EFFECT OF TEMPERATURE AND IRRADIANCE ON GROWTH OF HAEMATOCOCCUS PLUVIALIS (CHLOROPHYCEAE)¹

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ABSTRACT

The growth characteristics of Haematococcus pluvialis Flotow were determined in batch culture. Optimal temperature for growth of the alga was between 25° and 28°C, at which the specific growth rate was 0.054 h⁻¹. At higher temperatures, no cell division was observed, and cell diameter increased from 5 to 25 μm. The saturated irradiance for growth of the alga was 90 μmol quanta m⁻² s⁻¹; under higher irradiances (e.g., 400 μmol quanta m⁻² s⁻¹), astaxanthin accumulation was induced. Growth rate, cell cycle, and astaxanthin accumulation were significantly affected by growth conditions. Careful attention should be given to the use of optimal growth conditions when studying these processes.

Key index words: astaxanthin; Chlorophyceae; growth; Haematococcus pluvialis; irradiance; O₂ evolution; temperature

Of the various environmental factors influencing growth, temperature and light are considered to be the main physical elements that directly control the growth rate of algae. The present study was aimed at optimizing the growth requirements of H. pluvialis with respect to temperature and irradiance.

MATERIALS AND METHODS

Haematococcus pluvialis Flotow obtained from the Culture Collection at the University of Göttingen was cultivated in modified BG-11 (Stanier et al., 1971) containing 1.5 g NaNO₃ L⁻¹. The alga was grown in a 600-mL glass column, 4-cm width, containing 500 mL of medium. The culture was stirred by bubbling air containing 15% CO₂ from the bottom of the column. Temperature was controlled by inserting the culture column in a temperature-regulated water bath. Illumination was provided by cool-white fluorescent lamps (20 W) from one side of the water bath. Irradiance was measured in the center of the column with a quantum meter (Lambda LI-185). For all the experiments on the effect of temperature, growth irradiance was maintained at 130 μmol quanta m⁻² s⁻¹.

Samples were taken at indicated times, and the following growth parameters were measured immediately; cell number was counted using a hemacytometer. Absorbancy at 550 nm was measured as turbidity using a Klett Summerson spectrophotometer with a green filter. Dry weight was measured by filtering a 5-mL sample through preweighed Whatman GF/C filters and drying the cell mass at 80°C overnight.

For chlorophyll, a 5-mL culture sample was centrifuged, and the pellet was extracted with dimethylsulfoxide in 70°C for 5 min. The absorbance of the supernatant was measured at 672 nm, and chlorophyll concentration was calculated using the formula E₅₇₀ = 870 (Seely et al., 1972).

Astaxanthin was determined as previously described by Boussiba et al. (1992).

The initial chlorophyll concentration for all growth experiments was about 1.5 mg L⁻¹, corresponding to about 2 x 10⁸ cells L⁻¹. This relatively low concentration was used in order to avoid problems of self-shading at least during the early stages of growth. Growth rate was calculated from the initial logarithmic phase of growth for at least 48-72 h, by using the following equation:

\[ \mu = \frac{\ln X_f - \ln X_i}{dt} \]

where X is biomass concentration and dt is the time required for the increase in biomass from Xᵢ to Xᶠ.

Oxygen evolution rates were measured with a Clark-type O₂ electrode. During the measurement, the temperature was kept constant by means of a water bath, and illumination was provided by a slide projector lamp with an irradiance of 100 μmol quanta m⁻² s⁻¹. Samples were harvested by centrifugation and suspended in fresh BG-11 medium (containing an additional 20 mM NaHCO₃) to give a chlorophyll concentration of 2.2 mg L⁻¹.

The photosynthesis–light response curves of Haematococcus cultures were obtained by exposing culture samples (4 μg chlorophyll mL⁻¹) to 20 different irradiances from 0 to 3000 μmol quanta m⁻² s⁻¹ and measuring the initial rates of light-depen-

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The specific growth rate ($\mu$) of the cultures was calculated as the mean of the last three values in the asymptotic region of the photosynthesis–irradiance (P-I) curve. Light-limited slopes of the P-I curves ($\alpha$) were calculated by regression using the rates at the lowest 6–10 photon flux densities. The strictly linear region of the curves was judged on the basis of the maximum $r^2$. $I_i$ corresponded to the intersection of the extrapolated linear part of the curve with the horizontal line at $P_{\text{max}}$.

All data represent the means of at least three independent experiments.

**RESULTS AND DISCUSSION**

To determine the effect of temperature on the growth of *H. pluvialis*, cells previously grown at 28°C were inoculated at 20°, 22°, 25°, 28°, 31°, and 33° C. Cultures were allowed to adapt for at least one generation. The specific growth rate ($\mu$), as calculated from the increase of cell number or chlorophyll concentration, increased with a rise in temperature from 20° to 28° C (Fig. 1A); a further increase in temperature caused a decline in $\mu$. At 33°C, cell division was totally inhibited, but the decrease in growth rate based on chlorophyll was not as great (Fig. 1A). When $\mu$ was calculated from measurements of dry weight or protein concentration, a pattern similar to that obtained for cell number and chlorophyll was observed in the lower temperature range (20°–28° C). However, in the higher temperature range (28°–33° C), $\mu$ did not decline but, rather, stayed at a level similar to that at 28°C (Fig. 1B). The results suggest that the cell division process in *H. pluvialis* is much more sensitive to the increase in temperature compared to protein and chlorophyll synthesis. Based on cell number, the highest $\mu$ was obtained at 25°–28° C and had a value of 0.053–0.054 h⁻¹, which corresponds to a doubling time of 12–13 h.

These results correlated well with the response of photosynthetic $O_2$ evolution rates at different temperatures (Fig. 2). Cells grown at 28°C for at least five generations were incubated for 30 min at different temperatures, and the $O_2$ evolution rate of the cells was measured. The highest photosynthetic activity was recorded in the temperature range of 25°–30° C, with a slight drop at 35°C. A further increase in temperature resulted in a drastic decrease in $O_2$ evolution.

The turbidity of an algal culture is a function of cell volume and cell number. Dividing the value of turbidity of the culture by the cell concentration may serve as an indicator of the average cell volume in the culture. When cells were grown at 22° or 25° C, turbidity per cell was constant but increased markedly when grown at a higher temperature (33°C), indicating an increase in cell volume (Fig. 3). These results corresponded to measurements of minimum cell diameter: the diameter of the smallest cell in the culture increased slightly from 3 to 5 μm, with the increase in growth temperature from 20° to 28° C, and increased sharply from 5 to 25 μm, with a further increase in temperature from 28° to 33°C. For each temperature treatment, at least 100 cells were counted.

The optimal temperature for growth was found to be 25°–28° C based on the increase in cell number or the increase in chlorophyll concentration. From the measurements of $O_2$ evolution rate, we suggest
that 28°C is the optimal temperature for photosynthesis as well. At lower temperatures (20°C and 22°C), some metabolic processes, such as protein and chlorophyll synthesis, seem to be reduced, so that growth rates were lower than those at optimal temperatures. However, at higher temperatures (31°C and 33°C), contradictory results were observed: the rates of cell division and increase in chlorophyll concentration were much lower at 33°C than those at 28°C and 25°C, while the rates of protein increase were higher at 33°C than those at 25°C and 28°C. It seems that high temperature severely impaired cell division and to a lesser extent chlorophyll synthesis, but not protein synthesis. Indeed, as might be expected from such an unbalanced growth, the relative cell volume was reduced with the decrease in growth temperature or increased with an increase in temperature (Fig. 3). This was probably due to the different rates of synthesis of the different cell components and the rate of cell division (e.g. at 22°C the doubling time of chlorophyll, protein, and dry weight was 25.7, 28.9, and 34.7 h, respectively, but the doubling time of cell number was only 14.4 h). This discrepancy resulted in smaller cells. At higher temperatures, cell division nearly stopped, but dry weight, turbidity, and protein content increased continuously, so that each cell grew larger (up to 25 μm in diameter). It is interesting to note that such large cells stayed green during the growth periods and they did not accumulate astaxanthin.

The differences in specific growth rates (μ) calculated on the basis of different growth parameters show that the growth pattern of *H. pluvialis* is unbalanced and that the life cycle of this alga is complicated, so that steady-state conditions are difficult to maintain, especially in batch-grown cultures. This is mainly observed when cells are grown at temperatures below or above the optimal range (i.e. below 22°C or above 28°C). This is further supported by the observation that the parameter of turbidity·cell⁻¹ stays constant at cultures grown at 22°C or 25°C and changes when grown at 33°C.

To determine the effect of irradiance on the growth of *H. pluvialis*, cells grown at 100 μmol quanta·m⁻²·s⁻¹ were harvested at their exponential phase of growth and resuspended in fresh medium to a final chlorophyll concentration of 1.5 mg·L⁻¹. This low concentration was designed to minimize the effect of self-shading of the cells. The cultures were exposed to irradiances ranging from 50 to 400 μmol quanta·m⁻²·s⁻¹. The growth rate of each culture was calculated after allowing the cells to adapt to the new light regime for at least one generation. Exposing the cultures to a growth irradiance of up to 90 μmol quanta·m⁻²·s⁻¹ resulted in an increase in μ as calculated on the basis of increase in chlorophyll concentration in the culture (Fig. 4). Growth was saturated at about 90 μmol quanta·m⁻²·s⁻¹. A similar pattern was observed when μ was calculated on the basis of dry weight. Some increase in μ was observed to a maximum at 200 μmol quanta·m⁻²·s⁻¹. In addition, μ calculated from chlorophyll was generally higher than that calculated from dry weight. Such differences between chlorophyll and dry weight measurements showed again the unbalanced growth pattern of *H. pluvialis*. These results revealed that *H. pluvialis* can adapt to irradiances ranging from 90 to 400 μmol quanta·m⁻²·s⁻¹. The ability to adapt to different irradiances was further demonstrated in the photosynthetic light–response curves of two cultures grown at 60 and 130 μmol quanta·m⁻²·s⁻¹ (Fig. 5). Cells grown at 60 μmol quanta·m⁻²·s⁻¹ for 3 days showed a lower light saturation point (Iₚ = 212 ± 3 μmol quanta·m⁻²·s⁻¹) than those grown for 3 days at 130 μmol quanta·m⁻²·s⁻¹ (270 μmol quanta·m⁻²·s⁻¹). Although the interpretation of the light saturation point is somewhat questionable (Henley 1993), it is traditionally used as an indicator for photoacclimation in algae. In both cases, the initial slopes (α) were the same (1.73 ± 0.05 μmol quanta·m⁻²·s⁻¹ [μmol O₂·mg chlorophyll⁻¹·h⁻¹]), indicating that both cultures have the same photosynthetic efficiency. Yet the light-saturated photosynthetic rate of the cells grown
at 130 μmol quanta·m⁻²·s⁻¹ was higher (370 ± 10 μmol O₂·mg chlorophyll⁻¹·h⁻¹) as compared to that of the cells grown at 60 μmol quanta·m⁻²·s⁻¹ (280 ± 6 μmol O₂·mg chlorophyll⁻¹·h⁻¹). This phenomenon of change in Pmax without a change in the initial slope (α) is an indicator of light acclimation of the cells without photoinhibition, as recently reviewed by Henley (1993).

Irradiance affected the ratio between astaxanthin and chlorophyll. This effect was studied by growing H. pluvialis at irradiances ranging from 50 to 400 μmol quanta·m⁻²·s⁻¹. Under the higher irradiances (200 or 400 μmol quanta·m⁻²·s⁻¹), an increase in the astaxanthin/chlorophyll ratio was observed during the first 40–48 h of exposure until a new steady state was reached, the response being considerably greater under the higher irradiance (Fig. 6). The higher the irradiance, the greater the astaxanthin accumulation rate. After the first 48 h, the ratio decreased and then the cells started to divide (as observed by microscopic observation). Under lower irradiances (50 or 100 μmol quanta·m⁻²·s⁻¹), the ratio was practically constant, with no astaxanthin accumulation (Fig. 6). The changes in the ratios may imply a possible role of astaxanthin in the response of H. pluvialis to high irradiance (i.e. the higher the irradiance to which cells are exposed, the higher the rate and level of accumulation of astaxanthin).

We conclude that the growth of H. pluvialis is limited by an irradiance of up to 90 μmol quanta·m⁻²·s⁻¹ and that the fastest growth rate is achieved at the temperature range of 25°–28°C. The increased interest in H. pluvialis as a source of astaxanthin is reflected by the increased number of publications on this organism in the last few years. Unfortunately, many of those studies (Yong and Lee 1991, Hagen et al. 1993) are using suboptimal growth conditions (20°C and 50 μmol quanta·m⁻²·s⁻¹ in Zlotnik et al. 1993) or light/dark cycles with temperature cycles of 20° and 15°C, respectively (Borowitzka et al. 1991), resulting in slow-growing cultures so that the whole process of carotenogenesis is also significantly slowed down, making the comparison of data almost impossible. Studying the role of environmental conditions on astaxanthin production in H. pluvialis and its interrelation with growth rate should be directed to comparing data on the basis of optimal growth conditions, where the maximal specific growth rate is obtained, and astaxanthin inductive conditions that are a shift from the optima.

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EFFECTS OF TEMPERATURE ON GROWTH, LIGHT ABSORPTION, AND QUANTUM YIELD IN DUNALIELLA TERTIOLECTA (CHLOROPHYCEAE)

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ABSTRACT

The effects of growth temperature on the marine chlorophyte Dunaliella tertiolecta Butcher were studied to provide a more mechanistic understanding of the role of environmental factors in regulating bio-optical properties of phytoplankton. Specific attention was focused on quantities that are relevant for modeling of growth and photosynthesis. Characteristics including chlorophyll a (chl a)-specific light absorption \(a_{\text{ph}}(\lambda)\), Cchl a ratio, and quantum yield for growth \(\Phi_g\) varied as functions of temperature under conditions of excess light and nutrients. As temperature increased over the range examined (12°–28°C), intracellular concentrations of chl a increased by a factor of 2 and \(a_{\text{ph}}(\lambda)\) values decreased by more than 50% at blue to green wavelengths. The lower values of \(a_{\text{ph}}(\lambda)\) were due to both a decrease in the abundance of accessory pigments relative to chl a and an increase in pigment package effects arising from higher intracellular pigment concentrations. Intracellular pigment concentration increased as a consequence of higher cellular pigment quotas combined with lower cell volume. At high growth temperatures, slightly more light was absorbed on a per-cell-C basis, but the dramatic increases in growth rate from \(\mu = 0.5 \text{ d}^{-1}\) at 12°C to \(\mu = 2.2 \text{ d}^{-1}\) at 28°C were primarily due to an increase in \(\phi_g\) (0.015–0.041 mol C (mol quanta)\(^{-1}\)). By comparison with previous work on this species, we conclude the effects of temperature on \(a_{\text{ph}}(\lambda)\) and \(\phi_g\) are comparable to those observed for light and nutrient limitation. Patterns of variability in \(a_{\text{ph}}(\lambda)\) and \(\phi_g\) as a function of growth rate at different temperatures are similar to those previously documented for this species grown at the same irradiance but under a range of nitrogen-limited conditions. These results are discussed in the context of implications for bio-optical modeling of aquatic primary production by phytoplankton.

Key index words: absorption; Chlorophyta; Dunaliella tertiolecta; modeling primary production; phytoplankton optics; quantum yield; temperature limitation

Accurate modeling of aquatic primary production is a goal that currently has broad implications not only for many ecological questions posed by limnologists and biological oceanographers but also for geochemistry, climate change research, and other earth sciences. As algorithms for this purpose are being developed and evaluated (e.g. Kiefer and Mitchell 1983, Platt and Sathyendranath 1988, Balch et al. 1989, Sakshaug et al. 1989, Smith et al. 1989, Morel 1991), the need to adequately describe the effects of environmental factors such as light, nutrients, and temperature on phytoplankton physiology is becoming more apparent. Specifically, the magnitude and variability of optical and photosynthetic properties must be understood and quantified so that they can be accurately incorporated into models and algorithms.

Temperature has been shown to affect the rates of phytoplankton growth and photosynthesis in laboratory studies (see Raven and Geider 1988, Davison 1991) and has been implicated to explain patterns of chlorophyll a (chl a)-specific photosynthetic rates in the ocean (Epplley 1972). Phytoplankton cultures fully adapted to lower temperatures exhibit decreases in the chl a-specific photosynthetic rate at light saturation \((P_{\text{max}})\), whereas little variability is...
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