Net photosynthetic growth as controlled by dynamic light regimes

CHRISTOPHER D. HEWES*
MARINE BIOLOGY RESEARCH DIVISION, SCRIPPS INSTITUTION OF OCEANOGRAPHY, UC, SAN DIEGO, LA JOLLA, CA 92038-0202, USA

*CORRESPONDING AUTHOR: chewes@ucsd.edu

Received March 28, 2017; editorial decision September 6, 2017; accepted September 7, 2017

 Corresponding editor: Pia Moisander

The photosynthetic response of the marine diatom Thalassiosira pseudonana to a matrix of dynamic light regimes is described. Ash-free dry weight and chlorophyll-a were measured as a function of dynamic irradiance having maximum intensities of 250, 500, 1000 and 2000 μmol photons m⁻² s⁻¹ with lengths of day being 6, 9, 12, 18 and 24-h periods. Incident irradiance followed a Gaussian (normal) distribution, which provided a statistical standard for the integrated quantum flux into a 20 cm water column. The matrix of conditions resulted with growth occurring in three groups (low, high and intermediate light) as a function of the amount for residual irradiance estimated at the mixing depth of 20 cm. The low light group displayed shade-limited (“linear” from self-shading), and the high light group displayed light-limited (“exponential” with no self-shading) phases of the light-controlled growth dynamic. For the same range of integrated daily incident irradiances, cultures growing under the phase of “linear” growth had higher biomass yields per quantum than cultures growing under an “exponential” phase of the growth dynamic. The difference in net quantum efficiency due to phase of the growth dynamic entails the relationship between photic zone and mixing depth, and provides a new perspective for interpreting Sverdrup’s Critical Depth Model.

KEYWORDS: dynamic light regime; critical depth; compensation depth; quantum yield; SolarStat™; Thalassiosira pseudonana

INTRODUCTION

The photosynthetic growth dynamic of the marine diatom Thalassiosira pseudonana in a substrate (carbon and nutrient) replete environment can be described as having exponential-to-linear-to-stationary phases of growth in batch culture under a constant illumination regime (Hewes, 2015a, 2015b, 2016a). The phase of exponential growth occurs when cell density is low so that a quantum flux is in excess (regardless of light intensity) for the culture cell density, and the rate of growth is constant. Light-limiting, exponential growth is known as Blackman kinetics. The stationary growth phase occurs when the quantum flux (regardless of light intensity) is only sufficient for maintenance metabolism; the carbon fixed by photosynthesis is
equaled to that lost through respiration for the entire culture, and no net growth results. Between exponential and stationary phases of growth is the phase of linear growth for substrate-replete culture. During this phase, rates of net production (P) become constant as dependent upon the incident quantum flux, while specific rates of growth (µ) decline as a power to the arithmetic increase of biomass (B) retained by the cells, and µ = P/B (Hewes 2015a, 2015b, 2016a). Growth rates during the phase of linear growth, therefore, become limited by the inverse of biomass (i.e. “shade-limited”) contained within some volume that is mixed. The entire light-controlled growth dynamic from exponential through stationary phases can be observed for cultures grown under sub-optimal (i.e. “light-limiting”) intensities (Hewes, 2015a). In this context, the term “shade-limited” is used to describe growth that occurs during algal self-shading in a water column, and results with the linear phase of the light-controlled algal growth dynamic (light availability is limiting). Therefore, “light-limited” growth will here be referred to as a related phenomenon that occurs during Blackman kinetics (light intensity is limiting; see Introduction in Hewes, 2016a).

For algal culture, any measurable irradiance usable for photosynthesis above that is required for “maintenance metabolism” should be considered as being in excess, and this describes the photic zone as that region of the water column where excess light occurs (including irradiances below saturation of photosynthesis). During the transition between exponential to linear phases of growth for T. pseudonana under constant illumination and 20 cm mixing depth, all but 4–5 μmol photons m⁻² s⁻¹ of the quantum flux for photosynthetically active radiation (PAR, 400–700 nm) entering the culture is absorbed; this flux being dependent upon the spectral components remaining of the irradiance (Hewes, 2015a; 2016b; also see Platt and Jasby, 1976; Huismann, 1999; Sinetova et al., 2012). This ~5 μmol photons m⁻² s⁻¹ represents the energy requirements of the culture for maintenance metabolism, and the depth at which this intensity occurs will be considered “compensation” depth. Changes in the dynamic of culture growth from exponential to linear phases correspond as when compensation depth becomes equal with the mixing depth (Hewes, 2016b). A depth that the culture is mixed, within which a photic zone of all consumable irradiance for growth is contained, thus characterizes shade-limited conditions through self-shading to provide the phase of linear growth with constant illumination. In contrast, exponential growth occurs when the photic zone exceeds mixing depth; irradiance in the mixed layer (regardless of intensity that controls µ) is in excess of the minimum required for maintenance of the culture. Obviously, there comes a “critical” point where linear growth stops and the stationary phase begins (see Fig. 2 in Hewes, 2016a).

However, such simple interpretation of the growth dynamic in batch culture under constant illumination becomes confused when interpolating toward a dynamic light regime, such as would be encountered in natural, outdoor environments. Under a dynamic light regime such as the diel solar cycle, incident irradiance will result in a considerable variation in penetration depth of given intensity through the water column over a 24-h period. Assuming conditions under constant illumination, exponential growth would result when the photic zone is greater than depth of mixing, while linear growth would result when depth of mixing is greater than the photic zone. Yet, since the solar cycle dynamic might have exceedingly bright irradiance at noon and darkness at midnight, what defines photic zone depth when there is such high variability for intensity within the surface mixed layer? Photosynthetic rate certainly has a dynamic that follows the solar cycle, so an exponential growth phase cannot be expected to have a growth rate that is constant, as is the case for non-dynamic (constant) light intensity. Is it the average of light exposure in the surface mixed layer over a 24-h period, or maybe just values as obtained at noon, that defines compensation depth for the population in this water column? Also, does the light-controlled growth dynamic and compensation depth have any consequence for the efficiency of net primary productivity under such conditions? Most importantly, how can such dynamics be studied in a quantitative manner to begin to answer these questions?

The diel solar cycle, coupled with fluctuating light due, for example, to surface water turbulence, are likely the most influential environmental variables for phytoplankton growth in the natural world (Kirk, 1994; Iluz et al., 2012), even under nutrient-deplete conditions. Classical studies have entailed on/off (square wave) illumination with periods ranging between hours (i.e. “day length”) to milliseconds (i.e. “flashing light”). However, these are quite artificial conditions compared with, for example, the solar cycle having a pattern of incident irradiance during daytime likened to a truncated sinewave (Kirk, 1994). A few laboratory studies of natural dynamic irradiance patterns have been examined for their influence upon algal growth, but are difficult to compare because of the complexities imposed by the dynamic of light itself (for lists of historical studies involving dynamic and fluctuating light, refer to Litchman, 2000; Iluz et al., 2012; Hewes and Hewes, 2016; Jallet et al., 2016). Furthermore, even with the extremely limited number of laboratory studies of dynamic irradiance regimes to date, there is increasing evidence that the kinetics of net photosynthesis under such light climates are different from those as obtained with constant (non-dynamic) irradiance conditions.
Havelková-Doušková et al., 2004; van de Poll et al., 2007; Ibuz et al. 2012, Hewes and Hewes 2016; also see Flynn and Raven, 2017).

For laboratory studies of shallow water columns, conditions as found, for example, in the synthetic environments of raceway ponds, it was suggested that the dynamic of irradiance be modeled to follow a Gaussian (normal) distribution (Hewes and Hewes 2016). Although a Gaussian pattern does not describe a diurnal solar dynamic in its strictest sense, the distribution itself is central to statistical theory and concerns the area under its curve. This later point is emphasized, since water column studies that involve the phase of linear growth assume all light available for photosynthesis above maintenance is consumed (Hewes, 2015a, 2015b, 2016a, 2016b), and standard deviation ensures the proportion of integrated light that culture is exposed as independent of photoperiod (Hewes and Hewes, 2016). Although the sine equation is often used to model the solar dynamic, this too is artificial. A “truncated” sine might best describe downwelled (cosine) irradiance at the earth’s surface, but sun angle (among other things) changes how light penetrates a surface layer, and also it is preferable to measure underwater light for photosynthesis in terms of scalar irradiance (Kirk, 1994). For a shallow water column, the irradiance dynamic having a Gaussian distribution therefore allows the integrated light to be treated as a statistic that can be dissected and compared across a variety of conditions (Fig. 1). For example, time during the progress into the photoperiod can be replaced by amount of standard deviation; thereby the proportions of the daily-integrated light exposure per fraction of standard deviation remain the same when the photoperiod of the dynamic irradiance varies. Hence, the photosynthetic response by algae of a water column can be measured in relation to a dynamic light regime that is normalized regardless of photoperiod or intensity, with biological similarities and/or differences discerned from such normal variability defined of an environmental factor.

Hypothesized here is that the manner light is delivered to the water column influences the algal growth dynamic and related net productivity; this manner being the combination of photoperiod and maximum irradiance. A general assumption in phytoplankton ecology is that primary productivity for the water column is in proportion to the absorption of light by cells as integrated through that water column over a period of time (Platt et al., 1991). This has bearing on Sverdrup’s (1953) Critical Depth Model, by which integrated primary productivity (governed by photo-biological absorption of light) is greater than integrated “respiration” within a wind-mixed surface layer for body of water in order to effect blooming. However, what has been neglected in discussions of photosynthesis, light absorption in the water column, and critical depth is the manner irradiance is delivered and the phase in growth dynamic the algal population is in. The same daily-integrated irradiance can be obtained with high intensity over short duration, or low intensity over long duration. Reported here are results of an experimental matrix study whereby semi-continuous culture growth rate was held as a constant mean to provide differences in water column productivity as reflected in concentrations of ash-free dry weight (AFDW) and chlorophyll-a (Chl-a). Incident irradiance was modeled after a Gaussian distribution that allowed normalized portions of photoperiods to be scrutinized. Growth was compared as a function of dynamic light regimes that varied both in maximum intensity and duration throughout the 24-h day.

**METHODS**

The SolarStat™ (Hewes and Hewes, 2014) was used to grow cultures of the marine diatom *T. pseudonana* under dynamic light (Hewes and Hewes, 2016). Culture was irradiated from the bottom by light emitting diode (daylight) as focused through a 20 cm water column (172 ml), and held at 22 ± 1°C. The light climate was dynamic, with incident irradiance following a Gaussian (normal) distribution having maximum intensities of PAR at 250, 500, 1000 and 2000 μmol photons m−2 s−1, determined by *in situ* PAR sensor (QSL-100, Biospherical Instruments, San Diego); these maximum intensities were of scalar irradiance, measured in distilled water from the inside bottom of the culture vessel, and with irradiances

![Fig. 1](https://academic.oup.com/plankt/article-abstract/39/6/930/4209328){:width=600px}  
**Fig. 1.** Pattern of dynamic irradiance modeled by a Gaussian (normal) distribution. Example of the incident irradiance for a 12-h day having maximum PAR of 250, 500, 1000 and 2000 μmol photons m−2 d−1 (refer to inset for line descriptions). Day length = ±3 standard deviations from high noon, while ±1 (vertical dashed line) and ±2 (vertical stippled line) standard deviations are indicated.
attenuated by neutral density screens from 2000 μmol photons m$^{-2}$ s$^{-1}$. Hence, programming of the SolarStat™ involved 0–100% irradiance to maximize the number of bits that described the Gaussian distributed incident light, and thus minimize the step difference per bit between changing light levels to <1, 2, 4 and 9 μmol photons m$^{-2}$ s$^{-1}$ for the 250, 500, 1000 and 2000 μmol photons m$^{-2}$ s$^{-1}$ maximum intensities, respectively. Thereby, the proportion of the step change in irradiance was made the same in relation to the maximum irradiance exposing the cultures. The length of day was ±3 standard deviations for a Gaussian distribution, being 6, 9, 12, 18 and 24 h of irradiance; at the ±third standard deviation, irradiance occurred as 1% maximum incident PAR. Except for the 24-h cycle, irradiance went to 0 μmol photon m$^{-2}$ s$^{-1}$ at ~≤0.5% maximum incident PAR (Hewes and Hewes, 2016).

Double strength Guillard’s F-medium (ProLine® F/2 Algae Feed, Aquatic Eco-Systems, Inc.) with silicate added in Guillard proportions, as based on aged local seawater, and pH adjusted to ~7.8, was used to ensure nutrients were not limiting algal growth (Hewes, 2015a, 2015b). Algae Feed, Aquatic Eco-Systems, Inc.) with silicate was combusted by natural gas flame and weighed to obtain weight of remaining ash and salt. The difference between weights of the oven dried (whole biomass) and TD-700). To measure AFDW, samples (20–50 ml depending upon cell concentration) were filtered onto pre-weighted, oven-dried (100°C, overnight) 2.4 cm Whatman GF/F glass fiber filters. The filtered material was then dried overnight at 100°C, and afterwards weighed by analytical balance (Mettler, model B3, Greifensee, Switzerland). Subsequently, the filters were combusted by natural gas flame and weighed again to obtain weight of the combusted (ash) material. Whatman GF/F filters were used for nutrient replete conditions, it is assumed that C:N and C:P did not vary, and that C ≈ 50% AFDW.

Hewes (2016b) described the optical characteristics of T. pseudonana, with cultures grown under similar conditions (but constant light) as for the current study. Attenuation of in situ scalar PAR for the cultures at mixing depth (20 cm) was calculated from Chl-a concentration providing the percent transmission (%T) extrapolated to 20 cm (i.e. mixing depth) as follows:

\[
%T = (-0.1905 \text{Ln}(\text{Chl-a}) + 0.4588)^4
\]

for a phase of exponential growth, and

\[
%T = (-0.1289 \text{Ln}(\text{Chl-a}) + 0.3513)^4,
\]

for a phase of (shade-limited) linear growth, with Chl-a concentrations in mg l$^{-1}$ (Hewes, 2016b). With the null hypothesis of linear growth, and that mean growth rate was one doubling d$^{-1}$, the increase of Chl-a concentration for the second midnight was equal to the first midnight (Hewes 2016a), and %T can be estimated for each midnight. The %T of the first and second midnights were used to estimate residual light at 20 cm, with the differences between 20 cm and incident irradiance intensities used to calculate number of quanta absorbed. The quanta absorbed of both midnights (at 24 and 48 h) were averaged to obtain the amount absorbed the second day.
Similarly, the amount of AFDW produced the second day was estimated from the difference between the first and second days (assuming linear growth). Both estimated quanta absorbed and AFDW produced the second day were used to calculate quantum yield for net biomass (g AFDW (mol photon)$^{-1}$).

**RESULTS**

For all lengths of day, maximum incident irradiances, and daily values (Table I), Chl-$a$ concentrations increased at ~2.5% relative to AFDW concentrations (Fig. 2a). Therefore, the Chl-$a$ quota (to AFDW) increased in a curvilinear fashion with biomass that reached a plateau of ~2.5% (Fig. 2b). Since mean growth rates were the same for all cultures (1 doubling d$^{-1}$), the Chl-$a$ quota (to AFDW) increased with the increase of net primary productivity (i.e. a function of biomass) for these cultures. Since the Chl-$a$ quota (to AFDW) was the same at start and harvest for each of the different cultures (i.e. being a semi-continuous method), it might be assumed that little change occurred during the cycle period.

Dynamics of the irradiance to which growing cultures were exposed can be understood in statistical terms. Since a defined maximum irradiance occurred at high-noon having ±3 standard deviations for the duration of the light dynamic (Fig. 1), the total light flux that exposed cultures for a water column is the area beneath the Gaussian curve during the phase of linear growth. When Chl-$a$ concentration is known, the photon flux at depth is predicted (see Methods), and because the pattern of irradiance at depth nearly follows a Gaussian distribution (increasing pigment concentration with depth will skew the spectral distribution),

| Day length (h) | PAR$_{max}$ (μmol photons m$^{-2}$ s$^{-1}$) | End of Day 1 | End of Day 2 |
|---------------|------------------------------------------|-------------|-------------|
|               | Daily Incident PAR (mol photons m$^{-2}$ d$^{-1}$) | 24 h average 20 cm PAR (μmol photons m$^{-2}$ s$^{-1}$) | 24 h average 20 cm PAR (μmol photons m$^{-2}$ s$^{-1}$) |
|               | 2000 1000 500 250 | 2000 1000 500 250 | 2000 1000 500 250 |
| 6             | 18 9 4 2 | 14.8 17.9 7.4 7.2 | 9.1 12.2 4.9 5.1 |
| 9             | 27 13 7 3 | 3.7 10.5 8.6 15.7 | 1.7 6.4 5.6 11.6 |
| 12            | 36 18 9 4 | 1.4 1.8 1.3 4.3 | 0.4 0.6 0.8 2.7 |
| 18            | 54 27 13 7 | 2.2 1.9 1.5 2.9 | 0.7 0.7 0.6 1.6 |
| 24            | 71 36 18 9 | 2.6 1.5 2.6 0.9 | 0.8 0.5 1.1 0.4 |

**Fig. 2.** The ranges of Chl-$a$ and AFDW measured of cultures under the different dynamic light climates. (a) Mean ± standard deviation (error bars) of Chl-$a$ relative to AFDW. (b) The Chl-$a$ quota (to AFDW) has a curvilinear increase with AFDW.
the amount of light exiting the water column at 20 cm over a 24-h period can also be estimated. The difference between incident irradiance and the residual at 20 cm is the amount of PAR absorbed of the water column. Since the incident irradiance for all cultures of this experimental matrix followed a normalized (Gaussian) distribution, the biological responses to this light climate (i.e. Chl-a and AFDW concentrations) were unbiased with respect to the photoperiod and/or intensity as applied.

As a rough guide for the light attenuated at depth, the Chl-a concentration measured at harvest of the cultures can be used to estimate %T at 20 cm depth. Since mean growth rate was 1 doubling d⁻¹, 50% of the total increase in Chl-a occurred at midnight on the first of the 2-day cycle period, the other 50% on the second day, assuming the linear phase of growth. The Chl-a values were used to estimate residual PAR at 20 cm from %T for the first and second days, and the results group into high, low, and intermediate light categories (Table I). The maximum PAR at 20 cm vs. integrated daily PAR (Fig. 3) also clustered these data into three groups. Going one step further, the ±1 standard deviation occupies 68% of the total area under the Gaussian distribution, while taking ~33% of total daylight hours (Fig. 1). The ± 1 standard deviation for PAR of the 20 cm depth (StD₂₀) based on Chl-a concentration at harvest also resolves a clustering into three groups of values for the second day. Although the total daily PAR covered a very large range of values (Table I), the mean of StD₂₀ for the second day was narrowly focused at 59 ± 27 μmol photons m⁻² s⁻¹ for what will be called the “high light”, 2 ± 1 μmol photons m⁻² s⁻¹ for that to be called the “low light” and 9 ± 4 μmol photons m⁻² s⁻¹ for that to be called the “intermediate light” groups. The high light group consisted of those cultures grown with short day lengths, and the low light group consisted of those cultures grown with the longest day lengths, and these groupings were not necessarily related to maximum incident PAR or daily incident PAR (Table I).

Both Chl-a (Fig. 4a) and AFDW (Fig. 4b) increased with rising daily incident PAR. However, the high light group contained the lowest concentrations with an
apparent linear relationship(s) ($r^2 > 0.79$), whereas the low light group contained the highest concentrations with an apparent curvilinear relationship(s) (log-linear relationships drawn in Fig. 4, $r^2 > 0.73$). With all groups considered together, both Chl-a (Fig. 5a) and AFDW (Fig. 5b) had negative curvilinear relationships to the mean PAR within StD$_{20}$. However, neither high light nor low light groups were correlated with PAR within StD$_{20}$, with the curvilinear pattern reflecting their respective grouping (i.e. Fig. 3), and each group lacking a correlation with incident irradiance. These data are indicative that the amount of PAR available at 20 cm for each of the cultures is a function of light absorbance through the water column, and since average growth rate for all of the cultures was 1 doubling d$^{-1}$, this absorbance is related to net productivity of the culture as a function of its biomass. Thus the high light group had the lowest biomass resulting with little attenuation and high ambient irradiance, compared with the low light group having highest biomass resulting with greatest attenuation and low ambient irradiance.

Since the relationships of both Chl-a and AFDW behaved differently between daily incident PAR (Fig. 4) and mean PAR of StD$_{20}$ (Fig. 5) for each of the three groups, their respective Chl-a quotas (to AFDW) did so as well (Fig. 6). The Chl-a quota (to AFDW) was significantly ($t$-test, $P < 0.001$) lower ($1.2 \pm 0.4\%$) for the high light group than for the low light group ($2.4 \pm 0.4\%$) with respect to daily incident PAR. No relationship between Chl-a quota (to AFDW) and daily incident PAR was found for the low light group, while only a slight positive increase was found for the high light group ($r^2 = 0.36$; Fig. 6a). However, a negative curvilinear relationship (power function, $r^2 = 0.71$) occurred between Chl-a quota (to AFDW) and mean PAR for StD$_{20}$ when all groups were considered together (Fig. 6b). This was the result of neither high light nor low light groups having a Chl-a quota (to AFDW) correlated with mean PAR for StD$_{20}$.

Collectively, the data presented in Figs 2–6 indicate the amount of residual light at 20 cm depth discriminates between the net productivity for cultures as independent of total photon flux (Table I) and mean growth rate. Since the daily light flux both at incident PAR and 20 cm can be calculated for midnight of each day from start (Table I), the mean between these amounts of daily quanta absorbed were used to provide number of quanta absorbed for the water column the second day (see Discussion). From AFDW and absorbed quanta, a net quantum yield can be estimated (Fig. 7a). For both high light and low light groups, quantum yield for net AFDW declined in non-linear fashion with increased irradiance. However, the low light group had ~70% higher net quantum yield than the high light group for the common range of daily incident PAR $5–20$ mol photons m$^{-2}$ d$^{-1}$ as based on regression coefficients. This occurred as a function for the combination of maximum incident irradiance and day length, while independent of total daily incident quantum flux (Table I) and mean growth rate.

**DISCUSSION**

**General considerations**

For this study, *T. pseudonana* cultures were grown under a range of dynamic light regimes. They were cultivated in shielded tubes, irradiated only from the bottom, and

![Fig. 5. Mean Chl-a and mean AFDW as a function of mean PAR for ±1 standard deviation at 20 cm depth (mixing depth). With all data in consideration, both Chl-a (a) and AFDW (b) concentrations decreased in curvilinear fashion (lines) with the increase in mean StD$_{20}$ PAR, however, no light group by itself had any relationship to mean PAR for StD$_{20}$, with the apparent relationships a result of their clustering (i.e. Fig. 3). Symbols are as in Fig. 3.](https://academic.oup.com/plankt/article-abstract/39/6/930/4209328/fig?FIGREF1)
had coherent light passing upwards through a 20 cm water column. This way, irradiance through the water column was greatly attenuated between the exposed surface (being incident irradiance) and depth forming a light gradient. The cells were mixed by sparging with air, with the result that individuals passed through this light gradient in a 2–4 s cycle. Thus, the light climate encountered by individual cells within the culture was a rapid (i.e. seconds) fluctuating light regime superimposed upon a dynamic light regime having periodicity measured in hours. It is not known what effect on growth fluctuating light of this frequency has on productivity (see Laws et al., 1987; Iluz et al., 2012), being much too slow to include the flashing light effect (Terry, 1986) and much too rapid to impose physiological changes associated with mixing through a wind-mixed layer (Litchman, 2000; Havelková-Doušová et al., 2004; Wagner et al., 2006; van de Poll et al., 2010). However, this fluctuation in light regime was the same for all cultures in this study.
The semi-continuous cultures had a mean growth rate of 1 doubling d\(^{-1}\) (\(\mu = 0.69\) d\(^{-1}\)), utilizing a cycle period/harvest frequency of 75% for harvest and 25% for dilution to the starting volume every other day. The growth dynamic between start and harvest of shade-limited, semi-continuous culture under constant illumination resembles that of the linear growth phase in batch culture under shade-limited conditions (Hewes, 2016a). In this case, AFDW and Chl-a increase arithmetically with time between start and harvest under constant illumination, while the rates of growth decline as a power of AFDW (Hewes 2015a, 2015b). Thus, under constant illumination, \(\mu\) is predictable for semi-continuous culture, with the mean rate as the average of all rates encountered between start and harvest (Hewes, 2016a). However, the current study did not utilize constant, but rather, dynamic irradiance.

Under a dynamic light regime, it would be expected that photosynthetic rates vary as a function of light intensity that changes through duration of the day. Hewes and Hewes (2016) described growth during the linear growth phase under an incident light regime having a Gaussian distribution. They compared batch culture with semi-continuous culture under shade-limited growth, and found productivity to vary with semi-continuous culture as a function of mean \(\mu\), but growth was still predictable. Namely, that \(\mu\) might vary with light intensity during the time of day, however, growth was “linear” between periods of cycle for the dynamic illumination (also see Hewes, 2016a). In other words, with a mean growth rate at 1 doubling d\(^{-1}\) during a phase of linear growth and having a harvest frequency of two days (this experiment), 50% of the AFDW and Chl-a concentration increase occurred the first day (sampled at apparent midnight), and a 50% increase the second day (again, sampled at apparent midnight). During the linear phase of growth in batch culture, as is similar with semi-continuous culture, biomass increases arithmetically with time (mean net productivity is constant), and only the instantaneous \(\mu\) varies. For semi-continuous culture under a constant light regime and during shade-limited growth, change in the cycle period time influences the range of instantaneous \(\mu\) that the culture experiences, while keeping the same mean \(\mu\) (Hewes, 2016a). This occurs because all the quanta usable for net photosynthesis are consumed, and net productivity is in proportion to the daily, depth-integrated flux of incident irradiance. With the null hypothesis that all cultures for this current study were experiencing shade-limited (linear) growth, the attenuation of light for any depth, being depth of mixing for the culture (see Methods) reported here, is also predictable for T. pseudonana culture based on Chl-a concentration for a given day (Hewes, 2016b; Table 1).

The Chl-a concentration for cultures at apparent midnight can be used to get an idea of the light climate during the photoperiods of cultivation (Table 1). For the first day (24 h) at 20 cm depth, the high light group averaged an estimated 11.7 ± 4.4, the low light group 1.9 ± 0.9, and the intermediate group 3.6 ± 0.7 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\). For midnight the second day (48 h) at 20 cm depth, the high light group averaged an estimated 7.8 ± 3.1, the low light group 0.7 ± 0.5, and the intermediate group 2.0 ± 0.6 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\). These average values would be higher if high noon (12 and 36 h) Chl-a concentrations, rather than those from midnight, had been considered, but calculation of high noon concentrations is not self evident for light regimes that vary during the 24 h period.

Yet, a high noon estimate of residual PAR at 20 cm depth for the second day might be assumed halfway (i.e. their average if growth is linear during the photoperiod) between each of the midnight values (Table 1) yielding 24-h averages of 9.8, 1.3 and 2.8 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) for the high, low and intermediate light groups, respectively. However, it is not known at this time what dynamic of growth occurs during the daytime having a dynamic light climate (however, see Jallet et al., 2016). Nonetheless, since cultures are growing, attenuation at high noon second day would be between those values for midnight of each day (Table 1); furthermore, since 25% of harvest was used to start the cultures, a similar estimate could be made for high noon of the first day. Thus, low light and high light groups distinguish themselves regardless of whether Chl-a concentrations were measured at midday or midnight. If linear growth occurs when all PAR minus that required for maintenance in the water column is consumed, and maintenance requires \(\sim 5 \mu\)mol photons m\(^{-2}\) s\(^{-1}\) under similar growth conditions (Hewes 2016b; also see Jasby and Platt, 1976; Huisman, 1999; Sinetova et al., 2012), the high light group occurred with 24-h averaged PAR higher and the low light group lower at 20 cm than an intensity as necessary to result with linear growth (Table 1). The daily averaged PAR at 20 cm for the intermediate light group on the first day was a borderline for intensities of maintenance.

**Phase of growth dynamic a function of irradiance delivery**

Since the high light group contained average daily PAR at 20 cm depth (\(\sim 10 \mu\)mol photons m\(^{-2}\) s\(^{-1}\)) for 24 h average with high noon, second day; \(\sim 60 \mu\)mol photons m\(^{-2}\) s\(^{-1}\) for Std\(_{20}\), second day; Fig. 3; Table 1) much higher than required for maintenance, the photic zone was greater than the depth of mixing, and PAR was in...
excess as averaged over 24 h during both days of growth in the cycle period. Therefore, cultures of the high light group must have experienced Blackman (light-limited, “exponential”) growth kinetics. For cultures of the high light group, growth could not be undergoing shade-limited dynamics whereby biomass otherwise would increase “arithmetically”. In contrast, the low light group contained a PAR at 20 cm depth below that necessary for maintenance metabolism (~1 μmol photons m$^{-2}$ s$^{-1}$ for 24-h average with high noon, second day; ~2 μmol photons m$^{-2}$ s$^{-1}$ for StD20, second day; Fig. 3; Table 1), and PAR was limited via photic zone depth during both days of growth in the cycle period, therefore, cultures were experiencing a shade-limited (“linear”) growth dynamic. This being the case, the intermediate group was possibly growing “exponentially” on the first day, and “arithmetically” on the second day of the cycle period.

That the high light group must be growing “exponentially” disproves the null hypothesis that growth of all cultures was in the linear phase of growth. The difference in attenuation for T. pseudonana between phases of linear and exponential growth was suggested to occur due to packaging effects in the chloroplasts that decreases absorbance per Chl-a, while total cellular concentrations increase, during shade-limited growth (Hewes, 2016b). Re-calculation of PAR at 20 cm to account for exponential growth (see Methods) of the high light group would not change groupings of the cultures, since the high light group would have higher irradiances than presented in Table 1 and some figures.

However, a lower absorbance would affect the quantum yield for net biomass of the high light group (Fig. 7a), since less light was absorbed for growth attained. Therefore, the Chl-a and AFDW concentrations for the high light group at midnight for the first day were re-calculated based on exponential growth (rather than linear growth), with the differences of the absorbed integrated PAR (as described above) for exponential growth used to obtain the corrected quantum yields for net biomass (Fig. 7b). This correction increased quantum yield for net biomass of the high light group ~50% above uncorrected values, but these were still ~25% (based on regression coefficients) below the values for the low light group in the range 5–20 mol photons m$^{-2}$ d$^{-1}$. These results are put in the perspective of a previous finding (Hewes, 2015b), that under non-dynamic light, net productivity of semi-continuous culture is higher than continuous culture for given mean, sub-maximum $\mu$. Continuous culture is forcing exponential growth, while semi-continuous culture can replicate a linear phase of the light-controlled growth dynamic.

Instantaneous growth rates for all the cultures were dynamic as a function of the diel cycle for light intensity, and therefore do not fit the classical definitions for phases of the light-controlled growth dynamic (Hewes, 2013a); instantaneous $\mu$ was not constant for the “exponential” phase, and not declining as a power of biomass for the “linear” phase. Rather, and important from an ecological perspective, the photosynthetic growth dynamic can be classified based on the optical characteristic of the water column, and not some instantaneous behavior of individuals for a parcel of water circulating in the surface mixed layer. In other words, if the depth of the photic zone is less than the depth cells are mixed in a uniform Chl-a concentration of nutrient replete water (i.e. compensation depth is shallower than mixing depth), the population experiences shade-limited (“linear”) growth if growth occurs, and otherwise it does not. Therefore, the phase of the growth dynamic for a population in a turbulent mixed layer (in sensu Huisman et al., 1999; e.g. uniform Chl-a concentration in the wind-mixed layer), of nutrient replete water column, appears to be dependent upon the compensation depth and photoperiod, and likely independent of $\mu$.

### Critical depth and growth dynamics

It is generally considered that light-limited (Blackman) growth kinetics, determined with optically thin cultures under laboratory photosynthesis/irradiance (PE) analytical conditions, can be used to model the growth of algal cells during excursions (via turbulence) through a surface layer for a body of water that is deeper than a photic zone (Platt et al., 1991; MacIntyre et al., 2000). But, it should also be apparent (Fig. 7) that a large difference in photophysiology occurs between shade-limited and light-limited growth. Such difference is further reflected in the Chl-a quota (to AFDW; Figs 2b and 6). This difference is likely due to packaging within the chloroplasts and the increase of cellular Chl-a that occurs during the transition between exponential and linear growth (Hewes, 2016b). Difference in photosynthetic yield that occurs between shade-limited and light-limited growth as functions of irradiance dynamic and phase of the growth dynamic may change how we interpret such models, but also might add a new perspective to understand Sverdrup’s (1953) Critical Depth Model.

For the current study, the depth of mixing (i.e. “turbulence” as per Huisman et al., 1999; Franks, 2015; at 20 cm) and average growth rate (1 doubling d$^{-1}$) were both held as experimental constants. The dynamic of irradiance was also fixed, being modeled as a Gaussian distribution for incident light, and therefore the proportions of low-to-high illumination within the irradiance
dynamic were the same regardless of maximum irradiance or length of day (i.e. the “68, 97 and 99.7%” rule of standard deviations). The variability in this experiment came through the different maximum incident irradiance and day lengths, and the various combinations that resulted in overlapping daily-integrated values (Table 1). Apparently, some combinations (i.e. short day lengths) did not provide sufficient conditions for the culture to enter into shade-limited growth with a mean growth rate of 1 doubling d\(^{-1}\), even though the daily incident quantum flux was the same as found for cultures that did experience shade-limited growth dynamics. Therefore, it may not be sufficient to only know a daily quantum flux and depth of mixing (turbulence) in natural bodies of water to predict the spring bloom, as per Sverdrup’s (1953) Critical Depth Model, since phase of the growth dynamic influences efficiency via quantum yield of net biomass (Fig. 7).

The actual dynamic for the light climate could be pivotal in determining the quantum yield of net biomass for photosynthesis at a given quantum flux of incident irradiance. This goes beyond Eilertsen et al. (1995) who suggested that photoperiod initiates germination of phytoplankton spores that begins spring blooming in high latitude coastal waters. Although net growth occurred for all conditions of the dynamic irradiance, the quantum yield of net biomass was \(\sim\)25% higher for shade-limited growth than for light-limited growth having the same range of daily incident irradiance (Fig. 7b). “Critical depth” (in sensu Sverdrup) was not measured for any of the cultures in this study, since this would have resulted with a no net growth condition akin to a stationary phase. But mixing depth was greater than compensation depth for the low light, shade-limited group, while being less for the high light, “exponential” phase group. This \(\sim\)25% difference in net quantum yield would seem important outside lab conditions for bloom formation, since grazing is included as a component of respiration for the Critical Depth Model (Sverdrup, 1953; Platt et al., 1991; Smetacek and Passow, 1990; Behrenfeld, 2010), with microbial grazers having significant impact upon the productivity for natural ecosystems (Azam et al., 1983; Hewes et al., 1985; Hewes, 2009). Since net primary production (i.e. after including phytoplankton respiration and excretion) was measured in this study, the \(\sim\)25% difference would be available for export through the food web, or not exported to begin blooming. A population entering shade-limited growth could create a shallower depth where depth-integrated “respiration” equals depth-integrated net photosynthetic production (i.e. “critical depth”), as compared with a population that was growing exponentially. In this sense, it is net productivity, and not growth rate, that should be of interest for bloom formation (Flynn and Raven, 2017). Change in the growth dynamics, from “exponential” to “linear” phases, of a single population might also modify the bio-optical characteristics of the turbulent mixed layer to the disadvantage of other species in the community. Essentially, quantum yield for net biomass increase of a particular irradiance dynamic at some daily incident photon flux (hence, phase of the growth dynamic) is an important factor to consider in relation to the Critical Depth Model.

**Quantum efficiency and light intensity**

It is well accepted that the quantum yield for photosynthesis decreases as intensity increases; PE is a curvilinear function as described by numerous mathematical models (Béchet et al., 2013). However, since light saturation of photosynthesis for *T. pseudonana* occurs at 200–300 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) (reviewed in Hewes, 2015a), without photoinhibition most of the super-optimal incident irradiance during the length of day would not increase productivity between the different culture conditions; yield for net biomass would decline with increased irradiance, since productivity does not change much with the increase of photon flux above some plateau for production. Therefore, the quantum yield for net biomass would be expected to decline in a non-linear fashion as daily irradiance increases (i.e. low light group in Fig. 7). However, this cannot completely explain the curvilinear relationship of the low light group data.

Since a Gaussian distribution modeled the light climate, the proportions between standard deviations and maximum irradiance were the same for all the different cultures (Fig. 1). Overlap occurred between different maxima and day lengths providing the same daily-integrated light flux (Table 1). Therefore, the daily integrated light with a 12-h day length at maximum irradiances of 500, 1000 and 2000 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) was the same as for the 24-h day length having maximum irradiances of 250, 500 and 1000 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\), respectively. Since photosynthesis becomes saturating at 200–300 \(\mu\)mol photon m\(^{-2}\) s\(^{-1}\) (reviewed in Hewes, 2015a), the 12-h day length should be thought less productive than the 24-h day length because a greater proportion of its total illumination was super-optimal of photosynthesis, and would result with lower biomass yields per quanta. This, however, was not found, with both 12 and 24-h day lengths having nearly the same net production at equivalent daily quantum fluxes (Fig. 4b). The relationship between net quantum yield and daily incident PAR has relatively little variability.
about its regression (Fig. 7a; $r^2 = 0.976$) than might otherwise be expected from PE. Especially, considering the range of maximum intensities and day lengths having overlapping daily irradiances included in the study’s matrix (Fig. 3; Table I). Also excluded is the possibility that photoinhibition was involved, since a 12-h photoperiod would incorporate a higher daily percentage for dose of “toxic” irradiance than of a 24-h photoperiod having the same daily integrated PAR. The decline in net quantum yield with irradiance of the low light group (Fig. 7) probably has additional mechanisms at work that reduces photosynthetic efficiency, possibly non-photochemical quenching (NPQ; see Zijffers et al., 2010). Since the high light group contained higher ambient irradiance than the low light group (Table I), increased NPQ activity during “exponential” growth might help explain its lowered net quantum yield as well.

Various studies have emerged that show dynamic and fluctuating light regimes produce different physiological responses than obtained with constant or square-wave irradiance regimes (Litchman, 2000; Havelková-Doušová et al., 2004; Wagner et al., 2006; van de Poll et al., 2007; Iluz et al., 2012; Hewes, 2013a; Hewes and Hewes, 2016; and references within). Some of these differences do not appear related to efficiency of photosynthesis per se, but rather upon photoprotective mechanisms such as NPQ, which divert intense illumination away from photosynthetic reaction centers that would otherwise be damaging. If NPQ is initiated during brief periods of exposure to high irradiance intensity under fluctuating and/or dynamic light regimes, NPQ could reduce quantum yields of photosynthesis from their potential (i.e. becoming over-protective) during periods of low irradiance intensity if not de-activated in timely manner (Hewes, 2016b).

Solar incident irradiance varies daily between 0 and 2000–3000 μmol photons m$^{-2}$ s$^{-1}$, having day lengths of 0–24 h (depending on latitude and month of year; Kirk, 1994). Yet, most investigations involving photosynthetic responses utilize constant illumination and/or irradiances below saturation for photosynthesis, with cultures undergoing exponential growth. However, dynamic and fluctuating light regimes (Litchman, 2000; Havelková-Doušová et al., 2004; Wagner et al., 2006; van de Poll et al., 2007, 2010; Iluz et al., 2012; Hewes and Hewes, 2016), phase of growth dynamic (this study), or even the manner cultures are grown for experimental analyses (Hewes, 2015b; Hewes and Hewes, 2016), are indications that the ecology of photosynthetic organisms is much more complicated. The photosynthetic response outdoors is dependent upon the daily incident quantum flux. However, as shown by this study, the response can vary as a combination of maximum intensity and its duration during the day, even though yielding the same daily flux, which determines the phase of light-controlled growth dynamics and resulting quantum yield.

**CONCLUSIONS**

*Thalassiosira pseudonana* exposed to a matrix of dynamic light regimes in a 20 cm water column and cultured using a semi-continuous method were found to vary in net primary productivity dependent upon being in “linear” or “exponential” phases of the light-controlled growth dynamic. The matrix consisted of maximum irradiances 250–2000 μmol photons m$^{-2}$ s$^{-1}$ with 6–24 h photoperiods that resulted with growth occurring in three groups (low, high and intermediate light) as a function of the amount of residual irradiance estimated at the mixing depth of 20 cm. The low light group displayed shade-limited (“linear”), while the high light group displayed light-limited (“exponential”) phases of the light-controlled growth dynamic. For the same range of integrated daily incident irradiances (~5–25 mol photons m$^{-2}$ d$^{-1}$), cultures growing under the phase of “linear” growth had ~25% greater quantum yield for net AFDW than cultures growing under an “exponential” phase of the growth dynamic. This difference provides evidence for a new perspective of Sverdrup’s Critical Depth Model by suggesting that both photoperiod and maximum irradiance, not just the daily integrated value, maybe crucial for bloom development. NPQ might be involved in this difference between productivities in “linear” vs. “exponential” phases of the growth dynamic, whereby it is initiated under strong light but remains residual during periods of low light to hinder net photosynthesis under dynamic light regimes.

**ACKNOWLEDGEMENTS**

Many thanks to the Marine Biology Research Division, Scripps Institution of Oceanography, and Oz Holm-Hansen for providing laboratory space and equipment. Eric Allen supplied F/2 chemicals, and Brian Palenik donated culture tubes. Thanks to the Editors and Reviewers of JPR for help making this a better article.

**REFERENCES**

Azam, F., Fenchel, T., Field, J. G., Grav, J. S., Meyer-Reil, L. A. and Thingstad, F. (1983) The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.*, **10**, 257–263.

Behrenfeld, M. J. (2010) Abandoning Sverdrup’s Critical Depth Hypothesis on phytoplankton blooms. *Ecology*, **91**, 977–989.
