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What does the ^{14}C method for estimating photosynthetic rates in the ocean really measure?

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WHAT DOES THE ^{14}C METHOD FOR ESTIMATING PHOTOSYNTHETIC RATES IN
THE OCEAN REALLY MEASURE?

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Oceanography and Coastal Sciences

by

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ABSTRACT

The ^{14}C method has been used extensively by both limnologists and oceanographers to measure photosynthetic rates in aquatic systems, and the large database of ^{14}C measurements that now exists is the ground truth with which satellite algorithms for estimating marine photosynthetic rates on a basin and global scale have been calibrated. However, disconcerting uncertainties still remain with respect to whether and under what circumstances the ^{14}C method provides an estimate of net or gross photosynthesis, or something in between. My study combined batch and continuous culture studies to clarify this ambiguous issue.

The batch culture work with seven species of marine phytoplankton indicated that the ^{14}C method should estimate net photosynthesis for *Isochrysis galbana* and *Dunaliella tertiolecta*, gross photosynthesis for *Chlorella kessleri*, and a rate in between for the remaining four species. 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.

For *I. galbana* the photosynthetic rates estimated by ^{14}C uptake were in good agreement with the actual net fixation, but for *C. kessleri* the ^{14}C method overestimated TOC fixation by roughly 50–100%, the degree of overestimation depending on incubation length and growth condition. Time-course studies with *C. kessleri* indicated that at a high nitrate-limited growth rate recently fixed carbon began to enter the respiratory substrate pool after a time interval of about four hours. Results of 12:12 L:D cycle incubations were not as straightforward to interpret as the continuous culture results, but the calculated photosynthetic rates relative to net carbon fixation were clearly a function of species and growth rate. The fact that the specific activity (SA) of organic carbon respired in the dark period was less than the SA of the inorganic carbon in the growth medium implies that carbon respired in the dark was a combination of recently fixed carbon and old carbon. These results imply that in field studies

the uptake of ^{14}C during the photoperiod overestimates net photosynthesis, the degree of overestimation depending on the growth conditions and composition of the phytoplankton community.

CHAPTER 1: INTRODUCTION

1.1 Background

In 1952 Steeman Nielsen reported the first results of the use of inorganic carbon labeled with tracer amounts of ^{14}C to estimate photosynthetic rates in the ocean (Steemann Nielsen 1952). The method was so much more sensitive than the oxygen light-and-dark-bottle methodology that it was rapidly adopted by virtually all oceanographers and limnologists with an interest in measuring photosynthetic rates in aquatic systems (technique outlined by Saunders, Trama et al. 1962; Vollenweider, Talling et al. 1969; Strickland and Parsons 1972). Nevertheless, from the very beginning there was uncertainty as to what the so-called “ ^{14}C method” was actually measuring (Steemann Nielsen 1955; Ryther 1956; Ryther and Menzel 1965; Peterson 1980; Laws, Landry et al. 2000; Marra 2009).

Much of the controversy revolved around the question of whether the ^{14}C method measured net or gross photosynthesis, or something in between (reviewed by Peterson 1980; Falkowski and Raven 2007; Marra 2009). In an early publication Ryther (1956) pointed out that the ^{14}C method would measure net photosynthesis if

- a) recently fixed carbon is the substrate for respiration (i.e., respired carbon has the same specific activity as the inorganic carbon in the seawater), **or**
- b) all respired carbon is reassimilated

Alternatively, the ^{14}C method will measure gross photosynthesis if

- c) old carbon is the substrate for respiration (i.e., respired carbon contains essentially no ^{14}C), **and**
- d) no respired carbon is reassimilated

To follow up on these hypotheses, Ryther (1956) uniformly labeled a culture of *Dunaliella euchlora* with ^{14}C and then resuspended the cells in a fresh medium containing no inorganic

^{14}C . Cells incubated in the light for several days did not lose their ^{14}C activity, whereas the cells incubated in the dark for the same length of time lost approximately 20% of their ^{14}C activity. The fact that the cells incubated in the light retained all their ^{14}C activity implied to Ryther that either hypothesis (a) and/or (b) above was correct. Based on the work of Calvin (1949) and Weigl, Warrington et al. (1951), Ryther rejected hypothesis (a) and concluded that hypothesis (b) must be correct. In any case, the conclusion was that the ^{14}C method measures net photosynthesis.

Interestingly, Steemann Nielsen (1955) performed a similar experiment but with different results. *Chlorella pyrenoidosa* cells uniformly labeled with ^{14}C and resuspended in a ^{14}C -free medium actually lost some of their ^{14}C activity when they were subsequently incubated in the light, but not as fast as cells treated likewise but subsequently incubated in the dark. Steemann Nielsen concluded that the ^{14}C method gives a result between net and gross photosynthesis.

A lively debate between Ryther & Vaccaro on one side and Steemann Nielsen and co-workers on the other began (Peterson 1980). Since then, a number of studies have been carried out to try to determine what the ^{14}C method measures. Up to now, it has been six decades since the introduction of the ^{14}C method for estimating the primary production of natural planktonic communities. Some informative comparisons have been made between results obtained with ^{14}C versus alternative methods: comparisons with the oxygen light-and-dark-bottle technique, ^{18}O evolution techniques, and direct measurement of changes in particulate carbon both for cultures and for natural plankton populations (Peterson 1980). Insights concerning the mechanisms of carbon fixation have been obtained from these studies. However, disconcerting discrepancies still remain, especially the question of whether and under what circumstances the ^{14}C method provides an estimate of net or gross photosynthesis, or something in between (Laws, Landry et al. 2000; Marra 2002; Marra 2009). Our inability to answer this question reflects in part the fact that our understanding of intracellular carbon

flow within algal cells is incomplete (Williams, Robinson et al. 1996). 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, Esaias et al. 2002). Despite advances in the use of oxygen-based methodologies, the ^{14}C method remains the gold standard for measuring rates of photosynthesis in the ocean, and remote sensing techniques are validated based on their correspondence with ^{14}C uptake data (Carr, Friedrichs et al. 2006).

As noted by Williams, Robinson et al. (1996), “under most conditions, these uncertainties (in the interpretation of ^{14}C uptake measurements) give rise to errors less than a factor of two in the estimation of plankton production; however, with present-day requirements for extrapolations to global scales, and verification of biological models, our aim should be the measurement of phytoplankton photosynthesis to within a factor of 20–50%.” I agree that a more informed assessment of what the ^{14}C method measures is long overdue. The experimental tools that can be utilized to address this question today are much more sophisticated than those available to scientists like Ryther and Steemann Nielsen more than 50 years ago. To the best of my knowledge, continuous culture systems have never been used to assess what the ^{14}C method measures.

1.2 Objectives

My proposed study was to determine what the ^{14}C method measures using continuous culture systems. Batch culture studies involving cells uniformly labeled with ^{14}C were used to survey phytoplankton species and to identify patterns in carbon fixation. Continuous culture studies were used to focus on two different species chosen on the basis of the batch culture results. In the continuous culture work the net rates of carbon fixation were known precisely

from the dilution rate of the continuous culture system and the concentrations of particulate organic carbon (POC) and total organic carbon (TOC) in the growth chamber. Thus a rigorous comparison between the rates calculated from ^{14}C uptake and the known net rates of carbon fixation (the '**control method**') was possible. The continuous culture studies were carried out under light- and nutrient-limited conditions, under continuous irradiance and a 12:12 L:D cycle, and at high and low growth rates. The combination of growth conditions and species carefully selected on the basis of the batch culture studies provided a much more informed assessment of what the ^{14}C method could be expected to measure and hence a much more quantitative assessment of results from field studies than is currently possible.

1.3 Dissertation Organization

This dissertation is organized into seven chapters. Chapter 2 presents a literature review summarizing the history of the ^{14}C method and the issues related to it, and contemporary related studies. Chapter 3 shows in detail the rationale for my study, the hypotheses to be tested, the experimental design and its advantages, the detailed sampling methods, and some other related topics. Chapter 4 is short but provides important results of the batch culture studies that influenced the following continuous culture experiments. Chapter 5 provides the results of the continuous cultures under nitrate- and light-limited conditions and the discussion related to this topic. Chapter 6 presents the results of incubations on 12:12 L:D cycle and the related discussion. There are several subsections in Chapter 5 and Chapter 6 since these two chapters are the main body of the dissertation. Chapter 7 is a summary of the conclusions of the work in which possible future research directions are discussed.

CHAPTER 2: REVIEW OF LITERATURE

2.1 Historical Overview

Since the early experiments of Ryther (1956) and Steemann Nielsen (1955), a number of studies have been carried out to try to determine what the ^{14}C method measures. Ryther and Menzel (1965) demonstrated that the uptake of ^{14}C was in good accordance with the net increase in particulate carbon even though no corrections for respiration, dark uptake, or isotope discrimination were applied. In a batch culture study of *Chlamydomonas*, photosynthesis measurements by the ^{14}C method under conditions of both exponential growth and nutrient starvation agreed with net photosynthesis as measured by the increase of oxygen in light bottles (Ryther 1954). Using a mass spectrometer to measure oxygen exchanges, Bunt (1965) found that respiration in the light decreased to as little as 25% of that in the dark and concluded that the ^{14}C method measures net photosynthesis if excretion is negligible. Nonetheless, interpretation in terms of gross photosynthesis was not possible unless the precise extent of inhibition of respiration by light was known (Peterson 1980).

The most consistent conclusion of the studies in the early 1960s was that ^{14}C uptake measures the net increase in particulate carbon in the light, according to results from both laboratory cultures and field experiments (Antia, McAllister et al. 1963; McAllister, Shah et al. 1964; Eppley and Sloan 1965; Ryther and Menzel 1965; Steemann Nielsen 1975). A quantitative measurement of either gross or net daily production was still impossible because of the lack of techniques to estimate photorespiration and the respiratory loss of organic matter in the dark (Peterson 1980).

After the late 1960s and early 1970s the emphasis in productivity research shifted back to field studies. Various studies were conducted under a wide variety of culture and field conditions (Parsons, Stephens et al. 1969; Sutcliffe Jr, Sheldon et al. 1970; Ryther, Menzel et

al. 1971; Sheldon, W. H. Sutcliffe et al. 1973). The oligotrophic oceans were particularly troublesome regions for comparisons between the ^{14}C method and other techniques (Sorokin 1971; Sorokin 1971; Sieburth 1977; Sieburth, Johnson et al. 1977). Some results supported the idea that ^{14}C uptake might underestimate production in oligotrophic waters (Eppley, Renger et al. 1973; Berman and Pollinger 1974; Eppley and Sharp 1975; Verduin 1975; Venrick, Beers et al. 1977; Gieskes, Kraay et al. 1979; Bower, Kelly et al. 1987). Some other studies showed that ^{14}C uptake overestimated particulate carbon production (Ganf and Blazka 1974; Peterson 1978). The interpretation of these ^{14}C uptake experiments was confounded by a number of factors, including photoinhibition, photorespiration, and dark respiration. Most of these studies have been discussed at length in several reviews (Peterson 1980; Li and Goldman 1981; Li 1987).

Additional studies focusing on this issue have been carried out since the 1980s, and numerous discussions have emerged (Carpenter and Lively 1980; Eppley 1980; Morris 1981; Malone 1982; Bender, Grande et al. 1987; Grande, Marra et al. 1989; Williams, Robinson et al. 1996; Marra 2002 and 2009; Falkowski and Raven 2007). Advances have been made in the understanding of light respiration (a problem that is shared with the ^{18}O and ^{14}C techniques), dark respiration, phytoplankton growth rate, length of incubation, and some technological problems, such as sample filtration.

Unfortunately, none of these studies came to an unambiguous and conclusive interpretation about what the ^{14}C method actually measures. For example, Dring and Jewson (1982) concluded that the measurements of photosynthesis based on ^{14}C uptake overestimated net photosynthesis in incubations of up to 6–12 h and that ^{14}C uptake approximated gross photosynthesis. In contrast, Williams, Robinson et al. (1996) came to the same conclusion with Ryther (1956); their observational data were consistent with the complete refixation of respired CO_2 . Marra (2002) compiled published Joint Global Ocean

Flux Study (JGOFS) measurements comparing gross photosynthesis based on ^{18}O with the uptake of ^{14}C and concluded that the ^{14}C method underestimated gross production as measured by the ^{18}O method and that ^{14}C uptake appeared to approximate net community production. Bender, Orchardo et al. (1999) also reported comparisons of ^{14}C production rates with new/export production rates estimated *in vitro* from rates of gross and net O_2 production during JGOFS cruises in 1992. They concluded that ^{14}C production rates in samples incubated for 24 h were about 45% of gross carbon production rates calculated from gross O_2 production. This difference was compatible with expected rates of the Mehler reaction, photorespiration, excretion, and community mitochondrial respiration. Unfortunately only particulate organic ^{14}C -labeled carbon (PO^{14}C) was sampled *in situ*, and dissolved organic ^{14}C -labeled carbon (DO^{14}C) was ignored during JGOFS cruises.

In situ experimental data collected from the PRPOOS project in 1985 indicated that ^{14}C uptake results were in best accordance with net photosynthesis in dawn-to-dusk experiments, and that ^{14}C uptake was less than or equal to gross O_2 production in 24-h experiments (Williams and Purdie 1991; Marra 2002). Some of the experiments reported by Bender, Grande et al. (1987) showed that ^{14}C assimilation estimated net production. Aristegui, Montero et al. (1996) reported that total daily water-column-integrated carbon incorporation measured by ^{14}C uptake in the particulate fraction underestimated net community production measured by the oxygen method by 29–54% when oxygen consumption and production and carbon fixation by micro-organisms were measured simultaneously in coastal surface waters near the Antarctic Peninsula. Based on the measurements of gross primary productivity (GPP) *in vitro* and GPP and net community production (NCP) *in situ* on four cruises to the Hawaii Ocean Time series (HOT) station ALOHA during 2002–2003, Juranek and Quay (2005) concluded that *in vitro* GPP, determined by ^{18}O labeling, yielded integrated production (0–100 m) that was on average 1.5 times the ^{14}C integrated production.

Laws, Landry et al. (2000) compared estimates of photosynthesis based on the incorporation of ^{14}C -labeled inorganic carbon into particulate carbon with estimates of gross photosynthesis based on net O_2 production and the production of $^{18}\text{O}_2$ from H_2^{18}O during the JGOFS Arabian Sea process cruises and calculated the ^{14}C uptake:gross photosynthesis ratios as a function of optical depth. They took into consideration the combined effects of the Mehler reaction, photorespiration, dark respiration, excretion, grazing effects, and UV light effects on the two estimates of photosynthesis. Taking all factors into account, their model predicted that the ratio of ^{14}C uptake to gross carbon production would be 0.48.

2.2 Contemporary Related Studies

A promising way to explore the question of what the ^{14}C method actually measures is the assay for reassimilation of recently respired carbon, which was perhaps first suggested by Ryther in 1956 to explain culture experiments (Marra 2009). Raven (1972) described further evidence of refixation of respiratory CO_2 . Such reassimilation is the most likely explanation for why the ^{14}C method might estimate net production (Marra 2009). As noted by Marra (2002, p. 98), “Accepting the refixation of respired CO_2 solves most of the problems in the interpretation of the ^{14}C method and does not require ad hoc explanations of respiratory losses (e. g. Laws, Landry et al. 2000)”. In the study of Williams, Robinson et al. (1996), ^{14}C uptake into the particulate plus the dissolved fractions approximated net photosynthesis in the physiological window studied (10–20% of the intrinsic generation time and gross photosynthesis/respiration ratios of 2–3), and over the time scale studied, the ^{14}C technique was measuring net photosynthesis, consistent with essentially 100% recycling of respiratory CO_2 . Some results reported by Bender, Grande et al. (1987) also agree with the assumption that respired carbon is refixed, in which case ^{14}C assimilation can be considered to estimate net photosynthesis.

However, the assumption of 100% refixation was rejected by Dring and Jewson (1982) based on the work of Bidwell (1977). And the assumption of refixation does not explain the results of the shipboard incubations of Williams, Heinemann et al. (1983) and Grande, Williams et al. (1989). Therefore, the evidence concerning refixation of respired carbon is not consistent. Other processes, such as release of dissolved organic matter or the existence of differing metabolic pathways for recently fixed and older carbon (Marra 2002), may contribute to the inconsistency of results. What the ^{14}C method measures is therefore still a source of ambiguity in quantifying organic carbon fixation rates in aquatic systems (Laws, Landry et al. 2000).

In fieldwork a number of issues confound the interpretation of ^{14}C uptake experiments. In addition to uncertainty about the substrate for respiration and fate of respired carbon, these issues include the following:

Excreted carbon. Typical ^{14}C experiments involve filtering water at the end of the incubation and counting the ^{14}C activity on the filter (Strickland and Parsons 1972; Peterson 1980; Arnold and Littler 1985). This obviously overlooks any ^{14}C that was fixed and subsequently excreted into the water, either directly by the cells or as a result of the grazing activities of heterotrophs. Laboratory and field data indicate that the excreted soluble organic carbon accounts for anywhere from a few percent up to 40% of the total amount of carbon that is fixed. This soluble fraction was ignored in most early studies of primary productivity. However it should be considered to represent an input into the ecosystem of energy-rich material that can be used for heterotrophic growth (Holm-Hansen 1971). Aristegui, Montero et al. (1996) reported that unaccounted-for exudation of dissolved organic carbon during ^{14}C uptake experiments might explain the fact that total daily water-column integrated carbon incorporation measured by ^{14}C incorporation into the particulate fraction was 29–54% less

than net community production measured by net oxygen production in their field study of coastal surface waters near the Antarctic Peninsula.

Light:dark cycle effects. ^{14}C incubations are often carried out for 24 hours from sunrise to sunrise. Carbon respired at night is clearly not reassimilated via photosynthesis, so hypothesis (b) does not apply at night. The specific activity of carbon respired at night after a twelve-hour incubation is unclear, but an upper bound on its specific activity is the specific activity of the inorganic carbon in the seawater. If it is less than the specific activity of the inorganic carbon in the seawater, then the ^{14}C method will tend to overestimate net photosynthesis at the end of a 24-hour incubation. Growth on a light:dark cycle can be associated with additional confounding effects that are not apparent during growth under continuous light. Ditullio and Laws (1986), for example, studied ^{14}C uptake by cyclostat cultures of five species of marine phytoplankton grown under NH_4^+ -limited conditions on a 12:12 L:D cycle and concluded that during the photoperiod the cells were excreting dissolved organic carbon with a specific activity less than that of the inorganic carbon in the medium, and that they reassimilated some or all of the DOC at night. They estimated the DOC excretion rates to be at least 14% of net photosynthetic rates. There is also compelling evidence from field studies for such diel periodicity in the excretion and uptake of DOC by phytoplankton (Li 1987; Taguchi, Ditullio et al. 1988; Allen, Kanda et al. 1996).

Dark uptake. Some uptake of CO_2 will invariably occur in the dark as a result of anapleurotic processes that replace intermediates in the tricarboxylic acid (Krebs) cycle (Krebs 1937), e.g., $\text{pyruvate} + \text{CO}_2 + \text{ATP} \rightarrow \text{oxaloacetate} + \text{ADP}$. Recognition of anapleurotic uptake was the original rationale for subtracting so-called dark bottle counts from light bottle counts before calculating photosynthetic rates. Theoretically at least, chemosynthesis could also account for uptake of CO_2 in the dark, but under the conditions in which most photosynthetic measurements are made, chemosynthesis is considered to be

completely negligible. Based on current understanding of metabolic processes, the magnitude of anapleurotic uptake would be expected to be only a few percent of light-saturated photosynthetic rates. However, remarkably high rates of dark CO₂ uptake have been reported in some fieldwork, particularly in the surface waters of tropical seas (Li 1987; Taguchi, Ditullio et al. 1988). The explanation for these high rates of dark uptake is presumably heterotrophic bacterial uptake of labeled dissolved organic carbon excreted by phytoplankton during the preceding photoperiod. So puzzling has dark bottle CO₂ uptake become that some noteworthy field programs simply ignore dark uptake.

Effects of nutrients, light, and grazing. Both light and inorganic nutrients have important effects on marine primary production under conditions in which growth rates are limited by one or a combination of these factors. Significant differences in the ability of various species or classes of phytoplankton to utilize low nutrient concentrations or low levels of light have been apparent in many studies (Ryther 1956; Ryther and Menzel 1959; Eppley and Thomas 1969; Carpenter and Guillard 1971; Stross and Pemrick 1974; Underhill 1977; Falkowski and Owens 1980). For example, the study of Bender, Orchardo et al. (1999) indicated that ¹⁴C production rates were similar to net carbon production rates in the upper half of the euphotic zone, but at lower irradiances, where net photosynthesis approaches zero, ¹⁴C production numbers lie between net community production and gross primary production. According to the results collected during the JGOFS Arabian Sea process cruises, Laws, Landry et al. (2000) made further analyses and reported that due to UV light effects (the bottles used for the oxygen incubations were transparent to UV light and the bottles used for the ¹⁴C incubations were opaque to UV light) the ¹⁴C uptake:gross photosynthesis ratio was distinctly higher (0.62) for samples incubated at the surface. This ratio decreased to 0.45 ± 0.1 at optical depths < 3 in accord with theoretical considerations of the combined effects of

the Mehler reaction, photorespiration, dark respiration, excretion, and grazing effects. The ratio further declined to an average of 0.31 for bottles incubated at optical depths > 3 .

At the time when Steemann Nielsen (1952) first introduced the ^{14}C method, oxygen light-and-dark bottle methods were too insensitive to provide accurate estimates of photosynthetic rates, at least in oligotrophic parts of the ocean. One of Steemann Nielsen's criticisms of Gordon Riley's work, for example, was the fact that Riley incubated his samples for several days because he could not detect significant changes in oxygen concentrations after only one day (Riley 1941; Steemann Nielsen 1952). However, within the last 20 years refinements in oxygen methodologies have allowed estimates of gross photosynthesis to be made by either of two methods:

(1) Simply measuring the difference in oxygen concentrations in light and dark bottles after a period of 24 hours gives an estimate of gross photosynthesis if one is willing to assume that respiration rates are the same in the light and dark (Grande, Williams et al. 1989; Marra 2002; Falkowski and Raven 2007). These estimates are now possible because of refinements in titration methodologies and the use of large numbers of replicate bottles (Bryan, Riley et al. 1976; Williams and Jenkinson 1982; Oudot, Gerard et al. 1988; Furuya and Harada 1995). For example, Williams and Jenkinson (1982) proposed a system based on a photometric endpoint detector with a precision of $0.1 \mu\text{mol L}^{-1}$ in the O_2 concentration. Using this system Williams, Heinemann et al. (1983) reported a comparison of planktonic photosynthetic rates based on ^{14}C assimilation with measurements of oxygen flux for an oligotrophic marine environment and concluded that there was no evidence of persistent errors unique to the ^{14}C technique.

(2) Addition of H_2^{18}O to incubation bottles. The rate of appearance of ^{18}O in $^{18}\text{O}^{16}\text{O}$ is a measure of gross photosynthesis (Bender, Grande et al. 1987; Falkowski and Raven 2007; Vernet and Smith 2007). The rationale for interpreting the result as gross photosynthesis is

that very little of the oxygen in the water is labeled with ^{18}O during the course of the incubation, so that respiration removes very little of the $^{18}\text{O}^{16}\text{O}$ produced by photosynthesis. One advantage of this approach is that there is no need to assume that respiration rates are the same in the light and dark. However, the Mehler reaction unfortunately consumes H_2^{18}O and produces $^{18}\text{O}^{16}\text{O}$ without contributing to carbon fixation (Asada 1999). Hence estimates of gross photosynthesis based on the production of $^{18}\text{O}^{16}\text{O}$ are actually an upper bound on the rate of gross photosynthesis. The magnitude of the Mehler reaction is generally assumed to be no more than about 5–10% of the rate of gross photosynthesis (Laws, Landry et al. 2000).

Regardless of whether one estimates gross production from changes in oxygen concentration or incorporation of ^{18}O from H_2^{18}O into $^{18}\text{O}^{16}\text{O}$, any implications with respect to carbon fixation require a knowledge of the photosynthetic quotient (PQ), i.e., the ratio of O_2 produced to CO_2 consumed, which is critically important for relating photosynthetic electron flow, obtained from measurements of oxygen evolution, to carbon fixation (Falkowski and Raven 2007). This is not a fixed number and will depend on the kinds of organic compounds being synthesized and (in particular) on the source of inorganic nitrogen. Growth on nitrate and ammonium are associated with PQs of about 1.4 and 1.1, respectively (Laws 1991; Laws, Landry et al. 2000).

Since Steeman Nielsen first introduced the ^{14}C method for estimating photosynthetic rates in aquatic systems (1952), a very large database of ^{14}C measurements has accumulated (Barber and Hiking 2002). Despite analytical and methodological advances in alternative techniques based on oxygen production, the very high sensitivity of the ^{14}C method will almost certainly argue for its continued use in primary productivity studies. Given the historical and likely widescale continued use of the ^{14}C method, it seems remarkable that more sophisticated controlled experiments have not been carried out to resolve the question of what the ^{14}C method measures. In the following chapters I report results from research that

has combined batch culture studies and experiments carried out with continuous culture systems in which net carbon fixation rates could be quantified in terms of both particulate and dissolved organic carbon production to determine what the ^{14}C method measures for several species and growth conditions.

CHAPTER 3: MATERIALS AND METHODS

3.1 Conceptual Framework

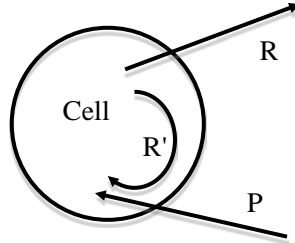


Figure 3.1. Conceptual model of carbon uptake by a phytoplankton cell.

In the conceptual model (Fig. 3.1) gross carbon uptake equals $P + R'$, and net uptake equals $P - R$. R is the rate at which respired carbon escapes from the cell back into the surrounding water, and R' is the rate at which respired carbon is re-assimilated by the cell. P is the rate at which dissolved inorganic carbon (DIC) from the surrounding medium is fixed.

The net uptake of ^{14}C is given by the equation

$$\text{net } ^{14}\text{C uptake} = \frac{P \cdot \text{SA}_{\text{DIC}}}{\text{ID}_P} - \frac{R \cdot \text{SA}_R}{\text{ID}_R} \quad (1)$$

where SA_{DIC} is the specific activity of the dissolved inorganic carbon (DIC), 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 $\text{SA}_{\text{DIC}} / \text{ID}_P$, and the specific activity of the respired carbon that escapes from the cell is $\text{SA}_R / \text{ID}_R$. ID_P is commonly assumed to equal 1.05. Photosynthesis calculated by the ^{14}C method equals the value of this expression multiplied by ID_P and divided by SA_{DIC} . Therefore

$$\text{calculated photosynthesis} = P - R \frac{\text{SA}_R}{\text{SA}_{\text{DIC}}} \frac{\text{ID}_P}{\text{ID}_R} \quad (2)$$

The right-hand side of Eq. 2 will equal net photosynthesis if (1) $R = 0$ (i.e., all respired carbon is recycled within the cell) or (2) $SA_R / ID_R = SA_{DIC} / ID_P$ (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. Alternatively, the right-hand side of Eq. 2 will equal gross photosynthesis if (1) $R' = 0$ (i.e., all respired carbon escapes from the cell) and (2) $SA_R = 0$ (i.e., all respired carbon is “old” carbon).

3.2 Rationale for Study

A continuous culture system (Fig. 3.2 shown in Sec. 3.4) provides an almost ideal system for addressing the question of what the ^{14}C method actually measures because net production of both particulate organic carbon (POC) and total organic carbon (TOC) can be accurately and repeatedly measured from the product of the dilution rate (μ) and the concentration of POC and TOC in the growth chamber.

If the inflowing medium (from the nutrient reservoir) contains no organic carbon, the net rate of production of POC and TOC in the growth chamber can be calculated from the product $\mu \cdot \text{POC}$ and $\mu \cdot \text{TOC}$, respectively. If the inflowing medium contains any POC or TOC, the rate of production in the growth chamber is $\mu \cdot (\text{POC}_{\text{out}} - \text{POC}_{\text{in}})$ and $\mu \cdot (\text{TOC}_{\text{out}} - \text{TOC}_{\text{in}})$, respectively, where the subscripts in and out refer to the inflowing and outflowing medium, respectively. Because the medium in the nutrient reservoir is sterile-filtered through a $0.2 \mu\text{m}$ filter, POC_{in} is essentially zero, and if the medium is prepared with artificial seawater, TOC_{in} will consist of little more than the total concentration of vitamins ($\sim 0.3 \mu\text{M}$) added to the growth medium.

Once the continuous culture system has come to steady state at a particular dilution rate and the rate of production of POC and TOC determined from repeated sampling of the

concentrations of POC and TOC in the growth chamber, inorganic ^{14}C can be added to the growth chamber and the subsequent incorporation of the label into POC and TOC determined from time-course sampling of the growth chamber. The relevant equation in the case of TOC is as follows:

$$\frac{d}{dt}(\text{TO}^{14}\text{C}) = U - \mu(\text{TO}^{14}\text{C}) \quad (3)$$

where μ is the dilution rate of the growth chamber, and U is the uptake rate of DI^{14}C . U is related to the calculated photosynthetic rate P_C by the equation

$$U = P_C \frac{\text{SA}_{\text{DIC}}}{\text{ID}_p} \quad (4)$$

where P_C is the calculated rate of production of TOC in the growth chamber, SA is the specific activity of the DIC, and the factor of 1.05 corrects for isotope discrimination (Strickland and Parsons 1972). SA_{DIC} is given by the equation

$$\text{SA}_{\text{DIC}} = \frac{\text{DI}^{14}\text{C}}{\text{DIC}} \quad (5)$$

Equation 3 can be numerically integrated over time to allow for changes in SA caused by dilution. If the ^{14}C method is measuring net production, then the value of P_C calculated by solving equations 3–5 will equal the rate of TOC production determined from the dilution rate and the concentration of TOC in the growth chamber. In the continuous culture system DI^{14}C will decline over time in a manner described by the following equation

$$\text{DI}^{14}\text{C} = \text{DI}^{14}\text{C}_0 e^{-kt} \quad (6)$$

where DI^{14}C_0 is the activity of the DIC at the beginning of a time interval of duration t . The value of k is given by the equation

$$k = \left(1 + \frac{\text{TOC}}{\text{ID}_p \cdot \text{DIC}} \right) \mu + \beta \quad (7)$$

in which β accounts for the minor loss rate of $^{14}\text{CO}_2$ associated with aeration of the growth chamber (see Fig. 3.2). $\text{DIC} = \text{DI}^{14}\text{C} + \text{DI}^{12}\text{C}$. We determined the value of k from the slope of a linear regression of the natural logarithm of DI^{14}C versus time and used that value of k to integrate Eq. 3 between time points.

In my study the data set for calculating ^{14}C uptake rates measured by the ^{14}C method came from a time series of measurements of radioactive counts of PO^{14}C and TO^{14}C . Photosynthetic rates so calculated were compared to net photosynthesis determined by “the control method”, i.e., a time series of measurements of POC and TOC concentrations before adding ^{14}C to the growth chamber, net production of POC and TOC being calculated from the equations:

$$\text{net POC production rate} = \mu \cdot (\text{POC}_{\text{out}} - \text{POC}_{\text{in}}) \quad (8)$$

$$\text{net TOC production rate} = \mu \cdot (\text{TOC}_{\text{out}} - \text{TOC}_{\text{in}}) \quad (9)$$

The null hypothesis was that the ^{14}C method was measuring net photosynthesis, and the decision to accept or reject that hypothesis was based on a comparison of production rates calculated from the ^{14}C method and from the control method.

The time series of measurements lasted 24 hours after addition of ^{14}C to the growth chamber. The sampling intervals ranged from 1 h to 6 h. I collected samples from the growth chamber hourly in the first 6 h to study the photosynthetic rates over short periods of time.

It is possible that ^{14}C uptake could estimate gross photosynthesis in short incubations if, during the short incubation, no respired carbon was recycled (i.e., $R' = 0$) and $\text{SA}_R = 0$. This is a scenario discussed by Marra (2002). If ^{14}C subsequently appeared in the respired carbon and if SA_R became identical to SA_{DIC} , then after sufficient time the ^{14}C method would begin

to measure net carbon uptake. Marra (2002) concluded that the length of the incubation should be taken into consideration when interpreting ^{14}C uptake results and that the time required for SA_R to equal SA_DIC might be a function of the growth rate of the phytoplankton.

In my study the growth rate of the phytoplankton was controlled via either nutrient or light limitation. One of the goals of the research was to determine whether there was any evidence that short-term ^{14}C incubations gave a higher estimate of carbon fixation than longer (up to 24 h) incubations, and if so, whether the difference between short-term and long-term results was a function of growth rate or the factor limiting growth.

To examine the effect of growth on a light:dark cycle, the continuous culture system was operated on a 12:12 L:D cycle. Carbon fixation during the light period was calculated using Eq. 3. However, during the dark period, the following equation described the rate of change of ^{14}C activity in organic carbon:

$$\frac{d}{dt}(\text{TO}^{14}\text{C}) = -R_\text{D} \frac{\text{SA}_\text{RD}}{1.05} - \mu(\text{TO}^{14}\text{C}) \quad (10)$$

where R_D is the dark respiration rate of TOC, and SA_RD is the specific activity of the respired carbon. The dark respiration rate R_D could be estimated independently from the rate of change of TOC in the growth chamber during the dark period:

$$\frac{d}{dt}(\text{TOC}) = -R_\text{D} - \mu(\text{TOC}) \quad (11)$$

By combining Eq. 10–11 I was able to determine SA_RD , the specific activity of the carbon respired during the dark period. This information gave me a good sense of the extent to which carbon fixed during the previous photoperiod was being used as a substrate for dark respiration.

Under light-limited conditions, both *I. galbana* and *C. kessleri* cultures were incubated under nutrient-saturated conditions but in relatively dim (low growth rate) and bright (high

growth rate) light. The two irradiances corresponding to dim and bright light were the same for both species, 20 and 60 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, respectively, measured at the center of the empty growth chamber.

The peristaltic pump was kept on in most of the chemostat experiments. To simulate conditions in a conventional bottle incubation, the peristaltic pump was turned off (dilution rate $\mu = 0$) when ^{14}C was added to the growth chamber during several light-limited continuous culture incubations and light-limited 12:12 L:D incubations. Because the algae were growing exponentially when the pump was off, the photosynthetic rate could be estimated with the following equation

$$\frac{d}{dt}(\text{TOC}) = P_{\text{TOC}} \cdot e^{vt} \quad (12)$$

where P_{TOC} is the net production rate of total organic carbon at the beginning of the time interval of duration t . The average value of the production rate during each time interval can be calculated by integrating the production rate over the time interval and dividing the result by the duration of the time interval. The growth rate (v) can be calculated from the change in cell concentrations measured with a model Z1 particle counter and the following *Malthus law* equation (Malthus 1826) related to exponential growth

$$\frac{d}{dt}(N) = v \cdot N \text{ or } N_t = N_o \cdot e^{vt} \quad (13)$$

where N_t is the cell count at time t , and N_o is the initial cell count at time zero.

The equation corresponding to equation Eq. 12 for the ^{14}C calculation is

$$\frac{d}{dt}(\text{TO}^{14}\text{C}) = P_{\text{TOC}} \cdot e^{vt} \cdot \frac{\text{SA}}{1.05} \quad (14)$$

where SA can be calculated from Eq. 5. The rate of production of TOC in the growth

chamber can be calculated from equations Eq. 12–13, i.e., $\frac{d}{dt}(\text{TOC}) = P_{\text{TOC}} \cdot \frac{N_t}{N_o}$.

3.3 Hypotheses To Be Tested

Hypothesis 1. The ^{14}C method measures net carbon fixation. If this hypothesis is true, reassimilation of recently respired carbon, as suggested by Ryther (1956), is the most likely explanation. I initially carried out batch culture experiments to test this hypothesis, the rationale for the experiments being as follows:

(1) for a specific algal species, the ^{14}C method would measure net photosynthesis if there were no decline of ^{14}C activity of uniformly labeled cells in a batch culture experiment when they were resuspended in fresh medium in the light. In other words, all respired carbon was reassimilated in the batch culture experiment.

(2) alternatively, the ^{14}C method would measure gross photosynthesis if the ^{14}C activity of cells uniformly labeled with ^{14}C declined when they were resuspended in fresh medium in the light, and the rate of decline was the same in the dark and light. In other words, old carbon was the substrate for respiration, **and** no respired carbon was reassimilated. If the rate of loss of ^{14}C activity of the cells was less in the light than in the dark, then the ^{14}C method would give an estimate intermediate between net and gross photosynthesis if the dark and light respiration rates were in fact the same.

Hypothesis 2. The factor limiting growth, whether nutrients or light, will have no effect on the relationship between carbon fixation estimated from ^{14}C uptake (Eq. 3) and net photosynthesis estimated from POC and TOC concentrations in the growth chamber (Eqs. 8-9). The results of my continuous culture experiments for testing this hypothesis would help to explain the discrepancies and resolve the uncertainties in numerous field studies (vide

supra) because the chemical and biochemical composition of microalgae are strongly influenced by the extent and nature (light versus nutrients) of growth rate limitation (Terry, Hirata et al. 1985).

Hypothesis 3. Phytoplankton growth rate would have no effect on the relationship between carbon fixation estimated from ^{14}C uptake and net photosynthesis estimated from POC and TOC concentrations in the growth chamber. Halsey, Milligan et al. (2010) investigated growth-rate-dependent photosynthate metabolism by monitoring six indices of photosynthetic activity in steady-state cultures of *Dunaliella tertiolecta* over a range of nitrate-limited growth rates and concluded that across all growth rates, O_2 -based chlorophyll-specific gross primary production, chlorophyll-specific net primary production, and F_v/F_m (index of photochemical efficiency) were constant, whereas chlorophyll-specific short-term C fixation showed a clear linear dependence on growth rate, a reflection of differential allocation of photosynthate between short-lived C products and longer-term storage products. They reported that ^{14}C incorporation into carbohydrates was five times greater in cells growing at 1.2 day^{-1} than at 0.12 day^{-1} .

Hypothesis 4. Growth on a 12:12 light:dark cycle would have no effect on the relationship between carbon fixation estimated from ^{14}C uptake and net photosynthesis estimated from POC and DOC concentrations in the growth chamber. The concern here was that carbon respired in the dark clearly is not reassimilated in the dark. Therefore ^{14}C uptake after 24 h would tend to overestimate net carbon fixation unless the carbon respired in the dark had the same specific activity as the DIC, i.e., all respired carbon was recently fixed carbon. Recently fixed in the context of this hypothesis would mean carbon fixed during the previous photoperiod. In other words, if cells were grown on a 12:12 L:D cycle, the ^{14}C method would estimate net carbon fixation if all the carbon respired in the dark were fixed during the previous photoperiod.

Hypothesis 5. The relationship between carbon fixation estimated from ^{14}C uptake and net photosynthesis estimated from POC and DOC concentrations is independent of the species of phytoplankton in the growth chamber. The alternative hypothesis, that the correct interpretation of ^{14}C uptake results varies between phytoplankton species, might explain some of the paradoxes about what the ^{14}C method actually measures, including the historical debate between Ryther & Vaccaro on one side and Steemann Nielsen and co-workers on the other (Peterson 1980), because different algal species were used in those early studies. Williams, Robinson et al. (1996) have reported a summary of comparisons of ^{14}C uptake and total carbon metabolism that is consistent with a species-dependent interpretation of ^{14}C uptake. If there is a species effect, information about what the ^{14}C method actually measures for common phytoplankton species would allow for a more informed interpretation of field results if information on species composition was available.

3.4 Experimental Design

3.4.1 Batch Culture Experiments

Hypothesis 1. I carried out an extensive series of batch culture experiments similar to those described by Ryther (1956) to determine whether uniformly ^{14}C -labeled algal cells lose activity when they are transferred to fresh medium in the light (*vide infra*). If labeled cells incubated in the light retain their activity, the ^{14}C method presumably measures net carbon fixation according to the hypotheses of Ryther (1956), i.e., all respired carbon is reassimilated in the light. If these cells lose some of their activity, then some respired carbon is not being reassimilated. In that case the ^{14}C method would estimate net carbon fixation only if recently fixed carbon were the substrate for respiration, an hypothesis rejected by Ryther (1956). I followed the same experimental design that I used in my preliminary studies described as following.

Batch culture studies were carried out on a total of seven species of marine phytoplankton. Cultures of *Isochrysis galbana*, *Chlorella kessleri* (strain 211-11h), *Tetraselmis suecica*, and *Pavlova lutheri*, were obtained from the culture collection of the Center for Marine Microbial Ecology and Diversity at the University of Hawaii (culture numbers CMMED 664, HRBP102, TETRA 01, and CMMED 1362, respectively). A culture of *Thalassiosira weissflogii* was obtained from the Provasoli-Guillard National Center for Marine Algae and Microbiota (culture number CCMP1050). *Dunaliella tertiolecta*, and *Synechococcus* sp. were obtained from the Culture Collection of Algae and Protozoa of the Scottish Association for Marine Science (culture number CCAP 19/27) and the Culture Collection of Algae at the University of Texas at Austin (culture number UTEX LB 2625), respectively. The cultures were maintained in a sterile-filtered (0.22 μm) medium made up with distilled water and seawater salts adjusted to a salinity of 33 with Instant Ocean®. Nitrate, phosphate, and vitamins (thiamine, biotin, and cyanocobalamin) were added at the concentrations specified for f/2 medium (Guillard and Ryther 1962). Trace metals were added in the EDTA-buffered formulation specified by Sunda and Hardison (2007).

Cultures were uniformly labeled with ^{14}C by adding a 10- μL aliquot of a log-phase culture to 5 mL of sterile growth medium to which 1.0 μCi of ^{14}C -bicarbonate had been added. The fraction of ^{14}C activity in the organic matter was assayed from time to time by pipetting small aliquots of the culture into a liquid scintillation vial and counting the activity on a Packard Tri-Carb model 3100 TR liquid scintillation counter with Ultima Gold LLT as a fluor. Inorganic carbon was driven off by adding 0.5 mL of 1 N HCl to the vial and allowing the CO_2 to degas overnight. Total activity was determined on samples in vials to which 0.5 mL of 1.0 N NaOH was added to ensure that no CO_2 escaped.

After an incubation period of about one week more than 90% of the ^{14}C activity had been incorporated into organic matter, and 0.1-mL aliquots of the culture were transferred to six

glass flasks containing 100 mL of fresh medium without added ^{14}C . The 1000-fold dilution of the specific activity of the remaining DI^{14}C effectively terminated ^{14}C uptake. Three of the flasks were incubated under continuous light ($440 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ of 400–700 nm wavelength light measured with a QSL 2100 quantum scalar light meter) provided by a bank of five daylight fluorescent lamps; the others were incubated in the dark. The ^{14}C activity in the organic matter was monitored on a daily basis for four days. In a subsequent set of experiments with *I. galbana*, the continuous light treatment was replaced by four days of growth on a 12-hour:12-hour light:dark (12:12 L:D) cycle.

3.4.2 Chemostat Experiments

Hypotheses 2–5. I operated a continuous culture system under both nitrate-limited and light-limited conditions (**Hypothesis 2**), at both high and low growth rates (**Hypothesis 3**) on continuous light and a 12:12 L:D cycle (**Hypothesis 4**), with two typical phytoplankton species (**Hypothesis 5**) to determine under what conditions the ^{14}C method gave a good approximation of net photosynthesis and under what conditions the results of ^{14}C uptake experiments deviated significantly from net photosynthesis and by how much.

For each species, I carried out eight continuous culture experiments, four under nitrate-limited conditions and four experiments under light-limited conditions as follows:

Table 3.1. The continuous culture experiments for each species.

Nitrate-limited		Light-limited	
Photoperiod	Growth rate	Photoperiod	Growth rate
Continuous	High	Continuous	High
Continuous	Low	Continuous	Low
12:12 L:D	High	12:12 L:D	High
12:12 L:D	Low	12:12 L:D	Low

The continuous culture system used in my studies was similar to those used previously (Laws and Bannister 1980; Laws, Popp et al. 1995; Laws, Bidigare et al. 1997; Laws, Pei et al. 2011; Laws, Pei et al. 2011). The growth chamber consisted of a Pyrex reaction flask with

a working volume of approximately 2.0 liters (Fig. 3.2). Light was provided continuously from a bank of six daylight fluorescent lamps with an irradiance of $440 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ as measured with a QSL 2100 quantum scalar light meter (Biospherical Instruments Inc.) at the center of the empty growth chamber. The temperature was 20 °C. The growth medium was identical to the batch culture medium (vide supra) for the light-limited studies. In the nitrate-limited work, the nitrate concentration was reduced from 882 μM to 45 μM .

The nutrient reservoir (40-liter glass carboy), sterile filter rig, and connecting silicone rubber and glass tubing were sterilized by autoclaving as a single unit, and the medium subsequently sterile filtered (0.22 μm) into the reservoir. The growth chamber and associated tubing was autoclaved separately and sterile-connected to the nutrient reservoir. The growth medium was pumped into the growth chamber via a peristaltic pump. The culture was stirred with a Teflon-coated magnetic stir bar and with sterile-filtered air bubbled into the growth chamber via an aeration tube. Aeration creates pressure in the headspace of the growth chamber, forcing medium through the overflow tube, which acts as an airlift. The growth chamber was initially inoculated with a sterile syringe inserted through a rubber septum in a port on the top of the growth chamber.

Samples were withdrawn from the growth chamber via a three-way stopcock on the aeration line (Fig. 3.2). Samples were taken at 1-h intervals during the first 6 h, 3-h intervals during the second 6 h, and 6-h intervals during the following 12 h. The input medium pump was left on throughout the experiment. In order to minimize the potential for perturbation to the steady state caused by sampling, the volume withdrawn from the growth chamber at each sampling time point never exceeded the amount of medium pumped into the growth chamber during the subsequent time interval and was always less than ~ 6% of the total volume of the growth chamber. During the time that the growth chamber was refilling following withdrawal of a small sample, the mathematical equations governing the rate of change of substances in

the growth chamber were virtually identical to those describing overflow conditions because the increase in culture volume due to the input of fresh medium dilutes the culture in a manner similar to that resulting from overflow under constant volume conditions (Laws 1985). I therefore assumed that sampling did not significantly perturb the steady-state condition of the phytoplankton population (Ditullio and Laws 1986).

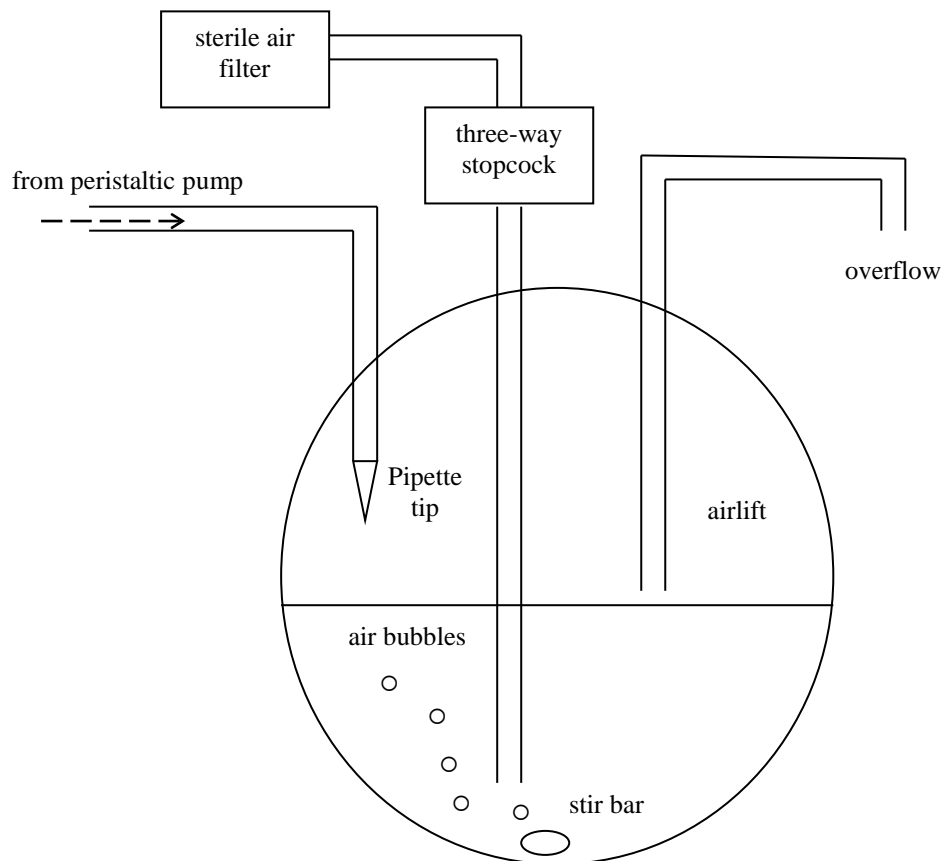


Figure 3.2. Schematic of continuous culture system.

3.5 Sampling Methods

Direct measurements before ^{14}C incubation. Cell counts in the chemostat were recorded daily with a model Z1 particle counter (Beckman Coulter), and dilution rates were controlled by a peristaltic pump and recorded daily. Sampling for characteristics other than cell counts in the growth chamber did not begin until four doubling times had elapsed at each dilution rate, by which time cell counts had typically stabilized with a coefficient of variation

of $\pm 2\%$ (Laws, Pei et al. 2011; Laws, Pei et al. 2011). Fifty milliliters of culture were filtered (glass fiber, GF/F) at selected time points for determination of POC (filter) and dissolved organic carbon (DOC) in the filtrate. POC was measured on an Exeter Analytical model CE 440 elemental analyzer and DOC with a Total Organic Carbon Analyzer (TOC-V CSN model, Shimadzu). TOC was calculated as the sum of POC and DOC. DIC concentrations were calculated from measurements of total alkalinity and salinity Strickland and Parsons (1972). This sampling was repeated daily for at least five days to permit accurate determination of the rates of production of POC and TOC via Eq. 8–9.

^{14}C incubations. At time zero the growth chamber was spiked with 500 μL of deionized water containing 10 μCi of $\text{NaH}^{14}\text{CO}_3$. Fifty-milliliter aliquots were withdrawn from the growth chamber at selected time intervals, and triplicate 5-mL samples were immediately dispensed into three vials with 0.5 mL 1-N NaOH to trap the total ^{14}C activity. To determine the activity of total organic ^{14}C (TO^{14}C), triplicate 5-mL samples were dispensed into three vials with 0.5 mL 1-N HCl and placed in a fume hood for 24 h to degas inorganic carbon. To measure the activity of particulate organic ^{14}C (PO^{14}C), triplicate 5-mL samples were filtered through 0.3 μm membrane filters, followed by a 5-mL rinse with filtered seawater to remove ^{14}C that had not been incorporated into phytoplankton cells. Each filter was placed in a vial containing 0.5 mL 1-N HCl and placed in the fume hood for 24 h to degas inorganic carbon. Samples for determination of ^{14}C activity were counted on a Packard Tri-Carb model 3100 TR liquid scintillation counter using Ultima Gold LLT as a scintillation fluor.

3.6 Improvements in My Experimental Design

Once in steady state, a continuous culture system can be sampled numerous times over the course of many days if necessary to obtain a very accurate estimate of the rate of carbon fixation in the system. Since the concentrations of POC and TOC in the nutrient reservoir are negligible compared to the concentrations in the growth chamber, the determination of fixation rates during growth with continuous illumination requires only knowledge of concentrations and dilution rates. In particular, one does not need to measure changes in concentrations between two points in time and estimate production from the difference. Furthermore, one has absolute control over the growth rate, the factor limiting growth, and the photoperiod.

When the cells are grown on a L:D cycle, there is an increase in the concentration of TOC in the growth chamber during the photoperiod and a decrease in the dark (Laws and Wong 1978). Under those conditions the uptake during the photoperiod (Eq. 3) is the sum of the change in TOC in the growth chamber during the photoperiod and the washout rate of TOC (i.e., $\mu \cdot \text{TOC}$). The dark respiration rate is the difference between the rate of change of TOC in the growth chamber in the dark and the washout rate. Although estimating the dark respiration rate requires calculating a difference of two numbers, the fact that the measurements can be made repeatedly, many times if necessary, allowed me to determine respiration rates with satisfactory accuracy (e.g., Laws and Wong (1978)).

With respect to the batch culture work, I feel my experimental protocol was a significant improvement over the methodology employed by Ryther (1956) and Steemann Nielsen (1955), who harvested their cells by centrifugation before resuspending them in a ^{14}C -free medium. As shown in Section 3.4.1, my method completely avoided the need for centrifugation, which could have been stressful to the cells (*vide supra*).

3.7 Algal Species Identification Using PCR

Since the species name of *Chlorella* sp. used in my experiment was unknown in the early period, Polymerase Chain Reaction (PCR) technique was used to identify its species name.

DNA purification procedure. DNA was purified according to a simple and rapid technique developed by Fawley and Fawley (2004). An aliquot of 1–2 mL of algal culture was centrifuged in a conical-bottom screw-top microcentrifuge tube of 2 mL at approximately 16,000 g for 1 min. The supernatant was discarded and 200 μ L of extraction buffer (1 M NaCl, 70 mM Tris, 30 mM Na₂EDTA, pH 8.6) was added and vortexed briefly. The suspension was then centrifuged at 16,000 g for 1 min. The supernatant was discarded, and then an aliquot of 200 μ L of fresh extraction buffer was added. A small quantity of glass beads (G-8772, Sigma Chemical Co., St. Louis, MO, USA) sufficient to fill the conical portion of the centrifuge tube was then added, followed by 25 μ L of 2% CTAB (Sigma Chemical Co.) and 200 μ L of chloroform. After agitation for 20 s, the mixture was then centrifuged at 2000 g for 2 min to separate the phases. An aliquot of 100 μ L of the aqueous phase was removed to a 1.5 mL microcentrifuge tube, 500 μ L of 100% alcohol was added and mixed, and after 10 min of centrifuging, the resulting solution was applied to a 70% alcohol which was then used to purify the DNA.

PCR amplification. Nuclear rDNAs and the internal transcribed spacer regions were amplified by the polymerase chain reaction in a process similar to that employed by Jasalavich, Morales et al. (1995). Similar Primers of NS12, CS12, and CS34 as the study of Wu, Hseu et al. (2001) were used. Amplifications of a 2 μ L target template were performed in 23 μ L buffer in each reaction tube, which included 7.3 μ L PCR H₂O, 5.0 μ L 5X Green Buffer, 5.0 μ L of 25 mM MgCl₂, 0.50 μ L of 10 mM dNTPs, 2.5 μ L of 10 μ M NS1 and NS2 primer (CS1 and CS2, or CS3 and CS4 were used for replicated samples), 0.20 μ L of 5 unites (μ L)⁻¹ Go-Taq polymerase. Each reaction consisted of an initial denaturation step of 5 min at 95 °C,

followed by 0.5 min denaturation at 94 °C, 1 min annealing at 50 °C, 2 min extension at 72 °C, and a final extension of 5 min at 70 °C. Thirty cycles of amplification were run. PCR amplification products were purified by agarose gel electrophoresis (Fig. 3.3).

DNA sequencing. PCR products were sequenced by the similar procedure as described by Johnson, Flowers et al. (2009) and the final result turn out to be *Chlorella kessleri* (strain 211-11h).

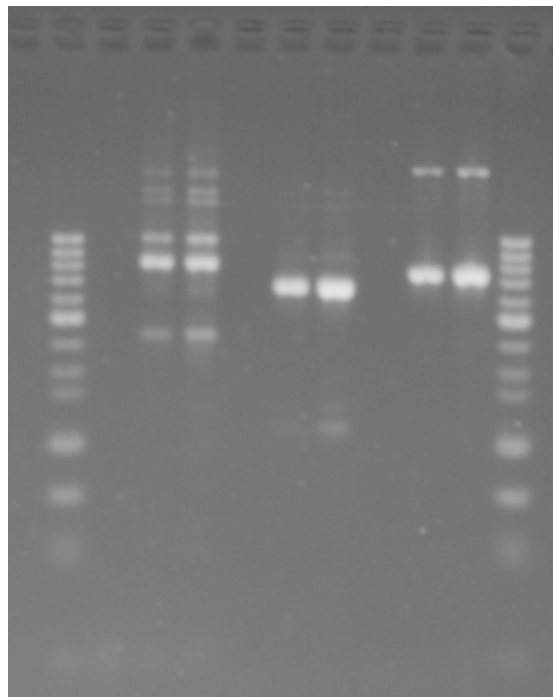


Figure 3.3. Agarose gel electrophoresis of 18S rDNA PCR products from algal isolates. (From left to right, DNA standard, NS12 primers was used for the first two replicated samples, CS12 primers was used for the middle two ones, and CS34 primers was used for the last two ones).

3.8 Assays for Heterotrophic Bacteria

Tests for the presence of heterotrophic bacteria were made using the spread-plate technique (Willey, Sherwood et al. 2010). Briefly, a sample was withdrawn from the chemostat. One-milliliter aliquots of the sample and of subsamples diluted by factors of 10 and 100 with sterile medium were spread onto Luria Bertani agar (Atlas 2010). The plates

with samples were incubated at a temperature of 30 °C for 24 hours, at which time the number of bacterial colonies was counted.

3.9 Statistics

Data were analyzed for patterns with the use of correlation, regression, and t-tests. Significant patterns were considered to exist if type I error rates were less than 0.05. Reported error bounds are in all cases 95% confidence intervals.

CHAPTER 4: BATCH CULTURE EXPERIMENTS

4.1 Results

The TO¹⁴C of the uniformly labeled *I. galbana* cells transferred to ¹⁴C-free media and incubated in continuous light remained constant for four days (Fig. 4.1A), whereas the TO¹⁴C of the same cultures incubated in the dark declined by about 15% per day (Fig. 4.1B). In similar batch culture experiments conducted with *Chlorella kessleri*, the TO¹⁴C activity of uniformly labeled cells declined in both the light and dark when they were transferred to ¹⁴C-free media (Fig. 4.1C and D). TO¹⁴C and PO¹⁴C counts declined at nearly the same rates in the light and dark, about 5% per day.

Of the other five species, only *D. tertiolecta* retained ¹⁴C activity when incubated in the light. The other four species lost ¹⁴C activity in both the light and dark, average loss rates being $2.5 \pm 0.6\% \text{ d}^{-1}$ and $5.4 \pm 1.5\% \text{ d}^{-1}$ in the light and dark, respectively (Table 4.1).

When the continuous light treatment was replaced with a 12:12 L:D cycle, labeled *I. galbana* cells transferred to ¹⁴C-free media lost no activity during the four subsequent 12-hour photoperiods. The ratio of TO¹⁴C activity at the end of each light period to the activity at the beginning of the same light period was 1.006 ± 0.022 during the following four days. The ratio of TO¹⁴C activity at the end of each dark period to the activity at the beginning of the same dark period was 0.963 ± 0.013 . In other words, about 4% of the TO¹⁴C was converted to DI¹⁴C during each 12-hour dark period. The TO¹⁴C of transferred *I. galbana* cells incubated in continuous darkness declined linearly over time at a rate of 3.7% per 12 hours, very consistent with the dark TO¹⁴C loss from the cells incubated on a 12:12 L:D cycle.

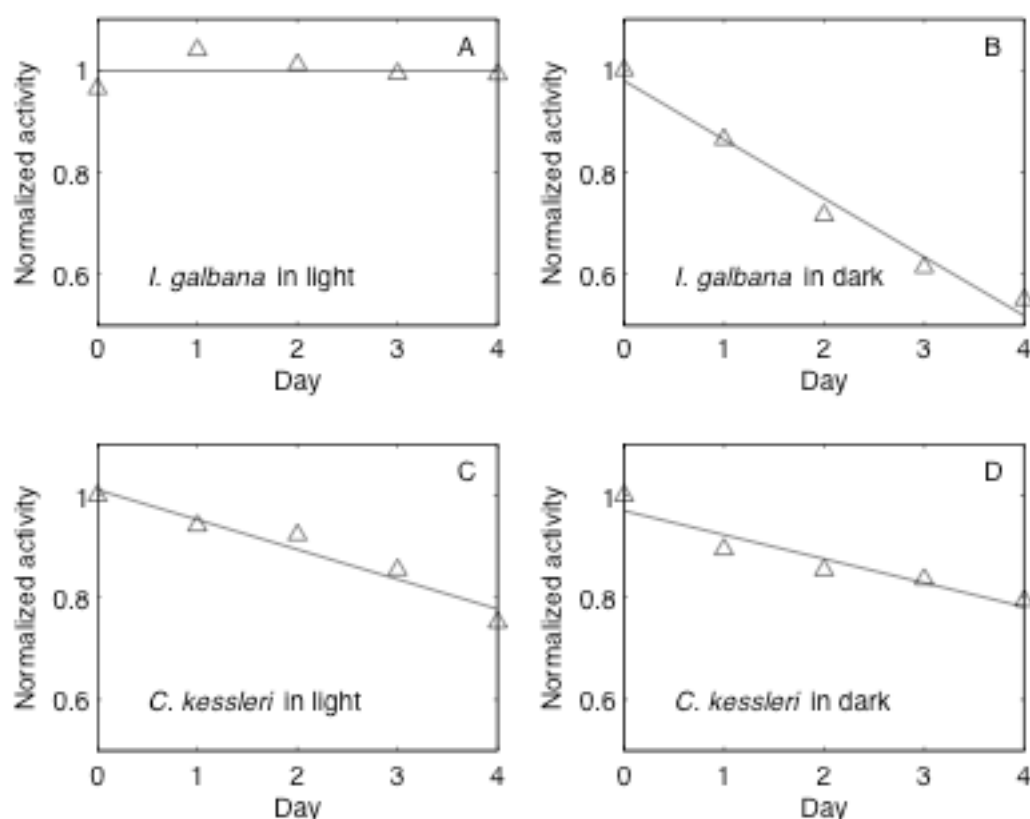


Figure 4.1. TO^{14}C activity of uniformly labeled cells after transfer to a ^{14}C -free medium and incubated for four days in the light or dark as indicated. In panel A normalized activity is the average of the activities at all times points. In panels B–C normalized activity is the activity at time zero.

Table 4.1. Loss rates of activity from cells uniformly labeled with ^{14}C and subsequently incubated in the light and dark for 4–5 days.

Species	Loss rate of ^{14}C in light (% d^{-1})	Loss rate of ^{14}C in dark (% d^{-1})
<i>D. tertiolecta</i>	0	12.3
<i>P. lutheri</i>	2.5	3.4
<i>T. suecica</i>	1.1	2.7
<i>T. weissflogii</i>	2.3	5.9
<i>Synechococcus</i> sp.	4.1	9.4

4.2 Discussion

The fact that uniformly labeled *I. galbana* and *D. tertiolecta* cells lost no activity when incubated in the light indicates that all respired carbon was being recycled and/or that new carbon was the substrate for respiration. In either case, the implication is that ^{14}C uptake

by these two species should estimate net photosynthesis during incubations in the light. The fact that uniformly labeled *I. galbana* cells lost activity in the dark when incubated on a 12:12 L:D cycle indicates that some old carbon (i.e., carbon fixed prior to the previous photoperiod) was being respired at night. The implication is that in fieldwork ^{14}C incubations lasting from sunrise to the following sunrise would tend to overestimate net photosynthesis, because the carbon respired at night would have a lower specific activity than the carbon fixed during the previous photoperiod.

The fact that uniformly labeled cells of the other five species lost activity when incubated in the light indicates that not all respired carbon was being recycled and that some of the respired carbon was old carbon. If respiration rates were the same in the light and dark, the fact that the loss rates were virtually identical in the light and dark in the case of *C. kessleri* implies that old carbon was the exclusive substrate for respiration and that no respired carbon was being recycled. The implication is that ^{14}C uptake by *C. kessleri* should estimate gross photosynthesis during incubations in the light. In the case of the remaining four species, the fact that loss rates in the dark were about twice loss rates in the light implies that ^{14}C uptake should estimate a rate intermediate between net and gross photosynthesis. If respiration rates were in fact the same in the light and dark, the implication is that $R' + R = 2R$, i.e., $R' = R$ as shown in Fig.3.1. If recently fixed carbon is not a substrate for photosynthesis (Calvin 1949; Weigl, Warrington et al. 1951), under these conditions ($R' = R$) ^{14}C uptake would estimate a rate (P) equal to the average of net ($P - R$) and gross ($P + R'$) photosynthesis.

A uniformly labeled culture of *Chlorella pyrenoidosa* studied by Steemann Nielsen (1955) lost some activity in the light, but the loss rate was lower than in the dark, which is similar as some species in my batch culture studies and suggests that some, but not all, respired carbon was being reassimilated. Comparatively, incorporation of ^{14}C into the particulate plus dissolved fractions by the culture of *Skeletonema costatum* studied by Williams, Robinson et al. (1996) approximated net photosynthesis.

CHAPTER 5: CONTINUOUS CULTURES

To test the implications of my previous batch culture studies, *I. galbana* and *C. kessleri* were grown respectively at high and low growth rates in the 24-h continuous culture and 12:12 L:D cycle chemostat systems under either nitrate- or light-limited conditions. In this chapter, we will focus on the 24-h continuous cultures. The results of continuous culture for each algal species will be shown respectively at high and low growth rate under nitrate-limited condition, and then the results of light-limited cultures will be shown subsequently. Finally, these results will be compared with previous batch culture works and the relevant implications will be discussed.

5.1 Results

5.1.1 Assays for Heterotrophic Bacteria

Although heterotrophic bacteria were present in my cultures, the number of colony forming units was less than 200 mL^{-1} . If the carbon content of these bacteria is assumed to be 20 fg cell^{-1} (Lee and Fuhrman 1987; Troussellier, Bouvy et al. 1997), the concentration of heterotrophic bacterial carbon was at most 4 ng L^{-1} , about 10^{-6} of the concentration of phytoplankton carbon in the batch culture flasks and chemostat growth chambers.

5.1.2 Nitrate-limited, High Growth Rate

When *I. galbana* was grown at a high growth rate of 0.972 d^{-1} under continuous light, the ^{14}C uptakes of TOC and POC in the photosynthesis process are shown in Fig. 5.1A. It seems that the uptake rates were slowing down in the second 12 hours (i.e. the 12–24 h as show in figure), which might be caused by the declining of specific activity (SA) of DIC in the medium along incubation time since ^{14}C in the growth chamber were continuously diluted by

pumping, assimilated by algae and blown away by aeration along incubation time. But this is not a problem for our calculation since the specific activity was corrected correspondingly with the variation of DI^{14}C concentration along time in Eq. 3–5.

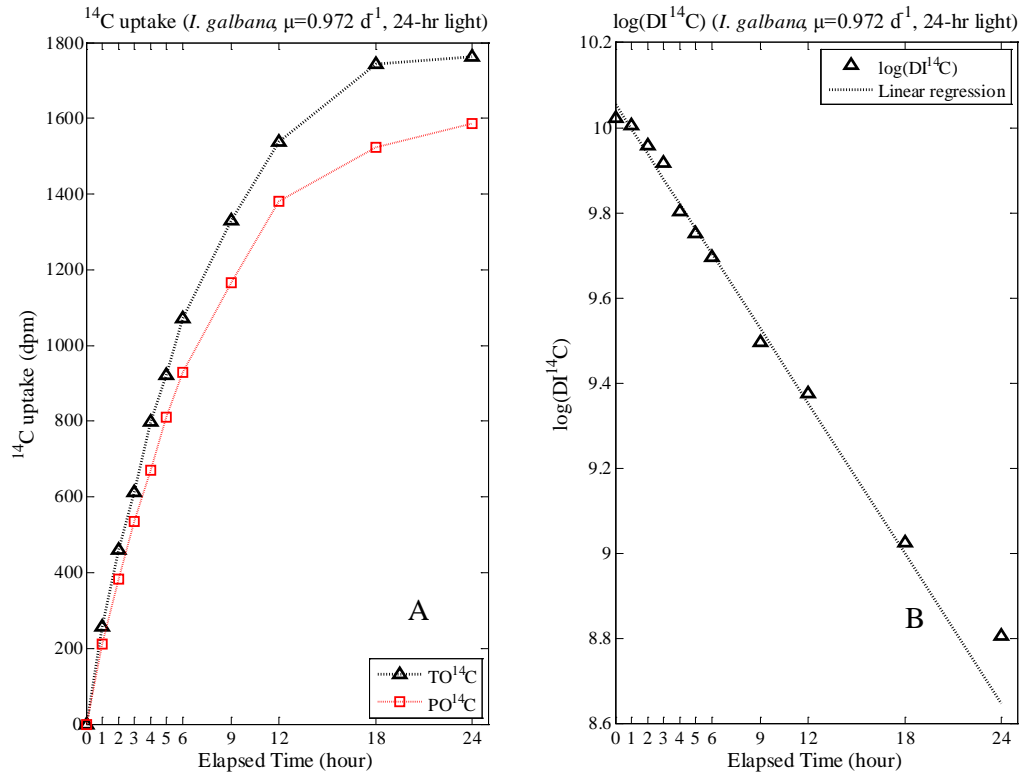


Figure 5.1. Radioactivity of ^{14}C uptake along incubation time (A) and linear regression for $\log(\text{DI}^{14}\text{C})$ along incubation time (B).

According to Eq. 6, there should be a linear relationship with negative slope between log-transformed values of DI^{14}C radioactivity ($\log(\text{DI}^{14}\text{C})$) and incubation time (t), and this was the case for all our incubations. For example, the regression line in Fig. 5.1B shows a significant linear correlation between $\log(\text{DI}^{14}\text{C})$ and t for the ^{14}C incubation of *I. galbana*. The p-value for linear regression analysis is close to zero indicating that the correlation is strongly significant. The regression slope (k) calculated by Eq. 6 is 1.286 d^{-1} for $\log(\text{DI}^{14}\text{C})$. It is reasonable to be a little higher than the growth rate of 0.972 d^{-1} since, as shown in Eq. 7, the calculated value of k should not only include the carbon fixation in photosynthesis

process but also the dilution effect (μ), and the minor $^{14}\text{CO}_2$ loss (β) associated with aeration of the growth chamber.

Fig. 5.2A shows the average production rates of *I. galbana* in each time interval estimated by the ^{14}C method and actual net production rates measured by the control method at the high growth rate of 0.972 d^{-1} . The average net TOC fixation rate calculated by Eq. 9 was $19.13\text{ }\mu\text{M h}^{-1}$ in 24 h. Corresponding rates estimated by the ^{14}C uptake after incubations of 0–24 hours averaged $16.91 \pm 1.28\text{ }\mu\text{M h}^{-1}$ (Table 5.1). The rates estimated by the ^{14}C uptake were about $(19.13 - 16.91)/19.13 = 11.60\%$ lower than the net fixation rate calculated with Eq. 9, a difference that was within the propagated analytical error of the TOC ($\pm 5\%$) and ^{14}C uptake ($\pm 5\%$) measurements. The results of the continuous culture work were, at least in this case, consistent with the implications of the batch culture studies.

The average photosynthetic rate calculated using Eq. 8 was $16.20\text{ }\mu\text{M h}^{-1}$ for POC in 24 h, and $2.93\text{ }\mu\text{M h}^{-1}$ for DOC (Table 5.1). The excretion rate of DOC accounts for about $2.93/19.13 = 15.32\%$ of the total photosynthetic rate of organic carbon calculated by Eq. 9. The photosynthetic rate of DOC calculated by the ^{14}C method was $1.64 \pm 1.64\text{ }\mu\text{M h}^{-1}$, a little lower than the actual rate directly measured by organic carbon analyzer. It accounts for $1.64/16.91 = 9.70\%$ of the total organic carbon uptake rate estimated by the ^{14}C method, a little lower than that measured by organic carbon analyzer as well.

To test whether the photosynthetic rates of TOC vary over incubation time, samples were collected hourly during the first six hours. After that, samples were collected with a time interval of 3 hours during the first 12 hours, and with a time interval of 6 hours during the second 12 hours.

Simple linear regression was used to test the relationship between photosynthetic rates and the incubation time. The regression line plotted in Fig. 5.3A suggests that there is no significant correlation between TOC uptake and incubation length ($p=0.9166$). The average

photosynthetic rates of TOC were $17.22 \mu\text{M h}^{-1}$ in the first six hours, $16.26 \mu\text{M h}^{-1}$ between 6–12 h, $16.66 \mu\text{M h}^{-1}$ between 12–18 h and $17.48 \mu\text{M h}^{-1}$ during 18–24 h.

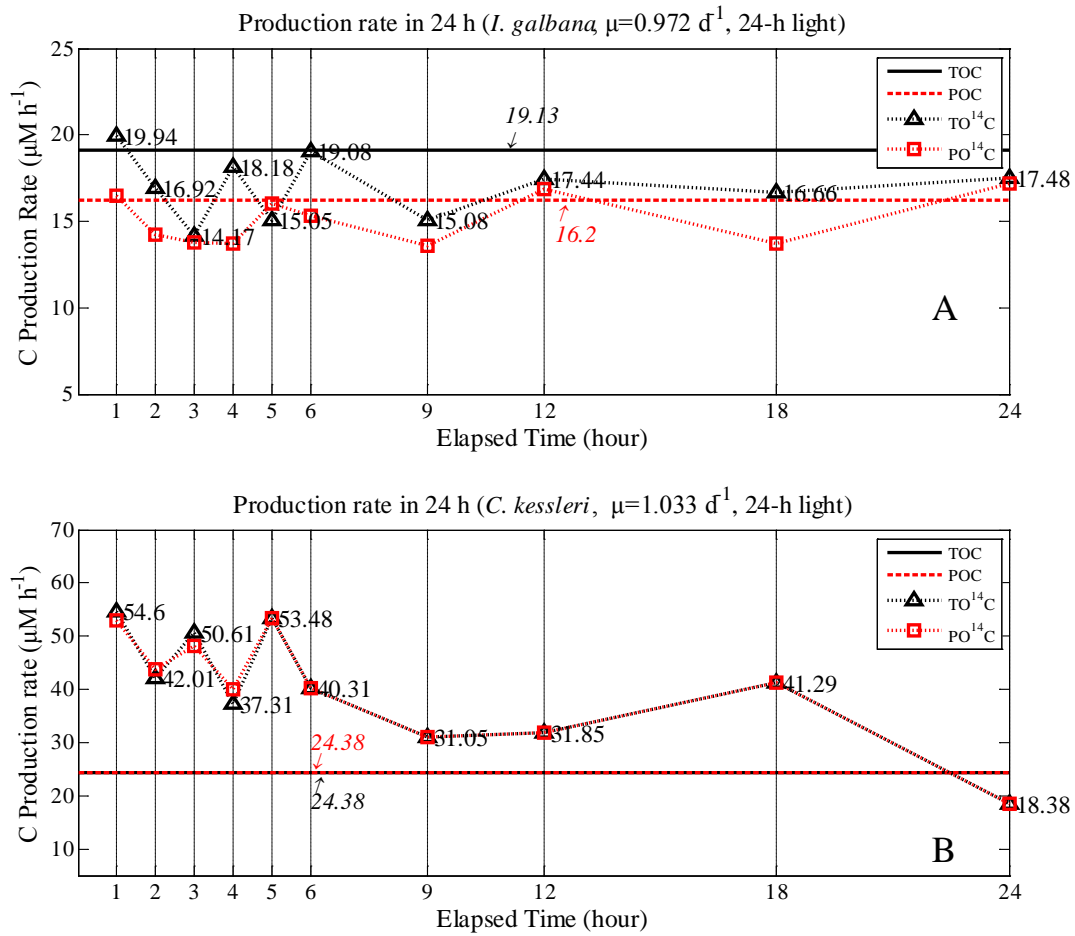


Figure 5.2. Results of continuous culture studies for *I. galbana* (A) and *C. kessleri* (B) at high growth rates. Solid black horizontal line and dashed red line are the actual net production rates of TOC and POC estimated by control method respectively (calculated by Eq. 8 and Eq. 9). Dotted black line with triangles and dotted red line with squares are the production rates of TOC and POC estimated by the ¹⁴C method. Both triangles and squares indicate the average values calculated for the time interval before that time points (for example 54.6 indicates the mean value between 0–1 h).

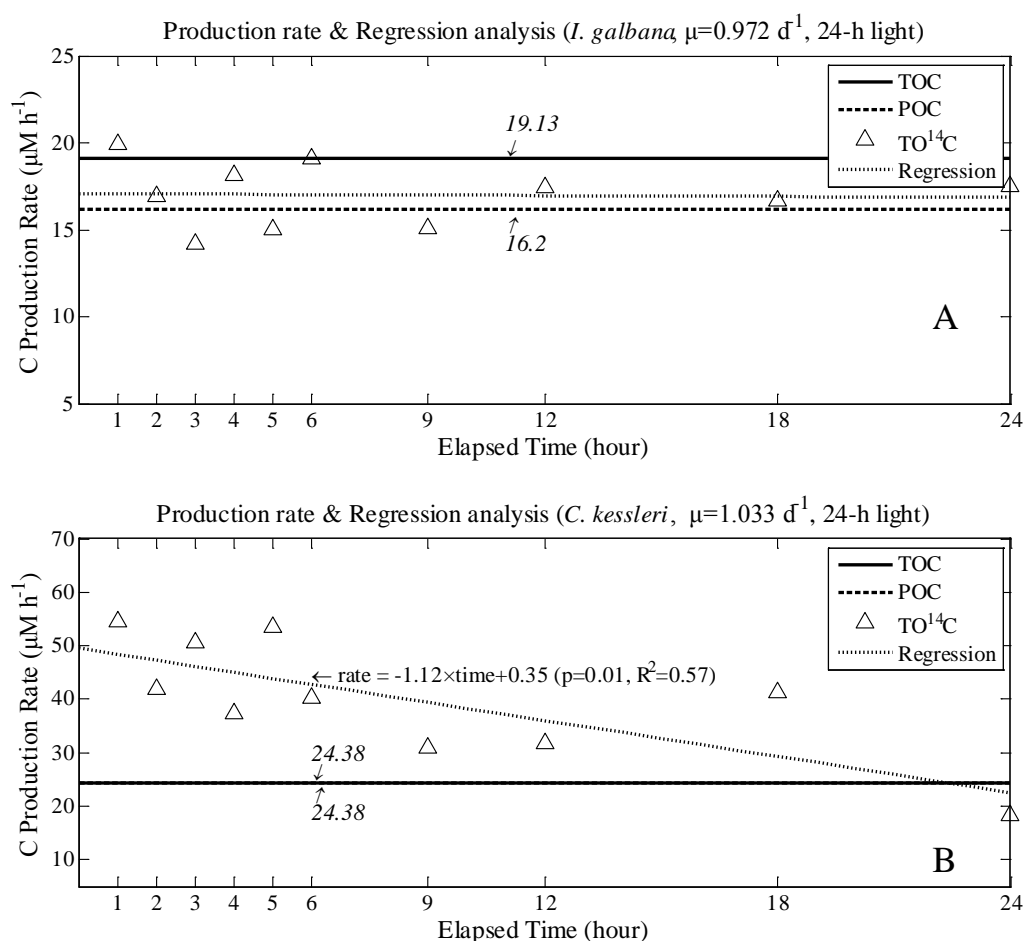


Figure 5.3. Regression analysis for relationship between TOC production rates measured by the ^{14}C method and the actual net production rates measured by control method at high growth rates. Solid black horizontal line and dashed black line are the actual net production rates of TOC and POC estimated by control method respectively (calculated by Eq. 8 and Eq. 9). Triangles indicate the production rate of TO^{14}C estimated by the ^{14}C method and the dotted black line is the regression line for these production rates.

C. kessleri was grown at a high growth rate of 1.033 d^{-1} under nitrate-limited continuous light conditions as well. Net photosynthetic rate calculated by Eq. 8–9 was 24.38 for POC and DOC and there was no evidence of DOC excretion.

Table 5.1 Production rates of POC and TOC measured by the ^{14}C method and control method for nitrate-limited continuous cultures.

Time (h)	<i>I. galbana</i> ($\mu=0.972\text{ d}^{-1}$)		<i>C. kessleri</i> ($\mu=1.033\text{ d}^{-1}$)		Time (h)	<i>I. galbana</i> ($\mu=0.318\text{ d}^{-1}$)		<i>C. kessleri</i> ($\mu=0.313\text{ d}^{-1}$)	
	P _{POC}	P _{TOC}	P _{POC}	P _{TOC}		P _{POC}	P _{TOC}	P _{POC}	P _{TOC}
0–1	16.49	19.94	52.95	54.60	0–3	11.39	11.39	15.31	15.31
1–2	14.22	16.92	43.72	42.01					
2–3	13.78	14.17	48.09	50.61					
3–4	13.73	18.18	40.02	37.31	3–6	8.83	8.83	15.90	15.90
4–5	16.01	15.05	53.48	53.48					
5–6	15.33	19.08	40.31	40.31					
6–9	13.61	15.09	31.05	31.05	6–9	12.20	12.33	14.48	14.65
9–12	16.87	17.44	31.85	31.85	9–12	10.58	10.44	17.19	18.73
12–18	13.71	16.66	41.29	41.29	12–18	9.14	9.14	16.05	15.18
18–24	17.21	17.48	18.38	18.38	18–24	11.16	11.16	13.74	13.74
Average (^{14}C)	15.27 \pm 2.11	16.91 \pm 1.28	34.39 \pm 9.75	34.38 \pm 10.09		10.45 \pm 1.43	10.45 \pm 1.46	15.31 \pm 1.39	15.30 \pm 1.41
Control	16.2	19.13	24.38	24.38		9.48	10.64	9.39	10.3
Deviation	-5.74%	-11.60%	41.06%	41.06%		10.23%	-1.79%	63.05%	48.54%
P-value (t-test)	0.0396	0.0052	0.0013	0.0005		0.1039	0.8751	0.0000	0.0006

The unit of production rate is $\mu\text{M h}^{-1}$.

μ = growth rate.

Average (^{14}C) line shows the average production rates measured by the ^{14}C method.

Control line indicates the average production rates measured by the control method.

Deviation is calculated by (Average (^{14}C)–Control)/Control.

After incubations of 0–24 h, both TOC and POC fixation rate estimated by the ^{14}C uptake averaged closely at $34.38 \pm 9.75 \mu\text{M h}^{-1}$ (Fig. 5.2B and Table 5.1). There was no distinct evidence of DOC production rate as well in the growth chamber. The average fixation rate of TOC estimated by the ^{14}C uptake was about $(34.38 - 24.38) / 24.38 = 41.02\%$ higher than the actual net fixation rate estimated by the direct measurement of TOC in the growth chamber. The significant difference (t-test, $p=0.0013$) indicates that the ^{14}C method overestimated the production rate for this species under nutrient-limited and high-growth-rate conditions. Such result of continuous culture was therefore again consistent with the implication of previous batch culture work, i.e., in the case of *C. kessleri*, ^{14}C uptake was providing an estimate of gross carbon fixation.

Linear regression shows that there is significant negative linear relationship between the TOC photosynthetic rates estimated by the ^{14}C method and the elapsed time ($p=0.0120$, $R^2=0.5662$) with a slope of -1.12 (Fig. 5.3B). The average uptake rates of TOC were $46.39 \mu\text{M h}^{-1}$ in the first six hours, $31.45 \mu\text{M h}^{-1}$ in the second six hour and $29.83 \mu\text{M h}^{-1}$ in the following 12 hours. Overall, the uptake rates measured by the ^{14}C method were declining over time and became in the rough range of the net photosynthetic rate at the end of incubation. The declining trend of carbon uptake rate over time for *C. kessleri* is obviously different from that of *I. galbana* as shown in Fig. 5.3A, suggesting that there might be different internal mechanism of carbon pathway for these two algae, which will be discussed latter in details.

5.1.3 Nitrate-limited, Low Growth Rate

Similarly, continuous culture experiments were carried out at low growth rates (0.318 d^{-1} for *I. galbana* and 0.313 d^{-1} for *C. kessleri* respectively) under nitrate-limited and continuous light conditions. For *I. galbana*, the average net TOC fixation calculated by Eq. 9 was $10.64 \mu\text{M h}^{-1}$ (Fig. 5.4A). Comparatively, the corresponding rates estimated by the ^{14}C method in the 24-hour incubation averaged at $10.45 \pm 1.46 \mu\text{M h}^{-1}$ for TOC (Table 5.1), about 1.79% lower than the net fixation rate calculated by Eq. 9, a difference that was within the propagated analytical error of the TOC ($\pm 5\%$) and ^{14}C uptake ($\pm 5\%$) measurements.

The results of the continuous culture work at low growth rate are consistent with those at high growth rate, and verify our batch culture studies again. The ^{14}C method was estimating the net production rates for *I. galbana*. Regression analysis indicates that there is no significant linear relationship between the photosynthetic rates of TOC and the elapsed time ($p=0.8957$ and $R^2=0.0048$, Fig. 5.5A).

For *C. kessleri* grown at a low growth rate of 0.313 d^{-1} under same conditions as *I. galbana*, net photosynthetic rates calculated by Eq. 8–9 were 10.30 and $9.39 \mu\text{M h}^{-1}$ for TOC and POC respectively. Comparatively, after incubations of 0–24 hours, both TOC and POC fixation estimated by the ^{14}C uptake averaged $15.31 \pm 1.41 \mu\text{M h}^{-1}$ (Fig. 5.4B). The fixation rates of TOC estimated from ^{14}C uptake were about $(15.31 - 10.30)/10.30 = 48.64\%$ higher than the net fixation rates estimated from measurements of TOC in the growth chamber, indicating the difference is significant ($p=0.0006$ for the one sample t-test). These results were therefore consistent with the implications of the batch culture work again, i.e., in the case of *C. kessleri*, ^{14}C uptake was providing an estimate of gross carbon fixation.

Linear regression analysis shows that there is no significant linear relationship between the production rates of TOC and the incubation time. In Fig. 5.5B it seems that there is a declining trend for TOC production rates along incubation time, but because the growth rate

was so low (doubling time = 2.21 d) that this trend is not statistically significant in our relatively short-period incubation of 24 hours.

In both incubations of *I. galbana* and *C. kessleri*, the production rates of DOC were not negative, which means there was net excretion for DOC from the algal cells into the medium. The DOC production rate and its proportion in TOC are calculated for each incubation and summarized in Table 5.4.

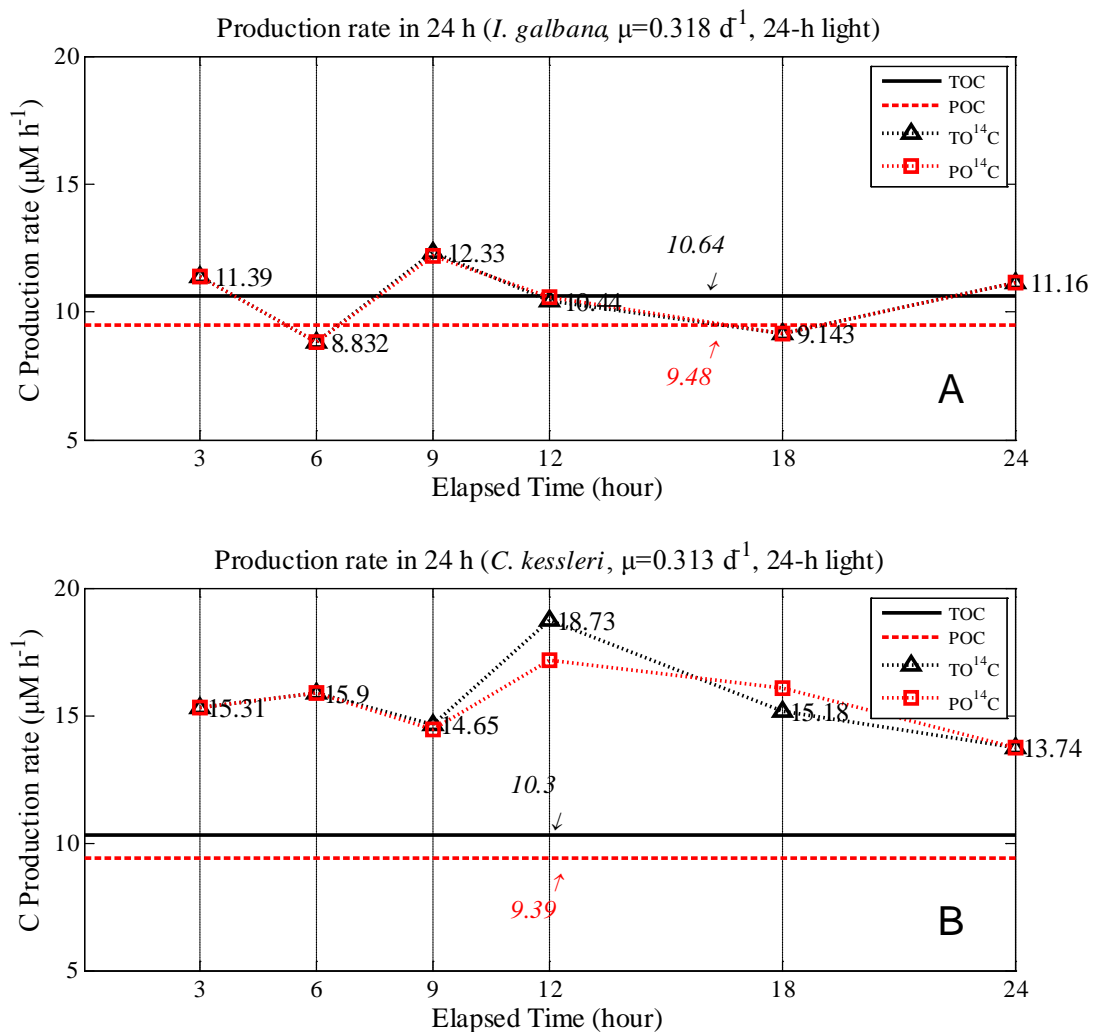


Figure 5.4. Results of continuous culture studies of *I. galbana* (A) and *C. kessleri* (B) at low growth rates. Solid black horizontal line and dashed red line are the actual net production rates of TOC and POC estimated by control method respectively (calculated by Eq. 8 and Eq. 9). Dotted black line with triangles and dotted red line with squares are the production rates of TOC and POC estimated by the ¹⁴C method. Both triangles and squares indicate the average values calculated for the time interval before that time points (for example 11.39 indicates the mean value between 0–3 h).

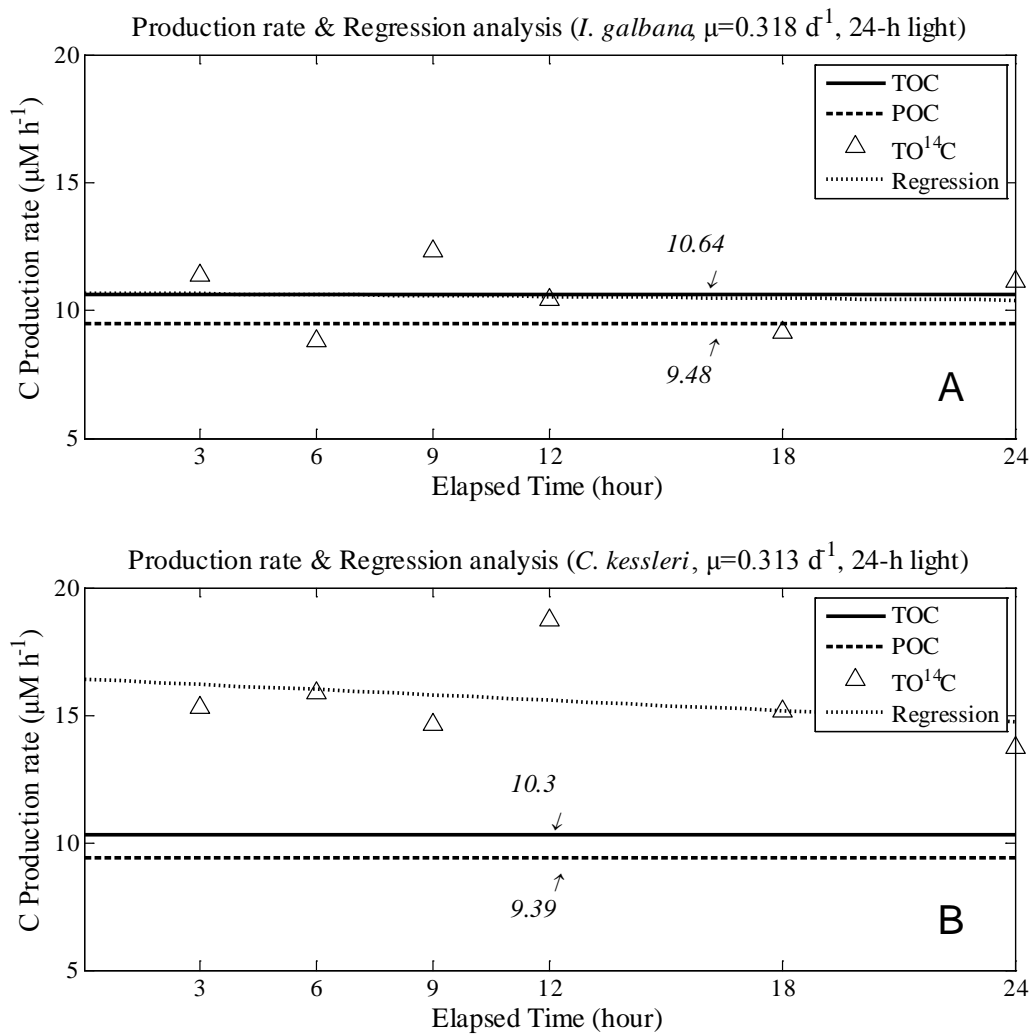


Figure 5.5. Regression analysis for relationship between TOC production rates measured by the ^{14}C method and the actual net production rates measured by control method at low growth rates. Solid black horizontal line and dashed black line are the actual net production rates of TOC and POC estimated by control method respectively (calculated by Eq. 8 and Eq. 9). Triangles indicate the production rate of TO^{14}C estimated by the ^{14}C method and the dotted black line is the regression line for these production rates.

5.1.4 Light-limited, High Growth Rate

When *I. galbana* were grown under 24-hour continuous irradiance of $60 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$, the continuous culture was run for about 1–2 weeks at a relative high growth rate of 0.855 d^{-1} before adding ^{14}C . To simulate the condition of regular bottle incubation, peristaltic pump

was turned off when doing ^{14}C incubation and the cell density was determined whenever collecting the ^{14}C samples.

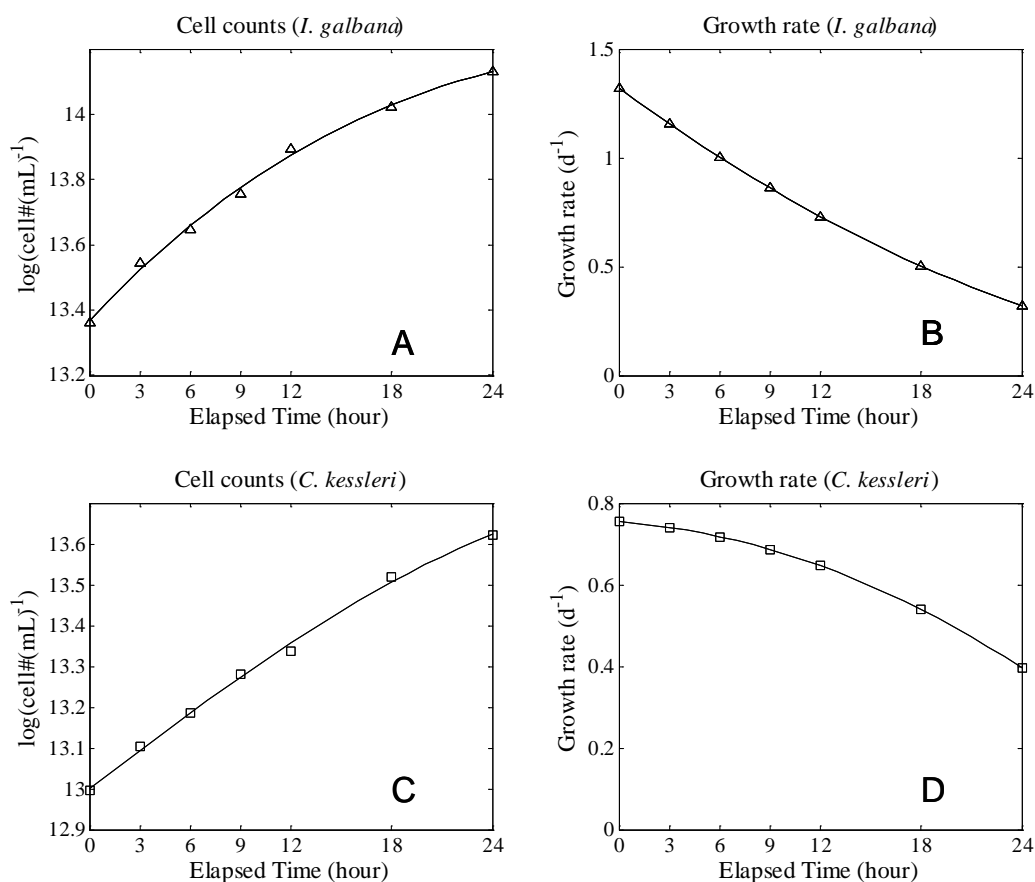


Figure 5.6. Cell density and calculated growth rates along incubation time. Plots A and B are for *I. galbana* and plots C and D are for *C. kessleri*. Both algae were incubated at high growth rates under continuous light-limited condition.

According to the cell densities and Malthusian growth model (also called the simple exponential growth model) (Malthus 1826), the average growth rates in each time interval are calculated by Eq. 13. Fig 5.6 A and C show the variation of cell density with incubation time and Fig. 5.6 B and D show the calculated growth rates corresponding cell density in each time interval. It can be seen that the growth rates were decreasing along time. The calculated growth rates are listed in the first column of Table 5.2.

In ^{14}C incubation, ^{14}C samples were collected every 3-hour during the first 12-hour, and every 6-hour during the second 12-hour. Initial DIC concentration at the beginning of ^{14}C incubation was $2733 \mu\text{M L}^{-1}$. Initial photosynthetic rates of POC and TOC at time zero were 19.53 and $20.27 \mu\text{M h}^{-1}$ respectively. The difference between TOC and POC rates is calculated to be the excretion rate of DOC, $0.74 \mu\text{M h}^{-1}$ in the light.

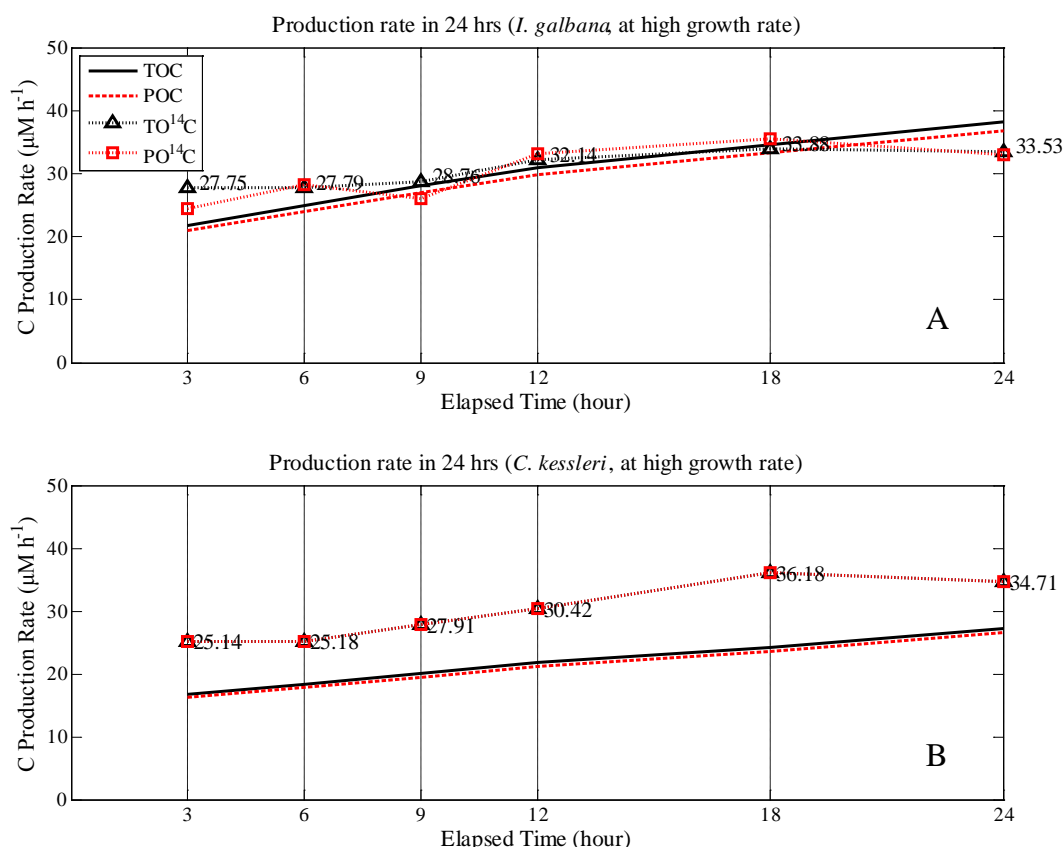


Figure 5.7. Results of continuous culture studies of *I. galbana* (A) and *C. kessleri* (B) at high growth rates under light-limited conditions (pump was off). Solid black line and dashed red line are the actual net production rates of TOC and POC estimated by control method respectively (calculated by Eq. 8 and Eq. 9). Dotted black line with triangles and dotted red line with squares are the production rates of TOC and POC estimated by the ^{14}C method. Both triangles and squares indicate the average values calculated for the time interval before that time points (for example 27.75 indicates the mean value between 0–3 h).

Since the algae were undergoing exponential growth when the pump was turned off, the photosynthetic rates of TOC and POC in each time interval could be calculated by combining Eq. 12–13. All results are plotted in Fig. 5.7 A and shown in Table 5.2. For comparison, the

photosynthetic rates of POC and TOC estimated by the ^{14}C method are calculated by Eq. 14 and listed in Table 5.2 as well. Two sample t-test is used to compare the average photosynthetic rates in each time interval estimated by the ^{14}C method with the rates measured by CHN and DOC analyzers (the control method). P-values, 0.3297 for POC and 0.5654 for TOC respectively, indicates that the differences are not significant in this case and the ^{14}C method estimated net production rates accurately. Regression analysis indicates that there is significant correlation between the TOC photosynthetic rates measured by two methods ($p=0.0061$).

C. kessleri was also grown under same irradiance and incubation conditions as *I. galbana*. The effect caused by the limited irradiances on the maximum growth rate was more significant for *C. kessleri* since its average growth rate was limited to be only 0.668 d^{-1} , much lower than 0.855 d^{-1} of *I. galbana* under the same irradiance. Since the pump was turned off when doing ^{14}C incubation, dilution effect can be ignored in the process of ^{14}C incubation.

The initial DIC concentration at the beginning of ^{14}C incubation was $1930\text{ }\mu\text{M}$ and the initial photosynthetic rates at time zero calculated by the control method were $15.57\text{ }\mu\text{M h}^{-1}$ and $15.99\text{ }\mu\text{M h}^{-1}$ for POC and TOC respectively. The corresponding initial excretion rate of DOC was $0.42\text{ }\mu\text{M h}^{-1}$. The calculated photosynthetic rates by the ^{14}C method (using Eq. 14) and by the control method (using Eq. 12–13) are shown in Fig. 5.7B and listed in Table 5.2. The p-values in two sample t-test are close to zero for POC and TOC respectively, indicating that the overestimation of production rates by the ^{14}C method is strongly significant.

Consequently, the results of the light-limited continuous cultures show that the ^{14}C technique estimates the net production rate for *I. galbana* and overestimate the net production rate for *C. kessleri*, which is consistent with our batch culture studies.

Table 5.2. Comparison of production rates for light-limited continuous cultures at high growth rates.

Time (h)	<i>I. galbana</i> ($\mu \sim 0.855 \text{ d}^{-1}$, pump was off)					<i>C. kessleri</i> ($\mu \sim 0.668 \text{ d}^{-1}$, pump was off)				
	$\mu \text{ (d}^{-1}\text{)}$	P_{POC}		P_{TOC}		$\mu \text{ (d}^{-1}\text{)}$	P_{POC}		P_{TOC}	
		^{14}C	Control	^{14}C	Control		^{14}C	Control	^{14}C	Control
0	1.319		19.53		20.27	0.754		15.57		15.99
0–3	1.155	24.47	21.01	27.75	21.81	0.740	25.14	16.31	25.14	16.75
3–6	1.002	28.19	24.04	27.79	24.95	0.718	25.18	17.87	25.18	18.35
6–9	0.861	26.00	27.00	28.76	28.03	0.686	27.91	19.51	27.91	20.03
9–12	0.730	33.21	29.82	32.14	30.95	0.646	30.42	21.20	30.42	21.77
12–18	0.503	35.58	33.25	33.88	34.51	0.539	36.18	23.63	36.18	24.26
18–24	0.321	32.94	36.85	33.53	38.24	0.397	34.71	26.55	34.71	27.27
Average	0.842	31.11	30.26	31.41	31.41	0.640	31.30	21.91	31.30	22.50
P (t-test)		0.3297		0.5654			0		0.0000	
Deviation		2.81%		0.00%			42.86%		39.11%	

μ = growth rate.

Average line shows the average production rates measured by the ^{14}C method and by control method.

^{14}C column indicates the average production rates in each time interval measured by the ^{14}C method.

Control column indicates the average production rates in each time interval measured by the control method.

To simulate the natural ecosystem, pump was turned off when adding ^{14}C into the chemostat and starting the 24-h incubation

Deviation is calculated by: (average rates measured by ^{14}C – average rates measured by control method)/ average rates measured by control method.

Both incubations were under same light irradiance.

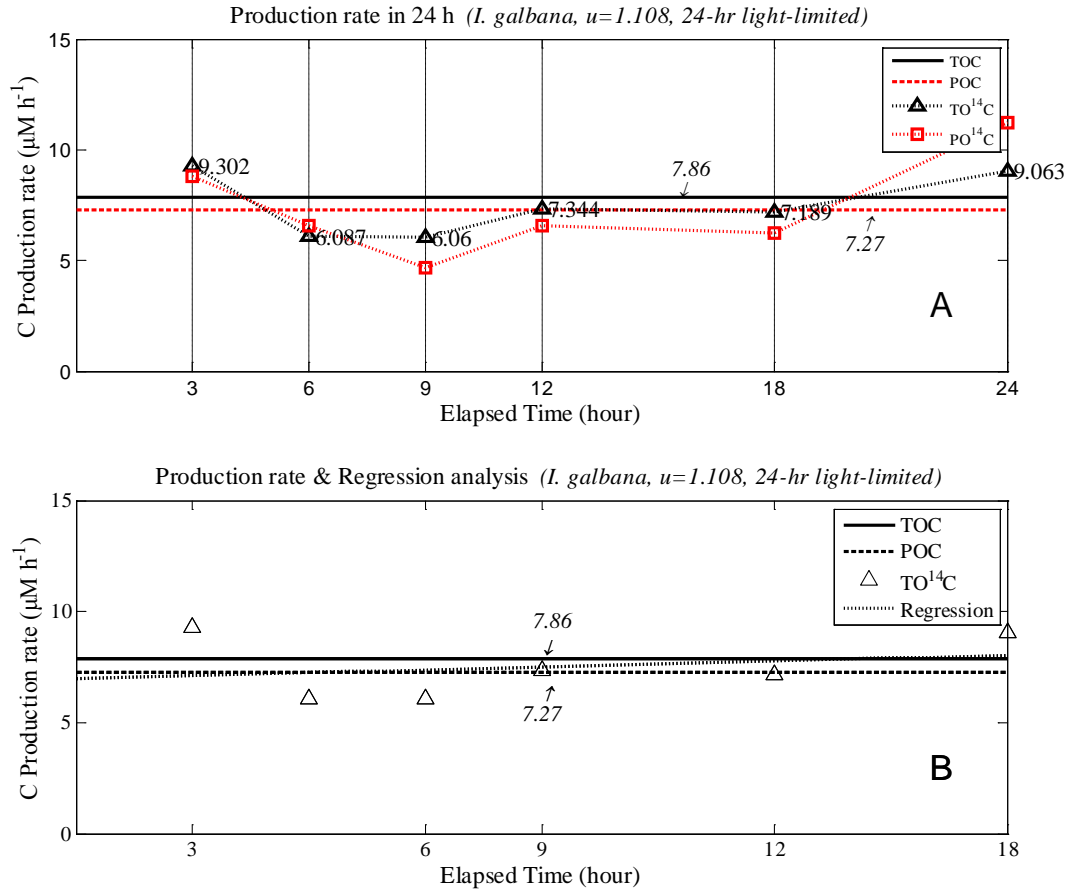


Figure 5.8. Repeated ^{14}C incubation of *I. galbana* at high growth rate under continuous light-limited condition (pump was on) and its regression analysis. In plot A, solid black horizontal line and dashed red line are the actual net production rates of TOC and POC estimated by control method respectively (calculated by Eq. 8 and Eq. 9). Dotted black line with triangles and dotted red line with squares are the production rates of TOC and POC estimated by the ^{14}C method. Both triangles and squares indicate the average values calculated for the time interval before that time points (for example 9.302 indicates the mean value between 0–3 h). In plot B, the dotted black line is the regression line for the production rate of TO^{14}C estimated by the ^{14}C method.

It is noteworthy that in previous ^{14}C incubations for *I. galbana* and *C. kessleri*, the pump was turned off. If the pump was kept on, would the ^{14}C method still estimate net photosynthetic rate for *I. galbana*? To answer this question, *I. galbana* was also incubated with pump kept on at a high growth rate of 1.108 d^{-1} controlled by the same continuous

irradiance of $60 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$. The production rates estimated by the ^{14}C method and those measured by the control method are shown in Fig. 5.8 and Table 5.3.

In this incubation, since the growth chamber was continuously diluted by fresh medium, the incubation process was similar as the nitrate-limited cultures shown in section 5.1.2 except that the limiting factor in this case was light rather than nitrate. The dilution rate (= growth rate) controlled by pump was 1.108 d^{-1} .

The initial DIC concentration at time zero was $2105 \mu\text{M}$ and the photosynthetic rates calculated by Eq.8–9 were $7.27 \mu\text{M h}^{-1}$ for POC and $7.86 \mu\text{M h}^{-1}$ for TOC. The excretion rate of DOC was about $0.59 \mu\text{M h}^{-1}$ and it accounts for about $0.59/7.86 = 7.51\%$ of the uptake rate of the total organic carbon (Table 5.4, P_{DOC} indicates the production rate of DOC; P_{TOC} suggests the production rate of TOC; and % of P_{TOC} is calculated by $P_{\text{DOC}}/P_{\text{TOC}}$).

The average production rate for each time interval is calculated and shown in Table 5.3. The total average production rate in 24 h estimated by the ^{14}C method was $7.69 \pm 2.61 \mu\text{M h}^{-1}$ for both POC and TOC, very close to the actual net production rates calculated by the control method, since the production rates calculated by the control method were $7.27 \mu\text{M h}^{-1}$ for POC, and $7.86 \mu\text{M h}^{-1}$ for TOC.

The t-tests with p-values of 0.9400 for POC and 0.5660 for TOC indicate that there is no significant difference between POC and TOC production rates measured by the ^{14}C method and those by the control method.

Therefore, either the pump is on or off in the ^{14}C incubation process, the ^{14}C method can provide an accurate estimation of net production rates for *I. galbana*. These results of continuous cultures are in good agreement with our previous batch culture experiments which imply that the ^{14}C techniques seems to estimate the actual net production rate for *I. galbana*, since no loss of activity was observed.

Table 5.3. Comparison of production rates for light-limited continuous cultures.

Time (h)	<i>I. galbana</i> ($\mu=1.108\text{d}^{-1}$)		<i>I. galbana</i> ($\mu=0.316\text{d}^{-1}$)		<i>C. kessleri</i> ($\mu=0.116\text{d}^{-1}$)	
	P _{POC}	P _{TOC}	P _{POC}	P _{TOC}	P _{POC}	P _{TOC}
0–3	8.80	8.80	8.76	9.41	10.57	10.57
3–6	6.55	6.55	7.21	7.11	9.76	9.76
6–9	4.67	4.67	8.05	11.55	9.12	9.12
9–12	6.57	6.57	7.76	5.29	11.49	11.49
12–18	6.23	6.23	9.36	10.99	9.43	10.60
18–24	11.23	11.23	10.29	8.03	8.62	9.35
Average (^{14}C)	7.69 \pm 2.61	7.69 \pm 2.61	8.89 \pm 0.65	8.93 \pm 2.41	9.63 \pm 0.8	10.11 \pm 0.96
Control	7.27	7.86	7.73	8.96	5.52	6.04
Deviation	5.78%	-2.16%	14.94%	-0.38%	74.46%	67.38%
P-value (t-test)	0.9400	0.5660	0.1272	0.8237	0.0002	0.0000

The first incubation was at high growth rate. The second and third ones were at low growth rate under same light irradiance.

In all three incubations, pump was kept on after adding ^{14}C .

Average (^{14}C) line shows the average production rates measured by the ^{14}C method.

Control line indicates the average production rates measured by the control method.

Deviation is calculated by: (average rates measured by ^{14}C – average rates measured by control method)/ average rates measured by control method.

5.1.5 Light-limited, Low Growth Rate

I. galbana and *C. kessleri* were also grown at lower growth rates (0.316 d^{-1} and 0.116 d^{-1} respectively) which were limited by the relatively dim light with an irradiance of $20\text{ }\mu\text{mol quanta m}^{-2}\text{ s}^{-1}$ at the center of the empty growth chamber. Different growth rate under the same light intensity indicates that the limiting effect of light is more significant on *C. kessleri* than on *I. galbana*, a same phenomenon as we have observed under relatively bright light. The pump was kept on while carrying on the ^{14}C incubation. Thus equations for nitrate-limited incubations could be used to calculate the production rates for these light-limited incubations here.

For *I. galbana*, the initial DIC concentration was $2130\text{ }\mu\text{M}$ at the beginning of incubation. The actual net production rates calculated by Eq. 8 and Eq.9 were $7.73\text{ }\mu\text{M h}^{-1}$ for POC and $8.96\text{ }\mu\text{M h}^{-1}$ for TOC (Table 5.3). Similarly, the DOC production rate was $1.23\text{ }\mu\text{M h}^{-1}$, so the DOC excretion rate accounts for $1.23/8.96 = 13.73\%$ of the total organic production rate (Table 5.4). Samples were collected every 3-h during the first 12 hours and every 6-h during the second 12 hours. The calculated photosynthetic rates by the ^{14}C method are shown in Fig. 5.9A and summarized in Table 5.3. The average production rates in 24 h estimated by the ^{14}C method were $8.88\text{ }\mu\text{M h}^{-1}$ for POC and $8.93\text{ }\mu\text{M h}^{-1}$ for TOC. So the deviations of production rates measured by two different methods are only 14.94% for POC and -0.38% for TOC. In addition, one-sample t-tests ($p=0.1272$ for POC and 0.8237 for TOC) show that for both POC and TOC, the differences in production rate estimation by the ^{14}C method and by the control method are not significant. Linear regression analysis shows that there is no significant relationship between the photosynthetic rate and the incubation time ($p=0.9946$).

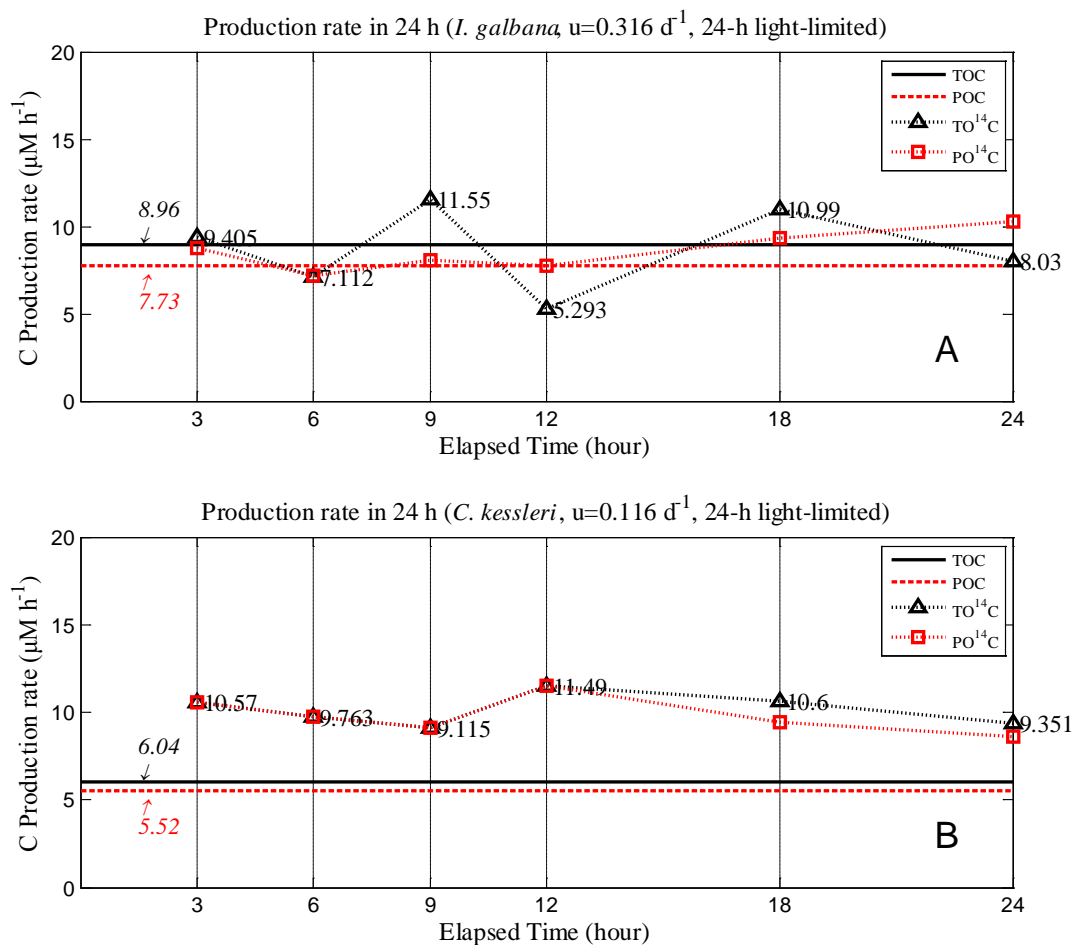


Figure 5.9. Results of continuous culture studies of *I. galbana* (A) and *C. kessleri* (B) at low growth rates under light-limited conditions. Solid black horizontal line and dashed red line are the actual net production rates of TOC and POC estimated by control method respectively (calculated by Eq. 8 and Eq. 9). Dotted black line with triangles and dotted red line with squares are the production rates of TOC and POC estimated by the ¹⁴C method. Both triangles and squares indicate the average values calculated for the time interval before that time points (for example 10.57 indicates the mean value between 0–3 h).

C. kessleri was incubated under same incubation condition as *I. galbana*. The calculated results are shown in Fig 5.9B and Table 5.3. Initial DIC concentration was 2560 μM L⁻¹ at the beginning of incubation. The net POC production rate calculated by Eq. 8 was 5.52 μM h⁻¹. The net production rate calculated by Eq. 9 was 6.04 μM h⁻¹ for TOC. Similarly, the excretion rate of DOC was 0.52 μM h⁻¹ and it accounted for 0.52/6.04 = 8.61% of the total production rate of organic carbon. Comparatively, the average production rates calculated by the ¹⁴C

method were $9.63 \pm 0.80 \mu\text{M h}^{-1}$ for POC, $10.11 \pm 0.96 \mu\text{M h}^{-1}$ for TOC. The difference of POC and TOC uptake rates measured by the control method and those measured by the ^{14}C method is strongly significant since the p-values of t-test are 0.0002 for POC and close to zero for TOC. The ^{14}C method overestimates the net photosynthetic rate by $(9.63 - 5.52)/5.52 = 74.46\%$ for POC and $(10.11 - 6.04)/6.04 = 67.38\%$ for TOC. It can be seen that the overestimated rates at lower growth rate are much larger than those at higher growth rate under light limited conditions.

To test whether the incubation length have an effect on the TOC uptake rates measured by the ^{14}C method, a linear regression analysis is carried out and the result indicates that there is no significant correlation between the uptake rates of TOC and incubation length ($p=0.7769$).

5.2 Discussion

5.2.1 Nitrate-limited Incubations

Comparison of Production Rates

The results of my batch culture studies (Fig. 4.1) implies that the ^{14}C method should measure net photosynthesis for *I. galbana*, because there was no decline of ^{14}C activity of uniformly labeled cells when they were resuspended in fresh medium in the light, i.e. all respired carbon labeled by ^{14}C was reassimilated in the batch culture experiment (Ryther 1956). Comparatively, the ^{14}C method should measure gross photosynthesis for *C. kessleri* since the ^{14}C activity of cells uniformly labeled with ^{14}C declines when they were resuspended in fresh medium in the light, and the rate of decline was nearly same in the dark and light, in other words, old carbon was the substrate for respiration, and no respired carbon was reassimilated.

My continuous cultures of two species at both high and low growth rates under nitrate-/light-limited conditions verified the implication of batch cultures. The comparisons of production rates estimated by the ^{14}C method and those measured by the control method are shown in Fig. 5.2 for high growth rate incubations and in Fig. 5.4 for low growth rate incubations respectively and also summarized in Table 5.1.

For *I. galbana* incubated at the high growth rate of 0.972 d^{-1} , the photosynthetic rates of TOC and POC measured by the ^{14}C method are no higher than the actual net rates calculated by Eq. 8–9. The ^{14}C method estimated the actual net production rates of POC and TOC in acceptable range (Table 5.1 and Fig. 5.2 A). When *I. galbana* was incubated at the low growth rate of 0.318 d^{-1} (Fig. 5.4A), the production rates measured by the ^{14}C method seems in good agreement with the net rates measured by the control method as well.

For *C. kessleri* incubated at a similar high growth rate of 1.033 d^{-1} , the ^{14}C method significantly overestimated the average photosynthetic rates of POC and TOC by about 41.02% (Table 5.1 and Fig. 5.2B). During the first few hours and last six hours they were roughly 200% and 75%, respectively, of the net rate of photosynthesis. At the low growth rate of 0.313 d^{-1} , these overestimation rates were 63.05% for POC and 48.54% for TOC (Fig. 5.4B). The fact that the ^{14}C method overestimated the actual net photosynthetic rate of *C. kessleri* verified the implication from previous batch cultures. The growth rate might have an effect on the overestimation caused by the ^{14}C technique, but it seems not very obvious under current conditions since the average overestimation rate for production by the ^{14}C method is only a little higher at low growth rate than that at high growth rate.

The effect of algal species is significant, which might explain some of the paradoxes about what the ^{14}C method actually measures, including the historical debate between Ryther & Vaccaro on one side and Steemann Nielsen and co-workers on the other (Peterson 1980), because different algal species were used in those early studies. And the information about

what the ^{14}C method actually measures for common phytoplankton species would allow for a more informed interpretation of field results if information on species composition is available.

Effect of Incubation Length

One of the goals of our study is to determine whether there is any evidence that short-term ^{14}C incubations give a higher estimate of carbon fixation than longer (up to 24 h) incubations, and if so, whether the difference between short-term and long-term results is a function of growth rate or the factor limiting growth.

When *C. kessleri* was incubated at the high growth rate of 1.033 d^{-1} (doubling time = $0.671\text{ d} < 24\text{ h}$), there is significant negative linear relationship between the TOC photosynthetic rate measured by the ^{14}C method and the elapsed time ($p=0.0120$, $R^2=0.5662$) as shown by the regression line in Fig. 5.3B. For *I. galbana* incubated under the same conditions (Fig. 5.3A), no such declining trend of TOC photosynthetic rate was observed. The discrepant trend between two algal species is discussed as following.

For *I. galbana*, the fact that the uptake rates calculated by the ^{14}C method were consistent with those calculated by Eq. 8–9 indicates that (1) $\text{SA}_R = \text{SA}_{\text{DIC}}$ or (2) $R = 0$ in Eq. 2 is true, i.e. the ^{14}C method measures net production rate for this algae. Since (1) $\text{SA}_R = \text{SA}_{\text{DIC}}$ was rejected by Ryther (1956), (2) $R = 0$ is correct, in other words, all respired carbon is reassimilated. So for this species, there should be no ^{14}C release from cells in the process of light respiration. Although the specific activity is always declining with time course, the Eq. 3–5 for calculating photosynthetic rate of TOC included the correction of specific activity along time. Consequently, the calculated rates were relative consistent during the whole incubation length of 24 h as shown in Fig. 5.3A and Fig 5.5A.

The results of the *C. kessleri* chemostat experiments were consistent with the batch culture experiments with one important caveat. At $\mu = 1.033 \text{ d}^{-1}$, the photosynthetic rates calculated from ^{14}C uptake during the first few hours after addition of ^{14}C were about twice the rate of net photosynthesis. It seems reasonable to postulate that during these first few hours old carbon with a specific activity of zero was being respired and lost from the cells. Thus these rates were probably very close to the rate of gross photosynthesis.

The fact that the calculated rates declined with time suggests that within a timeframe of a few hours some of the respired carbon included carbon labeled with ^{14}C . In other words, “old” carbon began to include carbon that had been fixed no more than a few hours prior to the incubations. The fact that the photosynthetic rate estimated from ^{14}C uptake during the last time interval (18–24 hours after the addition of ^{14}C) was about 75% of the net photosynthetic rate implies that $\text{SA}_\text{R}/\text{ID}_\text{R}$ was greater than $\text{SA}_\text{DIC}/\text{ID}_\text{P}$ during that time interval (Eq. 2). In fieldwork this condition would never occur, because for all intents and purposes SA_DIC remains constant during incubations in the field. However, in the chemostat DI^{14}C and hence SA_DIC declines exponentially with time (Eq. 6), and the rate of decline is positively correlated with the photosynthetic rate and growth rate (Eq. 7). In this particular case, SA of DI^{14}C declined by about 6% per hour calculated by combining equations 5–7. If $P = 2(P - R)$, as suggested by the ^{14}C results during the first few hours after addition of ^{14}C (Fig. 5.3B), then the fact that $P - R \frac{\text{SA}_\text{R}}{\text{SA}_\text{DIC}} \frac{\text{ID}_\text{P}}{\text{ID}_\text{R}} = 0.75P$ during the time interval from 18 to 24 hours implies that $\text{SA}_\text{R}/\text{ID}_\text{R}$ equaled $1.25(\text{SA}_\text{DIC}/\text{ID}_\text{P})$ during that time interval. In other words, the specific activity of the respired carbon that escaped from the cells was about 25% greater than the specific activity of the carbon fixed during that time interval. This conclusion combined with the fact that SA_DIC declined by about 6% per hour implies that the “old” carbon being respired had been fixed roughly four hours prior to its being respired. This conclusion is

consistent with the photosynthetic rates estimated from ^{14}C uptake being gross photosynthesis for the first few hours after ^{14}C addition and declining thereafter.

The *C. kessleri* results at $\mu = 0.313 \text{ d}^{-1}$ (doubling time = 2.21 d) are more consistent with the batch culture studies in that the rates determined from ^{14}C uptake overestimated net photosynthesis by roughly 50%, and there was no significant temporal pattern to the ^{14}C uptake results (Fig. 5.5B). The absence of a temporal pattern suggests that the carbon being respired had been fixed prior to the start of the incubation. The difference between the results in Fig. 5.3B and 5.5B appears to reflect differences in the turnover time of the pool of substrates used for respiration, the turnover rate presumably being higher in the case of Fig. 5.3B versus Fig. 5.5B. This result is consistent with compositional changes in phytoplankton grown under nutrient-limited conditions (Shuter 1979; Chalup and Laws 1990; Laws and Chalup 1990). At low growth rates large amounts of carbon are allocated to storage products that can serve as substrates for respiration. At high growth rates much less carbon is allocated to storage and more is allocated to components of the cell associated with the light and dark reactions of photosynthesis. These compositional differences combined with the fact that respiration rates are positively correlated with growth rates (Laws and Caperon 1976; Laws and Wong 1978) mean that the pool of respiratory substrates turns over much more rapidly at high versus low nutrient-limited growth rates.

On the other hand, it is proved that the incubation length have a significant effect on the interpretation of ^{14}C technique. Marra (2002) also concluded that the length of the incubation should be taken into consideration when interpreting ^{14}C uptake results and that the time required for SA_R to equal SA_{DIC} might be a function of the growth rate of the phytoplankton. Our results are in consistent with the previous studies by kinetic models of carbon incorporation and recycling which suggested that during an initial time course of carbon uptake, the ^{14}C fixation rate approximates gross photosynthesis (Landriau and Ducklow 1981;

Dring and Jewson 1982; Karl, Hebel et al. 1998) and the longer term rate of ^{14}C fixation should approximate net photosynthesis when ^{14}C isotopic equilibrium has been achieved (Karl, Hebel et al. 1998).

In sum, incubation length and growth rate might have a combined effect on species like *C. kessleri* in determining the production rates by the ^{14}C method, especially when they are grown at high growth rate, the accuracy of the ^{14}C technique might be affected by the incubation length significantly. In the practical usage of the ^{14}C method, the incubation time with ^{14}C ranges from less than 1 min (Lloyd, Canvin et al. 1977) to several hours (Dring and Jewson 1982; Williams, Robinson et al. 1996; Teira, Pazo et al. 2001), 24 h (Mingelbier, Klein et al. 1994; Letelier, Dore et al. 1996) or even longer (Ryther and Menzel 1965). If sample was only incubated for a short time (such as 1–6 h), then ^{14}C method would overestimated the net production rates. Take the average production rate in the first hour for example, the net production rate of TOC was overestimated by $(54.60-21.22)/21.22=157\%$ by the ^{14}C estimation. Dring and Jewson (1982) concluded that since several hours are required for cells to approach the equilibrium between $^{14}\text{C}:^{12}\text{C}$ ratio inside the cells and that in the external medium, all models in their study predicted that measurements of photosynthesis based on ^{14}C uptake overestimate net photosynthesis in incubations of up to 6–12 h, and that this overestimation were be especially severe close to the compensation point, or at depth in a water column.

DOC Uptake and Excretion

In addition to inorganic carbon assimilation by photosynthesis, phytoplankton is also able to utilize or excrete dissolved organic carbon (DOC) both in the light and dark period and play a role in organic carbon fluxes within aquatic food webs (Flynn, Clark et al. 2008). The net release from algae of dissolved organic matter (DOM) into the environment is still

one of these gaps in our knowledge that impact adversely on our ability to model primary production and hence the role of phytoplankton in global biogeochemical processes (Flynn, Clark et al. 2008). Measuring and modeling DOC production in planktonic ecosystem has long been studied and the biogeochemical interest in this topic is growing recently (del Giorgio and Peters 1993; Hansell, Bates et al. 1995; Teira, Pazo et al. 2001). Several processes, such as direct excretion from intact algal cells, cell lysis or microzooplankton grazing, are involved in the appearance of phytoplanktonic material in the dissolved organic compartment ultimately deriving from primary producers (Nagata 2008). In addition, DOC could also be taken up by algae in a process termed mixotrophy (Tittel, Bissinger et al. 2003), which occurs simultaneously with photosynthesis (Znachor and Nedoma 2010). Numerous algal and cyanobacterial taxa have been reported as potential consumers of DOC both in various environments (Paerl, Bebout et al. 1993; Lewitus and Kana 1994; Wood, Grimson et al. 1999; Vonshak, Cheung et al. 2000).

In our incubations, both excretion and uptake processes of DOC by algal cells have been observed. The DOC excretion rate and its percentage accounted for the total organic production rate in each incubation are summarized in Table 5.4. It can be seen that the positive excretion rates range from 2.63% to 15.32% for continuous cultures which are reasonably in the range of previous studies. The regression model of Baines and Pace (1991) based on 225 observations suggested that the average percent extracellular release (PER) of organic carbon to be 13% of total fixation.

If the calculated production rate of DOC by equations similar as Eq. 8 is positive, then the ^{14}C might be able to label the excretion of this part of DOC. For example, when *I. galbana* was grown at a high growth rate of 0.972 d^{-1} under nitrate-limited and continuous light conditions, the photosynthetic rate of DOC calculated by control method is $2.93\text{ }\mu\text{M h}^{-1}$ and it accounts for about $2.93/19.13 = 15.32\%$ of the total photosynthetic rate of organic

carbon calculated by Eq. 9. Comparatively, the photosynthetic rate of DOC calculated by the ^{14}C method is $1.64 \pm 1.64 \mu\text{M h}^{-1}$ and it accounts for $1.64/16.91 = 9.70\%$ of the total organic carbon uptake rate measured by the ^{14}C method.

Thus, although the ^{14}C method underestimates the actual DOC production rate a little, ^{14}C was indeed able to label the excreted part of dissolved organic carbon from the cell.

Our incubations and many evidence in previous studies (Williams 1990; Malinsky-Rushansky and Legrand 1996; Karl, Hebel et al. 1998) indicate that DOC production rates might represent a significant fraction of total organic production under certain environmental condition.

However, most standard procedures for ^{14}C technique involve filtering water at the end of the incubation and counting the ^{14}C activity on the filter (Strickland and Parsons 1972; Peterson 1980; Arnold and Littler 1985). Such typical ^{14}C experiments might underestimate the actual uptake rates of organic carbon because any ^{14}C that was fixed and subsequently excreted into the water, either directly by the cells or as a result of the grazing activities of heterotrophs, was obviously overlooked.

Besides the unlabeled DOC uptake, and DOC overlooking, some other issues related to DOC measurement are still the source of ambiguity in ^{14}C technique.

For example, convincing evidence was shown by Maske and Garcia-Mendoza (1994) to suggest that glass fiber filters may adsorb significant amounts of ^{14}C labeled DOC, and thus overestimate POC productivity and underestimate DOC productivity.

The study of Cibic and Virgilio (2011) indicated that inappropriate pH treatment of filters might damages the cell membrane with consequent loss of assimilated ^{14}C and result in the underestimation of POC productivity.

Table 5.4. DOC excretion or uptake in each incubation under continuous light.

24-h continuous light	Growth rate (d ⁻¹)	<i>I. galbana</i>			Growth rate (d ⁻¹)	<i>C. kessleri</i>		
		P _{DOC}	P _{TOC}	% of P _{TOC}		P _{DOC}	P _{TOC}	% of P _{TOC}
Nitrate-limited	0.972	2.93	19.13	15.32%	1.033	0.00	24.38	0.00%
	0.318	1.16	10.64	10.90%	0.313	0.91	10.30	8.83%
Light-limited	0.855	0.74	20.27	3.65%	0.668	0.42	15.99	2.63%
	0.316	1.23	8.96	13.73%	0.116	0.52	6.04	8.61%
	1.108	0.59	7.86	7.51%				

P_{DOC} indicates the production rate of DOC.

P_{TOC} suggests the production rate of TOC.

% of P_{TOC} is calculated by P_{DOC}/ P_{TOC}.

5.2.2 Light-limited Incubations

When two algal species were grown under continuous light-limited conditions, it is noteworthy that the limiting factor of light has more negative effect on the growth of *C. kessleri* than that of *I. galbana* because at the relative bright light of $60 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$, *C. kessleri* was limited to grow at a rate of 0.668 d^{-1} (average rate before pump was off), lower than 0.855 d^{-1} , the growth rate of *I. galbana* under same irradiance. Under relative dim light of $20 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$, the growth rate of *C. kessleri* was limited to be as low as 0.116 d^{-1} , comparatively, by contrast, it is 0.316 d^{-1} for *I. galbana* under same condition.

For *I. galbana* grown at a high growth rate under continuous light-limited condition, the algal cell undergone exponential growth because the pump was turned off when the ^{14}C incubation began (Fig. 5.7A). The growth rate slowed down with the incubation time (Fig. 5.6B). Average production rates of POC measured by the ^{14}C method and by the control method (directly measured by CHN and DOC analyzers) were $31.11 \mu\text{M h}^{-1}$ and $30.26 \mu\text{M h}^{-1}$ respectively, and for TOC they were both $31.41 \mu\text{M h}^{-1}$ (Table 5.2). Two-sample t-tests for comparing the average photosynthetic rates estimated by the ^{14}C method with the actual net rates measured by the control method indicate that the overestimation of production rates by the ^{14}C method is not significant in this case, since the P-values are 0.3297 for POC and 0.5654 for TOC respectively. Therefore, the ^{14}C method estimated net production rate accurately for both POC and TOC, which is consistent with our previous inference from batch culture experiment and the nitrate-limited chemostat experiments.

For *C. kessleri* grown under same continuous light irradiances and same incubation process as *I. galbana*, the algal cell and growth rate showed the similar trends as *I. galbana* (Fig. 5.6 C and D). However, the photosynthetic rates estimated by the ^{14}C method were always higher than those measured by the control method (Fig. 5.7B). It can be seen that the

^{14}C method overestimates the photosynthetic rates by 42.86% for POC and 39.11% for TOC respectively (Table 5.2). In addition, the p-values in two sample t-test are close to zero for POC and TOC respectively, indicating that the overestimation by the ^{14}C method is strongly significant.

Corresponding to the slowing down of growth rates for both *I. galbana* and *C. kessleri* along the incubation time observed in Fig 5.6 B and D, production rates estimated by the ^{14}C method also slowing down a little between 18–24 h. For example, in the incubation of *I. galbana*, ^{14}C method overestimated the actual net TOC production rate between 0–18 h, but then underestimated the TOC photosynthetic rate by $(38.24 - 33.53)/38.24 = 12.32\%$ between 18–24 h (Fig. 5.7 A and Table 5.2). It can also be observed that the production rates measured by the ^{14}C method for *C. kessleri* increased along time because of exponential growth. However, there is an obvious declining of ^{14}C estimation between 18–24 h (Fig. 5.7B).

The production rate underestimation between 18–24 h by the ^{14}C method might be associated with the tiny error in our calculation according to Eq. 12 in which the net growth rates was assumed to be related to cell density and was used for the calculations of control method. It is possible that the cell density increased by dividing along time, but the amount of carbon in each cell might decreased, especially when the cell density was very high and light intensity might be shielded by the thicken cell density at the end of the photoperiod (Caperon 1969). Therefore, the ^{14}C method should be more reliable in recording the uptake rate of carbon. Comparatively the values calculated by Eq. 12 should be the upper bound of actual net photosynthetic rate measured by the control method if the growth rate was estimated by Eq. 13 using cell density. The growth rate declining shown in Fig. 5.6 might also be caused by the shielded light irradiance and the lagged algal growth responses (Collos 1986; Duarte 1990). Similar slowing down of production rate in the latter period of incubation was also observed in the 12:12 L:D cycle incubations under nitrate-limited condition (Fig. 6.1 in the

Chapter 6) and the 12:12 L:D cycle incubations under light-limited condition (Fig. 6.2 in the Chapter 6).

The subsequently repeated incubation of *I. galbana* with pump on in the process of ^{14}C incubation (Fig. 5.8) is a supplemental evidence to show that either the pump was on or off in the incubation process, the ^{14}C method can provide an accurate estimation of net production rates at high growth rates. The production rates for this incubation listed in Table 5.3 were relative low compared with those in the previous *I. galbana* incubation where the pump was off. The reason might be the biomass (1.90×10^5 cells mL^{-1}) of this incubation was lower than the previous one whose cell density increased from 6.35×10^5 cells mL^{-1} to be 13.70×10^5 cells mL^{-1} . Linear regression analysis (with p-value of 0.64) indicates that there is no significant relationship between the uptake rates measured by the ^{14}C method and the incubation time (Fig. 5.8 B).

When *I. galbana* and *C. kessleri* were grown with pump on and at low growth rates of 0.316 d^{-1} and 0.116 d^{-1} respectively (Table 5.3), One-sample t-test for *I. galbana* shows that accurate estimations for both net POC and TOC production rates were given by the ^{14}C method ($p=0.1272$ for POC and 0.8237 for TOC). For *C. kessleri*, t-test (with p value = 0.0002 for POC and 0 for TOC) shows that the ^{14}C method significantly overestimated the net production rates by 74.46% for POC and 67.38% for TOC. The overestimated rates by the ^{14}C method at low growth rate and under relatively dim light are much higher than those at high growth rate and under relatively bright light, when light was the limiting factor (Table 5.2 and 5.3).

CHAPTER 6: The 12:12 L:D CYCLE INCUBATIONS

To test the implications of our previous batch culture and continuous studies and the effect of diel periodicity on the interpretation of ^{14}C technique while estimating photosynthetic rate, *I. galbana* and *C. kessleri* were also grown on 12-hour:12-hour light:dark (12:12 L:D) cycle under nitrate- and light-limited conditions, respectively. The compared results of nitrate-limited semi-continuous cultures estimated by the ^{14}C method with those measured by the control method will be shown in this chapter at high/low growth rate for each algal species, and then the results of light-limited cultures follows. Finally, these results will be compared with previous continuous cultures, and their implication to what the ^{14}C method is actually measuring will be discussed.

6.1 Results

When *I. galbana* and *C. kessleri* were grown on 12:12 L:D cycles with nitrate as a limiting factor, the nitrate concentration was reduced to be 45 μM in the growth chamber. When light was a limiting factor, although nutrients with a nitrate concentration of 883 μM were generally saturated for cell growth, relatively dim light (irradiance = 20 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) result in low growth rates and relatively bright light (irradiance = 60 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) resulted in relatively high growth rates.

6.1.1 Nitrate-limited, High Growth Rate

I. galbana was incubated at the nitrate-limited high growth rate of 1.015 d^{-1} on 12:12 L:D cycle (Fig. 6.1A). The growth rate was equal to the dilution rate controlled by peristaltic pump when the chemostat reached the steady state. After adding ^{14}C at the beginning of daytime, samples were collected with a time interval of 3 hours during the light period (the first 12 hours, i.e. the daytime), and soon with a time interval of 6 hours during the dark

period (the latter 12 hours, i.e. the nighttime). Initial DIC concentration in chemostat was 2313 μM at time zero. After 12-hour incubation under continuous light, DIC concentration decreased to be 1920 μM at the end of light period (Table 6.2).

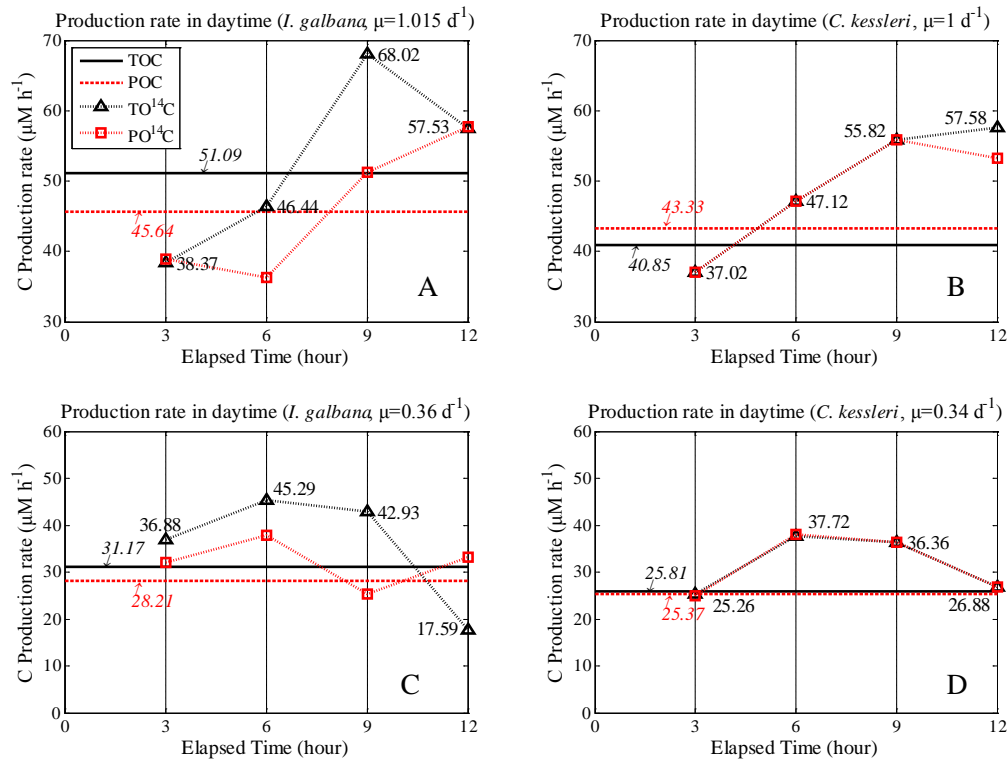


Figure 6.1. Results of incubations at high and low growth rates for *I. galbana* and *C. kessleri* on nitrate-limited 12:12 L:D cycles. Solid black horizontal line and dashed red line are the actual net production rates of TOC and POC estimated by control method respectively (calculated by Eq. 8 and Eq. 9). Dotted black line with triangles and dotted red line with squares are the production rates of TOC and POC estimated by the ^{14}C method. Both triangles and squares indicate the average values calculated for the time interval before that time points (for example 45.64 indicates the mean value between 0–3 h).

POC concentration increased from 305.20 μM at the beginning of daytime to be 613.20 μM at the end of daytime (Table 6.2). The average photosynthetic rate of POC in the light period calculated by Eq. 8 was 45.64 $\mu\text{M h}^{-1}$, and the average respiration rate of POC at night calculated by equation similar as Eq. 11 was 6.79 $\mu\text{M h}^{-1}$ (Table 6.3).

DOC concentration in the medium was 93.33 μM at the beginning of photoperiod, and it increased to be 107.50 μM at the end of photoperiod. Average photosynthetic rate or excretion rate of DOC calculated by the equation similar as Eq. 8 was 5.45 $\mu\text{M h}^{-1}$ during the first 12 hours of daytime. In the nighttime, the average loss rate for DOC calculated by the equation similar as Eq. 11 was $-3.04 \mu\text{M h}^{-1}$. The calculated negative value indicates the DOC production in the medium did not decrease but increase. The reason will be discussed later.

TOC concentration also increased from 398.53 μM at the beginning of photoperiod to be 720.70 μM at the end of photoperiod. So, the average TOC photosynthetic rate calculated by Eq. 9 was 51.09 $\mu\text{M h}^{-1}$ in the light period, and average respiration rate of TOC was 3.75 $\mu\text{M h}^{-1}$ at night which is calculated by Eq. 11 (Table 6.3).

It is noteworthy that since the value of DOC respiration rate is negative, the respiration rate of TOC is lower than that of POC. The reason might be that the only part of POC was respired in the dark, and actual the respiration rate should be $6.79 - 3.04 = 3.75 \mu\text{M h}^{-1}$. Part of POC was excreted to be DOC at a rate of $3.04 \mu\text{M h}^{-1}$ in the dark, which accounted for $3.04/45.64 = 6.67\%$ of POC average photosynthetic rate in the light period. The POC respiration rate of $6.79 \mu\text{M h}^{-1}$ calculated in Table 6.3 should be the total loss rate of POC in night time, which accounted for about $6.79/45.64 = 14.88\%$ of production rate in the daytime.

For comparison, the average uptake rates measured by ^{14}C method were $46.03 \pm 10.15 \mu\text{M h}^{-1}$ for POC, $6.56 \pm 8.43 \mu\text{M h}^{-1}$ for DOC, $52.59 \pm 12.94 \mu\text{M h}^{-1}$ for TOC in the light period (Table 6.1).

T-test is used to compare results measured from ^{14}C method with those measured by control method. The p-values of t-test are 0.9436 for comparing POC uptake rates, 0.8315 for comparing TOC uptake rates, indicating that there are no significant differences between the results measured by ^{14}C method and those measured by control method. As shown in Fig.

6.1A, the average uptake rate for TOC was $38.37 \mu\text{M h}^{-1}$ in the first three hours, and then it increased during 3–6 h, peaked at $68.02 \mu\text{M h}^{-1}$ during 6–9 h, and finally decrease during 9–12 h, indicating the steady state of continuous culture was interrupted by the cycle of light and dark periods.

Since the initial DIC concentration in chemostat was $2313 \mu\text{M}$ at time zero, the specific activity of DIC at this time point is calculated to be $2718 \text{ dpm } (\mu\text{mol})^{-1}$ according to Eq. 5 (Table 6.2).

Similarly, the DIC concentration was $1920 \mu\text{M}$ at the end of photoperiod and the corresponding specific activity at this time point was $1534 \text{ dpm } (\mu\text{mol})^{-1}$. It can be seen that the specific activity of DIC decreased about $(2718 - 1534)/2718 = 43.54\%$ because of photosynthesis assimilation, pump dilution and air bubbling.

In practical operation of ^{14}C technique, the specific activity of respired organic carbon in the dark period is unknown. But in our study it can be estimated by combining Eq. 10–11 and assuming the respiration rates of organic carbon estimated by the ^{14}C method are equal to those measured by the control method.

Since the respiration rates of TOC was $3.75 \mu\text{M h}^{-1}$, the specific activities (SA_{RD} in Eq. 10) of respired TOC were calculated to be $2012 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–18 h hour, $921 \text{ dpm } (\mu\text{mol})^{-1}$ between 18–24 h, and $1398 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–24 h in the dark period. For POC, the loss rate was $6.79 \mu\text{M h}^{-1}$, including the actual respiration rate of $3.75 \mu\text{M h}^{-1}$ and the excretion rate of $3.04 \mu\text{M h}^{-1}$ to be DOC.

Since the value of R_{D} calculated by equation similar as Eq. 10 was $6.79 \mu\text{M h}^{-1}$ for POC, the calculated SA_{RD} would be $1426 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–24 h, $979 \text{ dpm } (\mu\text{mol})^{-1}$ between 18–24 h and $1174 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–24 h in the whole dark period, if R_{D} is assumed to be equal to $6.79 \mu\text{M h}^{-1}$ in Eq. 10.

Table 6.1. POC and TOC production rates measured by the ^{14}C method and control method in the daytime on nitrate-limited 12:12 L:D cycles.

Time (h)	<i>I. galbana</i> ($\mu=1.015\text{ d}^{-1}$)		<i>C. kessleri</i> ($\mu=1.0\text{ d}^{-1}$)		<i>I. galbana</i> ($\mu=0.36\text{ d}^{-1}$)		<i>C. kessleri</i> ($\mu=0.34\text{ d}^{-1}$)	
	P _{POC}	P _{TOC}	P _{POC}	P _{TOC}	P _{POC}	P _{TOC}	P _{POC}	P _{TOC}
0–3	38.93	38.37	37.02	37.02	32.15	36.88	24.94	25.26
3–6	36.24	46.44	47.12	47.12	37.77	45.29	38.05	37.72
6–9	51.26	68.02	55.82	55.82	25.27	42.93	36.36	36.36
9–12	57.68	57.53	53.24	57.58	33.18	17.59	26.88	26.88
Average (^{14}C)	46.03 \pm 10.15	52.59 \pm 12.94	48.30 \pm 8.36	49.38 \pm 9.43	32.09 \pm 5.16	35.68 \pm 12.56	31.56 \pm 6.60	31.56 \pm 6.4
Control	45.64	51.09	43.33	40.86	28.21	31.17	25.37	25.81
Deviation	0.85%	2.94%	11.46%	20.87%	13.78%	14.44%	24.41%	22.28%
P-value (t-test)	0.9436	0.8315	0.3203	0.1682	0.2293	0.5254	0.1574	0.1699

The unit of production rate is $\mu\text{M h}^{-1}$.

μ = growth rate.

Average (^{14}C) line shows the average production rates measured by the ^{14}C method.

Control line indicates the average production rates measured by the control method.

Deviation is calculated by (Average (^{14}C)–Control)/Control.

C. kessleri was also grown at a nitrate-limited high growth rate of 1.00 d^{-1} under a 12:12 L:D cycle (Fig. 6.1B) and samples were collected similarly as *I. galbana*. DIC concentrations were $2150 \text{ } \mu\text{M}$ at the beginning of daytime, and $1700 \text{ } \mu\text{M}$ at the end of daytime (Table 6.2).

The POC concentrations were $283.00 \text{ } \mu\text{M}$ at the beginning of daytime, $580.83 \text{ } \mu\text{M}$ at the end of daytime, so photosynthetic rate calculated by the Eq. 8 was $43.33 \text{ } \mu\text{M h}^{-1}$ for the daytime. Respiration rate calculated by equation similar as Eq. 11 was $7.34 \text{ } \mu\text{M h}^{-1}$ for the nighttime (Table 6.3). Similarly, TOC concentrations increased from $162.17 \text{ } \mu\text{M h}^{-1}$ at the beginning of daytime to be $484.16 \text{ } \mu\text{M h}^{-1}$ at the end of daytime. So, the average photosynthetic rate calculated by Eq. 9 was $40.86 \text{ } \mu\text{M h}^{-1}$ in the daytime and the respiration rate calculated by Eq. 11 was $13.92 \text{ } \mu\text{M h}^{-1}$ in the nighttime.

For comparison, the average photosynthetic rates estimated by the ^{14}C method were $48.30 \pm 8.36 \text{ } \mu\text{M h}^{-1}$ for POC, $1.08 \pm 2.17 \text{ } \mu\text{M h}^{-1}$ for DOC, and $49.38 \pm 9.43 \text{ } \mu\text{M h}^{-1}$ for TOC during the daytime (Table 6.1). T-test results for POC and TOC photosynthetic rates (with a p-value of 0.3203 for POC and 0.1682 for TOC) indicate that there is no significant difference between ^{14}C method and control method in measuring the photosynthetic rates in the daytime. However, the average uptake rate of TOC estimated by the ^{14}C method ($49.38 \pm 9.43 \text{ } \mu\text{M h}^{-1}$) was $(49.38 - 40.86)/40.86 = 20.85\%$ higher than the rate of $40.86 \text{ } \mu\text{M h}^{-1}$ measured by control method.

Marra (2009) once pointed out that the ^{14}C uptake over daytime (dawn–dusk) periods estimates net primary production. Our results show that the difference between the ^{14}C method and net primary production in 12:12 L:D cycle is not as larger as that in the 24-hour continuous incubation for *C. kessleri* where the deviations between ^{14}C estimation and net TOC production rates are 41.06% at high growth rate and 48.54% at low growth rate (Table 5.1).

According to Eq. 5, the specific activity of DIC decreased from 2240 dpm (μmol)⁻¹ at the beginning of daytime to be 1391 dpm (μmol)⁻¹ at the end of daytime. By combining Eq. 10–11, the average specific activities of TOC were 868 dpm (μmol)⁻¹ between 12–18 h, 793 dpm (μmol)⁻¹ between 18–24 h, and 826 dpm (μmol)⁻¹ between 12–24 h in the dark period (Table 6.2).

For POC, they were 1639 dpm (μmol)⁻¹ between 12–18 h, 1562 dpm (μmol)⁻¹ between 18–24 h, and 1596 dpm (μmol)⁻¹ between 12–24 h. Both SA_{RD} of POC and TOC declined in the dark period, but they were a little higher than 1391 dpm (μmol)⁻¹, the SA of DIC at the time point of 12 h.

6.1.2 Nitrate-limited, Low Growth Rate

Both *I. galbana* and *C. kessleri* were grown at relatively low growth rates (0.360 d⁻¹ and 0.340 d⁻¹, respectively) on 12:12 L:D cycle as well. The ¹⁴C incubation procedures were similar as those at high growth rates. Samples were collected with a time interval of 3 hours during the daytime, and soon with a time interval of 6 hours during the nighttime. The calculated results are summarized in Fig. 6.1 and Table 6.1–6.3.

For *I. galbana*, the initial DIC concentrations in chemostat were 2410 μM at time zero and 2200 μM at the end of light period. POC concentration increased from 595.20 μM at the beginning of daytime to be 806.90 μM at the end of daytime (Table 6.2). The average photosynthetic rate of POC in the light period calculated by Eq. 8 is 28.21 $\mu\text{M h}^{-1}$, and its average respiration rate in the nighttime calculated by equation similar as Eq. 11 is 7.17 $\mu\text{M h}^{-1}$ (Table 6.3).

DOC concentration in the medium was 15.83 μM at the beginning of photoperiod, and it increased to be 45.83 μM at the end of photoperiod. Average photosynthetic rate of DOC calculated by the equation similar as Eq. 8 is 2.97 $\mu\text{M h}^{-1}$ during the light period, and the

average loss rate for DOC calculated by the equation similar as Eq. 11 is $2.04 \mu\text{M h}^{-1}$ in the nighttime. TOC at the beginning of photoperiod was $611.03 \mu\text{M}$, and it increased to be $852.73 \mu\text{M}$ at the end of photoperiod. So, the average photosynthetic rate of TOC calculated by Eq. 9 is $31.17 \mu\text{M h}^{-1}$ in the light period, and its average respiration rate calculated by Eq. 11 is $9.22 \mu\text{M h}^{-1}$ at night.

For comparison, the average uptakes rates of *I. galbana* measured by ^{14}C method were $32.09 \pm 5.16 \mu\text{M h}^{-1}$ for POC, $3.58 \pm 13.93 \mu\text{M h}^{-1}$ for DOC, $35.68 \pm 12.56 \mu\text{M h}^{-1}$ for TOC in the light period.

The p-values of t-test are 0.2293 and 0.5254 for comparisons of POC and TOC uptake rates respectively, indicating that there are no significant differences between the results measured by the two methods. More results are shown in Table 6.1.

According to Eq. 5, the specific activities of DIC are calculated to be $1541 \text{ dpm } (\mu\text{mol})^{-1}$ at time zero, $1249 \text{ dpm } (\mu\text{mol})^{-1}$ at the end of photoperiod and $983 \text{ dpm } (\mu\text{mol})^{-1}$ at the end of night time.

So the specific activity of DIC decreased about $(1541 - 1249)/1541 = 18.95\%$ in the light period because of photosynthesis assimilation, pumping dilution and air bubbling. In the dark period, it decreased by $(1249 - 983)/1541 = 17.26\%$ from the original specific activity.

By combining Eq. 10–11, the average specific activities of TOC are calculated to be $1261 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–18 h, $808 \text{ dpm } (\mu\text{mol})^{-1}$ between 18–24 h, and $1025 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–24 h in the dark period (Table 6.2). For POC, they were $1224 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–18 h, $731 \text{ dpm } (\mu\text{mol})^{-1}$ between 18–24 h, and $966 \text{ dpm } (\mu\text{mol})^{-1}$ from between 12–24 h in the dark period.

For *C. kessleri*, DIC concentrations at the time points of 0 h, 12 h, and 24 h were 2553, 2307 and $2553 \mu\text{M}$ (Table 6.2). The corresponding POC concentrations were $531.10 \mu\text{M}$ at

Table 6.2. Concentration and specific activity of DIC, respired POC and TOC under nitrate-limited conditions.

Time (h)	<i>I. galbana</i> ($\mu=1.015\text{ d}^{-1}$)			<i>C. kessleri</i> ($\mu=1\text{ d}^{-1}$)			<i>I. galbana</i> ($\mu=0.36\text{ d}^{-1}$)			<i>C. kessleri</i> ($\mu=0.34\text{ d}^{-1}$)		
	0	12	24	0	12	24	0	12	24	0	12	24
DIC (μM)	2313	1920	2313	2150	1700	2150	2410	2200	2410	2553	2307	2553
DIC SA	2718	1534	781	2240	1391	655	1541	1249	983	1790	1602	1193
POC (μM)	305.20	613.20	305.20	283.00	580.83	283.00	595.20	806.90	595.20	531.10	728.00	531.10
SA (12–18 h)		1426			1639			1224			651	
SA (18–24 h)			979			1562			731			1230
SA (12–24 h)		1174			1596			966			953	
TOC (μM)	398.53	720.70		162.17	484.16		611.03	852.73	611.03	546.10	745.50	546.10
SA (12–18 h)		2012			868			1261			653	
SA (18–24 h)			921			793			808			1234
SA (12–24 h)		1398			826			1025			956	

μ = growth rate.

SA = specific activity, the unit is dpm (μmol)⁻¹.

Table 6.3. Summary for photosynthetic rates and respiration rates for incubation on nitrate-limited 12:12 L:D cycles.

		<i>I. galbana</i> ($\mu=1.015 \text{ d}^{-1}$)		<i>C. kessleri</i> ($\mu=1.0 \text{ d}^{-1}$)		<i>I. galbana</i> ($\mu=0.36 \text{ d}^{-1}$)		<i>C. kessleri</i> ($\mu=0.34 \text{ d}^{-1}$)	
		^{14}C	Control	^{14}C	Control	^{14}C	Control	^{14}C	Control
POC	P (0–12 h)	46.03±10.15	45.64	48.30±8.36	43.33	32.09± 5.16	28.21	31.56±6.60	25.37
	R (12–24 h)	5.20	6.79	8.42	7.34	5.55	7.17	4.48	7.53
	R/P	11.30%	14.88%	17.43%	16.93%	17.29%	25.43%	14.19%	29.68%
	Total rate	20.41±5.08	19.42	19.94±4.18	18.00	13.27±2.58	10.52	13.54±3.30	8.92
	Deviation	5.10%		10.78%		26.14%		51.79%	
TOC	P (0–12 h)	52.59±12.94	51.09	49.38±9.43	40.86	35.68±12.56	31.17	31.56± 6.39	25.81
	R (12–24 h)	6.19	3.75	8.27	13.92	7.56	9.22	4.48	7.51
	R/P	11.77%	7.35%	16.75%	34.08%	21.18%	29.57%	14.19%	29.09%
	Total rate	23.20 ± 6.47	23.67	20.56 ± 4.71	13.47	14.06 ± 6.28	10.98	13.54 ± 3.20	9.15
	Deviation	-1.97%		52.64%		28.05%		47.98%	

P = photosynthetic rate in the light period.

R = Respiration rate in the dark period. The unit of production or respiration rate is $\mu\text{M h}^{-1}$.

R/P = the ratio of respiration rate to photosynthesis rate.

Control column indicates the average production rates measured by the control method.

Total rate is calculated by subtracting respiration rate from photosynthetic rate and then divided by 2.

Deviation is calculated by: (total rates measured by ^{14}C – total rates measured by control method)/ total rates measured by control method.

the beginning of daytime, and 728.00 μM at the end of daytime, so photosynthetic rate calculated by the Eq. 8 is 25.37 $\mu\text{M h}^{-1}$ in the daytime. Respiration rate calculated by equation similar as Eq. 11 is 7.53 $\mu\text{M h}^{-1}$ in the nighttime (Table 6.3).

Similarly, TOC concentrations increased from 546.10 $\mu\text{M h}^{-1}$ at the beginning of daytime to be 745.50 $\mu\text{M h}^{-1}$ at the end of daytime. So, the average photosynthetic rate calculated by Eq. 9 is 25.81 $\mu\text{M h}^{-1}$ in the daytime and the respiration rate calculated by Eq. 11 is 7.51 $\mu\text{M h}^{-1}$ in the nighttime (Table 6.3). DOC concentrations, average photosynthetic rates and respiration rate can be calculated as the difference of those of TOC and DOC.

For comparison, the average photosynthetic rates measured by the ^{14}C method are $31.56 \pm 6.60 \mu\text{M h}^{-1}$ for POC, and $31.56 \pm 6.39 \mu\text{M h}^{-1}$ for TOC during the daytime (Table 6.1). T-test results for POC and TOC photosynthetic rates (with a p-value of 0.1574 for POC and 0.1699 for TOC) indicate that the difference of measurements between ^{14}C method and control method is not significant in the daytime. However, the average uptake rate of TOC measured by the ^{14}C method is $(31.56 - 25.81)/25.81 = 22.28\%$ higher than the rate measured by control method.

According to Eq. 5, the special activity of DIC decreased by 10.50% from 1790 dpm $(\mu\text{mol})^{-1}$ at the beginning of daytime to be 1602 dpm $(\mu\text{mol})^{-1}$ at the end of daytime and then decreased by 22.85% to be 1193 at the end of nighttime (Table 6.2) because of photosynthesis assimilation, pumping dilution and air bubbling.

By combining Eq. 10–11, the special activities of TOC are calculated to be 653 dpm $(\mu\text{M})^{-1}$ from between 12–18 h, 1234 dpm $(\mu\text{M})^{-1}$ between 18–24 h, and 956 dpm $(\mu\text{M})^{-1}$ between 12–24 h in the dark period (Table 6.2). For POC, they were 651 dpm $(\mu\text{M})^{-1}$ between 12–18 h, 1230 dpm $(\mu\text{M})^{-1}$ between 18–24 h, and 953 dpm $(\mu\text{M})^{-1}$ between 12–24 h.

6.1.3 Light-limited, High Growth Rate

I. galbana was incubated at high growth rate under a 12:12 L:D cycle (Fig. 6.2A) and the light irradiance was same as incubations at the high-growth rates under continuous light ($60 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$). The dilution rate controlled by peristaltic pump was 1.038 d^{-1} , which was also the growth rate that the bright light was able to support. Several 12:12 L:D cycles were run before adding ^{14}C into growth chamber and the samples of POC and DOC were collected at a time interval of 12 hours. Pump was turned off when ^{14}C was added into the growth chamber and then samples were collected at a time interval of 3 hours during the light period (daytime), and then at a time interval of 6 hours during the dark period (nighttime).

The initial DIC concentration in chemostat was $2440 \mu\text{M}$ at time zero when the light was on and, meantime, ^{14}C was added into the growth chamber. DIC concentration decreased to be $2310 \mu\text{M}$ at the end of light period, and then it increased to be $2680 \mu\text{M}$ at the end of nighttime (Table 6.6).

TOC concentration increased from $429.00 \mu\text{M}$ in the beginning to be $661.70 \mu\text{M}$ at the end of daytime as a result of photosynthesis, and then decreased to be $613.17 \mu\text{M}$ at the end of nighttime because of respiration. The initial photosynthetic rates at time zero calculated by Eq. 12 were $14.00 \mu\text{M h}^{-1}$ for POC and $14.79 \mu\text{M h}^{-1}$ for TOC. In the daytime, since algal cells undergone the exponential growth, the average production rates of POC and TOC during each time interval can be calculated by Eq. 12 and they are summarized in Table 6.4. The average respiration rate of TOC at night calculated by equation similar as Eq. 11 (in which $\mu=0$) was $4.04 \mu\text{M h}^{-1}$. The average production rates in the light period were $18.35 \mu\text{M h}^{-1}$ for POC and $19.39 \mu\text{M h}^{-1}$ for TOC.

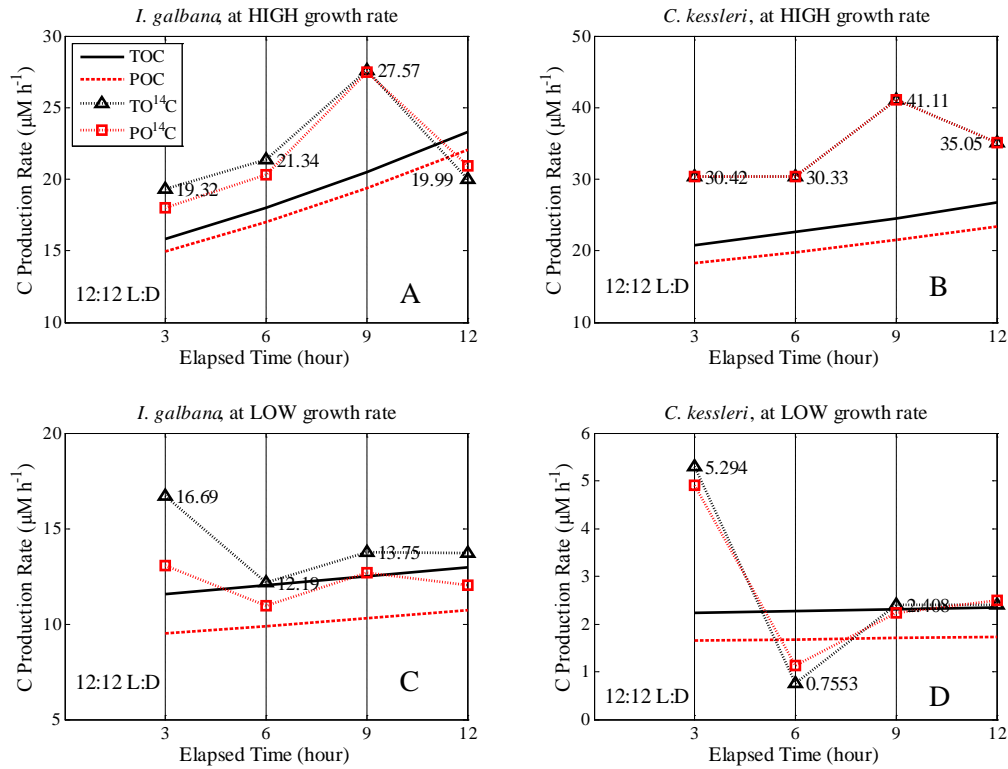


Figure 6.2. Results of incubations at high and low growth rates for *I. galbana* and *C. kessleri* on light-limited 12:12 L:D cycles. Solid black line and dashed red line are the actual net production rates of TOC and POC estimated by control method respectively (calculated by Eq. 8 and Eq. 9). Dotted black line with triangles and dotted red line with squares are the production rates of TOC and POC estimated by the ¹⁴C method. Both triangles and squares indicate the average values calculated for the time interval before that time points (for example 19.32 indicates the mean value between 0–3 h).

For DOC, the excretion rates from algal cells into the medium in the daytime and its loss rate from the medium in the nighttime can be calculated by the similar procedure as TOC. The net production rate of DOC at the beginning of daytime was about $0.79 \mu\text{M h}^{-1}$, and its average value in the daytime was about $1.04 \mu\text{M h}^{-1}$, which is only accounted for $1.04/19.39 = 5.36\%$ of TOC production rate (Table 6.7).

Comparatively, the average production rates estimated by ¹⁴C method in the light period were $21.68 \mu\text{M h}^{-1}$ for POC and $22.66 \mu\text{M h}^{-1}$ for TOC in the light period. The rates estimated for each time interval are shown in Table 6.4 and Fig. 6.2A. T-test is used to compare results estimated by the ¹⁴C method with those measured by the control method and the results with

p-values of 0.1748 for POC and 0.3079 for TOC indicate that there is no significant difference between the results estimated by ^{14}C method and those measured by control method in the light period.

The initial specific activity of DIC at time zero is calculated to be $2294 \text{ dpm } (\mu\text{mol})^{-1}$ according to Eq. 5. DIC concentration at the end of photoperiod was $2310 \mu\text{M}$ and the specific activity of DIC at this time point was $2121 \text{ dpm } (\mu\text{mol})^{-1}$. DIC concentration at the end of dark period was $2680 \mu\text{M}$ and the specific activity of DIC at this time point was $1751 \text{ dpm } (\mu\text{mol})^{-1}$ (Table 6.6). It can be seen that the specific activity of DIC were decreasing along incubation time by 7.54% in the light period and by 16.13% in the dark period.

In the practical operation of ^{14}C technique, the specific activity of respired organic carbon in the dark period is unknown for 12:12 L:D incubation. But we can calculate it by combining equations similar as Eq. 10–11 ($\mu=0$) in which both SA_{RD} of TOC and POC during the dark period can be calculated. In this case, the average specific activities of POC were $1382 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–18 h, $933 \text{ dpm } (\mu\text{mol})^{-1}$ between 18–24 h, and $1158 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–24 h in the dark period. The average specific activities of TOC were $1373 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–18 h, $588 \text{ dpm } (\mu\text{mol})^{-1}$ between 18–24 h, and $981 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–24 h in the dark period.

C. kessleri was also grown under a 12:12 L:D cycle with same irradiance as *I. galbana*. The light-limited high growth rate of *C. kessleri* was 0.668 d^{-1} (Fig. 6.2B), lower than that of *I. galbana* although the irradiance were exactly same. Pump was off while ^{14}C was added into the growth chamber and samples were collected with a time interval of 3 hours during the light period, and soon with a time interval of 6 hours during the dark period. The DIC concentrations at 0, 12 and 24 h were 1920, 1820 and $2090 \mu\text{M}$, which are also listed in Table 6.6.

Table 6.4. Summary for photosynthetic rates and respiration rates for incubation on light-limited 12:12 L:D cycle at high growth rate.

Time (h)	<i>I. galbana</i> ($v=1.038 \text{ d}^{-1}$)				<i>C. kessleri</i> ($v=0.668 \text{ d}^{-1}$)			
	POC		TOC		POC		TOC	
	^{14}C	Control	^{14}C	Control	^{14}C	Control	^{14}C	Control
0		14.00		14.79		17.48		19.93
0–3	18.00	14.95	19.32	15.80	30.42	18.23	30.42	20.79
3–6	20.30	17.02	21.34	17.98	30.33	19.82	30.33	22.60
6–9	27.46	19.38	27.57	20.48	41.11	21.54	41.11	24.57
9–12	20.95	22.06	19.99	23.31	35.05	23.42	35.05	26.71
P average (0–12)	21.68	18.35	22.06	19.39	34.23	20.75	34.23	23.67
Deviation	18.15%		13.77%		64.96%		44.61%	
T-test	0.1748		0.3079		0.0072		0.0138	
R (12–24)	1.49	2.73	1.87	4.04	1.35	3.00	1.35	4.53
R/P	6.87%	14.88%	8.48%	20.84%	3.94%	14.46%	3.94%	19.14%
Total (0–24)	10.1	7.81	10.10	7.67	16.44	8.88	16.44	9.57
Deviation	29.32%		31.68%		85.14%		71.79%	

v = average growth rate before ^{14}C incubation.

Both incubations were at the high growth rates under same irradiances.

P = photosynthetic rate in the light period (0–12 h).

R = Respiration rate in the dark period (12–24 h).

R/P = the ratio of average respiration rate to average photosynthesis rate, calculated for two method respectively.

^{14}C column indicates the production rate in each time interval measured by the ^{14}C method.

Control column indicates the production rates in each interval measured by the control method.

Total rate is calculated by subtracting respiration rate from photosynthetic rate and then divided by 2.

Deviation is calculated by: (total rates measured by ^{14}C – total rates measured by control method)/ total rates measured by control method.

TOC concentration increased from 652.00 μM at the beginning of daytime to be 936.00 μM at the end of daytime, and then decreased to be 881.67 μM at the end of nighttime. Initial photosynthetic rate of TOC was 19.93 $\mu\text{M h}^{-1}$ at the beginning of the light period. In the daytime, algal cells undergone the exponential growth and the production rates of TOC in each time interval were calculated by Eq. 12 and listed in Table 6.4. The average respiration rate of TOC at night calculated by equation similar as Eq. 11 (in which $\mu=0$) was 4.53 $\mu\text{M h}^{-1}$. The average production rates were 23.67 $\mu\text{M h}^{-1}$ for TOC and 20.75 $\mu\text{M h}^{-1}$ for POC in the daytime.

For DOC, the net excretion rate was about 2.45 $\mu\text{M h}^{-1}$ at the beginning of daytime, and the average excretion rate in the daytime was 2.92 $\mu\text{M h}^{-1}$ which is accounted for $2.92/23.67 = 12.34\%$ of TOC production rate and a little higher than that of *I. galbana* (5.36%, summarized in Table 6.7).

Comparatively, the production rates measured by the ^{14}C method in the light period are calculated by Eq. 14, and the results are summarized in Table 6.4 for comparison. The average production rates were both 34.23 $\mu\text{M h}^{-1}$ for POC and TOC in the light period. T-tests for comparing the production rates estimated by the ^{14}C method and by the control method gave a p-value of 0.0072 for comparing POC production rates, and a p-value of 0.0138 for comparing TOC production rates, indicating that there are significant differences between the results estimated by the ^{14}C method and those measured by control method in the light period.

According to the DIC concentration and radioactive measurement at 0 h, 12 h and 24 h, specific activities at these time points could be calculated by Eq. 5 and they were 2500, 2151 and 1889 dpm (μmol) $^{-1}$ respectively (Table 6.6). It can be seen that the specific activity of DIC decreased along incubation time by about $(2500 - 2151)/2500 = 13.96\%$ in the light period and by $(2151 - 1889)/2500 = 10.48\%$ in the dark period.

Based on the respiration rates of POC and TOC ($3.00 \mu\text{M h}^{-1}$ and $4.53 \mu\text{M h}^{-1}$ respectively), the specific activity (SA_{RD}) of respired POC and TOC during the dark period can be estimated by combining equations similar as Eq. 10–11 ($\mu=0$ in this case). The average specific activities of POC were $968 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–18 h, $964 \text{ dpm } (\mu\text{mol})^{-1}$ between 18–24 h, and $966 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–24 h in the dark period. The average specific activities of TOC were $642 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–18 h, $639 \text{ dpm } (\mu\text{mol})^{-1}$ between 18–24 h, and $640 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–24 h in the dark period.

6.1.4 Light-limited, Low Growth Rate

I. galbana and *C. kessleri* were also grown under relatively dim light and 12:12 L:D cycles. The irradiance was about $20 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ at the center of the empty growth chamber which was exactly same as 24-hour continuous cultures. The growth rates limited by dim light were 0.311 d^{-1} for *I. galbana* (Fig. 6.2C) and 0.128 d^{-1} for *C. kessleri* (Fig. 6.2D). It can be seen that *C. kessleri* was limited more seriously by dim light than *I. galbana*, the same phenomenon as we have observed in previous studies.

For *I. galbana*, DIC concentrations at 0 h, 12 h and 24 h were 2500, 2420 and 2550 μM (Table 6.6). TOC concentration increased from 698.57 μM at the beginning of daytime to be 845.70 μM at the end of daytime, and then decreased to be 788.33 μM at the end of nighttime. Initial photosynthetic rate of TOC at the beginning of the light period was $11.33 \mu\text{M h}^{-1}$. In the daytime, algal cells undergone the exponential growth, and the average production rates in each time interval of TOC are listed in Table 6.5. The average respiration rate of TOC at night was $4.78 \mu\text{M h}^{-1}$, which can be calculated by equation similar as Eq. 11 (in which $\mu=0$).

For POC and DOC, the production rates in the daytime and the respiration rate in the nighttime can be calculated by the similar calculations as TOC. All results are summarized in Table 6.5. Average production rates in all daytime were $12.26 \mu\text{M h}^{-1}$ for TOC and $10.11 \mu\text{M h}^{-1}$ for POC.

h^{-1} for POC. The net excretion rate of DOC at the beginning of daytime was about $1.99 \mu\text{M h}^{-1}$, and the average excretion rate in the daytime was about $2.15 \mu\text{M h}^{-1}$, which accounts for $2.15/12.26 = 17.54\%$ of TOC production rate, higher than DOC proportion of *I. galbana* (5.36%) at high grow rate under 12:12 L:D cycle (summarized in Table 6.7, P_{DOC} indicates the production rate of DOC; P_{TOC} suggests the production rate of TOC; % of PTOC is calculated by $P_{\text{DOC}}/P_{\text{TOC}}$).

Comparatively, the production rates estimated by the ^{14}C method in the light period are calculated by Eq. 14, and the results are listed in Table 6.5. The average production rates were $12.18 \mu\text{M h}^{-1}$ for POC and $14.09 \mu\text{M h}^{-1}$ for TOC in the light period. T-tests with p-values of 0.0339 for POC and 0.2022 for TOC indicate that there are no significant differences between the results estimated by the ^{14}C method and those measured by the control method in the light period.

According to the DIC concentration and radioactivity at the time points of 0 h, 12 h and 24 h, specific activities at these time points were 2000, 1937 and 1848 dpm $(\mu\text{mol})^{-1}$ respectively calculated by Eq. 5 (Table 6.6). It can be seen that the specific activity of DIC were decreasing along incubation time by 3.15% in the light period and by 4.45% in the dark period.

By combining equations similar as Eq. 10–11 ($\mu=0$ in this case) and the respiration rates of POC and TOC ($2.35 \mu\text{M h}^{-1}$ and $4.78 \mu\text{M h}^{-1}$ respectively), specific activity (SA_{RD}) of the respired POC and TOC during the dark period can be calculated and they are shown in Table 6.6.

The average specific activities of POC were 372 dpm $(\mu\text{mol})^{-1}$ between 12–18 h, 720 dpm $(\mu\text{mol})^{-1}$ between 18–24 h, and 546 dpm $(\mu\text{mol})^{-1}$ between 12–24 h in the dark period. The average specific activities of TOC were 559 dpm $(\mu\text{mol})^{-1}$ between 12–18 h, 295 dpm $(\mu\text{mol})^{-1}$ between 18–24 h, and 427 dpm $(\mu\text{mol})^{-1}$ between 12–24 h in the dark period.

Table 6.5. Summary for photosynthetic rates and respiration rates of incubation on light-limited 12:12 L:D cycle at low growth rate.

Time (h)	<i>I. galbana</i> ($v=0.311 \text{ d}^{-1}$)				<i>C. kessleri</i> ($v=0.1275 \text{ d}^{-1}$)			
	POC		TOC		POC		TOC	
	^{14}C	Control	^{14}C	Control	^{14}C	Control	^{14}C	Control
0		9.34		11.33		1.64		2.21
0–3	13.04	9.53	16.69	11.56	4.90	1.65	5.29	2.23
3–6	10.98	9.90	12.19	12.01	1.14	1.68	0.76	2.27
6–9	12.69	10.30	13.75	12.49	2.23	1.71	2.41	2.30
9–12	12.03	10.71	13.73	12.99	2.49	1.73	2.40	2.34
P average (0–12)	12.18	10.11	14.09	12.26	2.69	1.69	2.71	2.28
Deviation		20.47%		14.93%		59.17%		18.86%
T-test		0.0339		0.2022		0.3028		0.6839
R (12–24)	0.66	2.35	1.05	4.78	0.06	1.00	0.06	1.24
R/P	5.42%	23.24%	7.45%	38.99%	2.23%	59.17%	2.21%	54.39%
Total (0–24)	5.76	3.88	6.52	3.74	1.32	0.35	1.33	0.52
Deviation		48.48%		74.32%		280.32%		155.43%

v = average growth rate before ^{14}C incubation.

Both incubations were at the high growth rates under same irradiances.

P = photosynthetic rate in the light period (0–12 h).

R = Respiration rate in the dark period (12–24 h).

R/P = the ratio of average respiration rate to average photosynthesis rate, calculated for two method respectively.

^{14}C column indicates the production rate in each time interval measured by the ^{14}C method.

Control column indicates the production rates in each interval measured by the control method.

Total rate is calculated by subtracting respiration rate from photosynthetic rate and then divided by 2.

Deviation is calculated by: (total rates measured by ^{14}C – total rates measured by control method)/ total rates measured by control method.

For *C. kessleri* grown under same dim light irradiance as *I. galbana*, the DIC concentrations at 0 h, 12 h and 24 h were 2110, 2100 and 2150 μM (Table 6.6). It can be seen that the variation of DIC concentration at low growth rate was relative small compared with the variation of incubations at high growth rates, so as *I. galbana*.

TOC concentration increased from 402.75 μM at the beginning of daytime to be 430.16 μM at the end of daytime, and then decreased to be 415.25 μM at the end of nighttime. Initial photosynthetic rate of TOC at the beginning of the light period was 2.21 $\mu\text{M h}^{-1}$. In the daytime, algal cells underwent the exponential growth. The average production rates in each time interval of TOC are listed in Table 6.5. Average respiration rate of TOC at night was 1.24 $\mu\text{M h}^{-1}$ which is calculated by equation similar as Eq. 11 (where $\mu=0$).

For POC and DOC, the production rates in the daytime and the respiration rate in the nighttime can be calculated by the similar calculations as TOC and they are listed in Table 6.5. It can be seen that the average production rates in the daytime were 2.28 $\mu\text{M h}^{-1}$ for TOC and 1.69 $\mu\text{M h}^{-1}$ for POC. The net production rate of DOC at the beginning of daytime was 0.57 $\mu\text{M h}^{-1}$, and the average production rate in the daytime was 0.59 $\mu\text{M h}^{-1}$, which accounts for $0.59/2.28 = 25.88\%$ of TOC production rate (Table 6.7).

Comparatively, the production rates estimated by the ^{14}C method in the light period are calculated by Eq. 14 and listed in Table 6.5. The average production rates were 2.69 $\mu\text{M h}^{-1}$ for POC and 2.71 $\mu\text{M h}^{-1}$ for TOC in the light period. T-test result (with $p=0.3028$ for POC and 0.6839 for TOC) suggests that there are no significant differences between the results estimated by the ^{14}C method and those measured by control method in the light period.

According to the DIC concentration and radioactivity at the time points of 0 h, 12 h and 24 h, specific activity calculated by Eq. 5 were 2175, 2063 and 1789 dpm (μmol) $^{-1}$ correspondingly (Table 6.6). It can be seen that the specific activity of DIC decreased along incubation time by 5.15% in the light period and by 12.60% in the dark period.

By combining equations similar as Eq. 10–11 ($\mu=0$ in this case) and the respiration rates of POC and TOC ($1.00 \mu\text{M h}^{-1}$ and $1.24 \mu\text{M h}^{-1}$ respectively), the specific activities (SA_{RD}) of the respired POC and TOC during the dark period are calculated and shown in Table 6.6. The average specific activities of POC were $210 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–18 h, $35 \text{ dpm } (\mu\text{mol})^{-1}$ between 18–24 h, and $123 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–24 h in the dark period. The average specific activities of TOC were $113 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–18 h, $84 \text{ dpm } (\mu\text{mol})^{-1}$ between 18–24 h, and $99 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–24 h in the dark period.

6.2 Discussion

6.2.1 Nitrate-limited Condition

Comparison of Production Rates in the Light Period

DIC concentrations at the time points of 0 h, 12 h and 24 h under 12:12 L:D cycles are listed in Table 6.2. The total CO_2 concentration researched a maximum level at the beginning of the photoperiod and decreased to be a minimum level at the end of the photoperiod. The same phenomenon was also observed by Ditullio and Laws (1986) in the similar diel periodicity incubation of five species of marine phytoplankton. The reason might be that CO_2 was assimilated by photosynthesis in the light period and the CO_2 was released by respiration at night.

It is observed that in all 12:12 L:D incubations, photosynthetic rates of TOC and POC peaked between 3–6 h or 6–9 h as show in Fig. 6. For example, when *I. galbana* was incubated at a high growth rate of 1.015 d^{-1} (Fig. 6.1A), the average uptake rate for TOC increased from $38.37 \mu\text{M h}^{-1}$ in 0–3 h, peaked at $68.02 \mu\text{M h}^{-1}$ during 6–9 h, and then decreased to be $57.53 \mu\text{M h}^{-1}$ in 9–12 h (Table 6.1).

Table 6.6. Concentration and specific activity of DIC, respired POC and TOC under light-limited conditions.

Time (h)	<i>I. galbana</i> ($\mu=1.038\text{ d}^{-1}$)			<i>C. kessleri</i> ($\mu=0.668\text{ d}^{-1}$)			<i>I. galbana</i> ($\mu=0.311\text{ d}^{-1}$)			<i>C. kessleri</i> ($\mu=0.128\text{ d}^{-1}$)		
	0	12	24	0	12	24	0	12	24	0	12	24
DIC (μM)	2440	2310	2680	1920	1820	2090	2500	2420	2550	2110	2100	2150
DIC SA	2294	2121	1751	2500	2151	1889	2000	1937	1848	2175	2063	1789
POC (μM)	426.50	646.70	614.00	652.00	901.00	865.00	671.90	793.20	765.00	379.00	399.33	387.33
SA (12–18 h)		1383			968			372			210	
SA (18–24 h)			933			964			720			35
SA (12–24 h)		1158			966			546			123	
TOC (μM)	429.00	661.70	613.17	652.00	936.00	881.67	698.57	845.70	788.33	402.75	430.16	415.25
SA (12–18 h)		1373			642			559			113	
SA (18–24 h)			588			639			295			84
SA (12–24 h)		981			640			427			99	

μ = growth rate.

SA = specific activity, unit: dpm (μmol)⁻¹.

Table 6.7. DOC excretion or uptake in each incubation on 12:12 L:D cycle.

Limited by	Algae	Growth rate (d ⁻¹)	Daytime			Nighttime		
			P _{DOC}	P _{TOC}	% of P _{TOC}	R _{DOC}	R _{TOC}	% of R _{TOC}
Nitrate	<i>I. galbana</i>	1.015	5.45	51.09	10.67%	-3.04	3.75	-81.07%
		0.36	2.97	31.17	9.52%	2.04	9.22	22.13%
	<i>C. kessleri</i>	1.00	-2.48	40.86	-6.06%	6.58	13.92	47.27%
		0.34	0.44	25.81	1.70%	-0.02	7.51	-0.27%
Light	<i>I. galbana</i>	1.038	1.04	19.39	5.36%	1.32	4.04	32.67%
		0.311	2.15	12.26	17.54%	2.43	4.78	50.84%
	<i>C. kessleri</i>	0.668	2.92	23.67	12.34%	1.53	4.53	33.77%
		0.128	0.59	2.28	25.88%	0.24	1.24	19.35%

P_{DOC} indicates the production rate of DOC.

P_{TOC} suggests the production rate of TOC.

% of P_{TOC} is calculated by P_{DOC}/ P_{TOC}.

For *C. kessleri* incubated at a high growth rate of 1.00 d^{-1} (Fig. 6.1B), the photosynthetic rate of POC peaked at $55.82\text{ }\mu\text{M h}^{-1}$ during 6–9 h. At low growth rates, both *I. galbana* and *C. kessleri* had much higher photosynthetic rates of TOC during 3–9 h than the early period of incubation (0–3 h) and the ending period of daytime (9–12 h).

This trend is a little different from the 24-h continuous incubations shown in Fig. 5.2 and 5.4. The possible reason might be that the 12:12 L:D cycle disturbed the steady state of continuous culture system. The non-steady state ^{14}C incubation showed a trend of exponential growth in the light period. On the other hand, because of the absence of photosynthesis in the dark period, it is possible that nitrate, the limited-factor, would be accumulated in the dark. So, when the light was turned on in the morning, accumulated nutrients in the dark period might be able to support a faster growth until the extra nitrate concentration was consumed to be a limited level again. Similar trends as were also observed by Laws and Wong (1978) in the studies of carbon and nitrogen metabolism by three marine phytoplankton species in nitrate-limited continuous culture.

For the 12:12 L:D cycle incubations, although t-test results show that in the light period, there are no significant differences between the POC and DOC photosynthetic rates estimated by the ^{14}C technique and the actual rates measured by the control method (Table 6.1). The deviation of production rates measured by two methods ranges from 0.85% to 24.41% and the deviation at low growth rates is higher than that at high growth rate, which is true for both algae. For example, the TOC productivity deviation of *I. galbana* at a low growth rate of 0.360 d^{-1} is 14.44%, about five times higher than that (2.94%) at high growth rate of 1.015 d^{-1} . The similar trend is observed in *C. kessleri* incubations as well. Therefore, the effect of growth rate on the overestimation caused by the ^{14}C technique is much more obvious under the 12:12 L:D cycle than those incubations under 24 h continuous light (see Table 5.1).

In addition, under same conditions the deviations of TOC and POC production rates measured by two methods are much higher for *C. kessleri* than for *I. galbana*, which indicates that the ^{14}C method tends to overestimate the net photosynthetic rate in a relative larger range for *C. kessleri* than for *I. galbana*. At the similar high growth rates of $\sim 1 \text{ d}^{-1}$, the TOC production rate of *C. kessleri* was over estimated by 20.87%, comparatively, the overestimation was only 2.94% for *I. galbana*.

Therefore, although the 12:12 L:D incubation is not as straightforward as 24-hour continuous culture, it still reflect the fact that the ^{14}C overestimated the photosynthetic rates to some extent for *C. kessleri*. The effect of algal species on the ^{14}C technique accuracy is still significant in these incubations under light:dark cycles.

DOC Uptake and Excretion

The DOC excretion or loss rates in both light period and dark period were calculated according to the variation of DOC measurement in the medium using equations similar as Eq.9 or Eq. 11. The results are summarized in Table 6.7, in which P_{DOC} indicates the excretion rate from algal cells into medium caused by direct excretion from intact algal cells, cell lysis or microzooplankton grazing (Nagata 2008) in the process of photosynthesis. R_{DOC} indicates the loss rate of DOC in the medium in the dark period, which might be caused by several processes such as reparation, uptake by mixotrophy of algal cell (Tittel, Bissinger et al. 2003), or grazing effect of bacteria.

In most of our incubations, the calculated DOC excretion rates (P_{DOC}) in the light period were positive, suggesting a net excretion of DOC from algal cells into the medium. If the calculated excretion rate (P_{DOC}) was negative in the daytime, it means the DOC in the medium were assimilated by algal cells, or respired, or consumed by bacteria. One question raised here is where the DOC in the medium came from. In theory, because the medium in

the nutrient reservoir was sterile-filtered through a 0.2 μm filter, POC_{in} (in Eq. 8) should be essentially zero, and if the medium was prepared with artificial seawater, TOC_{in} (in Eq. 9) would consist of little more than the total concentration of vitamins ($\sim 0.3 \mu\text{M}$) added to the growth medium. The probable source of DOC in the medium might be the impurities in the base chemical compounds used to make the sea salt, because no manufacturer can afford to make a sea salt with absolutely pure chemical compounds. As reported by Atkinson and Bingman (2010), although the total organic carbon and the dissolved organic nutrients is lower than typical surface seawater, the TOC content of instant ocean which we used is tested to be about $29 \mu\text{mol kg}^{-1}$, more than half of the $50 \mu\text{mol kg}^{-1}$ of seawater.

Conversely, in the dark period, if the calculated loss rate of DOC (R_{DOC}) is positive, DOC in the medium was assimilated or consumed; if the calculated loss rate of DOC is negative, it indicates that DOC was excreted into the medium by the algal cells in the dark period.

When *I. galbana* was incubated at the high growth rate of 1.015 d^{-1} under a 12:12 L:D cycle, the average loss rate of DOC calculated by the equation similar as Eq. 11 is $-3.04 \mu\text{M h}^{-1}$ in the nighttime (Table 6.7). Since the negative loss rate of DOC in this case indicated that DOC production in the medium did not decrease but increase in the nighttime, although the apparent concentration of DOC was diluted from $107.50 \mu\text{M}$ at the beginning of dark period to be $93.33 \mu\text{M}$ at the end of dark period. Extra DOC might be excreted from the algal cells into the medium during the dark period. In the light period, DOC was also excreted at a rate of $5.45 \mu\text{M h}^{-1}$ (Table 6.7).

The condition was inverse for *C. kessleri* grown at a nitrate-limited high growth rate of 1.00 d^{-1} under a 12:12 L:D cycle, because DOC was assimilated (rather than excreted or produced) by the algal cells during the whole 12:12 L:D incubation period. In the daytime, the calculated excretion rate of DOC was $-2.48 \mu\text{M h}^{-1}$, indicating there was a net loss of

DOC from the medium in the growth chamber which might be caused by two possible reasons. One reason is related to the mixotrophy (Tittel, Bissinger et al. 2003), which occurs simultaneously with photosynthesis (Znavor and Nedoma 2010). Another reason might be related to the grazing effect of bacteria if there was significant amount of bacteria in our chemostat, since such excreted dissolved organic carbon has long been recognized as an important source to bacteria (Cole 1985). My assays for heterotrophic bacteria indicate that although heterotrophic bacteria were present in our cultures, the number of colony forming units was less than 200 mL^{-1} , which is trivial compared with the huge number of algal cells in the chemostat. So, DOC might be assimilated by the algal cells at a rate of $2.48 \mu\text{M h}^{-1}$, if the effect of bacteria grazing was neglected in this interpretation. In the nighttime, the uptake rate of DOC from medium into algal cell was $6.58 \mu\text{M h}^{-1}$ if the effect of bacteria in the medium was assumed to be small enough to be ignored.

For *I. galbana* incubated at the low growth rate of 0.360 d^{-1} on 12:12 L:D cycle, DOC was excreted at a rate of $2.97 \mu\text{M h}^{-1}$ from cells into medium in the daytime and then DOC in the medium was lost at a rate of $2.05 \mu\text{M h}^{-1}$ in the dark period. For *C. kessleri* grown at the low growth rate of 0.340 d^{-1} on 12:12 L:D cycle, the variation rates of DOC in the medium were not obvious since the calculated rates are very small in both light and dark period.

Special Activity Variation

Because of the effects of continuous dilution, air bubbling and carbon uptake or release by algal cells, the special activity of DIC in the medium showed a similar declining trend along incubation time under the 12:12 L:D cycle as those incubations under continuous light. The calculated specific activities for all incubations are listed in Table 6.2. Take *I. galbana* grown at 1.015 d^{-1} for example, the specific activity of DIC decreased from 2718 dpm

$(\mu\text{mol})^{-1}$ to be 1534 dpm $(\mu\text{mol})^{-1}$ at the end of daytime and then to be 781 dpm $(\mu\text{mol})^{-1}$ at the end of the nighttime.

The specific activities of respired POC and TOC in the dark period calculated by combining Eq. 10–11 are summarized in Table 6.2 as well. It seems that the specific activity of respired POC is more straightforward than that of respired TOC since the latter one was sometimes affected by DOC uptake or excretion. For example, the average specific activity of respired TOC in *C. kessleri* incubation at a high growth rate of 1.00 d^{-1} was 826 dpm $(\mu\text{mol})^{-1}$, only half of that of the respired POC (Table 6.2). The reason might be related to the high DOC uptake rate by algal cells in the dark period ($6.58\text{ }\mu\text{M h}^{-1}$ when bacteria grazing effect is ignored), which was nearly equal to the respired rate of POC ($7.34\text{ }\mu\text{M h}^{-1}$). Since the actual respiration rate of POC was $7.34\text{ }\mu\text{M h}^{-1}$, if DOC was assimilated into the cell and then respired at a rate of $6.58\text{ }\mu\text{M h}^{-1}$, the respiration rate of TOC became to be $7.34 + 6.58 = 13.92\text{ }\mu\text{M h}^{-1}$. As discussed above, DOC was assimilated rather than excreted by the cells in both daytime and nighttime for this incubation, the specific activity of assimilated dissolved organic carbon was as low as zero because only dissolved inorganic carbon rather than organic carbon in the medium was labeled by ^{14}C . Consequently, the calculated specific activity of TOC in Eq. 10 was reduced by half if this part of low-SA DOC was counted in the calculation.

As TOC specific activity was sometimes influenced by the DOC uptake or excretion, the specific activity of POC is used here for the following discussions.

Firstly, it can be seen that the variation of specific activity of respired POC in the dark period is different for *C. kessleri* and *I. galbana*. For *I. galbana* incubated at either high growth rate or low growth rate, the specific activity of respired POC was declining along the incubation time. At the high growth rate of 1.015 d^{-1} , the average specific activity of respired POC decreased from 1426 dpm $(\mu\text{mol})^{-1}$ in 12–18 h to be 979 dpm $(\mu\text{mol})^{-1}$ in 18–24 h. At

the low growth rate of 0.360 d^{-1} , it decreased from $1224\text{ dpm }(\mu\text{mol})^{-1}$ in 12–18 h to be $731\text{ dpm }(\mu\text{mol})^{-1}$ in 18–24 h (Table 6.2). In the study of Ditullio and Laws (1986), it was concluded that the excretion of DOC during the photoperiod and uptake of low-SA DOC at night may have been responsible for the decrease in specific activity of particulate ^{14}C at night. In our experiments, it seems true for the incubation of *I. galbana* at low growth rate of 0.360 d^{-1} , in which DOC was excreted at a rate of $2.97\text{ }\mu\text{M h}^{-1}$ from cell into medium in the daytime and then DOC in the medium was assimilated at a rate of $2.05\text{ }\mu\text{M h}^{-1}$ in the dark period if the bacteria grazing effect is ignored.

In addition to the effect of DOC uptake and excretion as suggested by Ditullio and Laws (1986), there might be several other possible reasons. One reason might be that the cells were preferentially respiring recently photo-assimilated carbon with high SA at night as opposed to their pre-existing carbon pools whose SA is close to zero. Since the doubling time for cell population were about 0.68 d for incubation of *I. galbana* at high growth rate and 1.93 d for incubation at low growth rate, there should be pre-existing old carbon left from light respiration in the cell when the dark period was coming. The specific activity of respired carbon might decrease when part of these pre-existing old carbon were respired in the latter period of nighttime (i.e. in 18–24 h) and a small fraction of pre-existing old carbon might be respired subsequently.

Another possible reason might be the minor increasing of DIC concentration, which could reduce the calculated value of specific activity according to Eq. 5. In the same incubation of *I. galbana*, DIC concentration increased from $1920\text{ }\mu\text{M}$ at the beginning of dark period to $2313\text{ }\mu\text{M}$ at the end of dark period (Table 6.2). The increasing rate is $(2313 - 1920)/1920 = 20.47\%$ in 12 hours. The same minor increase of DIC concentration was also observed in incubation at low growth rate of 0.360 d^{-1} .

For *C. kessleri*, the variation of specific activity of respired organic carbon is a little different from *I. galbana* probably because of different respiration mechanism within algal cells. When *C. kessleri* was grown under a 12:12 L:D cycle at a high growth rate of 1.00 d^{-1} , the average specific activities of POC were $1639\text{ dpm }(\mu\text{mol})^{-1}$ in 12–18 h, $1562\text{ dpm }(\mu\text{mol})^{-1}$ in 18–24 h, and $1596\text{ dpm }(\mu\text{mol})^{-1}$ in 12–24 h. The SA declining from the first six hour to the second six hour during the dark period was not as obvious as *I. galbana*. And the average specific activity of POC between 12–24 h was even a little higher than $1391\text{ dpm }(\mu\text{mol})^{-1}$, which was the specific activity of DIC at the time point of 12 h. The possible reason might be that the old carbon with low specific activity has been exhausted in the process of light respiration in the daytime, the recently fixed carbon with high specific activity were being repaired in the dark period. The specific activity for this recently fixed carbon could range between $1391\text{ dpm }(\mu\text{mol})^{-1}$ and $2240\text{ dpm }(\mu\text{mol})^{-1}$ which was the specific activities of DIC in the medium.

When *C. kessleri* was grown at the low rate of 0.340 d^{-1} , the average specific activities of respired POC were $651\text{ dpm }(\mu\text{M})^{-1}$ in 12–18 h, $1230\text{ dpm }(\mu\text{M})^{-1}$ in 18–24 h, and $953\text{ dpm }(\mu\text{M})^{-1}$ in 12–24 h. It can be seen that SA of POC increased from 12–18 h to 18–24 h but was still much lower than $1602\text{ dpm }(\mu\text{M})^{-1}$, the SA of DIC at time point of 12 h. Because the growth rate was so slow that the pre-existing old carbon (SA=0) was not thoroughly respired in the light period, this part of old carbon (SA=0) was left and mixed with the recently fixed carbon with high SA when the nighttime came. Both old carbon and the recently fixed carbon were combined to be respired in the dark period. The proportion of old carbon might decrease in the process of dark respiration, correspondingly the relative proportion of recently fixed carbon might increase along the incubation time although there was no net increase for this part of carbon in the nighttime. As a result, the average SA of POC increased from 12–18 h to 18–24 h.

When algal cells are grown on 12:12 L:D cycle, the ^{14}C method is expected to estimate net carbon fixation if all the carbon respired in the dark was fixed during the previous photoperiod, and ^{14}C uptake technique will tend to overestimate net carbon fixation unless the carbon respired in the dark has the same specific activity as the DIC, i.e., all respired carbon is recently fixed carbon. Both our *I. galbana* and *C. kessleri* experiments show that the average specific activities of repaired POC and TOC in the whole dark period were, to some extent, lower than those of DIC in the medium (Table 6.2), indicating that the ^{14}C method tends to more or less overestimate net photosynthesis at the end of a 24-hour incubation. It is also observed that the light:dark cycle have a significant effect on the relationship between carbon fixation estimated by ^{14}C uptake and net photosynthesis estimated by POC and DOC concentrations in the growth chamber. The overestimation will be discussed in details as following section.

Dark Respiration and Its Implication for Classic ^{14}C Technique

In the practical operations of the classic ^{14}C method on board, the specific activity of DIC in the medium is generally unknown in the dark period. If it is assumed to be as same as that at the time point of 12 h, what will the results be for our 12:12 L:D cycles incubations? The estimated results are summarized in Table 6.3. Photosynthetic rates in the light period are listed in the first line, respiration rates in the dark period are listed in the second line and the ratio of the two rates are listed in the third line. The total rates are calculated by subtracting respiration rate from photosynthetic rate and then divided by 2 (Laws and Wong 1978). The deviation is calculated by subtracting the rates measured by ^{14}C method from the rates measured by control method and then divided by the rates measured by the control method.

For *I. galbana* incubated at the high growth rate of 1.015 d^{-1} under 12:12 L:D cycle, the specific activity of DIC at the time point of 12 h was $1534\text{ dpm }(\mu\text{mol})^{-1}$ and the average

respiration rate of POC between 12–24 h was $5.20 \mu\text{M h}^{-1}$, which is calculated by equation similar as Eq. 10. The average photosynthetic rate of POC estimated by the ^{14}C method is $46.03 \pm 10.15 \mu\text{M h}^{-1}$. Therefore, the total production rate of POC in 24 hours estimated by the ^{14}C method would be $20.42 \pm 5.08 \mu\text{M h}^{-1}$. Comparatively, the corresponding total production rate of POC measured by control method was $(45.64 - 6.80)/2 = 19.42 \mu\text{M h}^{-1}$. It can be seen that there is no significant difference in the total production rates of POC between the ^{14}C method and the control method. Similarly, total production rate of TOC in 24 hours estimated by ^{14}C method was $23.20 \pm 6.47 \mu\text{M h}^{-1}$. The corresponding uptake rate of TOC measured by control method was $(51.09 - 3.75)/2 = 23.67 \mu\text{M h}^{-1}$. So in this case the TOC and POC production rate estimated by the ^{14}C method seems in good agreement with the actual net production rate.

For *C. kessleri* grown at the high growth rate of 1.00 d^{-1} under a 12:12 L:D cycle (Table 6.3), the specific activity of DIC at the time point of 12 h was $1391 \text{ dpm } (\mu\text{mol})^{-1}$. The average respiration rate of POC estimated by the ^{14}C method was $8.42 \mu\text{M h}^{-1}$ in the nighttime and the corresponding total production rate of POC in all day was $19.94 \pm 4.18 \mu\text{M h}^{-1}$. Comparatively, total production rate of POC measured by control method was $(43.33 - 7.34)/2 = 18.00 \mu\text{M h}^{-1}$. It can be seen that the ^{14}C method overestimated the actual net POC production rate by $(19.94 - 18.00)/18.00 = 10.78\%$. Similarly, the average respiration rate of TOC estimated by ^{14}C method was $8.27 \mu\text{M h}^{-1}$ in the nighttime and the corresponding total production rate of TOC in all day was $20.56 \pm 4.71 \mu\text{M h}^{-1}$. For comparison, the estimated total production rate measured by control method was $(40.86 - 13.92)/2 = 13.47 \mu\text{M h}^{-1}$. Thus, TOC photosynthetic rates estimated by ^{14}C method overestimated the actual net rates by $(20.56 - 13.47)/13.47 = 52.64\%$.

For *I. galbana* grown at a low growth rate of 0.360 d^{-1} under a 12:12 L:D cycle (Fig. 6.1C), the total production rate of POC in 24 hours estimated by ^{14}C method was $13.27 \pm$

2.58 $\mu\text{M h}^{-1}$, 26.14% higher than 10.52 $\mu\text{M h}^{-1}$, the total production rate of POC measured by the control method. The total production rate of TOC in 24 hours estimated by ^{14}C method was $14.06 \pm 6.28 \mu\text{M h}^{-1}$, 28.05% higher than 10.98 $\mu\text{M h}^{-1}$, the uptake rate of TOC measured by control method. For *C. kessleri* grown at a low rate of 0.340 d^{-1} under a 12:12 L:D cycle (Fig. 6.1D), the total production rate of POC in 24 hour estimated by ^{14}C method was $13.54 \pm 3.30 \mu\text{M h}^{-1}$, 51.79% higher than 8.92 $\mu\text{M h}^{-1}$, which is measured by the control method. The total production rate of TOC in 24 hours estimated by ^{14}C method was close to that of POC and 47.98% higher than 9.15 $\mu\text{M h}^{-1}$, which is the TOC total production rate measured by the control method.

Summarily, it can be seen that the effect of growth rate is significant, because for same species, the deviations between total production rates determined by two methods at low growth rates were much higher than those at higher growth rates. For example, for *C. kessleri* the ^{14}C method slightly overestimated the actual POC net production rates by 10.78% at high growth rate, but significantly overestimated that by 51.79% at low growth rate. And for *I. galbana* grown at high growth rate, the POC production rate estimated by the ^{14}C method was close to that measured by control method, however, the ^{14}C method overestimated the actual POC production rate by 26.14% at low growth rate. In the study of Ditullio and Laws (1986), the similar trend of higher overestimation at lower growth rates ($126 \pm 17\%$) than higher growth rates ($107 \pm 18\%$) was found for the 24-hour particulate carbon production.

On the other hand, the effect of algal species is significant as well, since at either high or low growth rate, the deviations between total production rates determined by two methods for *C. kessleri* are much larger than those for *I. galbana*, which could be observed by comparing the deviation values summarized in Table 6.3.

In addition to the effects of growth rate and algal species, the other possible reason might be that the special activity of DIC at 12 h was sometimes higher than the average special

activity of respired POC and TOC in our calculation above. If a relative higher value was assumed to be the special activity of respired POC and TOC and input Eq. 10, the calculated dark respiration rate would be underestimated by the ^{14}C method and thus the total production rate would be overestimated. However, the true value of specific activity of respired organic carbon was generally unknown since in Eq. 10, both variables of R_D and SA_{RD} are unknown. To calculate one of these two unknown variable, one of them must be assumed to be a reasonable value.

The ratios of respiration rate to photosynthetic rate are also calculated and listed in Table 6.3. It can be seen that the ratios of rates measured by the control method are mostly higher than those estimated by the ^{14}C method, which also reflect that the ^{14}C method underestimated the respiration rate in the dark period when specific activity of organic carbon are assumed to be a relative higher value than the actual value.

In sum, although the results of 12:12 L:D cycle incubations are not as straightforward as the 24-hour continuous cultures, they still reflect the effect of algal species and the effect of growth rate which has been shown in continuous culture incubation and batch culture. The overestimation phenomenon of the ^{14}C method also indicates that simply using a relative high special activity of DIC in the medium to represent the unknown special activity of respired POC and DOC for calculation will result in the underestimation of respiration rate and thus overestimate the total photosynthetic rate in the whole incubation period.

6.2.2 Light-limited Condition

Comparison of Production Rates in the Light Period

For *I. galbana* incubated at high growth rate under 12:12 L:D cycle, production rates in the daytime estimated by the ^{14}C method are compared by t-test with those measured by the control method. The P-values of t-test are 0.1748 for comparing POC production rates,

0.3079 for comparing TOC production rates, indicating that there are no significant differences between the results estimated by the ^{14}C method and those measured by the control method in the light period. However, in Fig. 6.2A it can be seen that production rates estimated by the ^{14}C method were higher than those measured by control method between 0–9 h. The ^{14}C method overestimated average net production rate in the daytime by 18.15% for POC and by 13.77% for TOC (Table 6.4).

It is noteworthy that the average production rate of TOC estimated by the ^{14}C method peaked between 6–9 h with a value of $27.57 \mu\text{M h}^{-1}$ (Fig. 6.2A), which is 34.62% higher than the actual net production rate during the corresponding time interval. However, it decreased to be $19.99 \mu\text{M h}^{-1}$ between 9–12 h, a little lower than the actual net production rate of $23.31 \mu\text{M h}^{-1}$ during the same period.

When *C. kessleri* was also grown under same conditions as *I. galbana*, the light-limited high growth rate of *C. kessleri* was about 0.668 d^{-1} before adding ^{14}C . T-test results (p-values=0.0072 for POC and 0.0138 for TOC) suggest that the ^{14}C method significantly overestimated the production rates by 64.96% for POC and by 44.61% for TOC in the daytime. The overestimation rates in this case are much larger than those for *I. galbana* (by 18.15% for POC and 13.77% for TOC as discussed above). The largest deviation between the production rates estimated by two different methods appears between 6–9 h (Fig. 6.2B), in which the ^{14}C method overestimated the net production rate of TOC by $(41.11 - 24.57)/24.57 = 67.32\%$. The same phenomenon is observed in the incubation of *I. galbana* as discussed above (Fig. 6.2A).

When *I. galbana* was grown at low growth rate under relatively dim light and 12:12 L:D cycles, the average production rates estimated by the ^{14}C method were $12.18 \mu\text{M h}^{-1}$ for POC and $14.09 \mu\text{M h}^{-1}$ for TOC in the light period. T-test with a p-value of 0.2022 for TOC indicates that there is no significant difference between the TOC production rates for each

time interval estimated by the ^{14}C method and those measured by the control method during the light period. In the whole daytime, the ^{14}C method overestimated the net average production rates by 20.47% for POC and by 14.93% for TOC.

For *C. kessleri* grown under same dim light condition as *I. galbana*, the average production rates estimated by the ^{14}C method were $2.69\ \mu\text{M h}^{-1}$ for POC and $2.71\ \mu\text{M h}^{-1}$ for TOC in the light period. T-test results (with $p=0.3028$ for POC and 0.6839 for TOC) suggest that there are no significant differences between the production rates estimated by ^{14}C method and those measured by control method in the light period. But in the whole daytime, the average deviations between production rates measured by two methods are manifest since they are 59.17% for POC and 18.86% for TOC.

For both *I. galbana* and *C. kessleri* incubated under relatively dim light, the highest overestimation of production rates by the ^{14}C method did not appear between 6–9 h as shown in Fig. 6.2 A and B, but appeared between 0–3 h (Fig. 6.2 C and D). For *I. galbana*, the ^{14}C method overestimated the actual net production rate of TOC by $(16.69 - 11.56)/11.56 = 44.38\%$ in this time interval and for *C. kessleri* it is $(5.29 - 2.23)/2.23 = 137\%$.

In sum, plots in Fig. 6.2 and calculated deviations (or overestimation rates by the ^{14}C method) show that in the daytime the ^{14}C method more or less overestimated the net production rates. For *I. galbana*, overestimation rate of incubations under relatively dim light is a little larger than that under relatively bright light (Table 6.4 and 6.5). For *C. kessleri*, the overestimation rate is nearly same under dim light and under bright light.

Under either dim light or bright light, the overestimation rates of TOC of *C. kessleri* (by 44.61% under bright light and by 18.86% under dim light) are higher than those of *I. galbana* (by 13.77% under bright light and by 14.93% under dim light).

Dark Respiration and Its Implication for Classic ^{14}C Technique

In the practical operation of the ^{14}C technique, the specific activity of respired carbon in the dark period cannot be calculated directly by Eq. 10 because both the dark respiration rate of TOC (R_D), and the specific activity of the respired carbon (SA_{RD}) are unknown. But SA_{RD} can be estimated by combining Eq. 10–11, in which the respiration rates estimated by the ^{14}C method were assumed to be equal to those measured by the control method.

For *I. galbana* incubated under relatively bright light and 12:12 L:D cycles, the respiration rates of POC and TOC measured by the control method were $2.73 \mu\text{M h}^{-1}$ and $4.04 \mu\text{M h}^{-1}$ respectively. By combining equations similar as Eq. 10–11 ($\mu=0$), the average SA_{RD} of respired POC during the dark period can be calculated to be $1383 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–18 h, $933 \text{ dpm } (\mu\text{mol})^{-1}$ between 18–24 h, and $1158 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–24 h (Table 6.6). Similarly, the average specific activities of respired TOC were $1373 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–18 h, $588 \text{ dpm } (\mu\text{mol})^{-1}$ between 18–24 h, and $981 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–24 h in the dark period.

Comparatively, the specific activities of DIC at the time points of 0 h, 12 h, and 24 h were $2294 \text{ dpm } (\mu\text{mol})^{-1}$, $2121 \text{ dpm } (\mu\text{mol})^{-1}$ and $1751 \text{ dpm } (\mu\text{mol})^{-1}$ respectively (Table 6.6). The specific activity of DIC decreased along incubation time by 7.54% in the light period and by 16.13% in the dark period. The specific activity of DIC was decreasing in the whole ^{14}C incubation, but not as fast as that in the nitrate-limited incubations because the pump was turned off when doing ^{14}C incubation and there was no dilution effect on the medium.

The calculated specific activities of respired POC and TOC seems much lower than those of DIC whose lowest value was $1751 \text{ dpm } (\mu\text{mol})^{-1}$, indicating that not only the recently fixed carbon with high-SA same as DIC and but also the old carbon with SA close to zero were respired simultaneously in the dark period. The average SA of respired POC decreased from $1382 \text{ dpm } (\mu\text{mol})^{-1}$ between 12–18 h to be $933 \text{ dpm } (\mu\text{mol})^{-1}$ between 18–24 h, so it seems

that the recently fixed carbon were preferentially respired in the first 6 h of dark period and then more old carbon were respired in the second 6 h during the dark period, or more low-SA DOC was assimilated by the cell as suggested by Ditullio and Laws (1986). Their study indicated that the respiration of recently photo-assimilated high-SA carbon only partially account for the decline in specific activity of POC and TOC, and the DOC taken up in the dark to be supplied by a daytime excretion rate of unlabeled DOC could explain the variation of SA as well.

For the whole ^{14}C incubation process of *I. galbana* in the period of 24 hours, what will the results be if specific activity was assumed to be equal to the value of DIC at the time point of 12 h ($2121 \text{ dpm } (\mu\text{mol})^{-1}$)? The average respiration rate of POC in the dark period was $1.49 \mu\text{M h}^{-1}$ which is calculated by equation similar as Eq. 10 ($\mu=0$). It can be seen that ^{14}C method underestimated the actual respiration rate because the actual respiration rate calculated by the control method was $2.73 \mu\text{M h}^{-1}$. The average photosynthetic rate of POC calculated by the ^{14}C method was $21.68 \mu\text{M h}^{-1}$. Therefore, the average production rate of POC in all day calculated by the ^{14}C method was $10.10 \mu\text{M h}^{-1}$, which is calculated by subtracting respiration rate from photosynthetic rate and then divided by 2 (Table 6.4). For comparison, the estimated production rate of POC calculated by the control method was $7.81 \mu\text{M h}^{-1}$, which is calculated by subtracting respiration rate from photosynthetic rate and then divided by 2 as well. It can be seen that the ^{14}C method overestimated the total production rate of POC in 24 h by 29.32%. Similarly, the average respiration rate of TOC in the nighttime was $1.87 \mu\text{M h}^{-1}$. The average production rate of TOC in all day calculated by ^{14}C method was $10.10 \mu\text{M h}^{-1}$. Comparatively, the actual respiration rate calculated by the control method was $4.04 \mu\text{M h}^{-1}$. The average production rate of TOC calculated by the control method was $7.67 \mu\text{M h}^{-1}$. Therefore, the ^{14}C method overestimated the total production rate of TOC in all day by 31.68%.

The SA of respired POC and TOC, the estimated respiration rate by the ^{14}C method and the total production rate in a whole day can be calculated by similar process as above for *I. galbana* grown at low growth rate and *C. kessleri* grown at high and low growth rate under 12:12 L:D cycles and all calculated results are summarized in Table 6.4, 6.5 and 6.6.

It can be seen that for *C. kessleri* grown at high and low growth rate, the SA of respired organic carbon did not decreased a lot from the first 6 h to the second 6 h during the dark period (Table 6.6), which suggested that old carbon might account for a large proportion of the respired organic carbon in the dark period.

The ratios of dark respiration rate to daytime net production rate calculated by the ^{14}C method and by the control method are also listed in Table 6.4 and 6.5. It can be seen that the ratios estimated by the ^{14}C method are always lower than those measured by the control method, which is also the evidence that the ^{14}C method underestimated the actual respiration rate. For example, when *I. galbana* was grown at high growth rate, the ratio of respiration rate to photosynthetic rate was about 6.87% calculated by the ^{14}C method, and it was 14.88% measured by the control method. In addition, at high growth rates, the ratios calculated by the control method are nearly same for *I. galbana* (14.88% for POC and 20.84% for TOC) and *C. kessleri* (14.46% for POC and 19.14% for TOC), but the ratios calculated by the ^{14}C method are much larger for *I. galbana* (6.87% for POC and 8.48% for TOC) than *C. kessleri* (3.94% for both POC and TOC). This reflects that the ^{14}C method underestimated the actual respiration rate much more for *C. kessleri* than for *I. galbana*, and thus overestimated the total production rate in all 24 h much more for *C. kessleri* than for *I. galbana*.

In Table 6.4, If the ^{14}C method was assumed to estimate the gross productivity for *C. kessleri* in the daytime of light-limited 12:12 L:D cycle, the average gross production rate of TOC would be $34.23 \mu\text{M h}^{-1}$ between 0–12 h and the net production rate would be $23.67 \mu\text{M h}^{-1}$ measured by the control method. If this is true, then the light respiration rate (light

respiration in the light period) of TOC should be $34.23 - 23.67 = 10.56 \mu\text{M h}^{-1}$, which is about two times higher than the dark respiration rate between 12–24 h ($4.53 \mu\text{M h}^{-1}$).

The ratio of respiration rate to gross photosynthetic rate was $10.56/34.23 = 30.85\%$ in the light period and was $4.53/34.23 = 13.23\%$ in the dark period, and totally the respiration rate account for $(10.56 + 4.53)/34.23 = 44.08\%$ in the whole 24-hour incubation. These ratios seems reasonable since previous studies indicated that the lowest respiration/photosynthesis ratio was found to be close to 10% for cyanobacteria (Langdon 1993) and the highest respiration/photosynthesis ratio was found to be about 60% for flagellates growing in a 12:12 L:D cycle with nitrate as a nitrogen source (Laws and Wong 1978), and in the case of diatoms growing on a 12:12 L:D cycle with ammonium as a nitrogen source, the dark respiration accounted for about 15% of the 24-h average photosynthetic rate (Laws and Wong 1978; Laws and Bannister 1980).

In our incubation of *C. kessleri* under dim light and 12:12 L:D cycles, it is found that the measurement for production rates and respiration rates is very sensitive to be influenced by the DOC excretion and experimental error, since the growth rate were so low that it is difficult to distinguish the calculated production rates very clearly with experimental errors sometimes. From Table 6.7, it can be seen that the ratio of DOC excretion rate to TOC production rate in the daytime was as high as 25.88%. Steemann Nielsen (1952) pointed out that the ^{14}C method could not be used for measuring the rate of photosynthesis at low light intensities where the rate of respiration is the same or lower as the rate of photosynthesis when describing the ^{14}C method for measuring organic productivity in the sea, since it is probable that CO_2 -uptake by photosynthesis and CO_2 -release by respiration do not take independently of each other. In our incubation, the photosynthetic rate ($2.28 \mu\text{M h}^{-1}$) was fortunately higher the respiration rate ($1.24 \mu\text{M h}^{-1}$), although not so much.

As discussed in Hypothesis 4 in section 3.3, if cells are grown on 12:12 L:D cycle, the ^{14}C method will estimate net carbon fixation if all the carbon respired in the dark was fixed during the previous photoperiod, and ^{14}C uptake after 24 h will tend to overestimate net carbon fixation unless the carbon respired in the dark has the same specific activity as the DIC, i.e., all respired carbon is recently fixed carbon.

My experiments of both *I. galbana* and *C. kessleri* show that specific activity of respired POC and TOC are generally lower than the specific activity of DIC (Table 6.6), indicating that the respired carbon in the dark period were mixed with the old carbon with low-SA and the recently fixed carbon with high-SA. Thus, the ^{14}C method will tend to overestimate net photosynthesis at the end of a 24-hour incubation.

And the relative lower specific activity in respired POC and TOC compared with that of DIC in medium also explained why the respiration rates calculated by the ^{14}C method (Eq. 10) were mostly lower than the actual respiration rate measured by the control method when the SA_{RD} in Eq. 10 is assumed to be equal to that of DIC. The assumed SA_{RD} was so high than the actual specific activity in respired POC and TOC that the actual respiration rate was underestimated by the ^{14}C method and thus contributed to the overestimation of total production rate in 24 h. Hypothesis 4 might be rejected, since growth on a 12:12 light:dark cycle have an significant effect on the relationship between carbon fixation estimated from ^{14}C uptake and net photosynthesis estimated from POC and DOC concentrations in the growth chamber.

CHAPTER 7: SUMMARY AND CONCLUSIONS

7.1 Summary

Batch culture studies were carried out with seven species of marine phytoplankton and followed up by chemostat studies with two of the seven species to determine whether and to what extent the species recycled respired carbon and their implication to interpret what the ^{14}C method really measure in estimating the photosynthetic rate in aquatic environment.

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, the implication being that the cells were recycling 100% of respired carbon, and hence that for these two species photosynthetic rates estimated from the uptake of ^{14}C would estimate net photosynthesis in the light. In similar experiments with the other five species, uniformly labeled cells lost activity when they were incubated in the light. Comparisons of the rates of loss of ^{14}C activity in the light and dark indicated that in four of these five cases an average of roughly 50% of respired carbon was being recycled in the light, the implication being that photosynthetic rates estimated from the uptake of ^{14}C in the light would estimate a rate equal to the average of net and gross photosynthesis. In the case of the remaining species, *Chlorella kessleri*, loss rates of ^{14}C were the same in the light and dark, the implication being that no respired carbon was being recycled in the light, and hence that uptake of ^{14}C in the light would estimate gross photosynthesis.

My following continuous culture experiments of two species at both high and low growth rates under nitrate-/light-limited conditions verified the implication of previous batch culture experiments and tested several hypotheses I inferred before experiments. For *I. galbana* incubated at a high growth rate of 0.972 d^{-1} or a low growth rate of 0.318 d^{-1} the photosynthetic rates of TOC and POC measured by the ^{14}C method are close to the actual net

rates measured by the control method. Comparatively, for *C. kessleri* incubated at a high growth rate of 1.033 d^{-1} and a low growth rate of 0.313 d^{-1} , the photosynthetic rates of both TOC and POC measured by the ^{14}C method are significantly higher than the actual net production rates measured by the control method. So, it seems reasonable to reject the hypothesis 5 in section 3.3 that the relationship between carbon fixation estimated from ^{14}C technique and net photosynthesis estimated from POC and DOC concentrations is independent of the species of phytoplankton in the growth chamber. This might explain some of the paradoxes about what the ^{14}C method actually measures, including the historical debate between Ryther & Vaccaro on one side and Steemann Nielsen and co-workers on the other, because different algal species were used in those early studies. And the information about what the ^{14}C method actually measures for common phytoplankton species would allow for a more informed interpretation of field results if the composition of the phytoplankton community is known.

Regression analysis indicates that for *C. kessleri* grown at a high growth rate of 1.033 d^{-1} , there is significant negative linear relationship between the TOC photosynthetic rate measured by the ^{14}C method and the elapsed incubation time. For *I. galbana* incubated under the same conditions, there is no such trend of declining. Under the lower growth rates (longer doubling time), the relationship between time course and photosynthetic rates was not observed for both species. Therefore, incubation length and growth rate might have a combined effect on species like *C. kessleri* grown at high growth rate in determining the production rates by the ^{14}C method. If sample was only incubated for a short time (such as 1–6 h) in field trips, then ^{14}C method would overestimated the net production rates. The hypothesis 3 might be rejected since phytoplankton growth rate, at least in this case, has a significant effect on the relationship between carbon fixation estimated from ^{14}C uptake and net photosynthesis estimated from POC and TOC concentrations in the growth chamber.

Results from the light-limited studies were consistent with the results of the nitrate-limited studies. The limiting factor of light has more negative effect on the growth of *C. kessleri* than that of *I. galbana* since under the same irradiance, the growth rate of *C. kessleri* was always lower than that *I. galbana*. For *I. galbana* grown at either high or low growth rate with pump either off or on, t-test results show that the ^{14}C method estimated net production rate very accurately for both POC and TOC. Comparatively, for *C. kessleri* grown under exactly same conditions as *I. galbana*, the ^{14}C method overestimates the photosynthetic rates significantly, and the overestimated rates at lower growth rate are much higher than those at higher growth rate under light-limited conditions.

When *I. galbana* and *C. kessleri* were incubated under nitrate-limited 12:12 L:D cycle, the effect of light:dark cycle is significant, since it disturbed the steady state of continuous culture system and the non-steady state ^{14}C incubation show a trend of exponential growth in the light period. In the daytime, the deviation of production rates measured by ^{14}C method and control method ranges from 0.85% to 24.41%, indicating the ^{14}C method overestimated the net production rates to some extent. Comparatively, the deviations of incubations at low growth rates are higher than those at high growth rate for both algae species. So, the effect of growth rate on the overestimation caused by the ^{14}C technique was more obvious in the 12:12 L:D cycle incubations than in 24 h continuous light incubations. In addition, at same growth rate, the ^{14}C method tends to overestimate the net photosynthetic rates of TOC and POC in a relative larger range for *C. kessleri* than for *I. galbana*. Therefore, the Hypothesis 4 in section 3.3 might be rejected, since growth on a 12:12 light:dark cycle have an significant effect on the relationship between carbon fixation estimated from ^{14}C uptake and net photosynthesis estimated from POC and DOC concentrations in the growth chamber.

The specific activity of DIC was always decreasing along the incubation time in 12:12 L:D cycle incubations. Incubations of both *I. galbana* and *C. kessleri* show that specific

activity of repaired POC and TOC are generally lower than the specific activity of DIC in the medium, indicating that the respired carbon in the dark period were mixed with the old carbon with low-SA and the recently fixed carbon with high-SA.

So, in practical operation of ^{14}C technique, the respiration rate would be underestimated by the ^{14}C method, and thus the total net production rate at the end of a 24-hour incubation would be overestimated by the ^{14}C method, if the special activity of respired organic carbon in the dark period is simply assumed to be equal to specific activity of medium DIC at the end of light period.

The deviations between final result from two different method show that for POC, the ^{14}C method overestimated the actual total production rate more for *C. kessleri* (by 51.79% at $\mu=0.340\text{ d}^{-1}$ and by 10.78% at $\mu=1.00\text{ d}^{-1}$) than for *I. galbana* (by 26.14% at $\mu=0.360\text{ d}^{-1}$ and by 5.10% at $\mu=1.015\text{ d}^{-1}$) at both high and low growth rates. And for the same species, the ^{14}C method overestimated these rates more at low growth rates than at higher growth rates. For TOC, there is the similar phenomenon. So, although the results of 12:12 L:D cycle incubations are not as straightforward as the 24-hour continuous cultures, they still reflect the effect of algal species and the effect of growth rate which has been shown in continuous culture incubation and batch culture.

It is noteworthy that the DOC uptake and excretion by the algal cells were both observed in our continuous culture and 12:12 L:D cycle incubations and has an noticeable effect on our calculations.

When *I. galbana* and *C. kessleri* were incubated at light-limited high/low growth rate under 12:12 L:D cycle respectively, the calculated deviations (or overestimation rates by the ^{14}C method) show that in the daytime the ^{14}C method more or less overestimated the net production rates. For *I. galbana*, overestimation rate is a little larger for incubation at low growth rate than that at low growth rates. For *C. kessleri*, the overestimation rate is nearly

same at low growth rate and high growth rate. In addition, at either high growth rate or low growth rate, the overestimation rates of TOC of *C. kessleri* are higher than those of *I. galbana*.

In the dark period, the special activities of respired organic carbon are always much lower than those of DIC in the medium. On the other hand, if the special activities of respired carbon are unknown and are assumed to be equal to that at the time points of 12 h, the ^{14}C method always underestimated the actual respired rate of organic carbon and thus overestimated the total production rate in the whole 24 h incubation. The fundamental reason for this overestimation is that in the dark period, not only recently fixed carbon with high SA but also a significant proportion of old carbon with low-SA (close to zero) were being respired together. Therefore, the Hypothesis 4 in section 3.3 might be rejected, since growth on a 12:12 light:dark cycle have a significant effect on the relationship between carbon fixation estimated from ^{14}C uptake and net photosynthesis estimated from POC and DOC concentrations in the growth chamber.

It is observed that the overestimation of TOC production rates by the ^{14}C method at low growth rate are much larger than those at high growth rate, and at either high growth rate or low growth rate, the overestimated TOC production rates of *C. kessleri* are much larger than those of *I. galbana*. Based on this conclusion, Hypothesis 3 and Hypothesis 5 in section 3.3 might be rejected, since both growth rate and algal species have effects on the usage of ^{14}C method and interpretation of its results. In incubations while pump was on, the mostly close value of specific activity of respired carbon is the DIC SA at the end of nighttime which has declined to be a very small value because of continuous dilution. But for incubations while pump was off, DIC SA in the medium was always higher than that those of respired organic carbon. Simply assuming the SA of respired organic carbon to be equal to SA of DIC in the medium will cause overestimation in a large range.

7.2 Future Research Directions

My current study only carried out the continuous culture experiment for two typical species. In future, if possible, I anticipate studying one species each from the Chlorophyceae, Coscinodiscophyceae, Bacillariophyceae, Cyanophyceae, Prymnesiophyceae, and Prasinophyceae. Several species are of particular interest because of results of previous work.

By surveying numerous species from several classes of algae, I expect to be able to identify patterns of carbon fixation, if they exist. My plan is to survey at least 50 species, with at least one species from each of the following genera:

<u>Bacillariophyceae</u>	<u>Chlorophyceae</u>	<u>Coscinodiscophyceae</u>	<u>Cyanophyceae</u>	<u>Dinophyceae</u>
Navicula	Chlorella	Chaetoceros	Prochlorococcus	Amphidinium
Nitzschia	Dunaliella	Coscinodiscus	Synechococcus	Gonyaulax
Phaeodactylum		Cyclotella	Synechocystis	Gymnodinium
Pseudo-nitzschia		Skeletonema		Karenia
		Thalassiosira		Peridinium
				Prorocentrum
<u>Prymnesiophyceae</u>	<u>Prasinophyceae</u>			
Emiliana	Tetraselmis			
Isochrysis				
Pavlova				
Phaeocystis				

In addition, I expect to schedule some field trips to coastal areas along the Gulf of Mexico and collect field samples for running chemostat. PCR technique would be used to identify the algal species in field samples. I hope to compare my results of continuous cultures with the remote sensing dataset which are intensively used by the physical oceanographers, with the oxygen light-and-dark-bottle methodology, and with the Fluorescence methods to get a better understanding about what the so-called “ ^{14}C method” was actually measuring, and hope to provide a clearer reference for oceanographers.

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