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# Combined influence of light and temperature on growth rates of *Nannochloropsis oceanica*: linking cellular responses to large-scale biomass production

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## Abstract

The interaction effects between irradiance and temperature on growth rates of *Nannochloropsis oceanica* were determined in both laboratory cultures and large-scale tubular photobioreactors. Growth responses were investigated in 48 batch cultures subjected to crossing light/temperature gradients ranging from 34–80 $\mu$mol photons $m^{-2}$ $s^{-1}$ and 14.5–35.7 °C respectively. Comparisons were made to growth responses observed in production systems (200 L biofences) operated in climate-regulated greenhouses with controlled temperature and artificial light gradients. Cellular responses showed increasing specific growth rates as a function of temperature, with a peak at 25–29 °C, after which the growth became increasingly unstable. The optimum temperature for growth increased with higher light intensities up to approximately 28 °C at 80 $\mu$mol photons $m^{-2}$ $s^{-1}$. At low light intensities the specific growth rate was less affected by temperature. The maximum daily production measured in the biofence systems increased proportionally with irradiation and reached approximately 0.7 g $L^{-1}$ $d^{-1}$ at 1030 $\mu$mol photons $m^{-2}$ $s^{-1}$ average daily radiation for a culture temperature of 24 °C. This corresponds to a daily yield of 140 g per day in a 200 L biofence system. When specific growth rates for the biofence cultures were measured at different densities and plotted against temperature, results showed a peak with the 24 °C temperature treatment. This peak became less pronounced as the density increased in the cultures. This is consistent with the laboratory results; increasing cell density in the biofence cultures resulted in less average light $cell^{-1}$, which produced the same temperature dependent response as seen by reducing the external irradiance exposure for the dilute laboratory cultures.

## Introduction

Marine microalgae are increasingly used as feed for marine organisms in aquaculture, constituting both a source of energy as well as providing the essential vitamins and polysaturated fatty acids (PUFAs). *Nannochloropsis*, a marine unicellular algae belonging to the Eustigmatophyceae, is one such microalgal group that is commonly cultivated in fish hatcheries as feed for rotifers due to it's high content of an essential PUFA, eicosapentaenoic acid (EPA, C20:5n3) (Chini Zittelli et al., 2004). Despite the widespread use of *Nannochloropsis* as aquaculture feed, it's industrial exploitation in mass culture systems as a source of EPA cannot yet compete with fish oil due to high production costs (Zhang et al., 2001). These costs can only be reduced by implementation of daily culture control to optimize production, which involves in-depth knowledge of independent and combined interaction effects of several key factors upon culture growth and dynamics.

Light and temperature are major processing factors that affect overall biomass productivity in photoautotrophic cultures (Goldman, 1979; Thompson & Guo, 1992; Carvalho & Malcata, 2003). Light provides the

energy source to the growing culture, and is indispensable to the photoautotrophic cell. The success of mass cultivation of microalgae is dependent on the efficient utilization of this light energy. Environmental temperature is a key parameter since it controls the basic rates of all chemical reactions in the microalgal cell. The effects of temperature on cell cultures relates both to the temperature dependence of the structural components of the cells (particularly lipids and proteins), as well as to the temperature coefficients of reaction rates. A consequence of these primary effects are significant changes in metabolic regulatory mechanisms, specificity of enzyme reactions, cell permeability and cell composition (Richmond, 1986).

There is evidence from both laboratory studies and mixed field population studies that light utilization during short-term responses of photosynthesis are strongly dependent on temperature (Collins & Boylen, 1982; Coles & Jones, 2000; Carvalho & Malcata, 2003). Understanding interaction effects of light and temperature on algal cultures will enable optimisation of growth in a controlled, production system by the implementation of culture temperature regulation as a function of irradiance. Effects of irradiance and temperature on microalgal growth have been investigated in numerous studies independently in the literature, and results have been extrapolated from such studies in which light or temperature have been held constant. However, there is evidence that some species may shift their acclimation strategies in response to combination of those parameters in a different way than if they acted independently (Dermoun et al., 1992), therefore indicating the need to investigate the combined effects of these factors.

An early study by Collins and Boylen (1982) clearly illustrated the physiological responses of cyanobacteria (*Anabaena variabilis*) to combinations of light and temperature. For each irradiance, there was a specific temperature at which the maximum photosynthetic rate was achieved and the optimal temperature for photosynthesis for this species increased with increasing irradiances. In a more recent study, Carvalho and Malcata (2003), investigated the combined effects of light and temperature on *Pavlova lutheri* using a kinetic modelling technique. They have generated mechanistic equations that allow the maximum growth rates and biomass for this species to be predicted in response to combined light and temperature conditions.

This experimental study aims to determine the combined influence of irradiance and temperature in the production of *Nannochloropsis oceanica*, in order to assess advantages of regulating culture temperature as a function of irradiance, relevant both on a daily and seasonal basis. The investigation includes studies at different scales, from controlled laboratory studies and large-scale, tubular biofences (200 L) used for mass culture of microalgae.

To achieve this objective, the following subgoals were identified:

- To determine the combined effects of irradiance and temperature on specific growth rates in controlled laboratory investigations.
- To investigate the effects of an artificial irradiance gradient and temperature variation on biomass production of *Nannochloropsis* cultured in large-scale photobioreators (biofences) operational in climate-regulated greenhouses.
- To couple the results between small and large-scale studies in terms of cellular reponses to light and temperature gradients.

## Materials and methods: Laboratory studies

### *Cultivation condition and experimental design*

*Nannochloropsis oceanica* was isolated from an operational hatchery in western Norway. The species was genetically determined by sequencing of 18S rDNA by CCAP, Scotland, U.K. and defined as a new species in their culture collection by CCAP 849/10 (Hart et al., 2005).

The growth of *Nannochloropsis oceanica* was determined in 48 batch cultures incubated in crossing light/temperature gradients. The temperature gradient was achieved by incubating the cultures in water-filled cylinders mounted on an aluminium plate, with cold (10 °C) and hot (40 °C) water circulated through channels drilled through opposite ends of the plate. The transfer of heat along the aluminium plate established a temperature gradient from 14.5 to 35.7 °C. The light gradient was achieved by inserting screens between the culture containers and the light source, which was fluorescent tubes mounted approximately 40 cm above the incubator plate. Six light levels were established from 34 to 80 $\mu$mol photons $m^{-2}$ $s^{-1}$.

An exponentially growing culture of *N. oceanica*, pre-incubated at intermediate levels of temperature and light (20 °C and 50 $\mu$mol photons $m^{-2}$ $s^{-1}$), was diluted to $30 \times 10^6$ cells $L^{-1}$ in the growth medium and distributed into $48 \times 50$ ml Erlenmeyer flasks, which were

placed on the incubator. The cell density in the cultures was monitored by daily counting with a Coulter Multisizer. The growth medium used in the experiment was Guillard's f/2 (Guillard, 1975) prepared from natural sea water diluted to a salinity of 17 g $L^{-1}$.

*Estimation of specific growth rates ($\mu$) in exponentially growing cultures*

The cell density of exponentially growing cultures as a function of time is described by the standard exponential growth equation:

$$N(t) = N_0 \exp[\mu(t - t_0)], \quad (1)$$

where $N$ is the number of cells, $t$ is the incubation time, $N_0$ is the cell number at $t_0$ and $\mu$ is the specific growth rate. For each growing culture, the specific growth rates ($d^{-1}$) were determined based on daily cell counts as

$$\mu = \frac{\ln(N(t)/N_0)}{t - t_0}, \quad (2)$$

which results from a linear fit in a semi-logarithmic plot of cell number against time. The specific growth rate is constant and at its maximal value for as long as the culture remains in the exponential growth phase.

## Materials and methods: Large-scale studies

*Cultivation conditions*

Three tubular photobioreactor biofence systems, each of 200 L volume, (Cellpharm, now Varicon Aqua, UK) were mounted in climate-regulated greenhouses at UMB as a research tool for the large-scale production of microalgae (Figure 1a). These biofences were used in a series of experiments that investigated the effects of light quality, irradiance and temperature on microalgal production during the period October 2003–December 2004. Real-time monitoring and automatic density control of large-scale microalgal cultures was achieved in experiments using near infrared (NIR) optical density sensors (Sandnes et al., 2005).

A schematic diagram showing the components of the biofence system is presented in Figure 1b. The fence serves to increase surface area for light capture, and consists of 48 PVC tubes of length 2.44 m and 30 mm internal diameter. The growing microalgal culture is circulated through the biofence system by a centrifugal pump (Calpeda SPAM 12, 0.45 kW) at a velocity of approximately 1 $ms^{-1}$ through the transparent tubes via a buffer-tank (degasser), which corresponds to a volume flow rate of 340 L $min^{-1}$. The volume ratio of the illuminated portion and the dark portion (buffer-tank and manifolds) is approximately a ratio of 40:60 respectively. The circulation of rubber beads through the tubular system prevent sedimentation and accumulation of algae on the plastic tubing. A controller unit continuously records culture temperature and pH measured by sensors (P14/Cap/PT100, supplied by Cellpharm, now Varicon Aqua, U.K.) submersed in the buffer-tank and is recorded every 15 s and averaged over 5 min. A constant supply of $CO_2$ was added into the outlet tube from each tank (approx. 10 L $h^{-1}$). This resulted in pH values increasing from approximately pH 6.5 at the start of the experiment to pH 7.8 by the end of the experimental period. Solar radiation was measured at 5 minute intervals by a pyranometer (Kipp & Sonen CM6B) mounted above the greenhouse. Microalgal batch cultures were

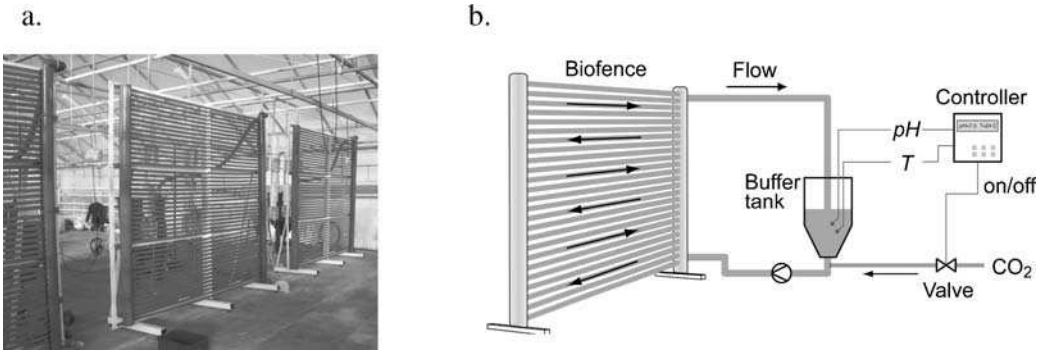


*Figure 1.* (a) Photograph of the biofence systems mounted in climate-regulated greenhouses. (b) Schematic representation of the biofence system. The 200 L algal culture is continuously circulated from a buffer tank through the fence of transparent tubes where incident light is absorbed by the algae. The controller unit records culture temperature and pH, and regulates $CO_2$ dosing.

grown for 2 week periods for each light-temperature treatment combination.

*Medium composition*

A nutrient feed consisting of a combination of agricultural fertilisers and urea represented a cost effective and time-saving alternative to Guillard's f/2 medium (Guillard, 1975). The dosage was based on the Redfield ratio for nitrogen:phosphate of 16:1. The nutrient combination used in the case studies described in the following section was: 1 g $l^{-1}$ Red Superba (Norsk Hydro, 7% N, 4% P, 21% K), 1 g $L^{-1}$ Urea, and Guillard's f/2 vitamins and trace metals (1 and 0.5 ml $L^{-1}$ respectively). In dilute batch cultures in this medium, growth rates were shown to be equal to, or higher than, standard f/2 nutrients at salinities between 20 and 35 g $L^{-1}$ and the higher phosphate concentrations in the fertiliser feed supported a high density culture. Filtered seawater (200 $\mu$m) that was treated to avoid contamination (acidified and subsequently neutralised) was used in all investigations. Batch culturing experiments were set-up in which the algae-seawater medium was dosed once with this nutrient feed at the beginning of the experiment.

*Experimental design*

The experimental trials that manipulated light levels were conducted in winter months to reduce the effects of natural ambient light. An artificial light gradient (up to summer light levels in greenhouses) was established using high pressure sodium lamps (Power Osram HQI-BT 400W/D), that are widely used in climate research in greenhouses and were tested during a preliminary study related to the use of supplementary lights in production of microalgae. The lamps were mounted vertically, directing the light perpendicular to the fence of photobioreactor tubes. The light gradient was set at the following levels (average of 48 measured set-points on the fence), for each fence and supplied 24 h $d^{-1}$: Low light (LL) = 309 $\mu$mol photons $m^{-2}$ $s^{-1}$ (68.7 $Wm^{-2}$), Medium light (ML) = 471 $\mu$mol photons $m^{-2}$ $s^{-1}$ (104.7 $Wm^{-2}$), High light (HL) = 921 $\mu$mol photons $m^{-2}$ $s^{-1}$ (204.7 $Wm^{-2}$). Shading curtains installed between biofences minimized interference from adjacent light treatments.

This artificial light gradient was run at 3 different temperature ranges as a 3 × 3 factorial design: low, mid and high temperature range that averaged: 18.5 °C ± 0.17 s.d., 23.7 °C ± 0.19 s.d. and 29.2 °C ± 0.12 s.d. respectively over a 14 d period. Temperature maintenance of the culture was achieved by a combination of climate control in the greenhouse and feed-back controlled sprinklers that sprayed cool water onto the fences.

*Biomass density measurements*

Culture biomass densities during the batch experiments were estimated daily by measuring the optical absorptance at 750 nm using a Unicam Helios Alfa spectrophotometer. Regression equations for this strain of *Nannochloropsis oceanica*, that had been established from over 30 samples taken under different environmental conditions in different studies, were used to convert absorptance measurements to biomass. Random samples were analysed for dry weight using standard methods (described below) and samples were taken for cell counting using a Coulter Multisizer to check the accuracy of optical methods. For determination of dry weight, algal cell culture (300 mL) was centrifuged at 8100 rpm for 15 min to produce a pellet. This was resuspended in ammonium formate (0.5 M solution) and centrifuged as above for salt removal, dried at 100 °C overnight, and weighed to determine algal dry mass.

The relationship between the measured absorptance coefficients and cell biomass of *Nannochloropsis oceanica* is illustrated in Figure 2. The calibration curve enabled fast and accurate assessment of biomass production and was used in the large-scale culture studies. The curve was divided into two sections to increase accuracy in biomass predictions: a linear area in the lower biomass range and an exponential range.

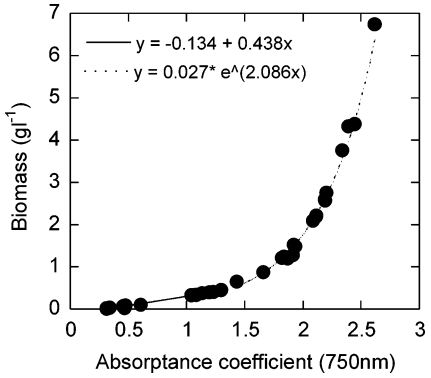


*Figure 2*. Relationship between cell biomass of *Nannochloropsis oceanica* and absorptance coefficients measured at a light wavelength of 750 nm.

*Estimation of daily productivity and specific growth rates ($\mu$) in the biofence system*

The derivatives of the measured biomass time series were used to determine the daily productivity ($g\,d^{-1}$) values . The highest productivity in the biofences occurs in the linear phase of growth, and the specific growth rate $\mu$ ($d^{-1}$) is determined here as the productivity ($dm(t)/dt$) divided by the biomass density ($m(t)$) at a particular time:

$$\mu = \frac{dm/dt}{m(t)} \tag{3}$$

Daily values for $\mu$ were thus obtained by dividing the daily increase in biomass density with the biomass density average during that time period. In a culture where the biomass density is increasing linearly with time, the specific growth rate is a decreasing function of time since the productivity remains constant while the biomass is steadily increasing. Maximum daily growth values ($p^*$) were calculated as a peak in production level and averaged over the three most productive days.

*Determination of total photosynthetic active radiation (PAR)*

The biofences are positioned in climate-regulated greenhouses and as such receive natural ambient light in addition to the artificial light gradient setup. The experiments were conducted in the winter months to minimise ambient light interference, however, during the low temperature experiments in February 2004 the ambient light levels were consistently and significantly higher than both previous experiments in January (Table 1). As such, the results were analysed as a function of the total photosynthetic active radiation (PAR) light for each experimental run.

The three fences were positioned facing south along the end wall of the greenhouse. A difference in light penetration due to the geometry of the greenhouse was corrected for by applying a correction factor specific for each fence, determined from an average of 20 measurement points on each fence at different times of day. The biofence positioned in the centre of the greenhouse, with low artificial light treatments, received the most natural light (56% light penetration), and the biofences either side received an average of 43% and 42% natural light for the mid and high artificial light treatments respectively during the experimental period. This light penetration factor was applied together with a general conversion factor for sunlight of 4.5 $\mu$mol photons $s^{-1}\,W^{-1}$ (PAR, between 400 and 750 nm) (Masojídek et al., 2004). This was added to the artificial lighting to give a total PAR light sum.

## Results

*Cellular responses to crossed light and temperature gradients: Laboratory scale*

The growth of *Nannochloropsis oceanica* was almost perfectly exponential during the first 100 h in most batch cultures, and the growth rate was calculated based on five observations during this period. However, in the upper temperature range (above 30 °C) where growth was less stable, the growth rates were calculated based on the increase in cell density during the first 50 h.

*Table 1*. Summary of main experimental biofence treatment parameters and biomass production results

| | Low temperature range | | | Mid temperature range | | | High temperature range | | |
|---|---|---|---|---|---|---|---|---|---|
| | LL | ML | HL | LL | ML | HL | LL | ML | HL |
| Max. daily production ($p^*$) (averaged over 3d) ($mg\,L^{-1}\,d^{-1}$) | 434 | 434 | 783 | 395 | 463 | 688 | 244 | 313 | 539 |
| Temperature range (average ± s.d.) | 19.28 ± 0.84 | 17.47 ± 0.64 | 18.70 ± 0.97 | 23.34 ± 1.09 | 23.77 ± 1.47 | 24.11 ± 1.21 | 28.53 ± 0.91 | 29.52 ± 1.12 | 29.43 ± 1.12 |
| Artificial light PAR ($\mu$mol photons $m^{-2}\,s^{-1}$) | 309 | 471 | 921 | 309 | 471 | 921 | 309 | 471 | 921 |
| Average natural light PAR ($\mu$mol photons $m^{-2}\,s^{-1}$) during 3d max. production period | 433 | 292 | 325 | 158 | 127 | 111 | 166 | 127 | 124 |
| Total light PAR ($\mu$mol photons $m^{-2}\,s^{-1}$) | 742 | 763 | 1246 | 467 | 598 | 1032 | 475 | 598 | 1045 |

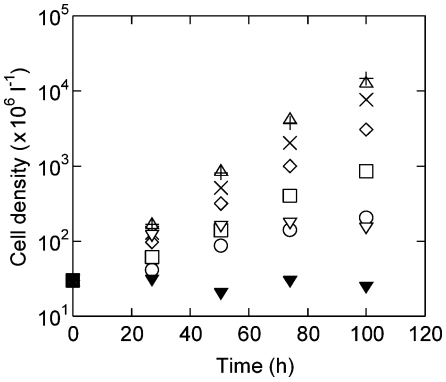


*Figure 3*. Growth curves of *Nannochloropsis oceanica* incubated with a constant irradiance of 80 $\mu$mol photons m$^{-2}$ s$^{-1}$ for the experimental temperatures (°C): 14.5 (○), 17.3 (□), 19.6 (◇), 22.7 (×), 25.6 (+), 29.1 (△), 32.4 (▽), 35.7 (▼).

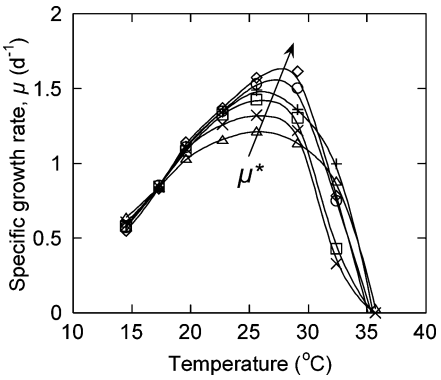


*Figure 5*. Specific growth rates ($\mu$) determined from the 48 batch culture experiments are plotted as a function of temperature for the experimental irradiance treatments ($\mu$mol photons m$^{-2}$ s$^{-1}$) : 34 (△), 44 (×), 54 (□), 61 (+), 72 (○), 80 (◇). The trend of maximum growth rates ($\mu^*$) is indicated by an arrow.

At 32.4 °C the growth stagnated after one day and at 35.7 °C no growth was observed.

Figure 3 shows examples of the growth curves of *N. oceanica* incubated at an irradiance of 80 $\mu$mol photons m$^{-2}$ s$^{-1}$. The cell densities are plotted as a function of time in a semi-logarithmic plot, and from these data points the specific growth rates ($\mu$) are determined as the gradient of the curves. Specific growth rates ($\mu$) were determined for all 48 cultures with different light and temperature combinations.

When growth rates ($\mu$) are plotted as a function of irradiance for the different temperatures (Figure 4), results show that for cells cultured at lower temperatures (from 14.5–17.3 °C), the effect of irradiance is minimal. For temperature treatments in the range from 17.3–25.6 °C, growth rates increase as a function of increasing light available and this gradient of increase is higher for the higher temperatures in this range. At 25.6 °C, for example, an increase in irradiance from 34 to 80 $\mu$mol photons m$^{-2}$ s$^{-1}$ results in an increase factor of 1.3 in the growth rate. At the highest light level of 80 $\mu$mol photons m$^{-2}$ s$^{-1}$ at this temperature, the growth rate corresponds to 2.3 doublings day$^{-1}$. In order to clarify the specific trends occurring in Figure 4, results for cultures above 32 °C are not plotted due to the instability in the cultures. It should also be noted that the irradiances tested in the laboratory experiments were considered below saturation level and as such, the growth rates measured in the exponentially grown cultures were below the maximum growth rates possible for *N. oceanica*.

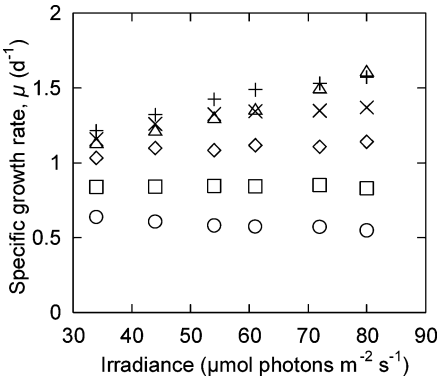


*Figure 4*. Specific growth rates ($\mu$) plotted as a function of irradiance for the experimental temperature treatments (°C): 14.5 (○), 17.3 (□), 19.6 (◇), 22.7 (×), 25.6 (+), 29.1 (△).

All specific growth rates determined from the 48 batch culture experiments are plotted as a function of temperature in Figure 5. The growth rate of *N. oceanica* increases rapidly with temperature up to approximately 25 °C, where a five degree increase in temperature in the range 15–20 °C result in almost a doubling of the specific growth rate for all light intensities. At 80 $\mu$mol photons m$^{-2}$ s$^{-1}$ the specific growth rate increased from approximately 0.6 d$^{-1}$ at 15 °C to 1.6 d$^{-1}$ at 25 °C. The influence of temperature on growth rates becomes less significant with decreasing light intensities and is illustrated in Figure 5 by the curves that flatten within the temperature range from approximately 19–29 °C. Above 29 °C the specific growth rates for all the irradiance treatments are seen to decrease dramatically with temperature, until the cultures become unstable around 35 °C.

The dilute, exponentially growing *N. oceanica* cultures show an optimal temperature ($T_{\mu^*}$) at which

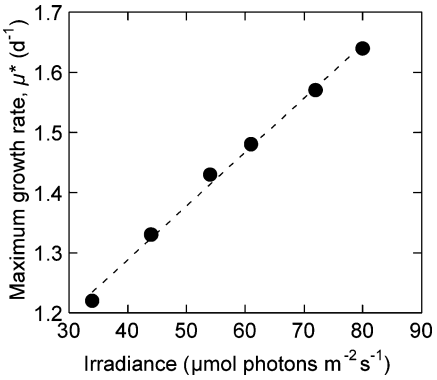


*Figure 6*. A linearly increasing relationship between maximum specific growth rates ($\mu^*$) and irradiance.

maximum specific growth rate ($\mu^*$) occurs. The arrow in Figure 5 indicates the increasing trend in $\mu^*$ as a function of both temperature and irradiance. In Figure 6 the maximum specific growth rates ($\mu^*$), determined from the line fit in Figure 5, are plotted against irradiance. The figure shows a linearly increasing maximum specific growth rate ($\mu^*$) as a function of irradiance in the range studied. The gradient is about 0.009 ($d^{-1}/\mu$mol photons $m^{-2}$ $s^{-1}$).

The optimal growth temperature $T_{\mu^*}$ for dilute *N. oceanica* cultures is seen to increase with increasing incident irradiance (Figure 5). Figure 7 shows the optimal growth temperatures, determined from the line fits in Figure 5, plotted as a function of irradiance. For the irradiance range from 30–80 $\mu$mol photons $m^{-2}$ $s^{-1}$, the optimal growth temperature increases by about 2 °C, from about 25.6 °C to 27.8 °C. The dashed line is provided to indicate what appears as a non-linear increase in optimal temperature as a function of light.

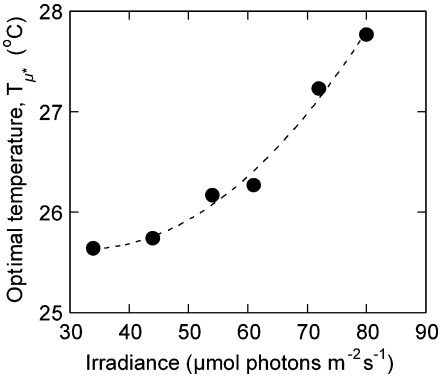


*Figure 7*. Optimal temperatures ($T_{\mu^*}$) plotted as a function of light.

*Effects of light and temperature in large-scale production of N. oceanica*

The biomass densities of the growing batch cultures in the biofence systems as a function of time are shown in Figure 8a–c. The cultures are started at low densities (approximately 10 mg $L^{-1}$) and the transition into the linear growth phases, where the culture is light-limited can be observed in the figures. At the end of the culture periods the biomass densities are seen to level off as the cultures approach the stationary phase of growth.

The daily biomass productions, determined from the derivative of the growth curves in the corresponding figures described above, are plotted as a function of biomass density in Figure 8d–f. At all temperatures investigated, the effects of increasing total irradiance resulted in significant increases in daily productivity up to a maximum recorded productivity of approximately 0.8 g $L^{-1}$ $d^{-1}$. All temperature treatments showed a relatively wide range of biomass values over which maximum daily biomass yield can be produced. The culture biomass at which maximum production occurred in the system (seen as the plateau on the graphs), occurred over a wider biomass density range at higher light levels from at least 1 g $L^{-1}$ to 4 g $L^{-1}$ in all temperature treatments compared to the lower irradiance treatments. At the lower light levels (at both low and mid light treatments) in the cultures maintained at both 19 °C and 24 °C, daily production levels were maintained constant at approximately 0.4 g $L^{-1}$ $d^{-1}$ in cultures densities between 1–3 g $L^{-1}$.

The main treatment parameters and results for the combined light and temperature treatments in the biofences are summarised in Table 1. Maximum daily biomass production ($p^*$) is given as the average over the three most productive days. As previously explained in the method section, the low temperature experiments received more natural light (more than the artificial low light treatment) and as such, the total PAR has been calculated based on the total amount of incident light. It should be noted that the natural ambient light disturbance in this low temperature trial resulted in similar total PAR values for the 'low' and 'mid' light gradient as explained in the Materials and Methods section, and similar maximum daily biomass productivity values of approximately 0.43 g $L^{-1}$ $d^{-1}$ for these treatments (Table 1).

The graph in Figure 9 shows maximum daily microalgal production ($p^*$) as a function of the total light (PAR) exposure for the different temperature treatments. The light gradient is representative for light

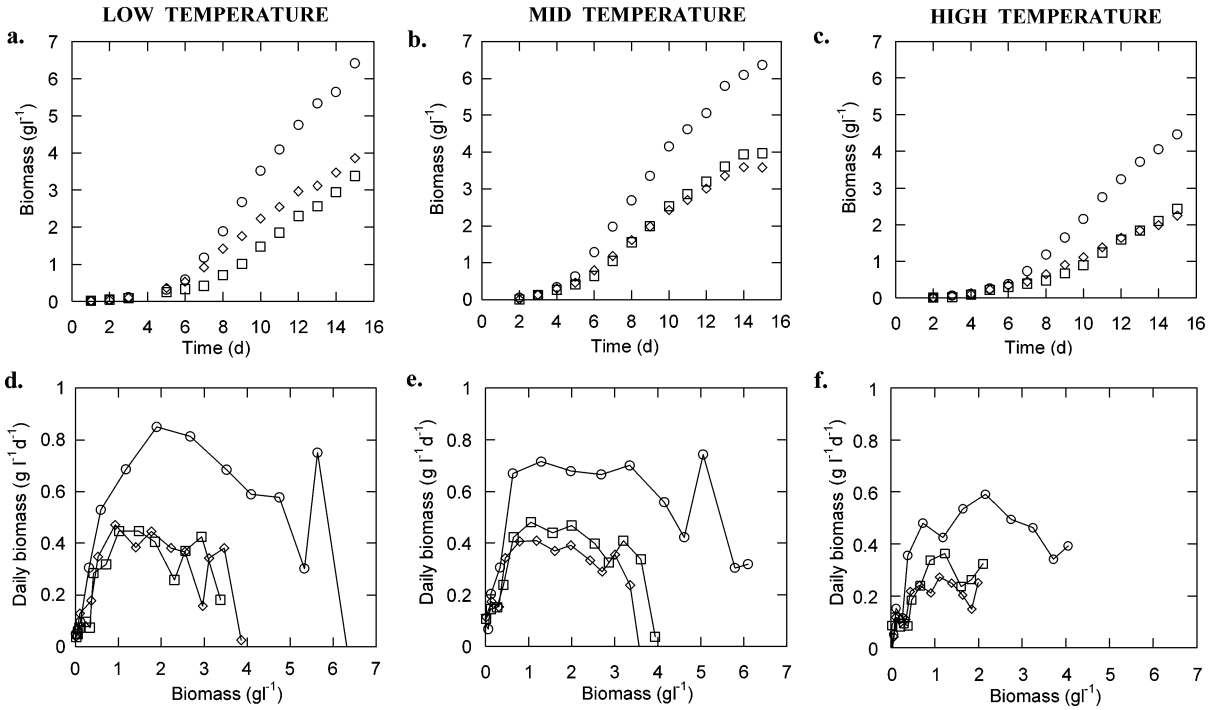


*Figure 8*. Figures a, b and c represent biomass production as a function of time for the low, mid and high temperature treatments respectively. For each temperature treatment, 3 artificial light intensities ($\mu$mol photons $m^{-2}$ $s^{-1}$) were investigated: 309 (◇), 471 (□), 921 (○). In a similar manner, the calculated daily biomass values ($g\,L^{-1}\,d^{-1}$) are plotted as a function of total biomass ($g\,L^{-1}$) for these respective treatments in Figures d, e and f. Refer to text explanation for natural light interference during the low temperature trials and Table 1 for the calculated total PAR levels for all experimental treatments in this figure.

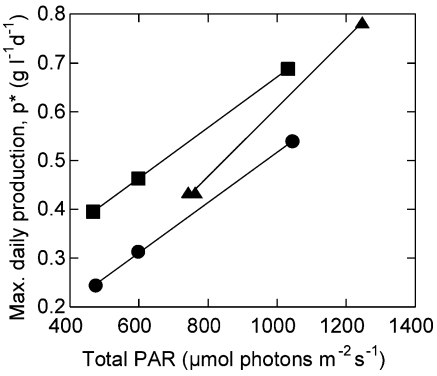


*Figure 9*. Maximum daily microalgal production ($g\,L^{-1}\,d^{-1}$) as a function of irradiance (total PAR, $\mu$mol photons $m^{-2}$ $s^{-1}$) for the experimental temperature treatments (°C): low (▲), mid (■) and high (●).

levels experienced in greenhouses in summer, which can reach typically 1000 $\mu$mol photons $m^{-2}$ $s^{-1}$ at this latitude (southern Norway). The figure indicates a linearly increasing maximum daily production ($p^*$) as a function of irradiance (PAR) for the three temperatures. The highest production rates occurred at the mid-temperature range (23.3–24.1 °C) given the same level of light exposure for the light gradient tested.

## Discussion

The mass culture of microalgae in photobioreactors is primarily concerned with maximising daily yield, which is achieved during the linear, light-limited phase of microalgal growth. Productivity in a photobioreactor is determined by both the culture density and the specific growth rate ($\mu$), which, for fixed temperature and fluid dynamics, is a function of the light profile within the reactor and the light regime to which the cells are subjected (Molina Grima et al., 1999). In dense microalgal cultures that are maintained in the light-limited linear growth phase to maximise production, light penetration is impeded by self-shading and light absorption (Rabe & Benoit, 1962), and as such denser cultures have lower specific growth rates. The culture density at which the cell mass reaches it's highest output rate of biomass for specific culture conditions is defined as the 'optimal population density (OPD)' and it is at this

cell density that the culture is most stable (Richmond, 2004). The aim for management of mass cultures is to sustain this optimal state in order to maximise yields and reduce costs.

The average irradiance ($I_{av}$) for each individual cell inside a reactor, expressed as light cell$^{-1}$, is the light level experienced by a single cell randomly moving inside the culture (Rabe & Benoit, 1962) and represents the most useful concept for understanding the effect of light on microalgal production in the light-limited, linear growth phase (Grima et al., 1995, 1999; Acien Fernandez et al., 1997). The light energy that each cell is exposed to at any given irradiance treatment is not only a function of the total illumination and cell density, but also determined by the culturing system configuration; factors that include: system light path as a function of the specific reactor geometry (Frohlich et al., 1983; Hu et al., 1996; Acien Fernandez et al., 1997), turbulence regime (Grobbelaar, 1991; Richmond, 2004) and cyclic changes in irradiance due to fluid movement between zones of different illumination within a photobioreactor system (Grima et al., 1999).

### *Effects of light and temperature on laboratory and biofence cultures of N. oceanica*

Effects of light and temperature on *N. oceanica* biofence cultures are interpreted in the context of the cellular responses measured in the exponentially growing laboratory cultures. The specific growth rate ($\mu$) is, in Figure 10, plotted as a function of culture biomass density for the high light treatment run at mid temperature range (24.11 °C ± 1.21 s.d). The specific growth rate ($\mu$) decreases as a function of the decreasing light

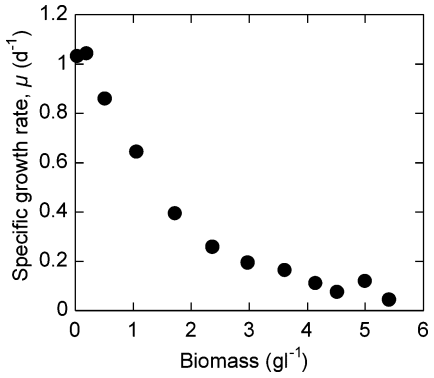


*Figure 10.* An example of the decrease in specific growth rates ($\mu$, ●) in the biofence as a function of culture biomass for the high light treatment run at mid temperature range (24.11 °C ± 1.21 s.d).

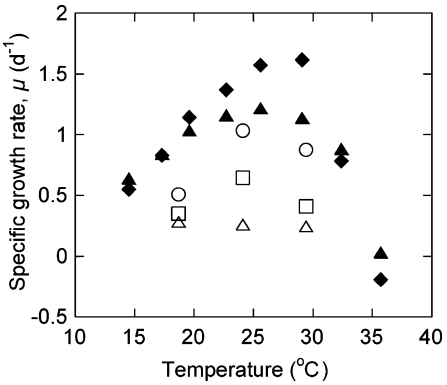


*Figure 11.* Relationship between specific growth rate ($\mu$) and temperature in exponentially grown cultures in the laboratory (filled symbols): 80 $\mu$mol photons m$^{-2}$ s$^{-1}$ (♦), 34 $\mu$mol photons m$^{-2}$ s$^{-1}$ (▲) and light-limited cells in the biofence system (open symbols): 0.02 g L$^{-1}$ (○), 1 g L$^{-1}$ (□) and 2 g L$^{-1}$ (△).

availability in the light-limited culture due to self-shading as the culture grows denser.

Specific growth rates for the biofence cultures grown at high irradiance and different temperatures, have been plotted together with the specific growth rates calculated from the exponential phase laboratory cultures (highest and lowest light levels plotted-data from Figure 5), and is shown in Figure 11. For the biofence data, specific growth rates were determined for the three temperature treatments at selected culture biomass densities in the growing batch cultures. As such, the biofence trend curves in Figure 11 show the temperature response in $\mu$ at measured culture densities. For the three biofence cultures maintained at different temperatures one observes the reduction in specific growth rate as the density increases (data points 20 mg L$^{-1}$, 1 g L$^{-1}$ and 2 g L$^{-1}$).

The highest specific growth rates were obtained in the mid-temperature range (24.11 °C ± 1.21) for the 20 mg L$^{-1}$ and 1 g L$^{-1}$ biofence cultures (Figure 11). This result reflects the outcome of the productivity analysis presented in Figure 9, where it was shown that the maximal daily production occurred in the mid-temperature range under equal light exposure.

The small-scale laboratory data from exponentially growing cultures showed that the peak in the maximum specific growth rates as a function of temperature became less pronounced when the irradiance was reduced (see Figure 5). In a similar manner, for the biofence cultures, the specific growth rate peak flattens with increases in culture densities. As the density increases in the light limited cultures, the average light

per cell exposure is reduced due to self-shading, which produces the same temperature response as seen by reducing the external irradiance incident on the dilute, exponentially growing cultures.

Whilst a peak in $\mu$ is seen between approximately 24–25 °C for both 20 mg $L^{-1}$ and 1 g $L^{-1}$ densities in the biofence cultures, the figure shows that there is no significant temperature effect on the specific growth rates for the highest density cultures at 2 g $L^{-1}$. This result was further investigated in an additional small-scale experiment whereby a high density (1 g $L^{-1}$) culture was maintained over a 40 day period in a 1.85 L internally illuminated bioreactor exposed to approximately 300 $\mu$mol photons $m^{-2}$ $s^{-1}$. The culture was diluted daily with a dilution rate of 0.4 $d^{-1}$, while the temperature was changed in intervals from 15–28 °C. Results showed no difference in the growth response of the culture over this temperature range, a result which is consistent with the (lack of) temperature response of the biofence cultures with specific growth rates of about 0.4 $d^{-1}$ (see Figure 11).

The data points plotted for the biofence cultures in Figure 11 show that, despite high incident light levels on the fences, all of the specific growth rates of the biofence cultures were lower than those investigated in the exponentially grown laboratory cultures. Although self-shading may explain reductions of specific growth rates in the denser, light-limited cultures, lower growth rates also occurred when the cultures were at low density. There are additional factors that contribute to the relatively low growth rates in the large scale biofence system compared to the lab cultures. The biofence culture is circulated between light and dark zones (40:60 respectively), which reduces the average artificial light exposure to approximately 368 $\mu$mol photons $m^{-2}$ $s^{-1}$. As the culture is circulated at high speed through the light and dark zones, the cells experience short periods (approximately 15 s) of high intensity light which is likely to be above the light saturation threshold for the algae cells. At low cell densities photoinhibition may therefore be a factor contributing to the low growth rates observed in the system. The laboratory cultures on the other hand are receiving a constant uniform illumination of relatively low intensity light. The effect of light saturation decreases as the culture becomes denser and the average irradiance in the culture volume is reduced due to absorption from other cells. Preliminary results also indicate that *N. oceanica* lab cultures exposed to equal light intensities show a lower growth rate when subjected to high frequency light/dark periods ($min^{-1}$) compared to the same total sum of light given over longer periods ($h^{-1}$ and $d^{-1}$) (Sandnes, unpublished results).

## Conclusions

Specific growth rates of *Nannochloropsis oceanica* increased with temperature in exponentially growing laboratory cultures, with a peak between 25–29 °C. Above 30 °C the cultures showed dramatic reductions in $\mu$, with no cultures growing at temperatures over 35 °C. A linear increase in the temperature-dependent maximum specific growth rate was observed as a function of irradiance over the range of 34-80 $\mu$mol photons $m^{-2}$ $s^{-1}$. The optimum temperature for growth increased with the irradiance to approximately 28 °C at 80 $\mu$mol photons $m^{-2}$ $s^{-1}$. At low light intensities the specific growth rate was less affected by temperature.

In the biofence systems, the specific growth rate declined as a function of increasing biomass density as expected in the linear phase of microalgal growth (light limited conditions), optimal for production systems. When specific growth rates measured at different densities were plotted against temperature, results showed a peak for the mid-temperature range (~24 °C). This peak became less pronounced as the density increased in the cultures. This is consistent with the lab results, as increasing cell density in the biofence cultures resulted in less average light per cell, and this produced the same temperature dependent response as seen by reducing the external irradiance exposure for the dilute lab cultures.

The maximum daily production measured in the biofence systems increased proportionally with the irradiation and reached maximum daily productivity levels of approximately 0.7 g $L^{-1}$ $d^{-1}$ at 1030 $\mu$mol photons $m^{-2}$ $s^{-1}$ average daily radiation for the mid-temperature treatment (~24 °C). This corresponds to a daily yield of 140 g per day in a 200 L biofence system. The range of biomass density at which maximum production occurs (the linear growth phase) is seen to increase with light exposure. It may be advantageous to run a production system at higher densities within this range as the culture is less sensitive to temperature variations, and less volume output may allow reductions in costs associated with harvesting.

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