

Growth of the Marine Haptophyte *Isochrysis* sp. (strain T.iso) in Response to Varying Light Intensity

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Abstract

The haptophyte microalga *Isochrysis* sp. (strain T.iso) was cultured for 7 days at 22°C under continuous light ranging from 17 to 183 $\mu\text{mol photons PAR m}^{-2}\text{s}^{-1}$ to examine the influence of increasing irradiance on cell growth. Cell density of replicate cultures was measured daily by in-vivo chlorophyll fluorescence to estimate the growth rate (μ). Growth rate increased with increasing irradiance but followed a saturating curve with a maximum growth rate (μ_{max}) of 0.71 d^{-1} and an irradiance half-saturation constant (I_k) of 32.25 $\mu\text{mol photons PAR m}^{-2}\text{s}^{-1}$. This indicates that saturating irradiance at these conditions is approximately 63 $\mu\text{mol photons PAR m}^{-2}\text{s}^{-1}$. As *Isochrysis* sp. (T.ISO) is commonly grown intensively in the aquaculture industry as shellfish larvae feed, this information can be used to optimize the light conditions required for maximum growth rate and cell production of T.ISO for molluscan hatcheries.

Introduction

Light can have very significant effects on the growth and internal composition of marine algae. The effects of varying light intensity range from the seasonal slowing/acceleration of growth rates in marine ecosystems, or marine microalgae sinking through the water column and out of the photic zone due to light attenuation (Barnes and Mann 1999).

Varying light intensity can also be used to control growth rates of algal cells. For example, the growth rate and time to harvest for large-scale bag cultures of microalgae can be controlled to meet varying larval production demands in the hatchery (Bolch 2004a,b). The internal composition of algal cells is also altered by varying light intensities. For example, pigment content (e.g. chlorophyll a) polyunsaturated fatty acid content and profile, carbohydrates and protein content can all change in response to increased or decreased light intensity (Thompson et al. 1990, Brown et al 1993).

The species of algae used in this study, *Isochrysis* sp. (T.ISO), is a marine haptophyte isolated from tropical waters surrounding Tahiti that is commonly used as a larval feed in the aquaculture industry due to its relatively wide temperature tolerance and its suitability as a live feed for many larval and juvenile molluscs, crustaceans and fish (Brown et al. 1993). The aim of this study is to examine the effect that varying irradiance has on the growth rate of T.ISO and determine saturating irradiance and maximum growth rates under typical hatchery growth conditions.

Materials and methods

To examine the effects of varying irradiance on growth rate, cells to be used as inocula were acclimatized at the appropriate light intensity for 1 week prior to the experiment. For each light intensity 0.5 ml of T.ISO cells were inoculated into 5 replicate screw-capped, 50 ml test tubes

containing 40mls of sterile f/2 growth medium. The inoculated test tubes were then placed upon a light box comprised of cool-white light fluorescent tubes with constant illumination at a temperature of 22°C with a diurnal deviation of 2-3°C. The light intensity was varied by using layers of nylon fly screen to establish seven different light intensities, ranging from 183 $\mu\text{mol photons PAR m}^{-2}\text{s}^{-1}$ (no fly screen) to 17 $\mu\text{mol photons PAR m}^{-2}\text{s}^{-1}$ (6 fly screens). Each group of tubes was covered by a aluminum foil lined box to reduce interference of other light sources and to provide an even light distribution in the boxes.

In-vivo chlorophyll fluorescence was measured daily using a fluorometer (Turner, model 10AU, Turner Designs CA, USA) for seven days, the class data collated, and a plot of growth rate versus irradiance, and an “inverse plot” (Line-Weaver Burke plot) constructed. A straight line regression was fitted to the inverse plot. From the regression line, values for μ_{max} and I_k can be derived by calculating the x- and y-intercepts of the line (x-intercept = $-1/I_k$; y-intercept = $1/\mu_{\text{max}}$), and from the gradient of the slope ($I_k = \text{slope} * \mu_{\text{max}}$) (Bolch, 2004b).

Results

The mean growth rate of T.ISO followed a saturating curve, increasing linearly with increasing irradiance to around 30 $\mu\text{mol photons PAR m}^{-2}\text{s}^{-1}$ and then leveling out at higher irradiance (Fig. 1). The highest mean growth rate of 0.58 $\text{d}^{-1} \pm 0.04$ was recorded at the highest light intensity of 183 $\mu\text{mol photons PAR m}^{-2}\text{s}^{-1}$. Mean growth rate of the T.ISO batch cultures did not increase significantly for irradiance levels above 76 $\mu\text{mol photons PAR m}^{-2}\text{s}^{-1}$ (Fig. 1). A significant decrease in fluorescence per cell was also noted at the higher light intensities, particularly at the highest light intensity examined in the experiment.

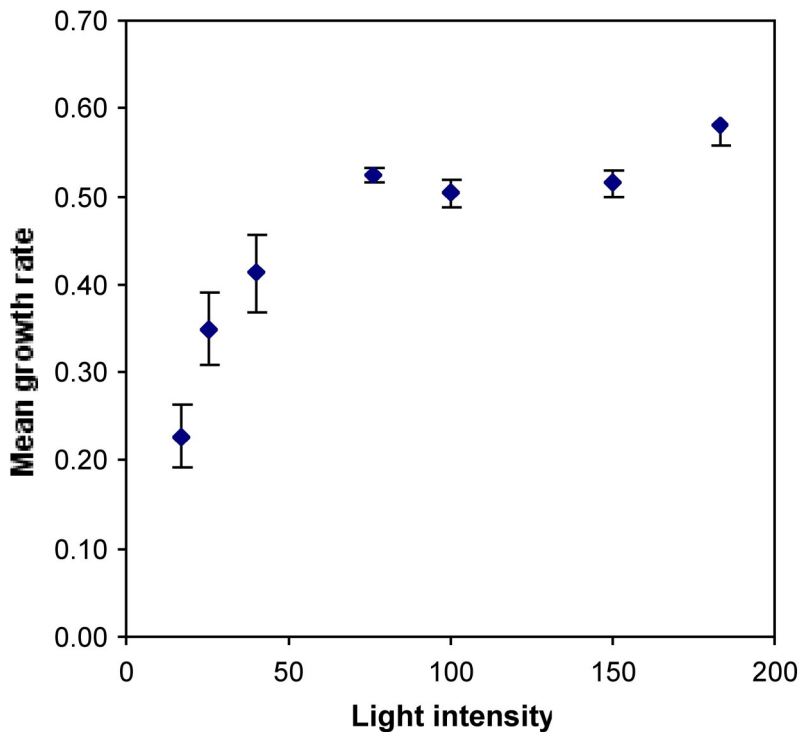


Figure 1. Mean growth rates of *Isochrysis sp. (T.iso)* at 22°C under constant illumination. Curve is hand-fitted to the data. Error bars represent standard error of five replicates.

The maximum growth rate (μ_{\max}) calculated from the Line-Weaver Burke plot was 0.71d^{-1} while the saturating irradiance was found to be $I_k=62.5 \mu\text{ mol photons PAR m}^{-2}\text{s}^{-1}$ (Fig. 2).

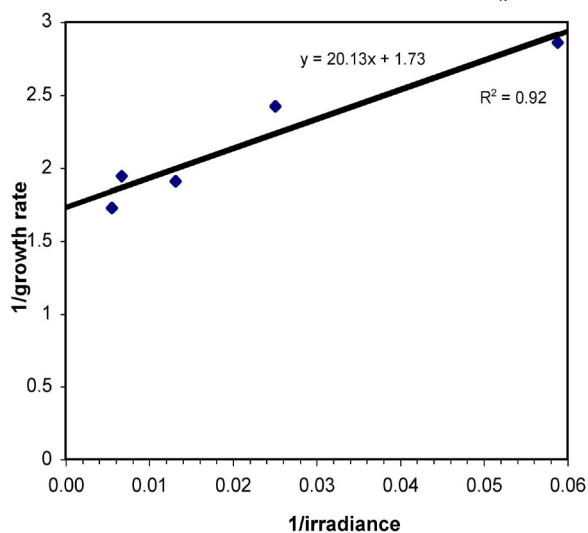


Figure 2. Line-Weaver Burke “inverse” plot, linear regression line and equation for the light and growth rate data. (Calculated values: $\mu_{\max} = 0.71\text{d}^{-1}$; $I_k = 31.25$)

Discussion

The data collected and examined from this practical shows clearly that increased irradiance leads to increased growth rate of *Isochrysis* sp. (strain T.iso) providing that the temperature is constant. This has already been established for other microalgal species in previous reports (e.g. MacIntyre *et al.* 2002, Richmond 1994). The observed maximum saturating irradiance ($62.5 \mu\text{mol photons PAR m}^{-2}\text{s}^{-1}$) is close to observations by previous students of intensive algal culture ($58 \mu\text{mol photons PAR m}^{-2}\text{s}^{-1}$, Bolch 2004a). The maximum growth rate (μ_{\max}) of 0.71d^{-1} is, however, considerably less than the published value (Table 1). It is possible that in 50 ml tubes that gas exchange was limited during the experiment and that the cultures may have become CO_2 limited (rather than light limited) at the higher light intensities. This would reduce the point at which light saturation occurs and explain the reduced value calculated for μ_{\max} .

When microalgal cells are exposed to high light intensity, photosynthesis is inhibited and therefore the growth rate of the algal culture is reduced, (Barnes and Mann 1999, Toro 1989). This process is known as photo-inhibition. This effect is caused by photo-oxidation reactions inside the cell due to excess light that cannot be absorbed into the photosynthetic apparatus; the increase in ultraviolet light also has detrimental effect on the cell (Barnes and Mann 1999).

While there was some reduction on growth rates evident between 100 and 150 $\mu\text{mol photons PAR m}^{-2}\text{s}^{-1}$ in this experiment (see Fig. 1), a similar growth rate was noted at the highest intensity, indicating that photo-inhibition is probably not the cause of the reduced μ_{\max} obtained in this experiment.

Table 1: Saturating irradiance and maximum growth rates for some common microalgal species at 18°C with 24 h light (after Bolch 2004a).

Species	μ_{\max} (day^{-1})	Saturating irradiance ($\mu\text{moles photons PAR m}^{-2} \text{s}^{-1}$)
<i>Chaetoceros calcitrans</i>	2.07	39
<i>Thalassiosira pseudonana</i>	2.11	38
<i>Dunaliella tertiolecta</i>	1.45	55
<i>Pavlova lutheri</i>	1.12	46
<i>Isochrysis</i> sp. (T.iso)	1.39	58

The saturating irradiance for T.ISO and most other microalgal species (39 to 58 μ mol photons PAR $m^{-2}s^{-1}$) is relatively low compared to typical light intensities used during laboratory and hatchery culture. Large scale algal bag cultures are commonly subjected to light intensities ranging from 200 to 1000 μ mol photons PAR M^2s^{-1} at the surface of the culture (Bolch 2004a), clearly higher than the saturating irradiance for T.ISO and other commonly cultured species. This is of particular concern when the cultures are inoculated and are at low cell concentration. The turbidity of culture is low therefore light penetration is high and can cause photo-inhibition, cell stress and reduced growth rates, and eventually cell death, resulting in loss of the culture.

Algal cells respond to increased light in three ways, an increase in growth rate and photosynthetic rate, changes in its photosynthetic capacity and changes in their cellular composition (Bolch 2004a, Thompson et al. 1990). Changes in cellular composition are important concerns for hatchery operations as the algal cells are often the sole feed source for larval marine animals species. Varying light intensities can effect the type and quantity of lipids and essential fatty acids, carbohydrates, chlorophyll, and protein present in the algae cultured, and it is of great importance to consider this when an a culture system is designed (Brown et al. 1993).

In conclusion, the experiment conducted has defined two important parameters that describe how the commercially important *Isochrysis* sp. (T.iso) responds to increasing light intensity. This knowledge allows algal culture staff to be able to avoid photo-inhibition and culture crashes, predict growth rates, and manipulate algal culture harvests to coincide with feed demand in a hatchery situation.

References

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