

A high-throughput method to measure photosynthesis-irradiance curves of phytoplankton

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Abstract

Photosynthesis-irradiance (P-E) curves are an important tool used to characterize the physiology of phytoplankton. In most marine environments, low biomass and photosynthetic rates make the carbon-14 tracer technique, used in conjunction with photosynthetrons to create the light gradient, the method of choice to measure P-E curves. However, conventional photosynthetrons are bulky and require large chilled-water recirculators to maintain temperature. In addition, setting the light levels is difficult. Here we describe a new type of photosynthetron, the microphotosynthetron, which avoids these problems. The microphotosynthetron is based on the standard 96-well plate format and allows precise light control using tunable light-emitting diodes (LEDs). Temperature is regulated using a Peltier device. Using monotypic cultures of several phytoplankton strains grown at different light levels and mixed field populations from different sampling sites and depths, we evaluated the utility of the microphotosynthetron by comparing the measured P-E curves to curves from standard photosynthetrons. The microphotosynthetron P-E curves were identical in initial slope (α) ($P > 0.1$) and light-saturated photosynthesis (P_{\max}) ($P > 0.1$) to P-E curves measured using conventional photosynthetrons. In addition, because the microphotosynthetron is based on a standard 96-well plate format, set-up and processing is greatly expedited in comparison to the conventional photosynthetron technique. Although the microphotosynthetron utilizes smaller sample volumes (200 μL) and therefore might not be suitable for highly heterogeneous samples, it has numerous advantages over the conventional protocol. We recommend the microphotosynthetron approach to measure P-E curves as a method for the rapid screening of photophysiological properties of phytoplankton, in particular, monotypic cultures.

Photosynthesis is the fundamental biological process driving aquatic food webs and biochemical cycles. As such, describing its variability and regulation is essential to characterizing aquatic environments. The photosynthesis-irradiance (P-E) curve describes the response relationship of the photosynthetic rate as a function of light. Since light drives photosynthesis, the P-E curve provides a basic tool to understand this important process (Jassby and Platt 1976; Kirk 1994). Various mathematical relationships have been proposed to describe the P-E relationship (Blackman 1905; Jassby and Platt 1976), but at present none fully capture its complexity (Johnson and

Barber 2003). Nevertheless, much of the variability in the P-E relationship can be characterized by its initial slope (α) and maximal value (P_{\max}). These parameters provide valuable insight into the regulation of photosynthetic rate (Geider and Osborne 1992). It is these parameters that are used to assess the influence of environmental variables on photosynthesis (e.g., light, nutrients, temperature, etc.) (Cullen et al. 1992; Davison 1991; Ryther 1956) and to model photosynthesis over larger space and time scales than can be directly measured (Bidigare et al. 1992; Longhurst et al. 1995; Platt and Sathyendranath 1988; Sosik 1996).

In practice, P-E curves are constructed using two basic methodological approaches. The first method uses measurements of oxygen concentration over time to quantify the photosynthetic rate. Typically these rate measurements are made at a defined light level over a 1–2 min period. The light level is then increased to the next point on the P-E curve, an oxygen evolution rate is measured, and so on, until all of the points on the P-E curve have been quantified (Walker 1988). Temperature is maintained by a regulated water jacket. This

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Acknowledgments

This work was supported by funds from NSF (#OCE05-26462 and #OCE05-50798) and the University of Hawaii. The authors would like to thank Prof. David Karl for the use of his liquid scintillation counter, Carli Bober and Anna Ritchie for assistance with coastal marine sampling, and two anonymous reviewers and the associate editor for helpful comments. This is SOEST contribution #7172.

approach has the advantages that the P-E curve is generated in real time, oxygen rate measurements measure net photosynthesis, and that the same sample is used for all points on the curve. The major disadvantages of this approach are that (1) there can be significant hysteresis in generating the curve—prior measurement light levels can significantly influence the observed photosynthetic rates, and (2) the oxygen evolution technique is not sensitive enough to measure P-E curves in most low biomass, low productivity environments common in the ocean (Falkowski and Raven 2007).

The second general approach to generating P-E curves is to use the C-14 tracer technique (Steemann-Nielsen 1952) to measure photosynthesis in combination with a photosynthetron used to incubate samples over a range of light levels (Lewis and Smith 1983). Several variations on the photosynthetron theme have been used (Babin et al. 1994; Johnson et al. 2002; Lewis and Smith 1983), but all use a single light source that is differentially attenuated to each position of the photosynthetron to create a light gradient. Temperature is regulated by a controlled water jacket. Each position of the photosynthetron corresponds to a different light-level resulting in numerous parallel incubations. Individual incubations, all of the same period, are assayed for C-14 incorporation, thus generating a P-E curve. Because of the sensitivity of the C-14 method for measuring photosynthesis, the photosynthetron-based approach is the conventional technique for measuring P-E curves in most aquatic environments (e.g., Cleveland et al. 1989; Hiscock et al. 2003; Johnson et al. 2002).

Although the C-14/photosynthetron approach is the favored technique in aquatic sciences because of its sensitivity, its utility is limited because of the nature of the photosynthetron. In particular, because the source of light emits a great deal of heat with the light, a large refrigerated water recirculator is required to maintain in situ temperatures. Another concern is that the combined water chiller/photosynthetron set-up requires a lot of space and electrical power, both of which can be limited in field or laboratory settings. The C-14/photosynthetron approach generally produces ~12 samples per P-E curve, which is sufficient to constrain parameter estimates for high quality data sets (Zimmerman et al. 1987). But, as the number of curves increases, the processing of the numerous parallel incubations also increases, thus making the generation of multiple P-E curves labor intensive. Finally, conventional photosynthetrons use different combinations of neutral density screening to modify the levels of light in each position (e.g., Johnson et al. 1999). Although technically sound, in practice it is time-consuming and difficult to precisely set the light level for each of the positions, often leading to inappropriate light gradients and ill-defined P-E curves.

We developed a novel photosynthetron with the goal of maintaining the advantages of the C-14/photosynthetron approach for generating P-E curves, while minimizing many of the disadvantages. To take advantage of existing high-throughput equipment and supplies, our approach is based on the standard

96-well microplate geometry, using the 96-well microplate as a massively parallel incubation vessel. Temperature is regulated using a Peltier device, which is specifically designed for accurately maintaining 96-well plate temperatures. Light is precisely controlled using tunable dials, obviating the need for the tedious trial/error method of standard photosynthetrons. The entire microphotosynthetron is small (~10 cm × 16 cm foot print) and requires relatively little electrical power. P-E curves generated from the microphotosynthetron are comparable with those from the standard photosynthetron, indicating that the advantages gained using this approach do not lead to reductions in data quality. This approach can dramatically increase sample throughput, ultimately leading to a better understanding of how environmental variables influence photosynthesis in aquatic environments.

Materials and procedures

Phytoplankton cultures and field sampling—Several strains of *Prochlorococcus* were grown in Pro99 media (Andersen 2005) at different light levels to create a wide range of shapes and magnitudes of P-E curves. *Prochlorococcus* strains were grown in a custom-designed incubator (Percival) that simulated the diel pattern of light in the tropical marine environment (12:12 h cycle). The *Prochlorococcus* strains used included MIT9211, SS120, MIT9313, NATL1A, MIT9515, and MIT9312 and represent a wide range of photophysiological adaptations (Johnson et al. 2006; Moore and Chisholm 1999; Rocop et al. 2002). All strains were grown at 25°C and the following noontime irradiance levels were used: 30, 75, and 100 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Culture biomass was monitored on a fluorometer (Turner 10-AU), and all photosynthesis measurements were made in mid-exponential growth phase.

To supplement the variability of P-E curves generated in the laboratory and to evaluate the microphotosynthetron approach on natural populations, P-E curves were measured on natural phytoplankton populations from the marine environment. One set of samples were collected from several locations within Kaneohe Bay, Hawaii (21.45N, 157.80W) in July 2006 as part of a coastal marine sampling program. Surface seawater samples were taken by hand-dipping opaque 1-L sample bottles from a small boat and storing the bottles in a cooler at ambient temperature until later analysis in the laboratory (~3-5 h later). Our previous work has shown that the phytoplankton community at this location is dominated by *Prochlorococcus*, with additional significant contributions from *Synechococcus*, diatoms, and other phytoplankton.

A second set of field samples were collected aboard the R/V *Kilo Moana* on a transect from Honolulu, Hawaii to Noumea, New Caledonia to Brisbane, Australia during January-February 2007. Using a CTD-equipped rosette with 12-L niskin bottles, water samples were collected from the 73%, 25%, and 6% of surface light isolumens, with the goal of achieving different levels of photoacclimation, and therefore, different shapes and magnitudes of P-E curves. All phytoplankton populations were

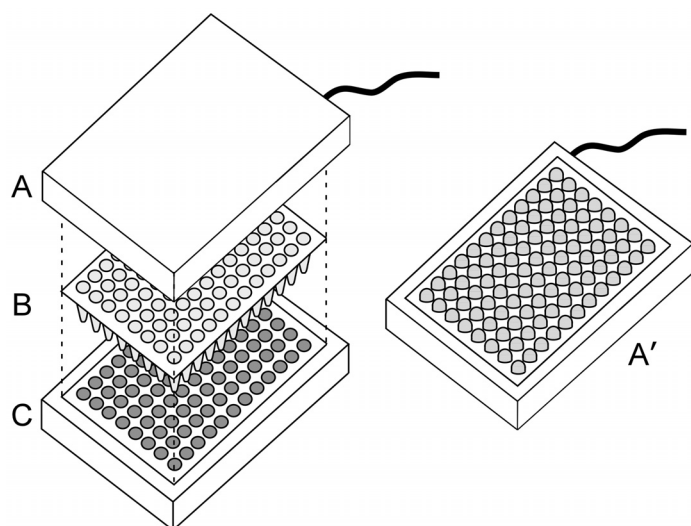


Fig. 1. Schematic diagram of the three major components of the microphotosynthesetron. (A) Lid containing 96 LEDs that acts as the light source, (B) 96-well black PCR plate used to hold the samples for incubations, and (C) Peltier device used to precisely regulate temperature of the PCR plate. During use, the three pieces are “sandwiched” together for optimal thermal conductivity and illumination. (A’ is the underside of the lid showing the 96 LEDs).

dominated by *Prochlorococcus* with significantly smaller contributions from *Synechococcus*, diatoms, and other phytoplankton.

Conventional photosynthesetron P-E curves—Replicate carbon-14-based photosynthesis-irradiance (P-E) measurements were made using custom-built temperature-regulated photosynthesetrons that have 13 positions, each at a different light level (Johnson et al. 1999). Light, which was supplied by a 250 W ENH projector bulb (Gray Supply), was attenuated and spectrally modified to match the spectral properties of the microphotosynthesetron using a combination of hot and cold mirrors (Optical Coating Laboratory) and neutral density screening (Roscolux). Photosynthetically active radiation (PAR) was measured using a 4π scalar irradiance meter (Biospherical Instruments - QSL-2101). Incubations of 1 mL, each inoculated with ~ 0.37 MBq H^{14}CO_3 were terminated after ~ 60 min (or ~ 120 min for open-ocean samples) with formaldehyde (3.1% final concentration), acidified to 0.1 N with HCl, and stored in the dark in the fume hood overnight. Carbon uptake rates were quantified using standard techniques (Barber et al. 1996) using the following equation:

$$\text{Photosynthesis (mgC m}^{-3} \text{ hr}^{-1}) = \frac{(\text{DPM}_{\text{sample}})1.05 * \text{DIC}}{\text{DPM}_{\text{tot}} * t} \quad (1)$$

where $\text{DPM}_{\text{sample}}$ is the amount of C-14 in the sample, DPM_{tot} is the total amount of C-14 added to the sample, t is incubation time in hours, 1.05 is the preferential uptake of C-12 over C-14, and DIC is the concentration of dissolved inorganic carbon, which here was assumed to be 24000 mgC m^{-3} in the waters sampled.

Microphotosynthesetron—Replicate C-14-based P-E measurements were made using a custom-built temperature-regulated micropho-

tosynthesetron (Fig. 1). The microphotosynthesetron is based on the standard 96-well plate format and uses 8 replicate rows each with 12 columns tunable to different light levels. Thus, a total of 12 different light levels can be generated, with each being replicated 8 times. In practice, we use 7 replicate rows with the final row reserved for the dark values, thus producing 13 point curves. Light is supplied from the top by blue (470/30 nm) light-emitting diodes (LEDs) (Panasonic - LNG901CFB). Each column is adjusted with a dial setting (Fig. 1A). PAR was measured using a quantum sensor (Li-Cor 190-SA). The quantum sensor was precisely positioned using a custom-built fitted collar to ensure that the sensor only received light from the well of interest. Temperature is regulated from the bottom with a Peltier device (Bio-Rad PTC0200) (Fig. 1C). Samples ($200 \mu\text{L}$), each inoculated with ~ 0.37 MBq H^{14}CO_3 , were placed in a HCl acid-cleaned (Fitzwater et al. 1982) black 96-well PCR-plate (VWR #83009-666) (Fig. 1B) and incubated for 60 min (or 120 min for open-ocean samples). Reactions were terminated with formaldehyde (3.1% final concentration), acidified to 0.1 N with HCl, and stored in the dark in the fume hood overnight. All pipetting was done using repeater or multichannel pipettes for increased speed. Radiocarbon incorporation was determined following standard techniques (Barber et al. 1996), and photosynthetic rates were calculated following Eq. 1.

P-E curve analyses—P-E curves were analyzed following Johnson and Barber (2003) using a custom-written optimization routine in MATLAB. Initial slopes (α) and light-saturated photosynthetic rates (P_{max}) of P-E curves were estimated from optimizations to a standard P-E model (Webb et al. 1974) and duplicate variables compared between the conventional and microphotosynthesetron approaches.

Assessment

Conventional photosynthesetrons typically use projector bulbs as a light source to create the light gradient. Most project bulbs (e.g., #ENH) installed in the conventional photosynthesetrons use 250 W of electricity. Because these bulbs produce a large amount of heat, a refrigerated water circulator is required to maintain temperature. The water circulator used in this experiment (VWR #1150S) uses 1560 W of electricity and has the cooling capacity to maintain temperature for ~ 3 photosynthesetrons at moderate temperatures ($\sim 20^\circ\text{C}$). By comparison, the microphotosynthesetron uses LEDs that require much less power; for all 96 positions of the microphotosynthesetron the total power consumption is 7 W. The Peltier device used to maintain temperature requires only 50 W for all positions. Thus, to generate six P-E curves, each of 13 points, conventional photosynthesetrons require ~ 4600 W, whereas the microphotosynthesetron requires only ~ 60 W. Since a typical electrical wall socket provides 1800 W, a minimum of 3 dedicated circuits is required to safely operate six conventional photosynthesetrons, whereas the microphotosynthesetron requires only one. Beyond saving electricity, these significant power savings are even more important for fieldwork (e.g., shipboard operations) where dedicated circuits are limited.

Many conventional photosynthetrons are custom-made and, therefore, their physical dimensions vary. However, as an approximation, the photosynthetrons used in this study have 13 positions and each requires $\sim 450 \text{ cm}^2$ of bench space. Each water circulator uses an additional 1750 cm^2 of floor space and is 64 cm high. For six photosynthetrons, a bench area of about 2700 cm^2 (or $\sim 90 \text{ cm} \times 30 \text{ cm}$) and floor space of about 3600 cm^2 (or $\sim 90 \text{ cm} \times 40 \text{ cm}$) is required. However, the microphotosynthetron only requires about 500 cm^2 (or $\sim 20 \text{ cm} \times \sim 25 \text{ cm}$) total. Thus, the space requirement for the microphotosynthetron is about an order of magnitude less than those for a comparable conventional photosynthetron system.

Like most conventional photosynthetrons, ours use complex light ray paths (including combinations of 90° hot mirrors, 45° cold mirrors, and screening) to produce the desired light quality and intensity. In addition, because light is not columnar at the source, accurately measuring light levels in conventional photosynthetrons requires a 4π scalar irradiance meter so that all angles are considered. However, because the light field is non-columnar, there can be light gradients within the incubation container. Taking these gradients into account, the precise placement of the collector sphere is crucial as small positional changes can influence the measured value. Conversely, the microphotosynthetron uses a simplified geometry to deliver light. Because the LEDs used have a half maximum luminous intensity angle of $\sim 25^\circ$ and are closely positioned to the sample ($\sim 5 \text{ mm}$), the majority of light is closer to being columnar. The use of black plates for incubation further reduces light scattering. As such, light is quantified using a cosine quantum sensor, and the measurements more reproducible. In addition to the actual measurements, LEDs provide a more stable light source because voltage is more easily regulated, and output is not substantially influenced by operating temperature. Conversely, the output of projector bulbs is influenced by power fluctuations (especially in the field) and changes in operating temperature. Thus, the microphotosynthetron light source is more stable and accurately quantified than for the conventional photosynthetron.

The P-E curves generated using the microphotosynthetron follow a classic P-E functional form (Blackman 1905; Jassby and Platt 1976; Johnson and Barber 2003) with an approximately linear increase in the low-light region, increasing to a maximal photosynthesis value at higher light levels (Fig. 2). The P-E curves are of high quality and follow a smooth functional form. A representative P-E model (Webb et al. 1974) optimized to microphotosynthetron P-E data are strongly correlated (mean $r^2 = 0.98$, $n = 36$) supporting the high data quality. Replicate microphotosynthetron P-E curves provide similar shaped P-E curves demonstrating the reproducibility of this approach (Fig. 2). For all of the replicate microphotosynthetron P-E curves generated, optimized P-E model parameters recovered were highly reproducible with an average %CV for α (13%) and P_{max} (11%) that were not significantly different ($P > 0.25$) from values measured for conventional photosyn-

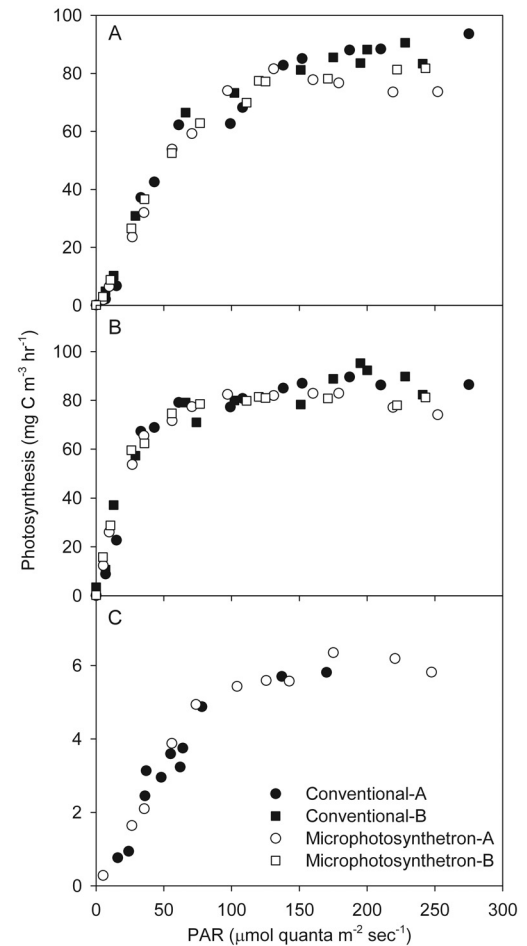


Fig. 2. Representative P-E curves measured using conventional photosynthetrons (filled symbols) and microphotosynthetrons (open symbols). Panel (A) *Prochlorococcus* MIT9312 at $100 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$, (B) *Prochlorococcus* MIT9312 at $30 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$, and (C) an environmental sample from the Western Pacific Ocean (32 25.3S, 159 3.4E) at 74 m ($\sim 6\%$ isolume). Conventional and microphotosynthetron P-E curves were run with the same number of light levels (points).

thetrons (α : 8% and P_{max} : 5%). These %CV values are near the minimum attainable for this size data set and the number of points on the P-E curve (Zimmerman et al. 1987). Overall, these results show that the microphotosynthetron generates high quality, reproducible P-E curves with errors of replication similar to P-E curves from conventional photosynthetrons.

The P-E curves measured using both the conventional and microphotosynthetron have similar shapes and magnitudes (Fig. 2). To quantitatively evaluate the two techniques, optimized model parameters were compared between the two types of curves (Fig. 3). Values of α are highly correlated ($r^2 = 0.96$) between the two types of curves and the means of the values are not significantly different ($P > 0.1$). Values of P_{max} are also highly correlated ($r^2 = 0.95$) between the two types of curves, and the means of the values are not significantly different ($P > 0.1$). Although the P-E curves from natural populations

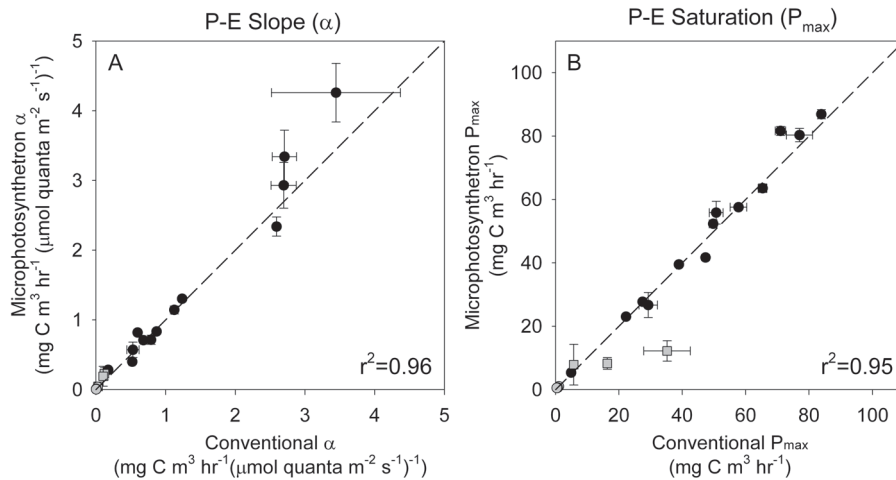


Fig. 3. Relationship between photosynthetic parameters, (A) α and (B) P_{\max} , derived from conventional and microphotosynthesetrons. Black circles are culture data and light gray squares are from environmental samples. The means of the values are not significantly different ($P > 0.1$) using a 2-tailed paired sample Student t test. Error bars represent standard deviations of duplicate measurements. The 1:1 line is plotted as a reference.

were less variable in the range of parameters recovered, these curves also exhibit similar relationships between the two types of photosynthesetrons. Thus there did not appear to be any dependency on physiological condition or sample origin. Overall the microphotosynthesetron-based approach generates data that is statistically identical to the conventional photosynthesetron and, therefore, the two techniques can be considered interchangeable.

Discussion

The development of the microphotosynthesetron was motivated by the desire to quickly and accurately measure multiple P-E curves simultaneously. Conventional photosynthesetrons can yield high quality data, and their utility has been demonstrated in numerous laboratory and field studies (Henley 1993; Marra et al. 2000; Sathyendranath et al. 1999). However, they have several shortcomings that limit their scalability and general utility. In particular, the size, power consumption, and difficulty of set-up make conventional photosynthesetrons an impractical tool when numerous P-E curves are required. These potential limitations are reduced because of the small physical size and reduced power consumption of the microphotosynthesetron. Further, because the microphotosynthesetron is designed around a standard 96-well microplate format, it has several advantages in terms of set-up and processing. First, setting the light levels in each well is trivial as the desired light level is “dialed in.” Unlike conventional photosynthesetrons, there is no trial/error system with neutral density screening. Light levels can quickly be adjusted between runs, something not possible for conventional photosynthesetrons. Second, multi-channel and repeater pipettes can be used to quickly and reproducibly load the microphotosynthesetron incubations wells. There is a large range of equipment and supplies, including robotic and automated systems, which are

specifically designed to increase sample throughput and reproducibility associated with the 96-well microplate format. The microphotosynthesetron leverages this equipment. Third, because the microphotosynthesetron is designed as a massively parallel incubator, replicate P-E curves can be generated to better constrain the values of the parameters recovered, or multiple cultured strains or environmental samples can be screened simultaneously for robust comparisons.

Beyond the improvements in set-up and sample throughput, the microphotosynthesetron has increased precision and accuracy for the environmental variables that it is simulating. The simplified geometry for the light field and simplified measurement improves the accuracy of quantifying the light levels. This is particularly important for low light levels, where small changes in the light field can dramatically influence the P-E curve (Johnson and Barber 2003). The precision and reproducibility of temperature regulation is also improved in the microphotosynthesetron. The Peltier device used in this study has temperature range of 4°C to 99°C, but other available models (e.g., Torrey Pines Scientific IC20XT) have a range of -20°C to 100°C, which cover the entire range of physiologically relevant temperatures. The thin-walled microplates used for the parallel incubations are specifically designed to conduct heat between the sample and the Peltier device, improving precision and accuracy. This compares with the conventional photosynthesetron where thick-walled vials, such as 20 mL polyethylene scintillation vials, are typically used that do not readily conduct heat (e.g., [Johnson et al. 1999]). This leads to differences between the temperature setting of the chilled water recirculator and the incubated sample and small differences in temperature can significantly affect photosynthesis, especially at higher temperatures (Eppley 1972).

Although there are some clear advantages to using the microphotosynthesetron to measure P-E curves, there may also

be some drawbacks. In particular, the microphotosynthetron uses a small volume format that assumes that the sample is homogeneous at the scale of these small volumes. This small volume homogeneity is a good assumption for monotypic laboratory cultures that are well-mixed. In addition, open-ocean samples that have a phytoplankton community dominated by unicell picoplankton are also likely homogeneous at the 200 μL volume scale (Cavender-Bares et al. 2001; Maranon et al. 2001). However, coastal marine environments with heterogeneous particle concentrations or areas that are dominated by large, chain-forming or mucous-producing phytoplankton (Hansen et al. 1995; Thornton 2002; Van Den Hoek et al. 1994) may violate this assumption. Indeed, the P-E curves from coastal marine sampling sites had significantly higher %CV than the P-E curves from cultured strains ($P < 0.01$). Open-ocean P-E curves most closely resembled cultured isolates and were not significantly different. Thus, for water samples that are expected to be heterogeneous at low volume scales, the advantages gained by the current microphotosynthetron approach may not compensate for the increased variability resulting from sample heterogeneity. This concern could potentially be addressed by increasing the number of replicates in each P-E curve, or by modifying the system from a 96-well based design to a larger volume well (e.g., 24, 14, or 9 well plates).

Another potential area of concern for the microphotosynthetron-based approach is related to the inoculum of C-14. Although the microphotosynthetron does reduce the volumes for the parallel incubations, it does not reduce the C-14 requirement. The same activity of C-14 is added to both the conventional photosynthetron and microphotosynthetron incubations, but the specific activity of the microphotosynthetron incubations is significantly higher because of the decreased volume. For large inocula, the C-14 "tracer" added as bicarbonate has the potential to appreciably alter the carbonate system, therefore violating the assumption that the addition is at tracer levels. The concentration of dissolved inorganic carbon is an important component to calculate the photosynthetic rate (Eq. 1). Many manufacturers provide C-14 bicarbonate at ~ 2 GBq/mmol, thus for the small incubation volumes of the microphotosynthetron, the added the "tracer" C-14 can significantly alter the DIC. For high-density cultures, this is not a concern because the amount of C-14 added is reduced because of the high biomass values. However, for open-ocean sites or other areas where photosynthetic biomass and rates are low, a larger inoculum must be used. For these sites, it is important to use a C-14 solution that has a sufficiently high specific activity to insure that the added C-14 is a small component of the total DIC pool.

There are other advantages to using the microphotosynthetron over conventional photosynthetrons beyond those realized for this study. For example, the microphotosynthetron reported here utilizes 8 replicate rows of 12 different light levels. However, alternative designs could control LEDs individually providing the capability of having 96 different light levels,

thus providing higher resolution along the light gradient to improve the estimation of α and P_{\max} (Zimmerman et al. 1987). Also, we have investigated using a more complex electronic circuit to fluctuate light during the course of the incubation to determine how light variability influences photoacclimation (Farmer and McNeil 1999; Lewis et al. 1984). Other colors of LEDs, of either narrow or broad spectrum, may be used to investigate the spectral response of photosynthesis (Rivkin 1989; Schofield et al. 1996) or associated properties such as enhancement effects (Emerson and Lewis 1943; Johnson 2000). Another potential advantage of the microphotosynthetron associated with the 96-well microplate format is for the quantification of incorporated C-14. Presently, there are multiple models of filter microplates and scintillation counters that are capable of directly quantifying C-14 from a microplate. In the current protocol, the rate-limiting step is the C-14 counting, thus using this technology would further enhance sample throughput. In short, the microphotosynthetron-based approach for generating P-E curves has the potential for further improvements to provide for additional functionality or to increase sample throughput.

The microphotosynthetron offers several advantages over conventional photosynthetrons for generating P-E curves to understand photosynthetic physiology. In particular, the microphotosynthetron increases sample throughput while providing a higher level of precision and accuracy for the incubation conditions (temperature and light). It does not obviate the need for use of C-14, which can be highly regulated in some jurisdictions, but this general approach remains the most sensitive and accurate technique for directly measuring photosynthesis. Because it uses small volumes, care must be taken when using the microphotosynthetron to ensure that the sample is homogeneous at the scale of incubation, a consideration for all types of comparative incubation techniques. Further care needs to be taken to ensure that the C-14 inocula do not significantly modify the carbonate system of the sample. Given these precautions, we recommend the microphotosynthetron for measuring P-E on monotypic laboratory strains and open-ocean phytoplankton populations. The advantages associated with this approach to understanding the photo-physiology of phytoplankton will help lead to a better understanding of the variability and regulation of photosynthesis in the aquatic environment.

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Submitted 15 May 2007

Revised 29 August 2007

Accepted 10 September 2007