

Measurement of daily primary production using 24 h incubations with the ^{14}C method: a caveat*

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ABSTRACT: Computer simulations and experiments with cultured and natural phytoplankton were used to study ^{14}C uptake kinetics and budgets over 24 h incubations. There was good agreement between experiments and simulations. After 24 h incubations and depending on the starting time of incubations, net ^{14}C uptake varied by a factor of 2 in the laboratory and by a factor of 3 in the field. Both simulated and experimental data showed lowest ^{14}C accumulation for incubations starting at sunrise (i.e. dawn-to-dawn incubation), while highest values corresponded to incubations beginning at sunset. Recommendations for field studies are as follows. For samples collected at night, incubations can be started at any time, but should be conducted for a full 24 h after dawn. Samples collected after dawn should be incubated for 24 h, and ^{14}C accumulation should be corrected in order to obtain dawn-to-dawn values.

KEY WORDS: Phytoplankton · Primary production · Respiration · ^{14}C · Daily production · 24 h incubation · Model

INTRODUCTION

Ever since the introduction of the method by Steemann Nielsen (1952), phytoplankton production has generally been measured by means of ^{14}C -bicarbonate incorporation. Incubations are carried out *in situ* or in incubators (simulated *in situ*) for different time courses. Following the traditional view, photosynthesis takes place in the light and respiration occurs in both light and darkness, so that short-term incubations (i.e. a few hours during daylight) should reflect gross production whereas long-term incubations (24 h) reflect both uptake and losses (net production) (Buckingham et al. 1975, Dring & Jewson 1982, Li & Harrison 1982). However, the problem is complicated by the facts that a significant amount of carbon may be lost in daylight and that carbon uptake may occur in darkness. Indeed, respiration may be enhanced by light, reflecting photorespiration (Tolbert 1974, Raven & Beardall 1981), or not (Grande et al. 1989). Alternatively, algal populations may also fix some CO_2 by dark processes

(Taguchi & Platt 1977, Cosper 1982, Legendre et al. 1983, Li et al. 1993). Other uncertainties affect production measurements. For example, losses of carbon occur in the form of extracellular release (exudation; Lignell 1990), during darkness (Berman & Gerber 1980) or daytime (Saunders 1972). In laboratory incubations, respiration in the light may be similar, or greater than in the dark (Grande et al. 1989). Respiratory CO_2 may be reassimilated during the incubation (Harris 1978, 1980). In algae, light-independent ^{14}C fixation is accomplished by β -carboxylating enzymes (Glover 1989). Anaplerotic carbon may be fixed by bacteria (e.g. Jones et al. 1958), or by adsorption on suspended matter (Romero & Arenas 1989). Geographical variations in dark uptake have been pointed out by Prakash et al. (1991). Several authors, including Doty & Oguri (1957), Curl & Small (1965), Malone (1971a, b), Falkowski (1980), Prézelin & Matlick (1980), and Vandavelde et al. (1989), reported diurnal rhythms in photosynthetic characteristics. This list of processes, which is not exhaustive (see the review by Peterson 1980), identifies large sources of variations which may cause major differences in estimates of daily primary production derived from single, short-term incubations (e.g. Sournia 1974, MacCaull & Platt 1977, Harding et

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al. 1982, Vandeveldel et al. 1989). Some of these problems can be circumvented by using dawn-to-dusk or 24 h incubations for determining daily carbon fixation.

Recently, JGOFS (1988) recommended 24 h dawn-to-dawn incubations for estimating the daily rate of primary production using the ^{14}C method. However, given logistics, it is not always possible to start *in situ* or simulated *in situ* incubations at dawn, especially when an extensive grid of stations must be sampled. If phytoplankton production was estimated from changes in oxygen concentration in light and dark bottles, values should be the same irrespective of the time of the day when incubation is started, assuming no day-to-day variability in other properties of the environment (e.g. irradiance). This is not the case, however, when primary production is estimated from the uptake of ^{14}C . In such a case, it is expected that the amount of labelled CO_2 and organic compounds lost during the dark hours will vary according to the time of the day the incubation starts. The present study investigates this problem using simple simulations combined with laboratory and field experiments with algal cultures and natural phytoplankton samples, in view of proposing practical solutions for field estimation of daily primary production.

MATERIALS AND METHODS

Model of ^{14}C uptake kinetics and budgets. A simple model was used to compute uptake kinetics during 24 h, and ^{14}C budgets after 24 h incubations. In the model, the change in ^{14}C over time is the difference between terms for gain (G) and loss (L), and the gain of tracer is proportional to solar irradiance and photosynthetic efficiency (α). Assuming no nutrient effect, the diurnal variation in ^{14}C uptake is modelled with a sine approximation for solar irradiance (e.g. Kirk 1983) multiplied by α ; the gain is zero during the night. To calculate the loss of tracer, it is assumed that there is a fixed lag (λ) between the uptake of ^{14}C and its respiration or/and exudation by phytoplankton. The loss of ^{14}C at a given time t is taken as a constant fraction of the amount of ^{14}C which was in the cells (instantaneous loss rate k) at time $t-\lambda$ (there is no loss during the initial period, of length λ).

The model is:

$$\Delta^{14}\text{C}/\Delta t = G - L \quad (1)$$

At any time t the G is estimated as:

$$G = \alpha \sin(\pi t/d) \quad (2.1)$$

during the daylight period (of length d), where α is the photosynthetic efficiency. Photosynthetic efficiency is estimated as:

$$\alpha = 0.02 + 0.01 \sin[\pi(t + f)/d] \quad (2.2)$$

where f is the phase of α with the light cycle. During the night,

$$G(t) = 0 \quad (2.3)$$

At any time t , L is estimated as:

$$L(t) = k^{14}\text{C}(t - \lambda) \quad (3)$$

where k is the instantaneous loss rate, and λ is the lag. Eq. (1) was numerically integrated with 1 min time steps.

Preliminary trials were performed to evaluate the impact of each parameter on ^{14}C uptake. Simulations for 24 h incubations were conducted with daylengths d varying from 12 to 18 h, lag values λ from 1 to 5 h, loss values k from 1×10^{-4} to $1 \times 10^{-3} \text{ min}^{-1}$, and photosynthetic efficiency α from 0.01 to $0.03 \text{ mg C mg}^{-1} \text{ chl a h}^{-1} (\mu\text{E m}^{-2} \text{ s}^{-1})^{-1}$ (according to observations made by Legendre et al. 1988 and Vandeveldel et al. 1989). In each case, ^{14}C budgets were calculated for 24 incubation windows, starting 0, 1, ..., 23 h after sunrise, respectively.

^{14}C uptake kinetics (Expts 1 and 2). Expt 1 was conducted in the laboratory with 4 cultures: (1) diatom *Thalassiosira weissflogii* ($1.6 \mu\text{g chl a l}^{-1}$), (2) flagellate *Isochrysis galbana* ($0.3 \mu\text{g chl a l}^{-1}$), (3) cyanobacteria *Synechococcus* sp. ($10^7 \text{ cells l}^{-1}$; strain WH 7805) whose pigments are dominated by phycoerythrin, and (4) a mixture of these 3 organisms ($0.8 \mu\text{g chl a l}^{-1}$) composed of 62% diatoms, 38% flagellates and $10^7 \text{ cells l}^{-1}$ of cyanobacteria. The biomass of diatoms and flagellates was estimated from chl *a* measurements using fluorometry (see below); cyanobacteria were counted by means of epifluorescence microscopy. Cultures were grown in f/2 medium (Guillard & Ryther 1962) in large volumes (20 l) kept under similar temperature (20°C) and light conditions (see below). Subsamples (500 ml) were collected and inoculated at 6:00, 9:00, 12:00, 15:00, 18:00, 21:00, 0:00 and 3:00 h and placed at 20°C in a simulated *in situ* incubator. The light source was composed of 10 fluorescence tubes (Spectralite) which were combined to give light intensities from 0, during the night (19:00 to 6:00 h), to $250 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ at noon, with steps of $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, producing an artificial day-night cycle of 13 h:11 h. Dark bottles were incubated starting at 6:00, 12:00, 18:00 and 0:00 h in order to follow carbon uptake of the cultures in dark conditions.

Expt 2 was conducted with natural samples from coastal waters off Gascons in Baie des Chaleurs (Gulf of St. Lawrence, Canada; $48^\circ 10' \text{ N}$, $65^\circ 55' \text{ W}$; 9–10 October 1992). A large volume of surface water (1000 l) was pumped into a tank (17:00 h), without adding nutrients. The sample was exposed to natural surface

light (13 h daylength). Subsamples of 6 l were collected at 19:00 and 6:00 h and incubated for 24 h under natural light and in dark conditions.

^{14}C uptake budgets (Expt 3). Water samples were collected every 2 h for 48 h at an anchor station in Baie des Chaleurs (Gulf of St. Lawrence, Canada; 48° 04' N 65° 38' W; 10–11 September 1991). Subsamples of 250 ml were incubated at surface temperature (13 °C) during 24 h in a simulated *in situ* incubator (see Expt 1).

Biomass measurements. Natural samples were pre-filtered on 200 μm Nitex. Phytoplankton biomass was estimated as chl *a* in all experiments. Samples (250 ml) were filtered on Whatman GF/F filters, and chl *a* concentrations were determined using the fluorometric technique (Holm-Hansen et al. 1965), after 24 h extraction in 90 % acetone at 4 °C in the dark.

Production measurements. After collection, samples were immediately inoculated with a solution of $\text{NaH}^{14}\text{CO}_3$. Two different methods were used to estimate ^{14}C kinetics and budgets, respectively. In the case of kinetics, ^{14}C was added to a final concentration of 80 $\mu\text{Ci l}^{-1}$, and ^{14}C accumulation was measured following the method of Schindler (1972). Subsamples of 1 ml were collected from each bottle every hour in Expt 1, and every 30 min in Expt 2, and placed in scintillation vials. Residual inorganic ^{14}C was removed by addition of HCl for 2 h (250 μl HCl 1 M ml^{-1} sampled water). For budget experiments, ^{14}C was added to a final concentration of 40 $\mu\text{Ci l}^{-1}$, and the 24 h incubations were terminated by filtration on Whatman GF/F filters. The filters were placed in scintillation vials and fumed during 20 min with concentrated HCl. In both types of experiments, 10 ml of scintillation liquid (Ready Safe™) was added to the vials. All samples were left at least 24 h in darkness prior to counting in a LKB scintillation counter.

In order to account for variations due to the growth of cultures during experiments, ^{14}C incorporation was expressed in production per unit biomass. In all cases, production values were corrected for dark uptake. The parameter values (k , λ and d) were adjusted to provide the best fit between experimental and simulated data. In order to plot simulated and observed values together in one figure using the same ordinate-scale, the latter were multiplied by the regression coefficient obtained by linear regression between corresponding simulated and field

data. The coefficient of determination (r^2) provides an estimate of the amount of variance in observations which is explained by the model.

RESULTS

Model simulations

When a 24 h incubation ($d = 12$ h) starts at sunrise (i.e. 6:00 h; Fig. 1a), the highest gain (G) is observed at noon and the maximum amount of accumulated ^{14}C is reached at sunset. Because $\lambda = 5$ h, loss (L) starts at 11:00 h, and causes, throughout the night, a decrease in the amount of accumulated ^{14}C . In contrast, if the incubation starts 1 h before sunset (i.e. 17:00 h,

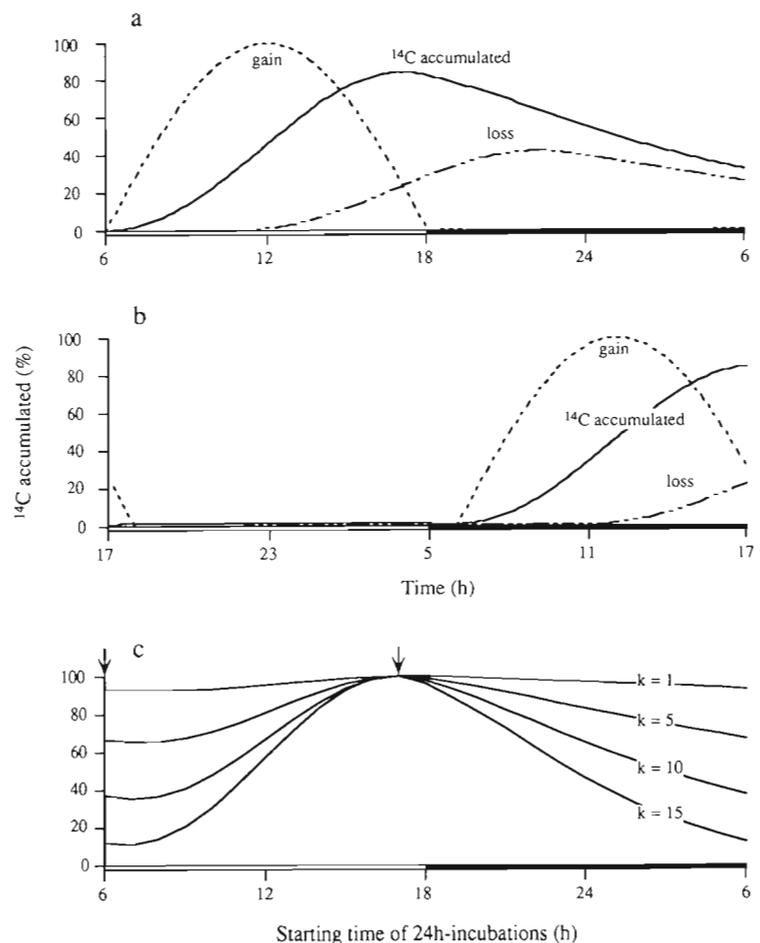


Fig. 1 Changes of simulated gain and loss terms and of the amount of accumulated ^{14}C during an incubation started (a) at sunrise (i.e. 6:00 h) and (b) 1 h before sunset (i.e. 17:00 h). (c) ^{14}C budgets for 24 h incubations started at different times of the day, and using 4 different loss rates [$k = 1, 5, 10$ and $15 \times 10^{-4} \text{ min}^{-1}$; arrows indicate the ^{14}C budget values over 24 h corresponding with simulations shown in (a) and (b)]. The relative amounts of accumulated ^{14}C are normalized to a maximum value of 100. Other parameters for the model are $d = 12$ h, $\lambda = 5$ h and $\alpha = 1$

Table 1. Ratios of the maximum to minimum amounts of ¹⁴C accumulated during a 24 h incubation, for 3 daylengths (*d*, in h), 5 time lags (λ , in h) and 3 instantaneous loss rates (*k*, in 10⁻⁴ min⁻¹) obtained with the model

λ	<i>d</i> = 12			<i>d</i> = 15			<i>d</i> = 18		
	<i>k</i> = 1	<i>k</i> = 5	<i>k</i> = 10	<i>k</i> = 1	<i>k</i> = 5	<i>k</i> = 10	<i>k</i> = 1	<i>k</i> = 5	<i>k</i> = 10
1	1.08	1.50	2.30	1.07	1.40	1.98	1.05	1.31	1.73
2	1.08	1.52	2.44	1.07	1.41	2.08	1.05	1.32	1.80
3	1.08	1.53	2.58	1.07	1.42	2.18	1.05	1.33	1.87
4	1.08	1.53	2.72	1.07	1.43	2.28	1.05	1.33	1.94
5	1.08	1.53	2.82	1.07	1.43	2.35	1.05	1.33	1.97

Fig. 1b), the total gain during the incubation is identical to the previous case (i.e. integral of the sine function during daylight, Eq. 2.1), but the total loss is much lower since it is a function of tracer accumulation (Eq. 3). As a result, the amount of ¹⁴C accumulated after the 24 h incubation is higher than in the previous case.

Combining gain and loss terms provides budgets of ¹⁴C accumulation. These were calculated for 24 incubations, starting at each hour of the day (Fig. 1c). The minimum amount of accumulated ¹⁴C corresponds to the incubation started 1 h after sunrise, and the maximum to the incubation started 1 h before sunset. The ¹⁴C budgets are quite similar for incubations started 1 h before or after sunrise (difference <2.4% for *d* = 13 h, *k* = 10 × 10⁻⁴ min⁻¹ and λ = 5 h). The same is true for incubations started 1 h before or after sunset (difference <2.9% for the same parameters). Using different values of *k* influences the amplitude of the curve, but it does not change the positions of extremes.

Minimum amounts of ¹⁴C accumulated during 24 h incubations simulated with daylengths of 12, 15 and 18 h are observed 1, 2 and 3 h after sunrise, respectively; corresponding maxima are observed 10 to 11, 12 to 13 and 14 to 15 h after sunrise, respectively. Timing of minimum and maximum accumulated ¹⁴C is mainly determined by daylength *d*, values of λ and *k* only playing a minor role.

Ratios of minimum to maximum amounts of accumulated ¹⁴C during a 24 h incubation were calculated for each of the 3 daylength, 5 lag and 10 loss values used in the simulations. Minimum ratios are quite similar for

the 3 daylengths (Table 1), and correspond to the smallest loss values (*k* = 1 × 10⁻⁴ min⁻¹). Maximum ratios decrease with increasing *d* and increasing λ ; maximum ratios correspond to the highest *k* values.

The introduction of a variable photosynthetic efficiency (α), out of phase with the irradiance sine function (Eqs. 2.1 & 2.2), creates an asymmetry in the ¹⁴C gain kinetics (not shown). When using high value for the phase (6 h), the displacement of maximum gain reached up to 2 h and the amplitude diminished by about 10% of that obtained with constant photosynthetic efficiency (Eq. 2.1). The budgets of accumulated ¹⁴C were only slightly influenced by varying α . Using a phase of 6 h moved the maximum amount of accumulated ¹⁴C by about 1 h and, for phase values of 2 to 3 h, variable α had only minor influence on the dynamics of ¹⁴C accumulation and on the budgets. Therefore, in subsequent simulations, α was kept constant.

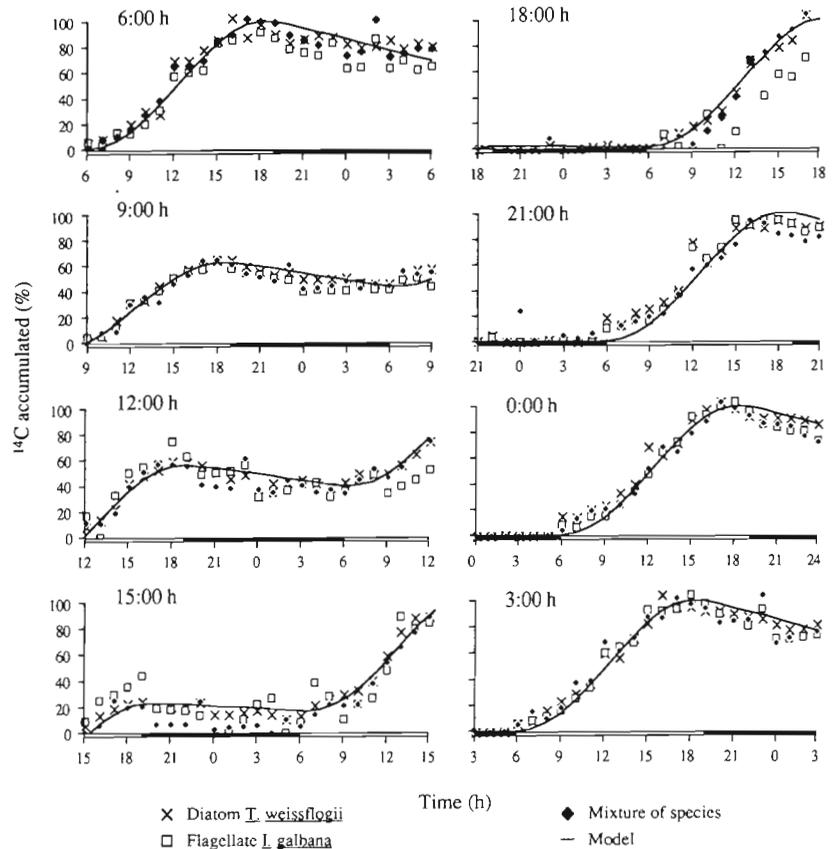


Fig. 2. ¹⁴C uptake kinetics of cultures for 8 incubations initiated at 6:00, 9:00, 12:00, 15:00, 18:00, 21:00, 0:00 and 3:00 h (Expt 1). Results are compared to those from a corresponding simulation. Parameters for the model are *d* = 13 h, *k* = 6 × 10⁻⁴ min⁻¹, λ = 5 h and α = 1

Experiments

^{14}C uptake kinetics

In Expt 1, uptake kinetics for the diatom and the flagellate were identical, and the mixture showed the same results as for cultures of individual species (Fig. 2). Given the low biomass of the cyanobacterium, the ^{14}C uptake was close to the background and non-significant (not shown). Incubations initiated at dawn showed accumulation during the day followed by loss during the dark period, whereas incubations started at dusk did not show any accumulation before light was turned on. Dark bottles displayed a slow and steady accumulation during the 24 h incubation, ranging from 2 to 16 % of the maxima in corresponding light bottles (not shown). Linear regressions showed highly significant relationship between experimental and simulated data ($p < 0.001$, $n = 24$; Table 2).

In Expt 2, as observed on cultures, incubations with natural communities started at 6:00 h showed an increase in the amount of accumulated ^{14}C during the day followed by a decrease beginning at sunset (Fig. 3a). The second incubation (started at 19:00 h) exhibited very low values during the night followed by an increase during the day (Fig. 3b). In both cases, values from dark bottles were close to background levels (not shown). Relationships between experimental and simulated data were highly significant ($r^2 = 0.89$ at 6:00 h and 0.96 at 19:00 h; $p < 0.001$). The 2 kinetics are similar to the corresponding ones in Expt 1 (see Fig. 2, 6:00 h and 18:00 to 21:00 h).

^{14}C budgets

Production budgets derived from Expt 1 (the last value of each kinetics in Fig. 2 representing the budget of a 24 h incubation) were compared with the model (Fig. 4). All correlations between experimental and simulated data are significant ($r^2 > 0.73$ and $p < 0.001$ for all cultures). The model shows that time variations of experimental budgets were minimum for incubation initiated just after dawn and maximum for incubation started just before dusk. Daylength in the model is 13 h, the best fit corresponded to $k = 6 \times 10^{-4} \text{ min}^{-1}$ and $\lambda = 5 \text{ h}$.

^{14}C budgets measured in the field over 24 h (Expt 3) showed 2 minima and 2 maxima within the 48 h sampling period, corresponding to incubations initiated at dawn and at dusk, respectively (Fig. 5). Despite a greater amplitude in variation for experimental data and a small phase difference, results are in good agreement with the model. Parameter values are nearly the same as for kinetics in Expt 1 ($d = 13 \text{ h}$, $k = 7 \times 10^{-4} \text{ min}^{-1}$ and $\lambda = 5 \text{ h}$; $r^2 = 0.37$ and $p < 0.001$).

Table 2. Coefficients of determination (r^2) between experimental and simulated data for 3 types of culture (Expt 1)

Starting time (h)	Diatom	Flagellate	Mixture
6:00	0.94	0.92	0.94
9:00	0.94	0.90	0.86
12:00	0.88	0.47	0.74
15:00	0.97	0.70	0.91
18:00	0.99	0.84	0.98
21:00	0.96	0.97	0.96
0:00	0.97	0.98	0.98
3:00	0.98	0.97	0.95

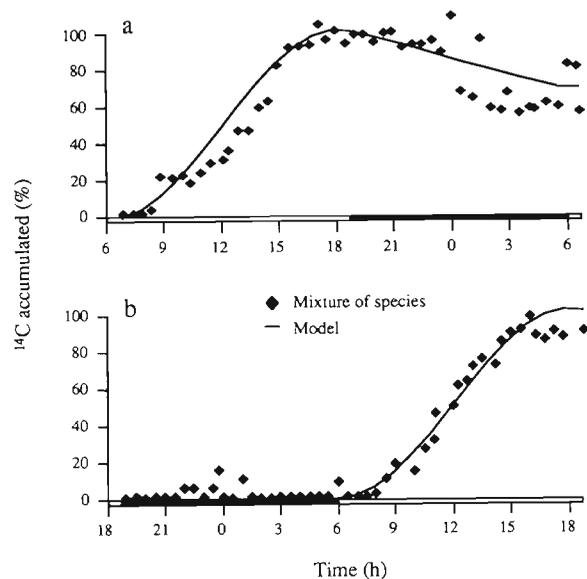


Fig. 3. ^{14}C uptake kinetics of natural phytoplankton, with incubation initiated (a) at dawn and (b) at dusk (Expt 2). Results are compared to those from a corresponding simulation. Parameters for the model are $d = 13 \text{ h}$, $k = 6 \times 10^{-4} \text{ min}^{-1}$, $\lambda = 5 \text{ h}$ and $\alpha = 1$

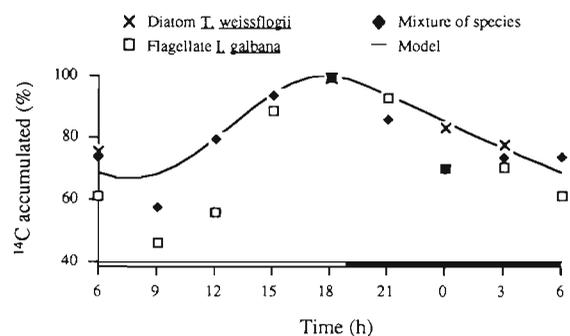


Fig. 4. Time variations of ^{14}C budgets, for incubations started at different times of the day (Expt 1). Values correspond to the last values of kinetics in Expt 1 (Fig. 2). Parameters for the model are $d = 13 \text{ h}$, $k = 6 \times 10^{-4} \text{ min}^{-1}$, $\lambda = 5 \text{ h}$ and $\alpha = 1$ (similar to Fig. 1c, with $k = 5 \times 10^{-4} \text{ min}^{-1}$)

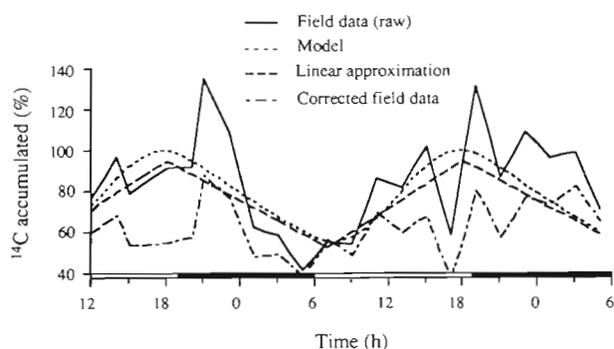


Fig. 5. Relative amounts (%) of ^{14}C accumulated in natural plankton samples, collected over 48 h at an anchor station, after 24 h incubations started at different times of the day (Expt 3). Comparison of results from the field (raw), the model, the linear approximation and corrected field data (corrected using the linear approximation). Parameters for the model are $d = 13$ h, $k = 7 \times 10^{-4} \text{ min}^{-1}$, $\lambda = 5$ h and $\alpha = 1$ (similar to Fig. 1c, with $k = 5$ and $10 \times 10^{-4} \text{ min}^{-1}$)

DISCUSSION

The simple model of ^{14}C accumulation used here explained a large fraction of the variation in experimental data. The model runs well, within the range of parameter values $d = 12, 15$ and 18 h, $\lambda = 1$ to 5 h and $k = 1 \times 10^{-4}$ to $1 \times 10^{-3} \text{ min}^{-1}$ (Table 1). However, when loss rates (k) are very high, the model may calculate losses even when the amount of accumulated ^{14}C has decreased to 0. This shows that the chosen time lag (λ) sets an upper limit on k . The length of the day influences the times at which minimum and maximum values occur as well as the interval between these times, whereas λ and k mainly determine the amplitude of curves as shown in Fig. 1c (high ratios correspond to steep slopes). Combining the different time lags and instantaneous loss rates results in losses ranging from 7% ($d = 13$ h, $\lambda = 5$ h and $k = 1 \times 10^{-4} \text{ min}^{-1}$) up to 65% ($d = 13$ h, $\lambda = 5$ h and $k = 10 \times 10^{-4} \text{ min}^{-1}$) of the ^{14}C uptake over the 24 h period (Fig. 1c). When comparing simulated to experimental results, the best fits were obtained with $\lambda = 5$ h and $k = 6 \times 10^{-4} \text{ min}^{-1}$ ($d = 13$ h corresponding with natural day-night cycle), and resulted in losses of 40%. This order of magnitude for the loss seems reasonable since light respiration is expected to not exceed 20% of the photosynthetic rates (Williams 1993), with dark respiration being much lower (see Sakshaug 1993 and references therein), and exudation is generally only 3 to 10% of the ^{14}C accumulation but may reach 60 to 90% in extreme cases (see Sakshaug 1993 and references therein). Even if the physiological history of samples plays an important role in primary production measurement (e.g. Harris 1978), it appears that photosynthetic efficiency, expressed as in Eq. (2.2), plays a

minor role in ^{14}C uptake model when compared to the effects of the lag or loss parameters.

Both ^{14}C uptake kinetics (Figs. 2 & 3) and budgets (Fig. 4) show a positive slope ($G > L$) during the day, which becomes negative ($G < L$) during the night. This resulted in maximum carbon accumulation at the end of the light period, which corroborated the assumption that ^{14}C is preferentially accumulated during the light period, proportionally to the solar irradiance, and that ^{14}C accumulation is negligible during darkness. Dark incubations in the laboratory confirmed this assumption, with a slow and steady accumulation during the 24 h incubations. However, higher dark fixation values were recorded in the field, suggesting higher β -carboxylase activity (Glover 1989), or higher heterotrophic activity (Gieskes et al. 1979, Li et al. 1993) in natural communities than in cultures from the laboratory. Approximately 40% of the accumulated ^{14}C was lost after 11 h in darkness during laboratory Expt 1 (Fig. 2, 6:00 h), and 60% during field Expt 3 (Fig. 5), leading to loss rates of 3.6 and 5.5% h^{-1} , respectively. These loss values agree with the range of estimates of 4.2% h^{-1} given by Eppley & Sharp (1975) and with the 2.5 to 10% h^{-1} from Ryther (1954, 1956a, b).

Kinetics performed in the laboratory were highly significantly correlated with the model (Table 2). The worst correlation, observed for flagellates incubated from 12:00 h ($r^2 = 0.47$), is due to a high signal/noise in the data. In this particular incubation, an abnormally low biomass resulted in low counts of labelled carbon. Uptake kinetics (Expt 1) of the diatom, the flagellate or their mixture were identical (Fig. 2). This was not expected, since field measurements of respiratory losses by Smith (1977) ranged from 13.4% for a diatom dominated assemblage to 78.4% for a community dominated by a dinoflagellate. The author explained these differences by higher respiratory demand due to the motility of the flagellates. Similarity between the 2 species, as observed in our laboratory experiment, may be explained by low (in the mixture) or null (in separated cultures) competition between species due to optimal conditions.

Since the results of kinetics measurements provide hourly estimations of gross production as well as total dark losses and net production (difference between gross production and total dark losses), these values may be compared to published results for time course experiments. For example, in the central gyre of North Pacific, 24 h noon-to-noon incubations gave only 12 to 15% more carbon incorporation than 6 h incubations (noon-to-sunset; Eppley & Sharp 1975). A similar calculation, derived from Expt 1, gives a comparable difference of 25%. In addition, the authors calculated a P6 h/P24 h ratio (6 h noon-to-sunset production/24 h noon-to-noon production), which varied from 0.66 to

1.30 depending on the sampling date. Ratios measured between March and June (≤ 1) were lower than those recorded in February (≥ 1). Since $d = 13$ h was used in Expt 1, our results are comparable to those from Eppley & Sharp (1975) measured between March and June. The P6 h/P24 h ratio from our Expt 1 is 0.75, agreeing well with their ratios from this period ranging from 0.66 to 1. Results from both field experiment and the model suggest that this ratio depends on the daylength, so that seasonal variations in its value should be observed independently of the sampling site.

Both ^{14}C budgets derived in the laboratory (Expt 1) and those measured in the field (Expt 3) agreed with the model (Figs. 4 & 5, Table 2). Values of the 24 h ^{14}C budgets are minimum for dawn-to-dawn incubations and maximum for dusk-to-dusk incubations. The difference between extremes represents the total losses of carbon during 24 h incubation, due to respiration and exudation processes. This difference reached 40 % for ^{14}C budgets measured in the laboratory (Fig. 4), and 60 % for budgets measured in the field (Fig. 5). Both variability and amplitude of the ^{14}C budgets were higher for field measurements. This is essentially due to differences in methods. Kinetics (Expt 2) were conducted in a well-controlled environment, using the same large water sample for filling all incubation bottles. This is contrary to budgets measured in the field (Expt 3), which used samples collected over 48 h at the anchor station (Fig. 5). In this last case, advection may have changed phytoplankton assemblages, and additional factors, such as hydrodynamics (e.g. Legendre & Demers 1984), solar irradiance (e.g. Côté & Platt 1983, Vincent 1992) and physiology (e.g. Harris, 1978), may have induced changes in the photosynthetic response of phytoplankton over the 48 h sampling. Finally, variables such as light saturation, day depression or circadian effects were not included in the model. All these factors may have caused the greater amplitude in production measurements during Expt 3, or/and the slight phase difference between observations and the model, and which would explain reduced correlation between field measurements and the model.

RECOMMENDATIONS

The above results indicate that ^{14}C values from dusk-to-dusk incubations would generally exceed those from the dawn-to-dawn measurements by at least 40 %. In order to allow comparisons among sampling areas and periods, 24 h ^{14}C budgets should be standardized. Our recommendations for field studies are as follows. The simplest approach for estimating daily rates of primary production using the ^{14}C method is to follow the recommendation of JGOFS (1988), and thus conduct 24 h

dawn-to-dawn incubations. Results from simulations and field experiments indicate (1) that sampling at sea could start 1 to 2 h before dawn, and continue for a period of 1 to 2 h after dawn, without influencing too much the estimated daily primary production, and (2) that incubations started before dawn should be conducted for a full 24 h period after dawn. When logistic constraints make it necessary to collect field samples at various times, it may be possible to get estimates of net primary production corresponding to dawn-to-dawn measurements, using the following approach. (1) Since ^{14}C uptake during darkness is minimal, samples collected during the night should be kept in darkness. They could be inoculated with $\text{NaH}^{14}\text{CO}_3$ immediately after collection or at any time before dawn without major effects. Incubations should then be conducted for a full 24 h period after dawn, and the resulting measurements of primary production do not need corrections. (2) For samples collected at other times, the accumulation of ^{14}C during 24 h incubations should also be determined on additional samples collected shortly after dawn (A_{dawn}) and before dusk (A_{dusk}). If phytoplankton in the sampling area is believed to be quite uniform, these measurements do not need to be repeated every day. Samples collected at time t should be inoculated shortly after sampling, incubated for a period of 24 h, and thus giving an accumulation value A_t . Assuming, as first approximation, linear accumulation of ^{14}C between dawn and dusk, estimates of dawn-to-dawn values can be calculated as:

$$^{14}\text{C}_{\text{dawn-to-dawn}} = \frac{A_t}{\left(\frac{A_{\text{dusk}} - A_{\text{dawn}}}{A_{\text{dawn}}}\right) \left(\frac{\Delta t_1}{\Delta t_2}\right) + 1} \quad (4)$$

where $\Delta t_1 = t - t_{\text{dawn}}$ and $\Delta t_2 = t_{\text{dusk}} - t_{\text{dawn}}$ for incubations started during the day, and where $\Delta t_1 = (t_{\text{dawn}} + 24) - t$ and $\Delta t_2 = (t_{\text{dawn}} + 24) - t_{\text{dusk}}$ for incubations started during the night. The difference in the correction for day and night initiated incubations is due, firstly to the asymmetry of the uptake curve in varying daylengths (d), and secondly to the change in the sign of the slope during day and night incubation.

For example, in the case of incubations started at 13:00 and 23:00 h, with the night extending from 20:00 to 4:00 h,

$$\frac{\Delta t_1}{\Delta t_2} = \frac{(13 - 4)}{(20 - 4)} = 0.56$$

$$\text{and } \frac{\Delta t_1}{\Delta t_2} = \frac{[(4 + 24) - 23]}{[(4 + 24) - 20]} = 0.63 \quad \text{respectively.}$$

The linear approximation (Fig. 5; Eq. 4) of the model (Figs. 1c & 5; Eq. 1) was used to correct all field data from Expt 3. Maximum and minimum values for computing the the approximation ($A_{\text{dawn}} = 53.6\%$ and A_{dusk}

= 92.3%) correspond to the averaged values from field incubations started at 6:00, 7:00 and 8:00 h and at 17:00, 18:00 and 19:00 h, respectively. The linear approximation, calculated from field measurements, differed by less than 10% from the model (Fig. 6; $r^2 = 0.98$ and $p < 0.001$). The mean value of the corrected field data is 60%, which is close to the 55% obtained with the model (Fig. 5). These corrected values do not show any daily variations which were present in the original data set.

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