

The effects of irradiance and photoperiod on the growth rate of three freshwater green algae isolated from a eutrophic lake

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ABSTRACT

The effects of irradiance and photoperiod on the growth rate of three freshwater green algae isolated from a eutrophic lake

In order to optimise algal growth in mass culture systems, the effect of irradiance and photoperiod on the growth rate of three freshwater green algae isolated from an eutrophic lake (*Selenastrum minutum*, *Coelastrum microporum f. astroidea* and *Cosmarium subprotumidum*) were studied in non axenic batch cultures, under non-nutrient limited conditions. Experiments were performed to determine a specific growth rate (μ max) and optimum light (I_{opt}) over a wide range of light intensities (30 to 456 $\mu\text{mol m}^{-2} \text{s}^{-1}$) at a temperature of 30° C, using a 15/9 (light/dark) photoperiod cycle. The maximum growth rates and optimum light intensities were 1.55 d^{-1} and 365 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for *Selenastrum minutum*, 1.59 d^{-1} and 390 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for *Coelastrum microporum f. astroidea* 0.88 d^{-1} and 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for *Cosmarium subprotumidum*. The photoperiod's effect was determined at 30° C and an incident light of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, under various light:dark cycles. The experimental values fitted by models of Belkoura et Dauta (1992) indicate an increase in the growth rate versus day length with a maximum at continuous light (1.84 d^{-1} for *Selenastrum minutum*, 1.72 d^{-1} for *Coelastrum microporum f. astroidea*, 0.88 d^{-1} for *Cosmarium subprotumidum*). However these experiments don't take into account the accumulated light intensities received by each culture (period of incubation: 24 hours). It was, therefore, not possible to independently appraise the real effect of the lengthened irradiance exposure. So more experiments were carried out, where all cultures under different (light/dark) photoperiod cycles at 30° C received the same cumulated irradiance (8.6 $\text{mol m}^{-2} \text{d}^{-1}$). The results showed that the growth rate is not constant but increased with day length with a maximum at continuous light. These results confirm the real effect of photoperiod on the microalgae growth rate.

Key words: Green algae, growth rate, irradiance, photoperiod.

RESUMEN

Los efectos de la irradiancia y el fotoperiodo en la tasa de crecimiento de tres algas verdes aisladas de un lago eutrófico

Con objeto de optimizar el crecimiento algal en cultivos de producción masiva, se han estudiado, en cultivos no estériles y sin limitación de nutrientes, el efecto de la irradiancia y el fotoperiodo sobre la tasa de crecimiento en tres algas de agua dulce de un lago eutrófico (*Selenastrum minutum*, *Coelastrum microporum f. astroidea* and *Cosmarium subprotumidum*). Los experimentos fueron diseñados para determinar una tasa de crecimiento específica (μ max) y un óptimo de luz (I_{opt}) en un amplio rango de intensidades de luz (30 a 456 $\mu\text{mol m}^{-2} \text{s}^{-1}$), a 30° C de temperatura y utilizando ciclo de fotoperiodo 15/9 (luz/oscuridad). Las tasas máximas de crecimiento y las intensidades de luz óptimas fueron 1.55 d^{-1} y 365 $\mu\text{mol m}^{-2} \text{s}^{-1}$ para *Selenastrum minutum*, 1.59 d^{-1} y 390 $\mu\text{mol m}^{-2} \text{s}^{-1}$ para *Coelastrum microporum f. astroidea* y 0.88 d^{-1} y 360 $\mu\text{mol m}^{-2} \text{s}^{-1}$ para *Cosmarium subprotumidum*. El efecto del fotoperiodo se determino a 30° C y luz incidente de 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, bajo varios ciclos luz:oscuridad. Los valores experimentales se ajustaron mediante modelos Belkoura y Dauta (1992) e indican un incremento en la tasa de crecimiento en relación con la duración del día, con un máximo a luz continua (1.84 d^{-1}

for *Selenastrum minutum*, 1.72 día^{-1} for *Coelastrum microporum* f. *astroidea*, 0.88 día^{-1} for *Cosmarium subprotumidum*). No obstante, estos experimentos no tuvieron en cuenta las intensidades de luz acumulada recibidas por cada cultivo (periodo de incubación de 24 horas). Además, no fue posible apreciar de forma independiente el efecto real del tiempo de exposición de la irradiancia. Por ello se realizaron otros experimentos en los que todos los cultivos bajo diferentes ciclos de fotoperiodo (luz/oscuridad) y a 30° C , recibieron la misma irradiancia acumulada ($8.6 \text{ mol m}^{-2} \text{ día}^{-1}$). Los resultados confirmaron el efecto real del fotoperiodo sobre la tasa de crecimiento de las microalgas.

Palabras clave: Algas verdes, tasa de crecimiento, irradiancia, fotoperiodo.

INTRODUCTION

The culture of microalgae requires a rigorous control of all growth factors: nutrients, pH, and temperature, concentration of CO_2 , O_2 , and light (Morris, 1981). The optimisation of the yield is the main factor in mass culture technology of microalgae. Thus, it is necessary to understand the behaviour of algal species under different environmental factors that determine the different growth parameters. The study of the interactions between these factors and growth modelling parameters allows finding the optimal conditions for selected species in large-scale productivity.

Overall, the growth of microalgal populations depends on three abiotic factors: available light, temperature, and level of nutrients such as nitrogen, phosphorus, and silicate (for diatoms). Among these factors, the light that directly influences photosynthesis mechanism is an important factor in defining optimal conditions for the culture (Falkowski *et al.*, 1985). In the presence of non-limiting nutrients, the efficiency of microalgal culture remained controlled mainly by the intensity of light and temperature.

In addition to temperature and light intensity that are among the main factors acting on the biomass productivity in large-scale microalgal cultures (Richmond, 1986a; De la Noüe & De Pauw, 1988), day length is the determinant factor on the microalgae development. Indeed, the day length influences the circadian rhythm of photosynthesis, respiration (Piquemal, 1990), cellular division (Hobson *et al.*, 1979), and the growth rate (Redalje & Laws, 1983). Moreover, this factor has also an affect on the enzymatic activities (Hobson *et al.*,

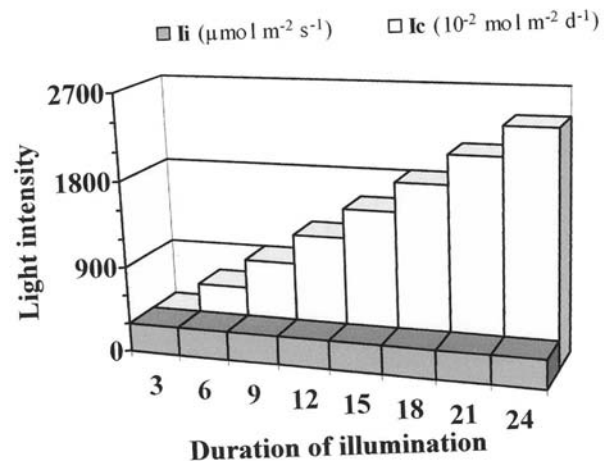


Figure 1. Variation of accumulated irradiance (Ic) in relation to the duration of illumination for a constant incident irradiance (Ii). *Variación de la irradiancia acumulada (IC) en relación con la duración de la iluminación para una irradiancia incidente constante (Ii).*

1979) and macromolecule syntheses (Foy & Smith, 1980). In order to optimise algal growth in applied mass culture systems, we investigated the effect of day length on the growth rate of three species: *Selenastrum minutum*, *Coelastrum microporum* f. *astroidea* and *Cosmarium subprotumidum*, under various conditions of light/dark photoperiod cycle and irradiance.

MATERIALS AND METHODS

Source of the organisms

Three different Chlorophyceae species were isolated from the eutrophic Takerkoust barrage's

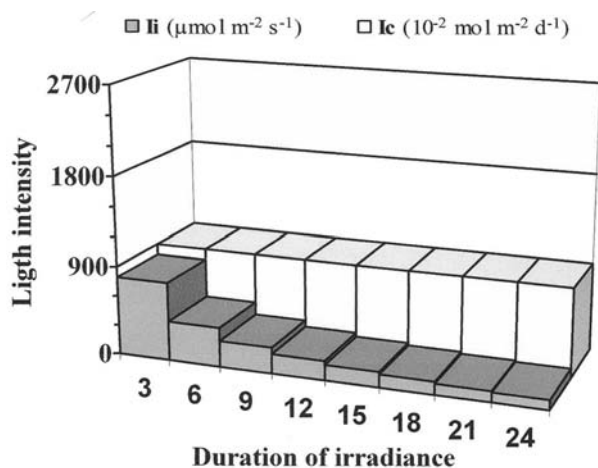


Figure 2. Variation of the incident irradiance (I_i) in relation to the duration of illumination for a constant accumulated irradiance (I_c). Variación de la irradiancia incidente (I_i) en relación con la duración de la iluminación para una irradiancia acumulada constante (I_c).

lake (Western Morocco) and were identified as *Coelastrum microporum* f. *astroidea* (DENOT) NyG, *Selenastrum minutum* (Naegeli) Collins and *Cosmarium subprotumidum* (Nordst) (Philipose, 1967). These species were chosen for our work because they were well adapted to laboratory conditions.

Culture conditions and measurements

Cultures were unialgal but not axenic. They were grown in batch cultures in a mineral medium (Dauta, 1982) with air bubbling ($0.5 \text{ l air l}^{-1} \text{ min}^{-1}$) using compressed and filtered air (Whatman filters GF/C of $1.2 \mu\text{m}$). Experiments were conducted under controlled light in a temperature-programmable chamber with a 15L/9D photoperiod. Phyto-Claude halogen lamps (400 Watt) were used to illuminate the chamber. The intensity of incident light was measured using a silicon sensor HD 8366. For all the experiments, initial strains were acclimated to the two experimental temperatures 25°C and 30°C before the experiment was started. In fact, for the two temperatures used, the cultures were maintained in exponential growth by frequent transfers (every 2 or 3 days). A concentrated culture was incubated in the dark for over 24 hours prior

Table 1. Values of the coefficients derived from Belkoura & Dauta's (1992) equation for *S. minutum*, *C. microporum* and *C. subprotumidum*. Valores de los coeficientes derivados de la ecuación de Belkoura & Dauta (1992) para *S. minutum*, *C. microporum* y *C. subprotumidum*.

Species	A	B	C	D	E
<i>S. minutum</i>	-2.26	0.278	0.9	-1.64	0.12
<i>C. microporum</i>	2.26	0.278	0.88	1.65	0.13
<i>C. subprotumidum</i>	-2.26	0.276	0.98	-1.7	0.12

to the experiment in order to induce synchronization of the algae population and avoid pre-adaptation to light. A sample of the concentrated culture was diluted in a new medium in order to avoid a self-shading effect. The diluted culture was then distributed among ten flasks and exposed simultaneously to ten different light levels ranging from 30 to $450 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Experiments (Fig. 1) for the photoperiod's effect were performed at 30°C and with an incident light of $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$, under various light:dark cycles (3/21, 6/18, ... 24/0, L/D). This temperature was chosen because the value is quite similar to that of Barrage's lake. This value is effectively characteristic of the three species, which dominate mainly during summer and early fall. However, these experiments don't take into account the accumulated light intensities received by each culture (period of incubation: 24 hours). It was, therefore, not possible to independently appraise the real effect of lengthening light exposure. Therefore, more experiments were carried out (Fig. 2), where all cultures under different photoperiod cycles at 30°C received the same accumulated irradiance ($8.6 \text{ mol m}^{-2} \text{ day}^{-1}$). The optical density method was used to measure the growth rate at 750 nm using 1 cm espectrophotometric cell. This method was chosen because preliminary data showed a significant correlation between cell number and light absorption at 750 nm ($r^2 = 0.99$ $n = 23$, $r^2 = 0.99$ $n = 27$, $r^2 = 0.92$ $n = 12$ for *S. minutum*, *C. microporum* and *C. subprotumidum* respectively). The experiment duration was 24 h for each temperature. Measurements were done at the beginning of the cycle (A_0) and after 24 hours (A_1). The growth rate was calculated; measurements were done at the beginning of the cycle (A_0) and after 24 hours

(A_1). The growth rate was calculated according to the equation,

$$\mu = \ln(A_1/A_0) \text{ day}^{-1} \quad (1)$$

To estimate physiological parameters for the growth-light intensities relationship, the experimental data were fitted to the model of Peeters & Eilers, 1978, according to the following equation,

$$\mu_{(T,15/9)} = \mu_{\max} * 2 * (1 + \beta) * I' / (I'^2 + 2 * I' * \beta + 1) \quad (2)$$

where μ , μ_{\max} and β are the growth rate, the maximal growth rate and the attenuation coefficient respectively. I and I_{opt} are the irradiance and optimum irradiance respectively, and $I' = I/I_{\text{opt}}$.

Experimental data for the growth and day length relationship was fitted to the model of Belkoura & Dauta, 1992, according to the following equation,

$$\mu_{\max(30,nH)} = \mu_{\max(30,15/9)} \left[A + 1 / (B + C \cdot e^{(D-E \cdot nH)}) \right] \quad (3)$$

where nH is the duration of illumination at $T = 30^\circ \text{C}$, A , B , C , D and E are not the physiological parameters but the model's coefficients for adjusting the observed data.

The coefficients (A , B , C , D and E) (Table 1) were calculated by the least squares methods. For all experiments, the results are means of three replicate flaks.

Table 2. Main growth parameters of *S. minutum*, *C. microporum*, and *C. subprotumidum* at 30°C . Adjusted to the model of Peeters & Eilers (1978). *Principales variables de crecimiento de S. minutum, C. microporum y C. subprotumidum a 30°C . Ajustadas al modelo de Peeters & Eilers (1978).*

Species	$\mu_{\max}(d^{-1})$	$I_{\text{opt}}(\mu \text{ mol m}^{-2} \text{ s}^{-1})$	GT (h)
<i>S. minutum</i>	1.55	365	10.7
<i>C. microporum</i>	1.59	390	10.4
<i>C. subprotumidum</i>	0.88	360	18.9

RESULTS

The results of the fitted data by Peeters & Filers's model (equation 2) described in material and methods, are presented in figure 3. The growth rates of the tree species increased with light until they reached a maximum value (μ_{\max}) associated with an optimal light intensity (I_{opt}). Beyond this intensity, which can be considered as a light saturated growth, μ decreased more or less rapidly. This photo-inhibition occurred at different intensity levels and depended on temperature conditions. The tolerance to light of the algae was higher at a temperature of 30°C than at 25°C .

Table 2 summarizes the main parameters associated with growth of the three species. Generation times (GT) have been determined from $\mu_{\max T}$ (at $T = 30^\circ \text{C}$) using the following relation: $GT = \mu^{-1} \ln 2$ (Reynolds, 1984).

Volumes and surface areas (Table 3) were estimated in order to investigate size effect on the growth rate using a standard geometrical formulae corresponding to an ellipsoid for the three species.

The influence of day length on growth at constant incident light is shown in figure 4. The experimental values indicate an important increase in the growth rate versus day length. A maximum growth for the three species is observed under continuous light (*S. minutum*: 1.84 day^{-1} ; *C. microporum*: 1.72 day^{-1} ; *C. subprotumidum*: 1.05 day^{-1}). On the other hand

Table 3. Volumes (V), Surface areas (SA), and growth rates (μ_{\max}) for *S. minutum*, *C. microporum* and *C. subprotumidum*. *Volúmenes (V), areas superficiales (SA) y tasas de crecimiento (μ_{\max}) para S. minutum, C. microporum y C. subprotumidum.*

Species	V ($\mu \text{ m}^3$)	SA ($\mu \text{ m}^2$)	$\mu_{\max}(\text{day}^{-1})$	$\mu^*(\text{day}^{-1})$
<i>S. minutum</i>	19	188	1.36	1.56
<i>C. microporum</i>	383	1091	1.16	1.27
<i>C. subprotumidum</i>	1738	4270	0.74	1.12

μ_{\max} , Maximum growth rate obtained from laboratory experiments at 25°C ;

μ^* , Maximum growth rate calculated by regression equation of Reynolds (1984) in relation with volume: $\mu = 1.855 - 0.226 (\log_{10} V)$.

the generation time decreased with an increase in irradiance length (Fig. 4).

The influence of day length on growth at constant cumulated irradiance ($8.6 \text{ mol m}^{-2} \text{ day}^{-1}$) is shown in figure 5. The results were similar to those observed in figure 4; the growth rate increased with irradiance length; a maximum growth rate for the three species was observed under continuous light (*S. minutum*: 1.76 day^{-1} ; *C. microporum*: 1.88 day^{-1} *C. subprotumidum*: 1.01 day^{-1}). However for the two experiments,

after 15 hours of irradiance, the growth rate barely increased and did not change significantly.

DISCUSSION

The results of the present work show that for a photoperiod of 15/9 L/D, *C. subprotumidum* exhibited the lowest μ_{\max} (0.88 day^{-1}) at a temperature of 30°C . Lower growth rate values ($0.13\text{-}0.33 \text{ day}^{-1}$) were reported in some species

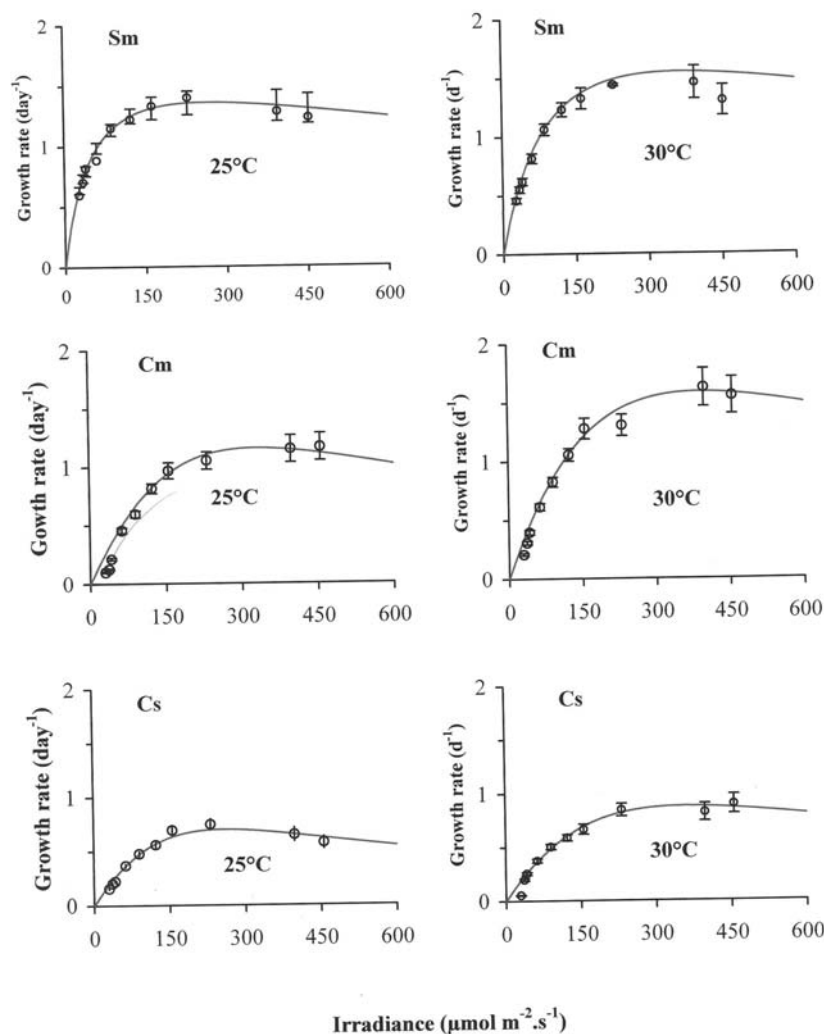


Figure 3. Effect of irradiance on the growth rate for *S. minutum* (Sm), *C. microporum* (Cm), and *C. subprotumidum* (Cs), the observed data (●) are fitted to the function of Peeters & Eilers (1978) (continuous line). *Efecto de la irradiancia en la tasa de crecimiento para S. minutum (Sm), C. microporum (Cm) and C. subprotumidum (Cs), Los datos observados (●) se han ajustado a la función de Peeters & Eilers (1978) (línea continua).*

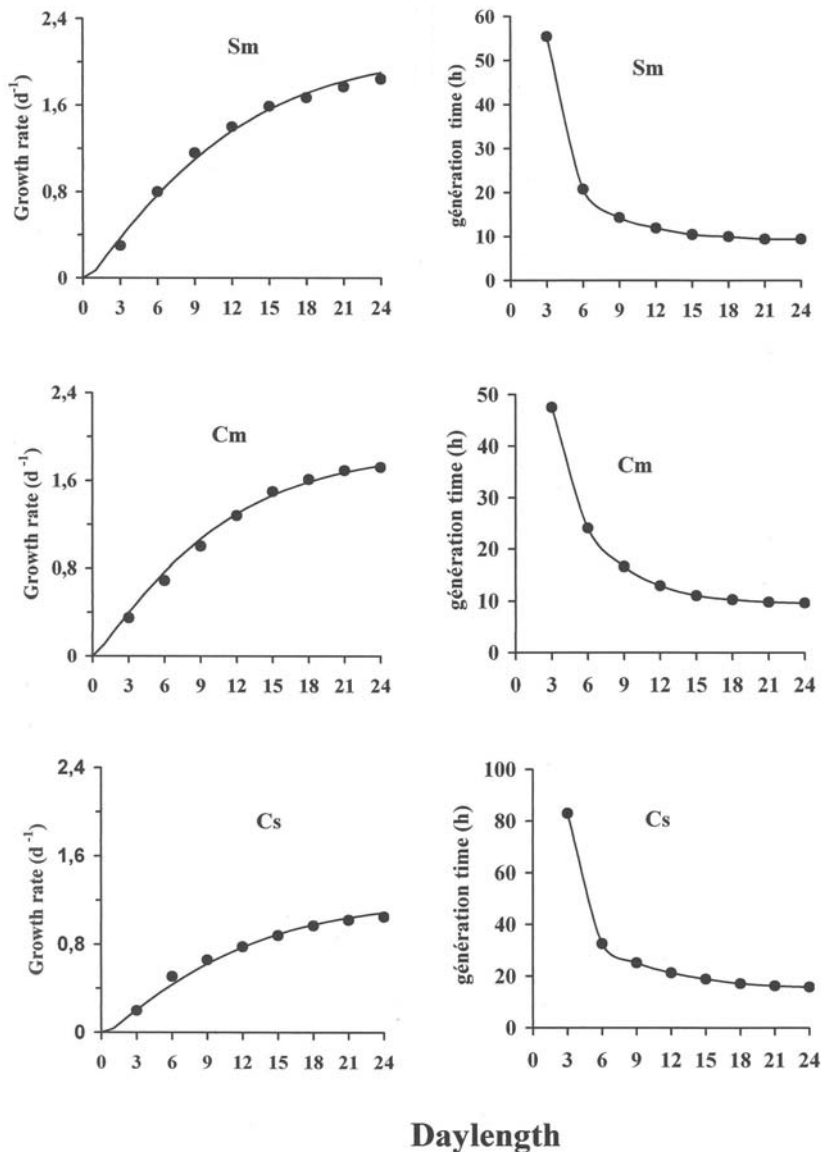


Figure 4. Variation of the growth rate and generation time of *S. minutum* (Sm), *C. microporum* (Cm), and *C. subprotumidum* (Cs) in relation to daylength for a constant incident irradiance ($I = 300 \mu\text{mol m}^{-2} \text{s}^{-1}$) at 30°C . The observed data (●) are fitted to the function of Belkoura & Dauta (1992) (continuous line). *Variación de la tasa de crecimiento y el tiempo de generación de S. minutum (Sm), C. microporum (Cm) y C. subprotumidum (Cs) en relación con las horas diarias de luz para una irradiación incidente constante ($I = 300 \mu\text{mol m}^{-2} \text{s}^{-1}$) a 30°C . Los datos observados (●) se han ajustado a la función de Belkoura & Dauta (1992) (línea continua).*

of desmids (Moss, 1973). Similarly, a lower growth rate (0.5 day^{-1}) was found for another desmid species, *Staurastrum pingue* (Dauta *et al.*, 1990). However, the maximum growth rate (μ_{max}) obtained for *C. subprotumidum*, is lower than those of *S. minutum* and *C. microporum*. The allometric relationship between growth rate

and cell size was the most plausible explanation of species' differences in maximum growth rate. Indeed our results indicate that for these species *S. minutum* (smaller size) grew faster than *C. subprotumidum* (larger size). Maximum growth rates of the three species at 25°C (Table 3) compared to the predicted values at the same

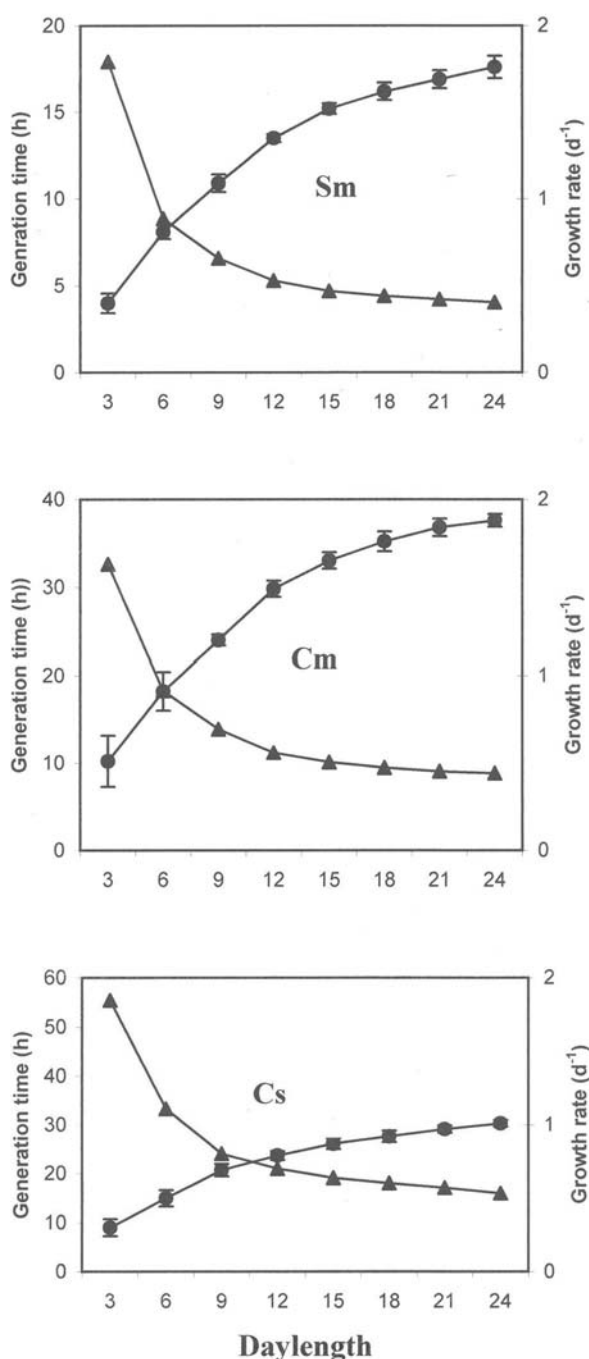


Figure 5. Variation of growth rate and generation time of *S. minutum* (Sm), *C. microporum* (Cm), and *C. subprotumidum* (Cs) in relation to day length for a constant accumulated irradiance ($I = 8.6 \mu\text{mol m}^{-2} \text{day}^{-1}$) at 30°C . Variación de la tasa de crecimiento y del tiempo de generación de *S. minutum* (Sm), *C. microporum* (Cm) y *C. subprotumidum* (Cs) en relación con las horas diarias de luz para una irradiancia acumulada ($I = 8,6 \mu\text{mol m}^{-2} \text{día}^{-1}$) a 30°C .

temperature (25°C) using Reynolds's equation (1984) were slightly below the regression line. This confirms that our results show an inverse relationship between cell size and growth rate. Similarly, several authors (Foy, 1980; Schlesing *et al.*, 1981; Reynolds, 1984; Stolte *et al.*, 1996) found a significant negative correlation between cell size and growth rate. This is due to the effect of various physiological and metabolic processes as well as the algae size and structure. In fact, small cells assimilate nutrients faster and incorporate carbon more efficiently than large ones (Reynolds, 1984).

Light is an essential resource often limiting the growth rate of algae and is also a major factor determining photosynthetic rate in algae. The optimal light intensity (I_{opt}) varied between $360 \mu\text{mol m}^{-2} \text{s}^{-1}$ for *C. subprotumidum*, $365 \mu\text{mol m}^{-2} \text{s}^{-1}$ for *S. minutum*, $390 \mu\text{mol m}^{-2} \text{s}^{-1}$ for *C. microporum*. The growth rate was reduced at light intensity values below or above those ranges. Previous studies revealed that low or high irradiances cannot sustain the maximum growth rate (Ojala, 1993; Belkoura & Dauta, 1994; Mouget *et al.*, 1995). Beyond the optimal light intensities, the growth seems to be limited by the phenomenon of photo-inhibition in the three species. The same results have been observed by other authors (Belkoura & Dauta, 1992; Lee & Rhee, 1999; Coles & Jones, 2000; Benider, *et al.*, 2001).

The influence of day length on the growth rate at a constant incident light ($300 \mu\text{mol m}^{-2} \text{s}^{-1}$) show that growth was maximum under continuous light. Accordingly, growth depends on the quantity of energy received by the cultures. The longer the duration of illumination; the shorter cell division is. The accumulated irradiance was indeed a factor that could limit cellular division in *S. minutum*, *C. microporum* and *C. subprotumidum*. However, beyond 15 hours of irradiance the variation in growth rate became less and less perceptible, confirming the variation of generation time. In this way, with a 15 hour/9 hour (light/dark) photoperiod cycle the cells seems to have accumulated enough energy for cell division in the closest time to the one observed in continuous culture. Hence, it seems

that 9 hours of darkness was sufficient for all the phenomena that occur in the absence of light. In this experience, the variation in growth rate could be related to the increase in the accumulated light intensity received during the period of incubation and not to the lengthened irradiance exposure. Therefore, in order to check this hypothesis, a second experiment was carried out, where all cultures received the same accumulated irradiance ($8.6 \text{ mol m}^{-2} \text{ day}^{-1}$). The results show that growth rate is not constant but increased with day length with a maximum at continuous light. These results confirm the real effect of the photoperiod on the microalgae growth rate. A similar response was reported by Nicklish, 1998 and Tahiri, 2000.

The relationship between microalgae growth rate and day length has been a neglected area of study. It is generally considered that algae exhibit a growth rate that is proportional to the duration of the effective light period. Several authors refer to this relationship (Foy 1976, Dermoun, 1987; Nielsen, 1992; Piquemal, 1990; Belkoura & Dauta, 1992; Foy & Gibson 1993, Mulyadi 1995).

Cultures under continuous light are often used because they achieve the maximal growth rate recorded. However, most works generally suggest the use of light/dark cycles instead of continuous light, which seems to be inappropriate. Indeed, a light/dark regimen allows for either an increase in final concentration or a lowering of production costs. The necessity of a dark phase was explained by the photosynthesis being governed by two reactions, a photochemical phase that is light dependent and another, a biochemical dark phase that is light independent. The compounds that are produced in the light dependent phase (ATP, NADPH) are used in the dark phase to synthesize metabolic molecules essential for growth. In addition, Laval & Mazliak (1995) have reported that some enzymes of the pentose cycle of photosynthesis and CO_2 fixation are inactive during the illumination. According to Roland & Joyard (1977), the affinity of carboxydismutase for CO_2 decreases dramatically in the dark when the pH decreases. Its activity can be completely

inhibited. This inactivation blocks the uptake of ribulose 1,5-diphosphate such as the total uptake could hinder the restarting of photosynthesis in the light. A dark phase remains necessary at least for the regeneration of cofactors (NAD^+ , NADP^+) required for phase I of photosynthesis.

With a 15/9 photoperiod cycle, using a series of photographs taken at every hour of the day, Dauta (1982) showed, that cell division occurs under dark conditions for many unicellular Chlorophyceae. Similarly, Dermoun (1987), working with a 16/8 photoperiod, has shown for *Porphyridium cruentum* that cell division occurs in the dark phase as well as in the illuminated phase. If the cell equilibrium that mitosis gives is possible under dark and illuminated conditions, cell divisions are more frequent after the interruption of the illuminated phase. It is therefore preferable to use photoperiod with a light duration of between 12 and 15 hours in order to allow for the equilibrium that is established between anabolic and catabolic phenomena during the photoperiod cycle. Furthermore, for industrial applications and considering the ratio between the cost of energy and the corresponding biomass productions, 12 to 15 hours duration for the illuminated phase is generally considered as optimal for algae growth. In addition, the algal species in this work have a natural photoperiod of 15/9 corresponding to the climate of arid and semi-arid regions of the Mediterranean.

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