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Does the ^{14}C method estimate net photosynthesis? Implications from batch and continuous culture studies of marine phytoplankton

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ABSTRACT

We carried out batch culture studies with seven species of marine phytoplankton and chemostat studies with two of the seven species to determine whether and to what extent ^{14}C uptake approximated net photosynthesis. In two of seven cases, *Isochrysis galbana* and *Dunaliella tertiolecta*, cells uniformly labeled with ^{14}C lost no activity when they were transferred to a ^{14}C-free medium and allowed to grow in the light. In similar experiments with four other species, uniformly labeled cells lost activity when incubated in the light, but the loss rates were only a few percent per day. Thus these six species appear to respire primarily recently fixed carbon. In the case of the remaining species, *Chlorella kessleri*, loss rates of ^{14}C in the light from uniformly labeled cells were about 29% per day, the apparent ratio of respiration to net photosynthesis being 0.4. Follow-up chemostat studies with *I. galbana* and *C. kessleri* grown under both light- and nitrate-limited conditions produced results consistent with the implications of the batch culture work: uptake of ^{14}C by *I. galbana* after incubations of 24 h yielded estimates of photosynthetic carbon fixation equal to the product of the chemostat dilution rate and the concentration of organic carbon in the growth chamber. Similar experiments with *C. kessleri* produced ^{14}C-based estimates of photosynthetic carbon fixation that exceeded the net rates of organic carbon production in the growth chamber by roughly 55%. Time-course studies with both species indicated that at high growth rates recently fixed carbon began to enter the respiratory substrate pool after a time lag of several hours, a result consistent with previous work with *D. tertiolecta*. The lag time appeared to be much shorter at low growth rates. The results with *C. kessleri* are similar to results previously reported for *Chlorella pyrenoidosa* and *Amphidium carteri*. Collectively these results suggest that ^{14}C uptake by species with relatively high ratios of respiration to photosynthesis may tend to substantially overestimate net photosynthesis, perhaps because a substantial percentage of the carbon respired by such species is old carbon.

1. Introduction

More than 60 years ago Steemann Nielsen (1951, 1952) reported the first results of the use of inorganic carbon labeled with tracer amounts of ^{14}C to estimate photosynthetic rates in the ocean. The method was much more sensitive than the oxygen light-and-dark-bottle methodology (Riley, 1939) and was rapidly adopted by virtually all oceanographers and limnologists with an interest in measuring phytoplankton photosynthetic rates in aquatic systems (Doty and Oguri, 1957; Koblentz-Mishke et al., 1970; Schindler and Holmgren, 1971). Large amounts of time and resources have been invested in the accumulation of an extensive database of ^{14}C measurements in the ocean, and those measurements have been used to calibrate satellite algorithms that are now being used to estimate marine primary production on a global scale (Behrenfeld et al., 2002). Despite advances in the use of oxygen-based methodologies, the ^{14}C method remains the most sensitive and therefore the first choice for measuring rates of photosynthesis in the ocean, and remote sensing techniques are validated based on their correspondence with ^{14}C uptake data (Carr et al., 2006). Nevertheless, from the very beginning (Ryther, 1956; Ryther and Menzel, 1965; Steemann Nielsen, 1955) and throughout the last 60 years (Hobson et al., 1976; Laws et al., 2000; Lloyd et al., 1977; Marra, 2009; Peterson, 1980; Williams and Lefevre, 1996; Williams et al., 1996; Williams, 1993), there has been uncertainty as to whether the ^{14}C method was measuring the rate of net or gross primary production or a rate intermediate between net and gross.

Some of the concerns regarding measurements of photosynthesis in the ocean have been methodological: (1) the size of incubation bottles (Gieskes et al., 1979; Laws et al., 1987); (2) the

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use of clean sampling (Fitzwater et al., 1982; Marra and Heinemann, 1987; Williams and Robertson, 1989) and incubation (Marra and Heinemann, 1984) techniques; and (3) the duration of incubations (Steemann Nielsen, 1952). These issues are not specific to the ^{14}C method but are relevant to any method that involves the collection and subsequent incubation of samples of seawater.

The issue that is specific to the ^{14}C method has been the question of whether the uptake and incorporation of inorganic ^{14}C into organic matter provides an estimate of net uptake, gross uptake, or a rate intermediate between these two. The nature of the problem is illustrated in Fig. 1. Respired carbon either escapes from the cell (R) and is returned to the surrounding medium or is recycled (R') within the cell. The gross uptake of inorganic carbon from the surrounding medium equals P. Gross photosynthesis equals $P+R'$, and net photosynthesis equals $P-R$. Fig. 1 is similar to Fig. 3 in Marra (2009), but in Fig. 1 we have omitted a mitochondrion and chloroplast to extend the conceptual model described by Marra (2009) to cyanobacteria. It is apparent from Fig. 1 that the net uptake rate of inorganic carbon from the surrounding medium equals $P-R$. In the case of ^{14}C uptake, P and R must be multiplied by the specific activities of the respective substrates and divided by the isotope discrimination factors associated with the respective processes. Therefore

$$\text{net}^{14}\text{C uptake} = \frac{P \times SA_{DIC}}{ID_P} - \frac{R \times SA_R}{ID_R} \quad (1)$$

where SA_{DIC} is the specific activity of the dissolved inorganic carbon (DIC) in the surrounding medium, SA_R is the specific activity of the carbon that is a substrate for respiration, and ID_P and ID_R are the isotope discrimination factors associated with photosynthesis and respiration, respectively. The specific activity of the carbon that is fixed is therefore SA_{DIC}/ID_P, and the specific activity of the respired carbon that escapes from the cell is SA_R/ID_R. ID_P is commonly assumed to equal 1.05, with an uncertainty of ± 0.03 (Williams et al., 1996). Photosynthesis calculated by the ^{14}C method equals the value of this expression multiplied by ID_P and divided by SA_{DIC}. Therefore

$$\text{calculated photosynthesis} = P - R\frac{SA_R}{SA_{DIC}}\frac{ID_P}{ID_R} \quad (2)$$

To the extent that respiratory carbon is recycled within the cell (i.e., $R' > 0$), the ^{14}C method cannot provide an estimate of gross photosynthesis ($P+R'$) because it provides no estimate of R'. The right-hand side of Eq. (2) will equal net photosynthesis if (i) $R=0$ (i.e., all respired carbon is recycled within the cell) or (ii) $SA_R/ID_R = SA_{DIC}/ID_P$ (i.e., the respired carbon that escapes from the cell has the same specific activity as the carbon fixed). The second condition implies that virtually all respired carbon that escapes from the cell is "new" (i.e., recently fixed) carbon (Williams, 1993). Alternatively, the right-hand side of Eq. (2) will equal gross photosynthesis if (i) $R'=0$ (i.e., all respired carbon escapes from the cell) and (ii) $SA_R=0$ (i.e., all respired carbon is "old" carbon).

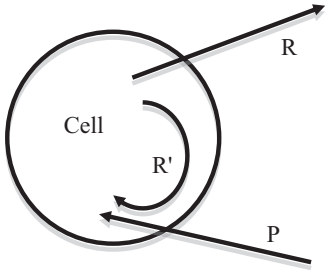

Fig. 1. Conceptual model of carbon uptake by a phytoplankton cell. R is the respired carbon that escapes from the cell, and R' is the respired carbon that is recycled within the cell. P is the gross uptake of inorganic carbon from the medium. Gross photosynthesis is $P+R'$. Net photosynthesis is $P-R$.

Ryther (1956) articulated these same conditions but rejected the possibility that new carbon was the substrate for respiration based on the work of Calvin (1949) and Weigl et al. (1951). In contrast, Halsey et al. (2011) found that roughly 60% of ^{14}C taken up by a culture of *Dunaliella tertiolecta* was incorporated into a transient carbon pool, most of which was catabolized within 20 min at low growth rates. At high growth rates the lifetime of ^{14}C in the transient carbon pool was much longer, but substantial amounts of the ^{14}C were respired and lost from the cell within 8 h. Halsey et al. (2010) concluded that short-term (20-min) ^{14}C uptake approximated net photosynthesis for slowly growing cells and gross carbon uptake for rapidly growing cells.

Both Ryther (1956) and Steemann Nielsen (1955) used batch culture experiments with phytoplankton uniformly labeled with ^{14}C to determine to what extent old carbon was respired and lost from the cells. The uniformly labeled cells were harvested, resuspended in media containing no added ^{14}C, and subsequently incubated either in the light or dark. Ryther (1956) found that *Dunaliella euchlora* cells lost no activity when incubated in the light, but after one day there was a 20% loss of the ^{14}C activity in cells incubated in the dark. He concluded that the ^{14}C method measured net photosynthesis. In contrast, Steemann Nielsen (1955) found that *Chlorella pyrenoidosa* cells incubated in the light lost ^{14}C activity, although at a lower rate (0.6–1.3% h^{-1}) than the loss rate in the dark (1.8–3.3% h^{-1}). He concluded that the photosynthetic rate calculated from Eq. (2) would be intermediate between net and gross photosynthesis.

Subsequent to these inconsistent results, a number of studies have been carried out to try to determine what the ^{14}C method measures. These studies have involved both laboratory cultures and field experiments and have provided comparisons between results obtained with ^{14}C versus alternative methods, including the oxygen light-and-dark-bottle technique (Bender et al., 1987; Grande et al., 1989; McAllister et al., 1964; Stanley et al., 2010), ^{18}O evolution techniques (Bender et al., 1987; Grande et al., 1989; Laws et al., 2000), and direct measurements of changes in particulate or inorganic carbon both for cultures (Ryther and Menzel, 1965; Smith and Platt, 1984; Williams et al., 1996) and for natural plankton populations (Antia et al., 1963; Eppley and Sharp, 1975; Eppley and Sloan, 1965; McAllister et al., 1961; Parsons et al., 1969; Ryther et al., 1971; Sheldon et al., 1973; Sutcliffe et al., 1970). It seems clear from studies that have compared estimates of gross photosynthesis with ^{14}C-based estimates of photosynthesis that the latter considerably underestimates the former (e.g., Grande et al., 1989; Laws et al., 2000), a not surprising conclusion given the strict conditions that would have to be satisfied if the ^{14}C method were to estimate gross photosynthesis (i.e., all respired carbon escapes from the cell, and no recently fixed carbon is respired). There remains the question of whether the ^{14}C method estimates net photosynthesis. Studies with natural populations include organisms other than phytoplankton, and the metabolism of carbon by those organisms confounds any comparison of changes in organic carbon with the implications of ^{14}C uptake. Measurements based on production of ^{18}O-labeled O_2 from $H_2{}^{18}O$ provide a measure of gross photosynthesis and net community production but do not provide a measure of net photosynthesis, and any comparisons with carbon metabolism require division or multiplication by assumed photosynthetic quotients or respiratory quotients, respectively.

The study of Ryther and Menzel (1965) provided a direct comparison between photosynthesis based on ^{14}C uptake and changes of particulate carbon in pure cultures. In incubations lasting either 6 or 12 h, their calculated photosynthetic rates estimated from ^{14}C uptake either equaled or exceeded changes