra (1983)

Date	Size-fraction	θ_P (±95% CI) mg C (mg chl *a*)$^{-1}$	μ_m^C d^{-1}	$\mu_m^{chl\ a}$ d^{-1}
2 Nov 94	<200 µm[a]	46.6[b]	1.17	1.18
17 Jan 95	<5 µm	21.5 (± 5.8)	1.56	1.46
17 Jan 95	5–200 µm	37.0 (± 8.7)	1.12	1.18
7 Feb 95	5–22 µm	29.6 (±12.0)	1.46	1.37
7 Feb 95	>22 µm	38.4 (±10.8)	1.49	1.41
16 Mar 95	<5 µm	28.1 (±14.6)	1.40	1.30
16 Mar 95	5–22 µm	24.1 (± 2.3)	1.96	1.85

[a]First experiment was not size-fractionated
[b]Regression slope biased due to midday start. Value used is mean of 2 light-saturated estimates; confidence intervals not available (see text)

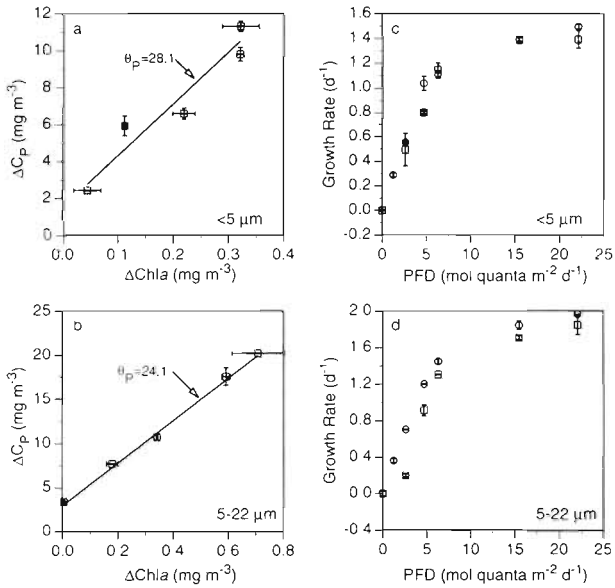

Fig. 3. (a) Plots of increment in carbon, ΔC_P, against increment in chlorophyll, Δchl *a*, for <5 µm size-fraction, 16-17 March 1995. θ_P are C:chl *a* production ratios estimated as the slope of a type II regression (plotted line). (b) As in (a) for 5–22 µm size-fraction. (c) Growth rates versus incident PFD calculated from increments in carbon (○) and chl *a* (□) plotted in (a). Initial phytoplankton carbon calculated from initial chlorophyll concentration and calculated θ_P. (d) As in (c), for <5 µm size-fraction calculated using incremental carbon and chl *a* and θ_P in (b). Error bars are range of duplicate measurements

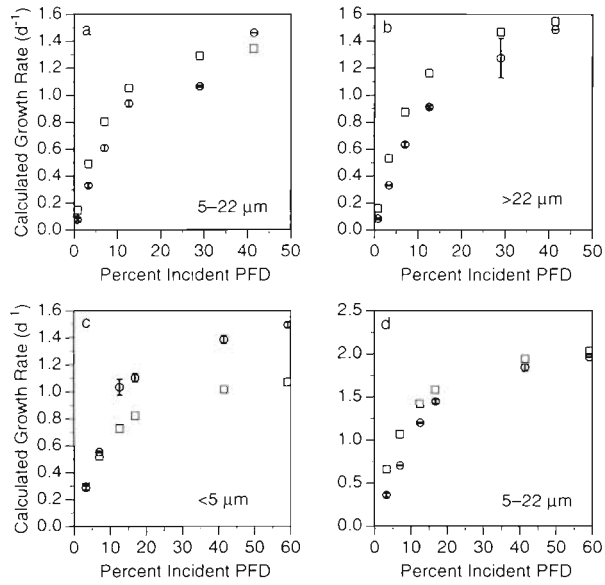

Fig. 4. Plots of phytoplankton growth rates calculated from light-saturation curve (□) based on 4 h measurements of ^{14}C uptake compared with 24 h incubations (○) as a function of percentage of surface photon flux density (PFD). (a) 7 February 1995, 5–22 µm size-fraction; (b) 7 February 1995, >22 µm size-fraction; (c) 16 March 1995, <5 µm size-fraction; (d) 16 March 1995, 5–22 µm size-fraction. Error bars are range of duplicate measurements

the abcissa may indicate that the loss rate of chl *a* was higher in the light than in the dark on that day.

Overall θ_P varied from 21 to 46 mg C (mg chl *a*)$^{-1}$, and there was no pattern with size-fraction or date (Table 2). Minimum coefficient of determination for the regressions was 0.92 (not shown), and 95% confidence limits for θ_P ranged from ±10 to ±50% of estimated values. Carbon-specific maximal growth rates varied from 1.12 to 1.96 d^{-1}, which is not a large range overall, probably reflecting the high nutrient status of the harbor. Chlorophyll-specific maximal growth rates varied over a similar range. The difference between maximal μ^C and $\mu^{chl\,a}$ ranged from 3 to 8% of the chl *a*-based estimate.

Short-term ^{14}C uptake

On 2 dates we compared growth rates estimated from 24 h incubations with those obtained from 4 h incubations centered at about midday (Fig. 4). Parameters of the light saturation curves are reported in Table 3. With the exception of the <5 µm size-fraction on 16 March (Fig. 4c) the short-term estimates of growth rate generally exceeded the 24 h estimates (Fig. 4a, b, d). The difference is greater in the low light treatments and the magnitude varies between days and size-fractions. Calculated rates at light saturation agreed more closely. Furthermore, a simple estimate of C-specific growth rate determined by (e.g. Eppley 1972) $\ln(1 + P_m^B \cdot 24D/\theta_P)$ where D = photoperiod (fraction of day) differed from the maximal 24 h rate by <20% (Table 3).

Table 3. Photosynthetic parameters, and calculated maximal growth rate based on light-saturated photosynthetic rate normalized to chlorophyll biomass compared with value based on 24 h incubation. $\ln(1 + P_m^B \cdot 24D/\theta_P)$ = maximal C-specific growth rate estimated by extrapolation of short-term P_m^B over the photoperiod, $24D$, (h); μ_m^C (d^{-1}) = maximal C-specific growth rate calculated from 24 h incubations. θ_P are given in Table 2. Units of I_k are µmol quanta m^{-2} s^{-1}; units of P_m^B are mg C (mg chl *a*)$^{-1}$ h^{-1}

Date	Size-fraction	I_k	P_m^B	$24D$	$\ln\left(1+\frac{P_m^B \cdot 24D}{\theta_P}\right)$	μ_m^C
7 Feb 95	5–22 µm	156	7.70	13	1.47	1.46
7 Feb 95	>22 µm	194	13.48	13	1.72	1.49
16 Mar 95	<5 µm	175	5.09	12	1.15	1.40
16 Mar 95	5–22 µm	239	15.95	12	2.19	1.96

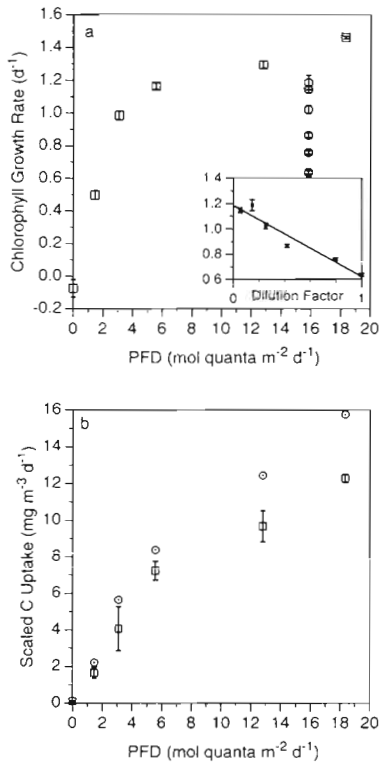

Fig. 5. Effect of dilution on estimates of chlorophyll-based growth rate and on C uptake, 16-17 January 1995, <5 µm size-fraction. (a) Chlorophyll-based growth rate as a function of photon flux density (PFD) on 17 January (□), dilution factor = 0.059, and 16 January (○) at a single PFD, dilution factor varying from 0.05 to 1.0. Inset shows net growth rate and fitted regression as a function of dilution factor on 16 January. (b) Carbon fixation as a function of PFD on 17 January in diluted (□) and undiluted (○) incubations multiplied by the dilution factor, 0.059. Error bars are range of duplicate measurements. Undiluted incubations in (b) were unreplicated

Effect of dilution

We conducted standard dilution experiments the day prior to the experiments reported here, except in November. Details of grazing results will be reported elsewhere. Here we illustrate the potential impact of grazing on estimates of $\mu^{chl\ a}$ by plotting the dilution series for 16 January, <5 µm size-fraction at its observed light intensity along with the μ-I curve measured the following day entirely on diluted water with dilution factor = 0.059 (Fig. 5a). Net growth rate in undiluted water on 16 January was only about 50% of that at dilution factor = 0.05. The net growth rate at dilution factor = 1.0 was roughly equivalent to growth rate in the diluted sample at PFD = 2 mol quanta m^{-2} d^{-1}, corresponding to the 11% light level. Maximal growth rates were similar between the 2 days. The full dilution curve (Fig. 5a, inset) shows that $\mu_n(0.05) \approx \mu$ is a good approximation.

Dilution had a contrasting effect on carbon uptake. Carbon uptake in undiluted water, multiplied by the dilution factor to correct for the higher biomass in undiluted water, was slightly higher than rates measured in diluted samples on 17 January in the <5 µm size-fraction (Fig. 5b). The difference increased at higher PFD and production rates. The tendency for either equal or higher rates (when multipled by the dilution factor) in the undiluted water was apparent on all dates.

MODELING TRACER FLUX

Model development

Determination of θ_P from diluted incubations requires that accumulation of pigment and carbon be balanced. Is phytoplankton growth in nature ever balanced? If so, under what conditions? We constructed a model of ^{14}C tracer flux to investigate errors incurred by assuming $\theta_P = \theta$ when θ_P is estimated using increments of C and chl *a* in a series incubations diluted to minimize grazing. In particular, we wished to determine whether our incubation procedure to estimate the μ-I relationship of phytoplankton induces photoadaptation so as to bias our estimates of θ.

The phytoplankton are considered to consist of 2 C pools: one, C_1, that is small and rapidly cycled which exchanges C with seawater; and another, C_2, that accumulates C from the exchanging pool (Fig. 6). A proportion of newly fixed C is synthesized into chlorophyll. A 2-pool model is the minimum that is consistent with detailed time course studies of ^{14}C labeling of

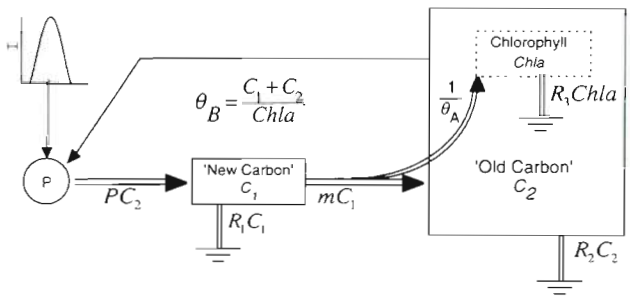

Fig. 6. Schematic diagram of 2-pool C flux model used to study effect of unbalanced growth on the C:chlorophyll production ratio. Double arrows trace the flux of C. 'Old Carbon', C_2, is that which produces biomass. 'New Carbon', C_1 represents recently fixed C. Chlorophyll *a*, chl *a*, is a subset of C_2. Specific photosynthesis rate, P, is function of incident irradiance, I_0, and m = transfer rate. The fraction of C transferred from C_1 to C_2 that is designated as chl *a* is governed by the C:chlorophyll allocation ratio, θ_A. R_1 and R_2 are, respectively, photosynthesis-dependent and biomass-dependent respiration rates. R_3 = metabolic breakdown rate of chl *a*. θ_B is the C:chlorophyll ratio of the biomass

total phytoplankton carbon (Smith & Platt 1984) and chl *a* (Goericke & Welschmeyer 1993). Our implementation is similar to that of Williams (1993) because we permit respiration to take place from both C_1 and C_2 at specific rates R_1 and R_2 respectively.

Following Williams (1993) the mass-balance equations for cellular carbon pools C_1 and C_2 are given by:

$$\frac{dC_1}{dt} = PC_2 - mC_1 - R_1 C_1 \quad (5a)$$

$$\frac{dC_2}{dt} = mC_1 - R_2 C_2 \quad (5b)$$

where P is the specific rate of photosynthesis (d^{-1}), and m (d^{-1}) is the rate constant for transfer from newly fixed carbon to biomass C. C_2 is assumed to be the producing pool that drives the accumulation of new C in C_1. P is modeled as a hyperbolic tangent function (tanh) of PFD (Jassby & Platt 1976) given by:

$$P(I) = P_{max} \tanh\left(\frac{I}{I_k}\right) \quad (6)$$

where P_{max} is the maximal C-specific photosynthesis rate (d^{-1}), I is the PFD (µmol quanta m^{-2} s^{-1}) and I_k (µmol quanta m^{-2} s^{-1}) is the light saturation parameter Irradiance was modeled as a half-sine wave raised to the power 1.3 (McBride et al. 1993)

$$I(t) = I_{max} \sin^{1.3}\left(\frac{\pi t}{D}\right) \qquad 0 \le t \le D \quad (7a)$$

$$I(t) = 0 \qquad D \le t \le 1 \quad (7b)$$

where D is photoperiod (d) and I_{max} (µmol quanta m^{-2} s^{-1}) is the maximal PFD at solar noon.

Equations analogous to (5a and 5b) are written for the accumulation of newly fixed carbon, F_1 and F_2, into pools C_1 and C_2 respectively (Williams 1993). These are pools traced by the uptake and transfer of ^{14}C. Dark ^{14}C fixation, ^{14}C excretion, and isotope discrimination are not considered. An experiment is assumed to commence with the inoculation with ^{14}C so that initial conditions for F are $F_1 = F_2 = 0$.

Chlorophyll is accumulated as a fraction of the transfer of C from C_1 to C_2 (Fig. 6),

$$\frac{d\,\mathrm{chl}a}{dt} = \frac{m}{\theta_A} C_1 - R_3\,\mathrm{chl}a \quad (8)$$

where θ_A is the C:chl *a* ratio for allocation of newly fixed C (Geider & Platt 1986), and R_3 is the degradation rate of chlorophyll. Because of its direct role in photosynthesis, we consider chl *a* to be a subset of the producing pool, C_2 (Fig. 6). We investigated cases with $R_3 = 0$ (Goericke & Welschmeyer 1992, 1993) and $R_3 = R_2$.

In the model we can identify 3 types of C:chl *a* ratios. θ_A is the ratio that governs the production of chl *a* from newly fixed carbon. We investigated cases in which θ_A remained constant or varied with ambient light intensity (Geider & Platt 1986). The instantaneous C:chl *a* in the biomass, θ_B, is $(C_1 + C_2)$/chl *a*. θ_B (previously denoted as un-subscripted θ) is the coefficient that is needed to estimate C_P from chl *a*, and C-specific growth rate from ^{14}C uptake. Note that $\theta_B = \theta_A$ only when $C_1 = 0$ (i.e. at the start of a simulation or after a dark period sufficiently long for all accumulated C_1 to transfer to C_2), and when $R_3 \approx R_2$. The C:chl *a* production ratio, θ_P, as previously defined is given in the model by $(F_1 + F_2)$/[chl $a(t)$ – chl $a(0)$], i.e. $\Delta C_P/\Delta$chl *a*.

As noted above, we wish to determine the effects of unbalanced growth on the validity of our assumption that θ_P, as would be measured experimentally in incubations free of grazing, gives an unbiased estimate of θ_B. Noting that increments in C must be measured operationally by the ^{14}C technique, we anticipate that 2 sources of unbalanced increments in C and chl *a* can result from methodological constraints of the ^{14}C technique, namely inability to accurately measure respiratory carbon losses, and synthesis of chl *a* from unlabeled C_1 accumulated prior to ^{14}C inoculation, i.e. $C_1(0) > 0$. Unbalanced increments in C and chl *a* may also occur due to photoadaptive changes in θ_A. Modeling scenarios were selected to examine these 3 sources of imbalance.

In principle θ_P may be estimated from the ratio of discrete increments in C and chl *a*, i.e. $\Delta C_P/\Delta$chl *a*. To better model our field experimental procedure by which we estimated both θ_P and the μ-I relationship, we took our estimate of θ_P from a series of 6 simulated 24 h incubations at a range of light intensities selected to give a spread of simulated ΔC_P and Δchl *a*. This was accomplished with the model by conducting six 1 d runs with identical initial conditions, varying only the parameter I_{max}. θ_P was determined as the slope of a regression of the 6 values of $F_1 + F_2 \equiv \Delta C_P$ against the respective Δchl *a* after 24 h. We also examined discrete ratios of $\Delta C_P/\Delta$chl *a* in relation to light levels to determine conditions under which discrete ratios provide satisfactory estimates of θ_B.

The model with constant θ_A was applied to 3 different starting times, i.e. sunrise, noon, and sunset. Appropriate starting values for θ_B for each of the start times were determined by running the model with I_{max} = 1000 µmol quanta m^{-2} s^{-1} until θ_B achieved a stable value at sunrise, with θ_A arbitrarily fixed at 40 mg C (mg chl *a*)$^{-1}$ For a sunrise start we took $C_1 = 0$ and arbitrarily set C_2 = 10 mg m^{-3} Values of C_1 and C_2 for noon and sunset start times were determined by running the model with the appropriately initialized value of θ_B for a quarter or half-day from a sunrise start to determine noon or sunset values of θ_B ('Modeling results', see below), C_1 and C_2. F_1 and F_2 were then reset to 0 for a

run lasting a further 24 h to simulate noon or sunset times of inoculation with ^{14}C. Because the size of the C_1 pool depends on m, we determined the effect of start time on estimated θ_P at 2 values of m. Values of parameters for the different simulation scenarios are given in Table 4.

Photoadaptation was incorporated into the model by allowing the allocation C:chl *a* ratio, θ_A, to vary (linearly) with PFD according to Eq. (1) of Geider (1987), using his parameter values for *Thalassiosira pseudonana*, a small diatom common in estuaries, i.e.:

$$\theta_A(t) = 19.7 + 0.0755 \quad I(t)$$

The model differs from that of Geider & Platt (1986) by the presence of the C_1 pool. Our intent was not to model any particular adaptation strategy or mechanism, but to investigate the consequences of such variability on estimated θ_P. For examining the case with adaptation we considered only sunrise start time and set $R_3 = R_2$, because if $R_2 >> R_3$ then $\theta_B << \theta_A$ regardless of PFD. That is, if there is substantial degradation of biomass ('old') carbon without degradation of chlorophyll, then the model generates unrealistically low values of θ_B that bear no relation to photoadaptation. Furthermore, because chl *a* is a subset of C_2, there would be considerable ambiguity in setting $R_2 >> R_3$.

Two scenarios were investigated using the model with variable θ_A. In the first case the model was run with I_{max} = 1000 µmol quanta m^{-2} s^{-1} until θ_B achieved a stable value at sunrise (see below). That value was

Table 4. Values of parameters used in simulations of increments in fixed C and chl *a* with the 2-pool C flux model

Parameter	Value	Units	Source
Fixed values			
P_{max}	4.0	d^{-1}	Scaled to make observed $\mu^C \approx 1\text{-}1.5$ d^{-1}
D	0.5	d	Annual average
I_k	175	µmol m^{-2} s^{-1}	Typical value, this study
Values for base run, varied as noted below for specific scenarios			
m	10.0	d^{-1}	Williams (1993); Goericke & Welschmeyer (1993)
R_1	0.5	d^{-1}	Williams (1993), 'diatom' value
R_2	0.05	d^{-1}	Williams (1993), 'diatom' value
R_3	0.05	d^{-1}	Goericke & Welschmeyer (1993)
[a]I_{max}	0 to 1000	µmol m^{-2} s^{-1}	Up to about 50% of growing season surface incident
Start time	0	d	Relative to sunrise
$C_1(0)$	0	mg m^{-3}	Williams (1993)—no new C before sunrise
$C_2(0)$	10	mg m^{-3}	Arbitrary value

Parameters varied for specific scenarios

Scenario	Parameter(s) varied	Remarks
θ_A constant: 40 mg C (mg chl *a*)$^{-1}$		
Resp.–I	R_3 = 0 d^{-1}	
Resp.–II	R_1 = 0.1 d^{-1}	
Noon start–I	Start time = 0.25 d	Starting C_1, C_2, and chl *a* Determined by 0.25 d initialization run
Sunset start–I	Start time = 0.5 d	Starting C_1, C_2, and chl *a* Determined by 0.5 d initialization run
Noon start–II	Start time = 0.25 d; m = 5 d^{-1}	Starting C_1, C_2, and chl *a* Determined by 0.25 d initialization run
Sunset start–II	Start time = 0.25 d; m = 5 d^{-1}	Starting C_1, C_2, and chl *a* Determined by 0.5 d initialization run
$\theta_A(t) = 19.7 + 0.0755 \quad I(t)$		
Photocycle Hi/Hi	I_{max} = 1000 µmol m^{-2} s^{-1}	For initialization
	[a]I_{max} = 0 to 1000 µmol m^{-2} s^{-1}	For incubation
Photocycle Lo/Lo	I_{max} = 40 µmol m^{-2} s^{-1}	For initialization
	[b]I_{max} = 0 to 40 µmol m^{-2} s^{-1}	For incubation
Light shift Hi/Lo	I_{max} = 1000 µmol m^{-2} s^{-1}	For initialization
	[b]I_{max} = 0 to 40 µmol m^{-2} s^{-1}	For incubation
Light shift Lo/Hi	I_{max} = 40 µmol m^{-2} s^{-1}	For initialization
	[a]I_{max} = 0 to 1000 µmol m^{-2} s^{-1}	For incubation

[a]0, 20, 100, 400, 800, and 1000 µmol m^{-2} s^{-1}
[b]0, 5, 10, 20, 30, and 40 µmol m^{-2} s^{-1}

used to determine starting chlorophyll concentration, assuming $C_2(0)$ = 10 mg m^{-3}, for 6 simulated 24 h incubations at I_{max} varying between 0 and 1000 µmol quanta m^{-2} s^{-1} (see Table 4). Similarly, we ran the model with I_{max} = 40 µmol quanta m^{-2} s^{-1} until θ_B achieved a stable (see below) value and used it to determine initial chlorophyll concentration for 6 simulated 24 h incubations with I_{max} varying from 0 to 40 µmol quanta m^{-2} s^{-1} (Table 4). We refer to these 2 simulations as the 'photocycle adaptation' scenario because PFD exposures used in the incubation runs never exceeded those to which the simulated assemblage was previously exposed in setting the starting values. We designated the ranges of PFD exposures, respectively, as 'Hi' and 'Lo' and refer to the simulated incubations by 'range used to initialize'/'range used to incubate'; e.g. 'Hi/Hi' refers to a simulation in which initial θ_B and chl *a* were determined with I_{max} = 1000 µmol quanta m^{-2} s^{-1}, and incubations were simulated with I_{max} varying from 0 to 1000 µmol quanta m^{-2} s^{-1}. For the second scenario we used the initial conditions as determined above with the Hi and Lo I_{max} values, but reversed the ranges of I_{max} used in the simulated incubations, leading to Hi/Lo and Lo/Hi simulations. We refer to these simulations as the 'light-shifted' scenario because the range of PFD exposure during the simulated incubation differed greatly from that upon which the initial θ_B were based.

Modeling results, θ_A constant

Diurnal variability in the size of the C_1 pool causes substantial variation in θ_B even when θ_A is constant (Fig. 7, solid line). The ratio increases rapidly after sunrise, then rises more slowly until just before sunset.

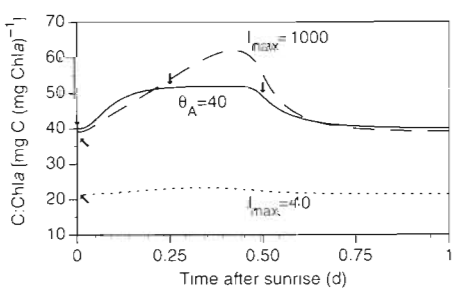

Fig. 7. Steady-state diel curves of C:chl *a* biomass ratios, θ_B, generated by the 2-pool C flux model with: (—) C:chl *a* allocation ratio, θ_A = 40 mg C (mg chl a)$^{-1}$ (constant) and m = 10 d^{-1}; (— —) θ_A variable and I_{max} = 1000 µmol m^{-2} s^{-1}; (····) θ_A variable and I_{max} = 40 µmol m^{-2} s^{-1}. Vertical arrows indicate starting values of θ_B for (left to right) sunrise, noon, and sunset start times. Angled arrows indicate starting values of θ_B for (— —) Hi/Hi and Hi/Lo, and (····) Lo/Lo and Lo/Hi photoadaptation scenarios

Simulated θ_B ranged from 40.04 to 51.9 mg C (mg chl *a*)$^{-1}$ with the minimum occurring at sunrise and the maximum 9 h later. The values at sunrise, noon, and sunset (Fig. 7, downward pointing arrows) encompass most of the observed diurnal variability of θ_B.

For the base run simulation (i.e. sunrise start, R_1 = 0.5 d^{-1}, and R_3 = R_2 = 0.05 d^{-1}) the discrepancy between θ_B at the start of the incubation and θ_P was nearly 0. The starting time of the simulated incubation had a marked effect on the calculated θ_P. Errors were 11 and 6% overestimates of θ_B for noon and sunset starting times, respectively (Table 5). The pre-incubation exposure to a half or full photoperiod of saturating PFD puts unlabeled carbon in the C_1 pool that is capable of simulating chlorophyll synthesis in the low light incubations, thereby steepening the slope of the ΔC_P versus Δchl *a* regression. The error increases with smaller values of m because the lower transfer rate allows more carbon to accumulate in C_1. Halving the value of m to 5 d^{-1} gave discrepancies between θ_B and θ_P of 23 and 19% respectively for noon and sunset start times (Table 5).

The simulated ΔC_P varied nearly linearly with Δchl *a*. Coefficients of determination for the regressions used to determine θ_P exceeded 0.98 in all of the simulations reported in Table 5. Ratios of discrete paired values of $\Delta C_P/\Delta$chl *a*, however, show varying patterns when plotted against simulated photon flux density (PFD) (log-transformed, on the vertical axis to resemble depth profiles; Fig. 8). At low PFD the ratio of $\Delta C_P/\Delta$chl *a* becomes highly dependent on the intercept on the Δchl *a* axis as ΔC_P approaches 0. Only in the case with θ_A constant and R_3 = 0 d^{-1} was the ratio invariant with

Table 5. Comparison of C:chl *a* ratios at start time, θ_B, and those estimated by regression of six 24 h simulated pairs of ΔC_P and Δchl *a* (slope = θ_P). The 2-pool C flux model was run with the parameter scenarios described in Table 4. Percent error = 100 × (θ_P – θ_B)/θ_B

	θ_B	θ_P	% error
	mg C (mg chl *a*)$^{-1}$		
θ_A = 40 mg C (mg chl *a*)$^{-1}$			
Base run	40.04	40.06	<0.1
Resp.–I	37.00	38.86	5.0
Resp.–II	40.04	40.06	<0.1
Noon start–I	51.48	57.37	11.4
Sunset start–I	49.06	52.07	6.1
Noon start–II	57.44	70.62	23.0
Sunset start–II	56.71	67.55	19.1
$\theta_A(t)$ = 19.7 + 0.0755 $I(t)$			
Photocycle Hi/Hi	39.07	37.60	–3.8
Photocycle Lo/Lo	21.44	21.43	–0.1
Light shift Hi/Lo	39.07	21.43	–46.2
Light shift Lo/Hi	21.44	37.60	75.7

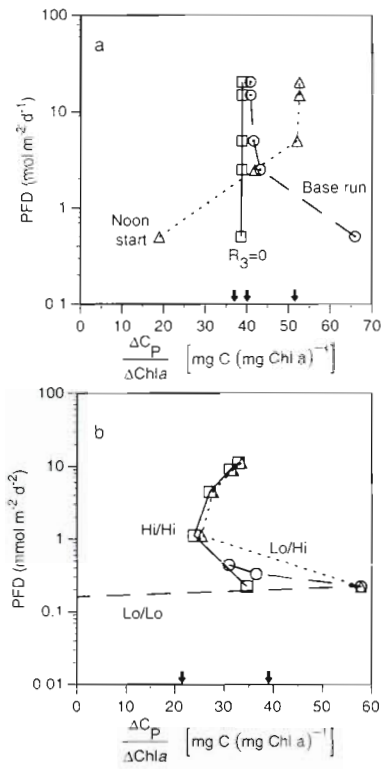

Fig. 8. Ratios of simulated increments in carbon, ΔC_P, to increments in chl *a*, Δchl *a*, as a function of PFD. Irradiance is plotted vertically on logarithmic scale to imitate depth profile. (a) Simulated C:chl *a* allocation ratio, θ_A, was constant and chlorophyll degradation rate constant, R_3, and start time were varied: (○) base run, i.e. $R_3 = R_2 = 0.05$ d^{-1}; (□) $R_3 = 0$ d^{-1}; (△) noon start time. Sunset start time (omitted for clarity) resembled (△). Arrows on abcissa show starting θ_B values for (left to right) $R_3 = 0$, base run, and noon start simulations. (b) Simulated θ_A was a function of irradiance to simulate photoadaptation. 'Photocycle', (□) Hi/Hi and (○) Lo/Lo, and 'light shifted', (△) Hi/Lo, scenarios are described in the text. 'Light shifted' Lo/Hi (omitted for clarity) resembled (□). Arrows on abcissa show starting θ_B for (left) Lo/Lo and Lo/Hi, and (right) Hi/Hi and Hi/Lo simulations. Other parameters are given in Table 1

depth (Fig. 8a, □). With noon or sunset start times (Fig. 8a, △) there is positive chl *a* production from unlabeled C at low and 0 PFD, causing the discrete ratio to decline. Interestingly, the discrete ratios obtained at the highest 2 light levels agreed well with the starting values of θ_B for the noon start time (cf. Fig. 8a, Table 5). With $R_3 = R_2 = 0.05$ d^{-1} (Fig. 8a, ○) there is loss of chl *a* at low or 0 PFD, so that the discrete ratio may become very large (potentially undefined) before going negative. That is, at low PFD the ^{14}C technique will always produce a positive estimate of C production, even when the net change in chl *a* is 0 or negative. Thus any time $R_3 > 0$ there may be a positive intercept on the ΔC_P axis (cf. Fig. 3a & b). These small increments in C_P and chl *a* fall on the same line formed by increments at higher PFD, thereby improving the overall estimate of θ_P as the slope; but the ratios of discrete increments measured at low PFD may be positive or negative, and do not give an accurate indication of the composition of the phytoplankton.

θ_A variable

When we included photoadaptation of θ_A in the model, both the daily minimum and the amplitude of the diurnal variation of simulated θ_B depended greatly on I_{max} (Fig. 7). At I_{max} = 1000 µmol quanta m^{-2} s^{-1}, θ_A varies over its entire range and there is ample C fixation for adjustments in θ_B to occur; θ_B thus varied from 39 to 62 mg C (mg chl *a*)$^{-1}$ over the diel cycle (Fig. 7, long dashes). Conversely, at I_{max} = 40 µmol quanta m^{-2} s^{-1} both the diurnal variation in θ_A and the fixed C available to adjust θ_B are reduced; θ_B only varied from 21.4 to 23.3 mg C (mg chl *a*) ^{1}.

In the photocycle adaptation scenario the estimated θ_P agreed closely with the starting value of θ_B (Table 5) regardless of whether the prior I_{max} was 1000 (Hi/Hi) or 40 (Lo/Lo) µmol quanta m^{-2} s^{-1}. Differences between θ_B and θ_P were <4%, with the error resulting from Hi/Hi simulation larger than the Lo/Lo. The simulated light shift scenario resulted in much larger discrepancies between θ_P and θ_B (Table 5). The worst case was the Lo/Hi simulation, where θ_B was overestimated by >70%. The values of θ_P in Table 5 indicate that, if photoadaptation occurs as formulated, the slopes obtained from regression of ΔC_P against Δchl *a* depend on the light regime used for the incubation and not on the initial θ_B. This occurs because the rate of adaptation is determined by the rate of photosynthesis at the light level to which the assemblage is shifted (Geider & Platt 1986). At low PFD both C and chl *a* production are light limited and adaptation occurs slowly, whereas at high PFD C is accumulated rapidly resulting in more rapid adjustment of θ_B toward θ_A.

Patterns of discrete ratios of $\Delta C_P/\Delta$chl *a* simulated with varying θ_A were qualitatively similar to those with θ_A constant (Fig 8b). Simulated incubation in the high light series (i.e. Hi/Hi) produced discrete ratios that decreased with depth as expected for photoadaptation (Fig. 8b, □). Incubation in a series of low PFDs produced erratic variations in discrete ratios (Fig. 8b, ○ and △) because the ratios come under the influence of the intercept as noted above for θ_A constant. Interestingly, simulations showing both regularly declining (Hi/Hi) and erratic (Lo/Lo) discrete ratios of $\Delta C_P/\Delta$chl *a* produced accurate estimates of θ_B when estimated by regression (Table 5).

Discrete ratios at saturating light were accurate estimates of θ_B in the Hi/Hi scenario (Fig. 8b, cf. □, arrow), suggesting the possibility of estimating θ_B from incuba-

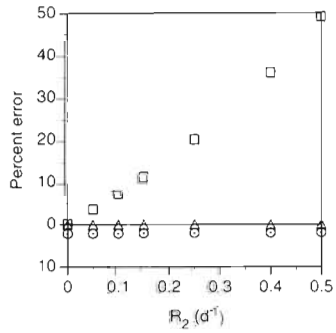

Fig. 9. Sensitivity of error in calculated θ_P to the magnitude of respiration rate of 'old' carbon, R_2, using the 2-pool C-flux model with photocycle Hi/Hi scenario (see Table 4): (□) calculated using discrete ratio from single incubation at saturating light; (△) calculated using discrete ratio from incubation at saturating light corrected for dark loss of chl *a*; (○) calculated using Type II regression of 6 pairs of simulated ΔC_P and Δchl *a*. Percent error: $100 \times (\theta_P - \theta_B)/\theta_B$

tions at a single, saturating PFD (e.g. Table 2, result for 2 November). This conclusion was, however, sensitive to R_2 (Fig. 9, □) because at high values of R_2 (assumed to equal R_3) respiration reduces ^{14}C fixation proportionately less than chl *a* production. Addition of the dark loss of chl *a* corrects the bias (Fig 9, △).

Model summary

We conclude that the C:chl *a* production ratio estimated from a regression of incremental values of C and chl *a* can provide accurate estimates of the initial C:chl *a* ratio of the phytoplankton biomass provided the situations in which the procedure is used reflect balanced growth. Chlorophyll production can be unbalanced with respect to *measured* carbon uptake when there is recently fixed unlabeled carbon available to produce incremental chl *a* prior to the start of an experiment as would occur with starting times substantially later than sunrise. Because the transfer from C_1 to C_2 is a first-order process, C_1 is empty and θ_B is minimal only after a dark period of ~3/*m* (e.g. after a quarter to half a day). This imbalance is particularly severe with incubations at low light. Growth is also unbalanced when the light levels used in the incubations differ greatly from those to which the phytoplankton have a history of prior exposure. The variations in discrete ratios of ΔC_P to Δchl *a* incubated at different light intensities are neither diagnostic of photoadaptation nor useful indicators of bias in estimates of θ_P made by regression of ΔC against Δchl *a*. Discrete ratios at saturating PFD may, however, give acceptable estimates of θ_B for starting times other than sunrise, provided correction is made for dark loss of chl *a*.

DISCUSSION

Balanced growth

The 2-pool model of carbon flux implies that short-term accumulation of C and chlorophyll must always be unbalanced as the accumulation of 'new carbon' is temporary and not yet proportionately allocated to pigment. The modeling (Table 5, Fig. 8) and experiment of 2 November (Fig. 1) indicate that the presence of new carbon at the start of an experiment, as would be expected for plankton sampled at noon from well-lit waters, permits synthesis of pigment at low light or in the dark that is not supported by fixation of radiolabeled carbon. The result is an overestimate in θ_P from diluted μ-*I* incubations (Table 5); but the modeling indicates that the discrete ratios of $\Delta C_P/\Delta$chl *a* at saturating PFD may give an acceptable estimate of θ_P (cf Table 5, Fig. 8a, △ at 2 highest PFD).

Subsequent experiments (Table 2; Figs. 2a, b & 3a, b) indicated that sampling plankton prior to sunrise eliminated the unbalanced production of chlorophyll at low light intensities. Only on 7 February was the calculated intercept on the Δchl *a* axis slightly positive at 0.075 mg chl *a* m^{-3} for the 5–22 µm size-fractions; but on that date we did not conduct a dark incubation and it is possible that with a dark incubation a zero or negative intercept would have been resolved. In the model, the amount of new carbon at the end of the dark period is sensitive to the magnitude of the transfer coefficient, *m*. Unreported experimentation with the model showed that values of *m* less than about 6 d^{-1} allow sufficient carbon remaining in C_1 at the end of 12 h dark for some slight production of chlorophyll in a simulated incubation in the dark. Using the chlorophyll labeling method Goericke & Welschmeyer (1993) found that growth rate-normalized transfer coefficients, analogous to *m*, for the precursor of the porphyrin subunit of chlorophyll ranged from 2 to 45 d^{-1}. The low values would permit imbalance of carbon and chlorophyll production even in incubations commencing prior to sunrise and lasting 24 h. Their typical value of 15 d^{-1}, however, is large enough to empty C_1 prior to sunrise. A negative chlorophyll intercept at $\Delta C_P = 0$ was calculated in all our other experiments commencing at sunrise. Residual new carbon does not appear to have caused unbalanced growth when we sampled plankton prior to sunrise.

Unbalanced growth due to photoadaptation can be of major concern. The discrepencies between θ_P and θ_B were large in the 'light shift' scenarios, i.e. when the range of PFD used in the simulated incubations diverged from that used to determine the initial θ_B for the assemblage (Table 5). When the highest light in the simulated incubation matched those used to fix the

initial C:chl *a* ratio, errors were <4% (Table 5, photocycle adaptation). This works in the modeled Lo/Lo scenario because the total change in θ_A is minimal, and the situation approaches the constant θ_A case. In the Hi/Hi scenario, the rate of adaptation in the incubations at low light is limited by the rate of photosynthesis (Geider & Platt 1986), whereas the incubations at saturating light intensities have not been greatly shifted with respect to conditions used to fix the starting values of the coefficients. Thus the modeling indicates that accurate estimates of θ_P can be obtained even when paired ratios of $\Delta C_P/\Delta$chl *a* indicate adaptation to light has occurred (cf. Fig 8b □, Table 5).

In our experiments we sampled from the surface of a well-mixed water column. Because of the strong tidal stirring in Manukau Harbour there is no opportunity for phytoplankton to become isolated from the surface for lengthy periods. Studies of *P-I* parameters in estuaries have indicated that estuarine phytoplankton do not photoadapt in the conventional sense, because of the requirements to withstand frequent exposure to surface PFD while being mixed through strong light gradients (Harding et al. 1987). We intentionally avoided incubating samples at intensities known from previous *P-I* measurements to be photoinhibiting (Vant & Budd 1993). Furthermore, photobleaching of chlorophyll, such as would be likely in 24 h incubations at the surface, would result in spuriously high estimates of θ_P. In fact, however, all of our estimates of θ_P based on sunrise collection were <40 mg C (mg chl *a*)$^{-1}$ (Table 2), typical of healthy, nutrient-sufficient phytoplankton (Parsons et al. 1984); and that determined with the midday start could well have been elevated above its sunrise value by normal diel variability (see Fig. 7, solid line). We conclude that major problems of short-term ΔC_P and Δchl *a* imbalance, photoadaptation, photoinhibition and photobleaching can be circumvented by avoiding light shifts and incubating for 24 h at non-inhibiting light levels. Midday starts with correction for dark chl *a* loss appear to give acceptable estimates of θ_P at saturating PFD, but if sub-saturating PFDs are excluded the opportunity to estimate the complete μ-*I* curve based on $\mu^{chl\,a}$ is foregone.

The possibility of unbalanced growth due to alteration of the nutrient regime has not been addressed here because of the high nutrient concentrations in Manukau Harbour. In more oligotrophic waters incubation in bottles might cut off allochthonous nutrient supplies. Dilution to eliminate grazing also cuts off regeneration of nutrients (Andersen et al. 1991). A highly diluted initial phytoplankton standing stock, however, places less demand on the dissolved nutrients, which, together with internal stores at the start of an incubation (Andersen et al. 1991), may often be sufficient to avoid artefacts of nutrient depletion. Addition of nutrients to nutrient-deficient phytoplankton is known to induce transient physiological changes such as increased respiration, reduced ^{14}C uptake and increased dark C uptake (Healey 1979, Elrifi & Turpin 1987) that would confound estimates of θ_P. The addition of excess nutrient commonly employed in standard dilution experiments is done to estimate microzooplankton grazing rate (Landry & Hassett 1982), and is not required or advised for the procedure described here.

Importance of dilution

The presence of grazing by microzooplankton inside incubation bottles (Eppley 1980) is the primary reason θ_P has not received widespread use as an estimate of the θ_B in the past (but cf. Eppley 1968). Welschmeyer & Lorenzen (1984) measured negative changes in chlorophyll concentration in Dabob Bay, Washington, USA, in the presence of active ^{14}C labeling of chlorophyll. They implicated microzooplankton grazing as one of several possible degradative processes. Similarly, Welschmeyer et al. (1991) found stable concentrations of carotenoid markers of picoplankton in the subarctic Pacific, but increasing concentrations of markers for a larger diatom in undiluted shipboard incubations. Collateral dilution experiments (Strom & Welschmeyer 1991) confirmed that growth rates estimated by ^{14}C pigment labeling were broadly consistent with those estimated by dilution experiments (Welschmeyer et al. 1991); but the picoplankters were selectively grazed by microzooplankton, so that changes in pigment concentrations in the bulk, undiluted incubations were not a reliable indicator of picoplankton growth.

In this work dilution experiments conducted the day prior to the μ-*I* incubations indicated that microzooplankton grazing on the <5 µm size-fraction was 35 to 47% of light-saturated growth rates. In the example shown (Fig. 5a inset) a grazing coefficient of 0.56 d^{-1} combined with a dilution factor of 0.05 indicates that grazing in the diluted μ-*I* incubations should reduce gross chl *a*-based growth rates by <0.03 d^{-1}, or <2% of observed maxima on that date. In these highly diluted incubations, therefore, pigment increment is a viable marker of phytoplankton growth.

More intriguing was the effect of dilution on ^{14}C uptake (Fig. 5b). Whereas grazed pigment is destroyed, grazed ^{14}C that is not respired contributes to the final measure of carbon uptake, even though it is transferred out of the producing pool (Jackson 1983). We would expect, therefore, that the effect of grazing on ^{14}C uptake would be less negative than that on pigment production. In 24 h incubations there may be excretion of dissolved organic ^{14}C, uptake by bacteria

(Jackson 1993), some of which would be retained on a 0.8 µm Nuclepore filter. Specific activity of the dissolved organic carbon (DOC) pool, and possibly also the biomass of bacteria growing on it, would be higher in undiluted samples. Any grazing on labeled bacteria that is not respired would also be retained in the particulate phase. We cannot determine whether these processes were quantitatively significant in our experiments, but they do provide a possible mechanism for ^{14}C fixation in undiluted samples (multiplied by the dilution factor) to exceed that in diluted samples. Dilution is, therefore, important for both eliminating grazing losses of pigments and for restricting measured ^{14}C gains to processes associated solely with phytoplankton growth.

Short-term *P-I* comparison

Maximal growth rates calculated from measured PFD and parameters of the light saturation curve generally agreed well with rates measured in 24 h incubations (Fig. 4). At PFD levels below the maximum used, however, the growth rates calculated from *P-I* curves were usually higher than those determined from 24 h incubations (Fig. 4). Some of this discrepancy may be due to our inability to correct for respiration in the calculation of 24 h growth rate from short-term *P-I* measurements. Short-term ^{14}C-uptake measurements are believed to more closely approximate gross production than 24 h incubations. Failure to include respiration would have a greater proportional effect on calculations at low PFD because of the logarithmic transformation to obtain growth rate from increments in C.

The largest deviations between rates based on 24 h incubations and those calculated from *P-I* measurements appeared to be due to an underestimate of P_m^B on 16 March, <5 µm size-fraction (Fig. 4c). Diurnal variability in *P-I* parameters is another process that can confound comparisons between short- and long-term incubations. Observations of diurnal periodicity in *P-I* parameters of natural assemblages of marine phytoplankton have shown that maxima can occur at various times of the day (MacCaull & Platt 1977, Harding et al. 1982). If diurnal periodicity were responsible for the differences between calculated and observed rates in Fig. 4c, the maxima would have to be either morning or afternoon, and be of sufficient magnitude to produce increment in C 40% greater than that calculated in the highest light treatment.

The simpler calculation of light-saturated growth rates by linear extrapolation of P_m^B over the photoperiod, $\ln(1 + P_m^B \cdot 24D/\theta_P)$ (Table 3), is inherently biased due (in addition to omission of respiration as noted above) to overestimation of C fixation rates when PFD is below saturating levels (e.g near sunrise and sunset). The magnitude of this bias depends on the measured PFD and the particular value of I_k. Here $\ln(1 + P_m^B \cdot 24D/\theta_P)$ exceeded the estimate based on the complete *P-I* curve by 7 to 10%. The largest discrepancy between $\ln(1 + P_m^B \cdot 24D/\theta_P)$ and the observed 24 h growth rate was an underestimate by 0.25 d^{-1} (Table 3), probably due to an underestimation of P_m^B (see above). Otherwise $\ln(1 + P_m^B \cdot 24D/\theta_P)$ was 1 to 15‰ higher than the estimate from 24 h incubations. The abbreviated calculation appears to be a useful approximation when complete PFD records are not available.

Manukau Harbour

Microscopic examination has confirmed that organic detritus is generally an important component of the seston in the harbor waters. Because of this, analyses of particulate C have produced marked overestimates of θ_B [e.g. values up to 600 mg C (mg chl *a*)$^{-1}$ following periods of wind-induced resuspension of harbor sediments; M. Gibbs unpubl. results]. Previous estimates of μ^C in these waters have, therefore, been based on assumed values of θ_B. For example, Vant & Budd (1993) used a value of 34 mg C (mg chl *a*)$^{-1}$ during a period of dominance by diatoms (Chan 1980), in good agreement with the estimates of θ_P obtained here (Table 2). Additionally, measured values of θ_B agree with a recently published (Cloern et al. 1995) empirical equation to predict C:chl *a* from light, temperature, and nitrogen to within 5 to 35%, well within the prediction limits of the equation with the data from which it was derived (see e.g. Fig. 1 in Cloern et al. 1995). We now plan to determine whether the expected higher values of θ are observed during future periods of dinoflagellate dominance of the harbor assemblage [e.g. Chan 1980, values up to ~100 mg C (mg chl *a*)$^{-1}$].

General applicability

Chlorophyll concentrations in Manukau Harbour are high by oceanic standards. Aside from nutrient considerations discussed above, can we expect diluted μ-*I* incubations to be sufficiently sensitive for estimating C:chl *a* ratios in more oligotrophic waters? A typical value for initial, undiluted chlorophyll in the <5 µm size-fractions in our experiments was about 3 mg m^{-3}. After 24 h we needed 500 ml of the 2400 ml available diluted sample, indicating capacity to work with 4× lower concentrations. In oligotrophic waters a dilution factor of 0.1 might be a sufficient reduction of grazing

if paired with full dilution experiments (cf. Fig. 5a) to more fully assess and correct for the effect of grazing. Sensitivity could be further increased by using 4 l bottles, so that the procedure might be used in waters having as little as 0.1 to 0.2 mg chl *a* m^{-3}. In oligotrophic waters in which small increments must be resolved, adequate stirring during sample dispensing and accurate determination of initial chlorophyll concentration would be essential. As indicated above, 24 h incubations of plankton and avoidance of light shifts and photoinhibiting PFDs are necessary. A sunrise start time is necessary to resolve the complete μ-I curve, because of the possibility of imbalanced chl *a* production at low irradiance resulting from midday sampling. In general, we would expect diluted μ-I incubations to be useful for estimating C:chl *a* ratios and phytoplankton carbon anywhere the dilution technique is feasible. We encourage cautious exploratory use of the method in other waters.

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