# Quantitative assessment of the major limitations on productivity of *Spirulina platensis* in open raceways

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## Abstract

This work represents an attempt to assess the relative contribution of the factors limiting productivity of *Spirulina platensis* in open raceways throughout the year. Temperature of the culture during daylight exerted the predominant effect on productivity and elevating the temperature resulted in a significant rise in productivity even in summer. Photoinhibition had a decisive role in summer in determining productivity of *Spirulina* in open raceways in that growth almost ceased after mid-day. Contamination by other microorganisms, particularly *S. minor* and *Chlorella* sp. was estimated to reduce the net biomass yield by at least 15 to 20%, but measures to curtail the establishment of these species in the raceway have been devised. The effect of harvest time during the day on the yield of dry mass was examined: no conclusion could be drawn except in mid-summer, when evening harvesting resulted in a significant increase in the output rate of dry weight over morning harvesting.

It was estimated that in a subtropical climate with little cloudiness, it should be readily possible to obtain an annual output rate of dry mass of ca. 60 to 70 t ha$^{-1}$. Such output, however, which would reduce very substantially the cost of production to-date, is possible only if the optimal temperature for *Spirulina* can be maintained throughout daylight, photoinhibition essentially controlled, harvesting in summer performed in the evening, and night-loss of dry mass as well as the extent of contamination by other cyanobacteria or microalgae can be kept low. The pronounced daily fluctuations in the output rate at peak productivity in summer suggest that when environmental limitations of growth are minimal, other limitations become dominant. These should be identified to facilitate an even greater increase in the productivity of *Spirulina* in outdoor cultures.

## Introduction

Commercial microalga culture today is to a large extent determined by production facilities which consist of outdoor open raceways. This mode of culture results in a rather low rate of production per unit of reactor area or investment capital. Cost of production becomes such as to make economic success dependent on marketing of costly and exclusive products, for which demand is naturally limited. Hence the dire need to evaluate quantitatively, throughout the year, the major factors which govern net productivity in outdoor cultures.

The basic role of environmental factors in effecting growth and productivity of microalgal cultures grown outdoors was reviewed by Richmond (1986), and more recently elucidated by Fontes *et al.* (1987), Bocci *et al.* (1987) and Boussiba *et al.* (1988). Richmond and Grobbelaar (1986) studied the limiting factors that affect productivity of *Spirulina* cultures grown outdoors, and in particular, the effect of modifying the light regime for the cells by manipulating the depth of culture as well as cell density, which was described earlier in detail by Vonshak *et al.* (1982, 1983). The effect of temperature on the productivity of *Spirulina* in a closed photobioreactor throughout the year was well described by Torzillo *et al.* (1986).

Nevertheless, the whole array of limitations and their interactions exerted on growth and productivity of *Spirulina* cultures in outdoor raceways throughout the year has yet to be elucidated. The following account attempts to assess quantitatively the contribution of the major factors influencing net productivity of *S. platensis* in open raceways throughout the year.

## Materials and methods

### 1. Growing the culture

Algal cultures were grown outdoors in a 2.5 m$^2$ raceway stirred with a paddle wheel as described by Boussiba *et al.* (1988). The pH was maintained at 9.8 to 10.3 by bubbling $CO_2$ through a porous tube. The growth medium was as described by Vonshak *et al.* (1982).

Growth in the culture was estimated by (a) measurement of the optical absorbance in klett photometer with a No. 54 filter (approximate spectral range: 500–570 nm) and (b) determination of dry weight (DW) or chlorophyll (Vonshak, 1986).

### 2. Modes of harvesting

Harvesting, i.e. removal of *Spirulina* cells from the growth medium, was carried out in either of the following modes:

(a) Filtration: A pre-set volume of *Spirulina*-laden medium was poured over a 300-mesh screen, and the wet mass thus collected was washed off the screen.

(b) Bleeding: A certain volume of the culture in the pond was removed and replaced by the same volume of freshly prepared growth medium.

### 3. Light and oxygen measurements

Photon Flux Density (PFD) was measured using a Li-Core instrument with a quantum sensor.

Oxygen concentration in the cultures was measured by a Clarke type electrode Oxi 56 (WTW W. Germany).

### 4. Estimation of degree of photoinhibition

Oxygen evaluation rates to estimate the degree of photoinhibition were carried out according to Vonshak *et al.* (1988).

### 5. Temperature control

#### (a) Measurement of temperature
Maximum-minimum thermometers were submerged in each pond and one thermometer was placed in the air. Pond temperatures were recorded three times daily: morning, noon, evening. Maximum and minimum temperatures were recorded at the evening measurement, when the thermometers were reset. The thermometers were calibrated once a month using a laboratory class-2 glass thermometer.

#### (b) Heating the cultures
The cultures were heated by submerged black polyethylene heat exchanger made of 5-mm tubing inserted in the pond. The heating liquid consisted of deionized water heated in a reservoir to 95 °C by 2 to 5 KW heating elements (depending on ambient temperature) and circulated continuously

by a 0.5 HP centrifugal pump which supplied the hot water to the two heat exchangers simultaneously.

The temperature in the cultures was controlled by a liquid sensor imerged in one of the heated ponds connected to a thermostat controller, which turned on/off the hot water circulating pump whenever the present temperature was reached.

The heaters and circulating pump were turned on between 0330 to 0530 (depending on season) aimed to achieve 28 °C at 0800 in the heated ponds. The average rate of temperature rise per hour in the heated ponds was 5.5 to 6.5 °C, which was considered moderate and non-damaging to the cells.

The heaters as well as the hot water circulating pump were turned off at 1530 at the latest. In the summer, they were turned off as soon as the desired pond temperature was reached. Temperature equalization of the two heated ponds was achieved by controlling the amount of hot water circulating through each of the heat exchangers, using two manual gate valves in the effluent lines. Heating was so controlled as to affect an elevation of pond temperature up to 35 °C, thus the difference in pond temperature between the heated and non-heated treatments was most pronounced in winter, ˙when the heated culture was heated throughout the day. In summer, the heated ponds reached 35 °C by 0930 to 1100 in the morning, while the non-heated cultures reached maximal temperature some two or three hours later.

## Results and discussion

### 1. Correlating the optical density with the dry weight throughout the year

A quick assessment of growth outdoors may be provided by following changes in the optical density of the culture. This has an added advantage of facilitating continuous, on-line estimation of growth, with a proper turbidity sensor. The correlation constant $(R)$ between the dry weight (DW) and the optical density of the culture throughout

the year was 0.72, a lower degree of correlation $(R = 0.62)$ having been observed between the optical density and the extracted cell-chlorophyll.

The conversion factor (mg DW per klett unit) varied significantly in the various experimental treatments and was particularly affected by the temperature of the culture, the season of the year and the population density. The largest conversion factor was found for cultures maintained at low cell density (150 klett units – ku), averages ranging from 3.03 in unheated ponds in winter to 2.45 in heated ponds in summer. Smaller conversion factors were found in cultures maintained at higher cell densities (225 ku and at 275 ku). This phenomenon was most prominent in summer in the heated cultures in which the lowest conversion factors, ranging between 2.45 to 2.20, were found. During the winter as well as in unheated cultures throughout the year, there was usually more DW per ku than there was during the summer or in the heated cultures throughout the year.

### 2. Effect of temperature on productivity

Increasing the temperature in the culture exerted a decisive effect on the productivity (Fig. 1). The optimal temperature for Spirulina under controlled conditions ranges between 35 to 37 °C (Richmond, 1987) and the highest effect of temperature on biomass production was clearly evident in winter, when it was impossible under our conditions to sustain the cultures unless they were either heated or covered with polyethelene sheets which affected a temperature rise of ca. 6 to 8 °C. The high ambient temperature in summer affected a rise in temperature of the unheated pond which by miday approached the optimal for Spirulina, i.e. 35 to 37 °C. Heating in summer was therefore provided only in the morning, resulting nevertheless in a significant increase in the output rate of dry weight. Thus, while the relative effect of heating in winter was much larger than in summer (Fig. 1), heating the culture in summer mornings affected some 20% increase in the output rate of biomass. The overall yield of biomass in the
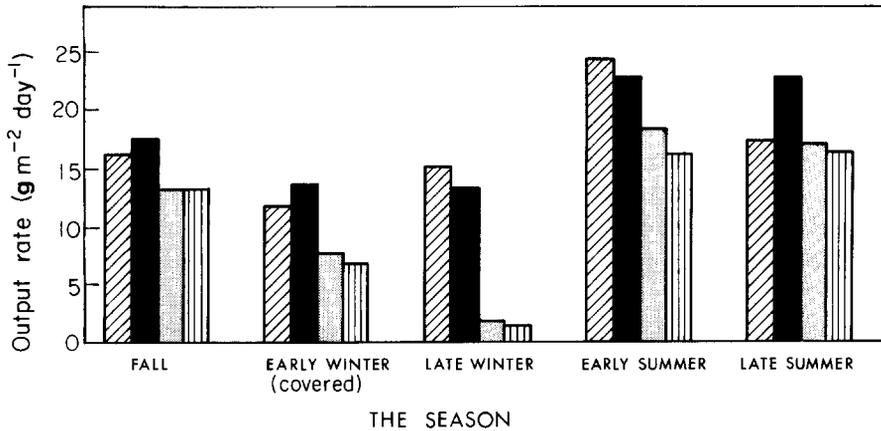
*Fig. 1.* Output rates as a function of temperature and the time of harvesting: ▨ morning harvesting, heated; ■ evening harvesting, heated; ☐ morning harvesting, unheated; ▥ evening harvesting, unheated.

heated ponds during 6 months in fall and winter was ca. one-half of that obtained in compareable cultures in late spring and summer. This was interpreted to result from the reduction in day length and radiation intensity along late fall and winter.

### 3. Morning vs evening harvest

Harvesting in the evening is thought to reduce nocturnal respiratory losses, particularly when the temperature in the pond remains relatively high during the entire diurnal cycle. Night loss of biomass was reported to be highly dependent on operational and environmental conditions such as cell concentration, temperature of culture and product formation (Torzillo, 1990; Vonshak et al., 1982). The rate of removal of dissolved $O_2$ during the night may also be significant, as in one preliminary experiment it was possible to reduce night loss of biomass by bubbling of $N_2$ during early evening (Guterman et al., 1989). The range of night loss of biomass in summer varied greatly from 5 to 12% of the total pond biomass, and a typical pattern of fluctuations in biomass concentration along a diel cycle is shown in Fig. 2. If however the nocturnal loss of dry weight is computed on the basis of the output produced in the preceeding day rather than on the basis of the overall cultures mass, the extent of nocturnal loss

may at times be very substantial, e.g. 30 to 50%, since the daily output represents only a fraction of the standing concentration of culture mass. There are indications that nocturnal loss of biomass may, under certain circumstances which we could not define, play an important role in reducing net productivity of outdoor cultures, particularly in the warm season (Torzillo and Materassi, 1990). In our experimental set-up, i.e. small 2.5 $m^2$ raceways, consistent differences between morning and evening harvesting through most of the year could not be discerned, except in late summer when evening harvest resulted in a significant increase in the output rate (Fig. 1). At this season, the ambient temperature was at its peak and
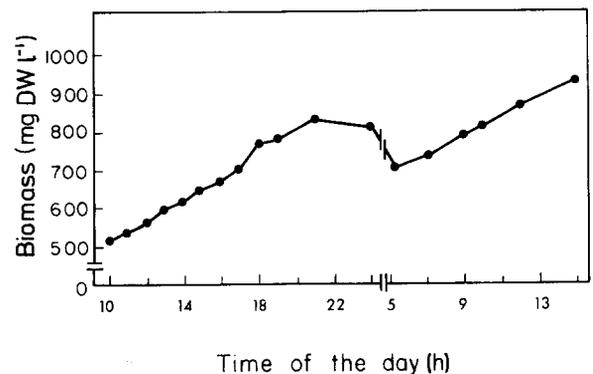


*Fig. 2.* Diel fluctuations of biomass concentration in a summer culture.

heating the culture did not increase productivity unless evening harvesting was performed (Fig. 1).

This finding is further supported by observations of two of us (A.R. and A.V.) in large-scale industrial operations (Ein-Yahav Algae, Israel, and Siam Algae, Thailand). When the time of harvesting in these commercial instalations was altered from the morning to late afternoon or evening, an increase in net productivity in the range of 15–20% was observed in comparison with the production obtained in a previous year when harvesting was carried out mainly in the morning (Vonshak & Richmond, unpublished data).

## 4. The effect of solar irradiance on the pattern and the rate of growth

### (a) Patterns of growth

The course of growth along the day in a *Spirulina* raceway was given special attention in this work. Since growth conditions outdoors change continuously, particularly in relation to light, true steady state growth cannot exist in outdoor cultures. A detailed daily pattern of *Spirulina* growth was thus studied in summer by estimating the 'relative growth rate' throughout the day as follows: The optical density and dry weight concentrations were measured in one to two hour intervals, and the increment increase per interval $(x_1)$ was calculated relative to the biomass concentration measured at the end of the previous measurement $(x_0)$, i.e. $(x_1 - x_0) x_0^{-1} h^{-1}$. The parameter thus calculated was termed the 'relative growth rate' (Fig. 3). This parameter should be viewed with some caution due to inaccurecies imbuded in measurement of small increments of growth in arbitrary intervals. Nevertheless, we believe it serves a useful purpose in amplifying the radical changes in growth rate which take place in outdoor *Spirulina* cultures throughout the summer days. In the heated treatments, a marked difference was observed between the daily patterns of growth which occurred in winter and in summer. In winter and early spring, a slow growth-rate observed in the morning remaining essentially
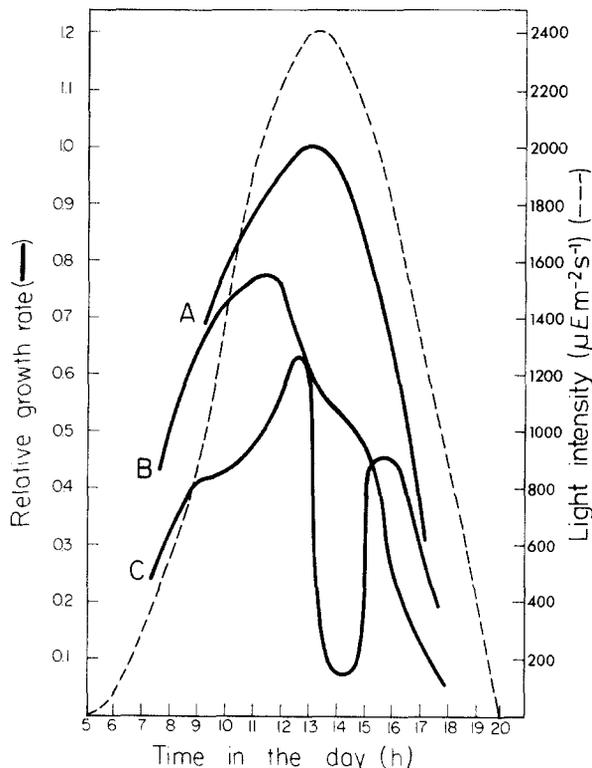


*Fig. 3.* Patterns of the relative daily growth rate in summer. ----- irradiance; ——— relative growth rate.

constant until declining on the afternoon with the fall in light intensity. In contrast, the fast exponential rate of increase in biomass which occurred typically in summer was evident only up to noon, slowing down sharply after-midday.

In heated cultures in mid-summer, when the output rate was maximal, three general patterns in daily growth could be discerned: In one (c), the relative rate of growth increased steadily with the increase in light intensity. Soon after midday growth was sharply curtailed for some 2 hours, the rate of growth increasing shortly at mid-afternoon and declining soon afterwards as irradiance rapidly diminished. Another frequently observed daily pattern of growth is depicted in (b): The relative rate of growth increases along the morning as in (a), peaking around early noon and gradualy decreasing thereafter. Still another pattern of growth, often observed particularly in cultures maintained at low population density (150 ku) and thus under a relatively lower light

limitation is shown in (a): The growth rate increasing very rapidly becoming very high at noon but diminishing very sharply thereafter.

### (b) Patterns of dissolved oxygen concentration

An outdoor algal culture represents, in effect, an open system for gaseous exchange, in which the oxygen concentration has an important role. Its concentration readily builds up in the culture as soon as photosynthesis starts in early morning. Oxygen concentration in the culture is thus essentially a function of both the rate of photosynthesis and the rate of its dissipation from the culture which in turn is greatly effected by the temperature in the culture as well as by the intensity of stirring (Richmond, 1986).

Previous findings indicate that measurements of the concentration of photosyntheticaly-produced oxygen in the culture as it increases following sunrise constitutes an essential and useful parameter for estimation the relative 'well being' of the culture or its production potential (Vonshak et al., 1982; Richmond, 1986). In the uncovered and unheated pond in late winter, little change in pond-$O_2$ was observed (Fig. 4d), reflecting the low photosynthetic activity prevailing under our winter conditions. Heating the culture resulted in increased photosynthetic activity as reflected in a significant rize in DO concentration, which decreased rapidly in the afternoon (Fig. 4b). In contrast, the DO concentration in summer generally followed more closely the daily temperature and irradiance curves (4a + 4c).

Measurements of DO concentration throughout the day in summer showed that, reminiscent of the pattern exhibited by the 'relative growth rate' during a summer day, the great surge in pond-$O_2$ which began in the early morning lasted only to about noon, decreasing rapidly thereafter. Although the maximal pond temperature was reached in summer at about 1300 in both heated (4a) and non-heated (4c) ponds, there was a marked difference in the daily pattern of DO concentration between these treatments: In heated cultures the rise in $O_2$ was moderate, yielding a wide plateau at the range of only 15 to 17 ppm, which represents a ca. 200 percent saturation. In the non-heated cultures, where pond temperature rose gradually with the increase in ambient temperature, pond-$O_2$ rose much shar-
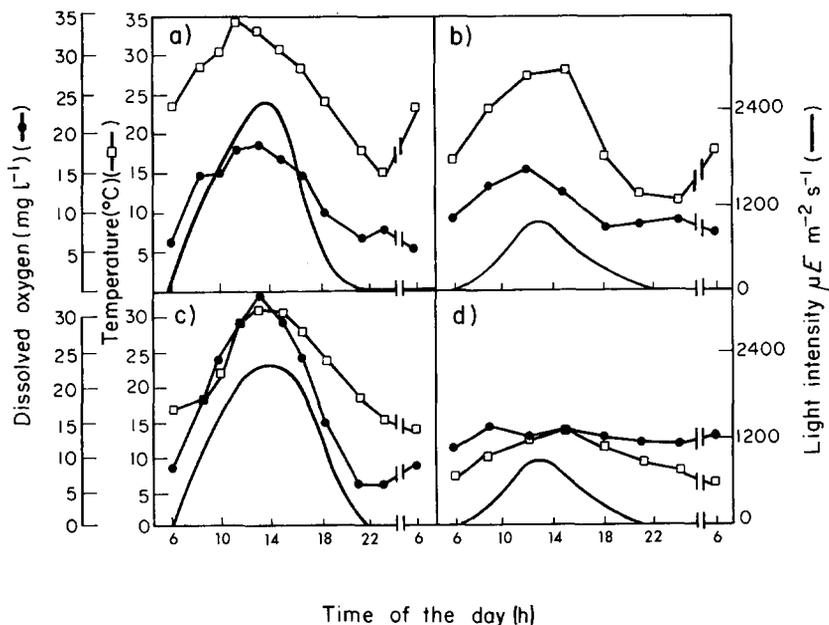


Time of the day (h)

Fig. 4. Diurnal pattern in dissolved oxygen concentration as a function of irradiance and culture temperature: (a) heated, summer; (b) heated, winter; (c) unheated, summer; (d) unheated, winter.

per, finally peaking with maximal pond temperature as was the case with the heated pond (4c). In contrast with the heated treatment, DO in the non-heated pond reached a steady-state at a much higher concentration – 30 to 33 mg l$^{-1}$ (close to 500% saturation). The sharp rise in DO in non-heated cultures in summer reflected the dominant effect the rising temperature and irradiance exerted on the photosynthetic activity. That rise was not observed in the heated ponds was apparently due to the relatively high temperature which prevailed from early morning curtailing the build-up in O$_2$ concentration.

### (c) Effect of Photo-inhibition

In an experiment carried out in the preceeding summer we measured the effect of the output rate of a polyethelene cover placed over a 100-m$^2$ raceway. The average outputs obtained were 15.3 and 19.2 g DW m$^{-2}$ d$^{-1}$ for the uncovered and covered ponds, respectively. The difference in the light cut-off of the plastic sheets between the covered and non-covered pond was about 30%, the former providing protection from high light intensity at mid-day. The increase in productivity in the covered pond was thus interpreted to result mainly from an arrest of photoinhibition. Indeed Vonshak et al. (1988) demonstrated that exposure of Spirulina cultures to high light intensities may readily lead to photoinhibition not only in laboratory but also in outdoor cultures, causing a loss as high as 30% of the potential output rate. The sensitivity of S. platensis to photoinhibition may be aptly demonstrated from measurements of the extent of photoinhibition in summer mornings in heated vs non-heated ponds (Table 1). Evidently, a relatively low morning temperature when

coupled with relatively high PFD (amounting to some 50% of maximal summer radiation) may already cause damage to the cells.

The decline in dissolved O$_2$ in both heated and non-heated ponds as afternon progresses reflects the reduction in photosynthetic activity which was clearly due to the decline in light intensity (Fig. 4). The very fast rate of decline, however, which is evident when the PFD is still a few times above saturation for single cells (e.g. at 1500) is worth noting: we propose that it reflects the photoinhibitory damage suffered by many cells at noon which are thus responding more sharply to the progressive reduction in light intensity. Indeed, Vonshak and Guy (1988a + b) were first to report sharply reduced rates of O$_2$ evolution by Spirulina cells damaged by photoinhibition as light intensity declines on the afternoon.

We thus propose that photoinhibition represents one major reason for the marked decrease in the rate of growth of S. platensis past midday, shown in Fig. 3. Accordingly, many cells of Spirulina grown in summer recuperate only at late afternoon and during the night from the damaging exposure to high PFD in midday, resuming their full growth potential on the next morning. Overall productivity of Spirulina in summer is thus significantly curtailed, the estimated loss in output rate amounting to some 30% in the relatively sensitive strain used. This appraisal is arrived at by computing the output that would be if the growth rate reached at early noon continued essentially unabated for a period of another 3 h after midday.

### 5. Effect of contaminants on overall productivity of Spirulina platensis throughout the year

Microalgal contaminants, algae different from the cultured species as well as protozoa and fungi, represent a major limitation to the overall net output of open outdoor cultures of S. platensis. It was therefore attempted to record the appearance and magnitude of contaminants throughout the year and estimate their overall effect on productivity. The observations summarized in Table 2 fit many years of experience in open outdoor cul-

*Table 1.* Effect of pond temperature on photoinhibition in summer mornings measured at 1000 in the morning, radiation range 1100–1500 $\mu$mol photon m$^{-2}$ s$^{-1}$.

|  | Pond temperature | Photon Flux density | % Photo-inhibition |
| --- | --- | --- | --- |
| Heated | 27 | 1300 | 6 |
| Non-heated | 18 | 1300 | 14 |

*Table 2.* Annual survey of the major contaminants of *Spirulina platensis* culture in heated and non-heated open raceway outdoors during 1987–1988.

| Heated or Non-heated | Date | Population density (klett units) | Pond Temp. typical daily range | *Chorella* | Diatom | *Micro-cystis* | *S. minor* | Protozoa/ Amoeba | | Treatment |
|---|---|---|---|---|---|---|---|---|---|---|
| H | 15–20 | 220 | 29–37 | + | | + | | | | |
| NH | Nov. | | 18–30 | + | | | | | | |
| | 29–18 | 220 | 26–32 | | + | + | | | | 1 mM NH₄⁺H |
| NH | Nov.-Dec. | | 13–24 | | | | | | | |
| H | 10–29* | 150 | 29–36 | + | | + | | | | |
| NH | Jan. | | 10–21 | + | | | | | | |
| H | 31–12 | 150 | 28–33 | + | | | | | | |
| NH | Jan.-Feb. | | 11–21 | | | | | | | |
| H | 6–18 | 225 | 25–30 | | + | | | | | |
| NH | March | | 11–22 | | + | | | | | |
| H | 20–31 | 225 | 24–31 | + | + | | | | + | |
| NH | March | | 11–24 | + | + | | | | | |
| H | 11–15* | 150 | 28–33 | + + | | | | | | 2 mM NH₄⁺ |
| NH | April | | 14–27 | + + | | | | | | |
| H | 17–22 | 150 | 25–33 | | + + | + | | | | |
| NH | April | | 13–26 | | + + | | | | | |
| H | 24–29 | 150 | 26– | + | + | | | | | 2 mM NH₄⁺ |
| NH | April | | – | | + | + | | + | + | |
| H | 8–19 | 150 | 26–35 | + + | + + | + | + | + | | 2 mM NH₄⁺ |
| NH | May | | – | + | | | | + | | |
| H | 29–10 | 225 | 26–35 | | | | | | | |
| NH | May-June | | 18–33 | | | | | | | |
| H | 12–17 | 225 | 27–35 | | + | | | | | |
| NH | June | | 21–32 | + | | | | + | | |
| H | 19–24* | 225 | 27–34 | + | + | | + | | | |
| NH | June | | 19–32 | + | + | | + | | | |
| H | 26–01 | 150 | 28–33 | | + | | + + | + | + | |
| NH | June-July | | 19–32 | | + | | + + | + | + | |
| H | 3–15** | 150 | 29–35 | | + | | | + | | |
| NH | July | | 21–34 | | + | | + | | | |
| H | 24–29 | | 29–34 | | + | | | + | + | |
| NH | July | | 22–33 | | + | | | + | + | |
| H | 31–19 | 225–275 | 27–33 | | + | | + | + | | |
| NH | July-Aug. | | 23–32 | | + | | + | + | | |
| H | 21–25 | 225–275 | 31–33 | | + | | | + | | |
| NH | August | | 22–32 | | + | | + | + | | |

* Culture was discarded due to contaminants.
** *Spirulina* filaments were filtered and used for inoculum in a new medium; the used medium was discarded.

tures of *Spirulina*, i.e. the only contaminants which seriously impede and endanger the culture are other microalgal species.

Of the less important contaminants, *Diatom* spp. appeared frequently both in winter and summer. Diatoms often became temporarily abundant when a new culture of *Spirulina* was initiated (Table 2), but diatoms were not particularly damaging since they would, as a rule, disappear from the culture within a few days of their appearance. In general, many observations reveal that at the lower population density (150 ku, i.e. - ca. 400 mg DW $l^{-1}$), there was much more contamination than at higher population densities (225 and 275 ku). This was especially true for the two most damaging contaminants for outdoor cultures of *S. platensis* under our conditions: *Chlorella* spp. and *Spirulina minor*.

A small species of *Spirulina*, measuring 40 $\mu$m in spiral width and between 180 to 240 $\mu$m in helix length and which was tentatively identified as *S. minor* (Richmond, 1986), constituted a very harmful contaminant. This species would rapidly overtake the *S. platensis* culture, since harvesting the culture by filtration affected its continuous enrichment due to their easy passage through the usual harvesting screen (325-mesh). As illustrated in Table 3, when filtration as a method for removal of biomass from the culture was replaced by 'bleeding' the culture (see Methods), the ratio between *S. platensis* and *S. minor* gradually changed, and *S. platensis* regained dominance

within 2 to 3 weeks. The temperature optimum for this strain under controlled laboratory conditions indicated an advantage to *S. minor* over *S. platensis* at a temperature range of 22 to 27 °C, and *S. minor* in open raceways was in previous experiments most harmful at spring, when temperature in the culture at midday was 25 to 27 °C. In the present work *S. minor* became most harmful in June and early July, indicating that temperature constituted only one determining factor for the appearance and bloom of this species in cultures of *S. platensis*. For lack of a filter that would separate entirely all the algal biomass from the culture medium in the course of harvesting, maintaining the population density at a relatively high concentration (i.e. 600 to 700 mg DW $^{-1}$) and harvesting by bleeding were the only preventative methods found useful to arrest the competition between the two *Spirulina* species and ensure the dominance of *S. platensis*.

*Chlorella* spp. represents another harmful contaminant in *Spirulina* cultures (Richmond *et al.*, 1982; Vonshak *et al.*, 1983). Although the pH optimum for most *Chlorella* species is below 8.0, there exist alkalophilic types of *Chlorella* which thrive in a *Spirulina* medium. In this work, the population of *Chlorella* spp. was observed to increase greatly in early spring (Table 3). At this period, the local strain of *Chlorella* enjoyed an obvious advantage over *S. platensis* in that its optimal growth temperature is some 10 °C lower than the optimum for *S. platensis*.

Our many observations (Vonshak *et al.*, 1983) and several unpublished observations in the years '84 to '86 from Ein-Yahav Algae (a commercial installation) indicate that when a drop in temperature occurred following an increase in the organic load of the medium, *Chlorella* spp. will gradually take over a culture of *S. platensis*. This process was accelerated if intensive harvesting by screening took place. Since *Chlorella* is ca. 3–5 $\mu$m in diameter, its population density is quickly enriched when the culture is filtered in the course of harvesting. *Chlorella* was particularly successful in becoming rapidly the dominant species in the pond at low (150 ku) population density, in which light was less growth-limiting.

*Table 3.* Effect of changing the mode of harvest from filtration to bleeding (see Methods) on relative abundance of *S. minor* and *S. platensis* in heated ponds in summer. First day was 6 July, 1988, at which time harvesting by filtration ceased and bleeding was initiated.

| Day* | % S. platensis | % S. minor |
| --- | --- | --- |
| 1 | 15 | 85 |
| 2 | 17 | 83 |
| 5 | 18 | 82 |
| 6 | 33 | 67 |
| 7 | 33 | 67 |
| 8 | 35 | 65 |
| 11 | 68 | 32 |

High bicarbonate alkalinity, i.e. – 0.2 M, was reported to prevent contamination by *Chlorella* in some of the cases (Richmond *et al.*, 1982). Once the contamination of *Chlorella* began to expand however, an effective treatment consisted of repeated pulses of 1 to 2 mM $NH_4^+$, followed by a 30% dilution of the culture. The present work revealed that treatment with ammonia which was not followed by dilution of the culture could not arrest the development of *Chlorella* once the growth conditions for this species were favourable. Thus relatively low temperature and population density as well as an increase in the organic load all affected a quick dominance of *Chlorella* in a *Spirulina* culture (Richmond, 1986). We concluded that in order to reduce contamination in *Spirulina* mass cultures, the population density should be maintained at a higher level than that which would be mandated by considerations intent on maximal output rate of biomass alone.

The overall effect of contaminants in reducing the annual output in outdoor mass cultures of *S. platensis* was difficult to estimate accurately. We calculated the potential productivity if no contamination had taken place by considering the time periods required for re-establishment of cultures which had to be discarded, and concluded that the overall annual loss of productivity fell in the range of 15 to 20%.

## 6. Correlating the productivity with limiting environmental factors

When the daily output rate in the heated ponds were correlated with daily irradiance, the correlation coefficient $(R)$ was 0.73 (Fig. 5,B), becoming highly significant $(R = 0.91)$ on the basis of a weekly average (Fig. 5,A). In contrast, the correlation of output rates with irradiance in unheated ponds was poor, the correlation coefficients were 0.42 on a daily basis $(5,B_2)$ and 0.53 $(5,A_2)$ on the basis of the weekly averages in both the output rate and total irradiance. The rather poor correlation of the output rate with irradiance in unheated ponds it to be expected: It portrays well the dependence of *Spirulina* on the ambient temperature, which as a rule is below optimal for growth during extended periods in all seasons. In unheated ponds the output rate correlated very
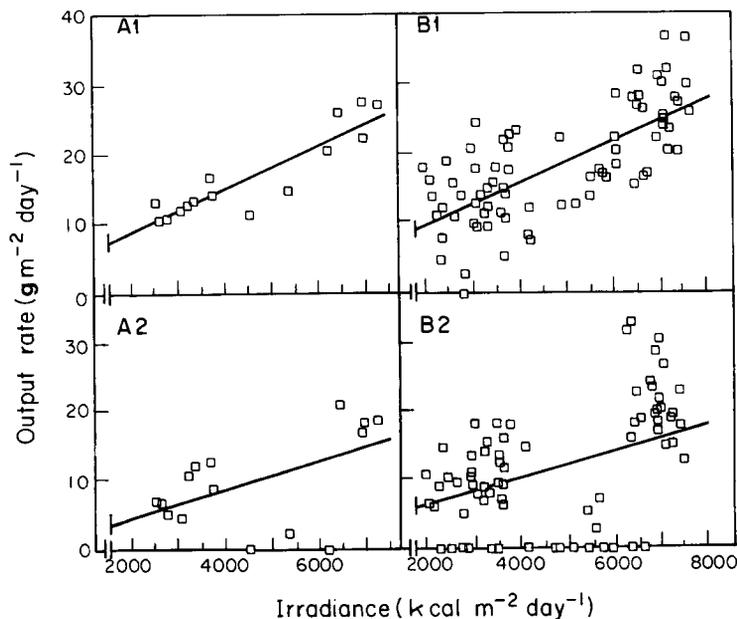


*Fig. 5.* Weekly output rate average (A) and the daily output rate (B) in summer, correlated with irradiance in heated- (1) and unheated-cultures (2).

well ($R$ = 0.89) with the temperature as measured by the number of hours of temperature above 18 °C during day-light (Fig. 6), this correlation being much poorer (0.58) in the heated ponds (not shown). Clearly, growth in non-heated cultures of *S. platensis* in open raceways was greatly dependent on the temperature whereas in the heated cultures, growth was to a major extent a function of irradiance, as would be expected of photoautotrophic mass-cultures (Richmond, 1986). This generalization however, sways attention from the role of limitations of growth other than environmental factors, and may lead to misleading conclusions: Thus the high correlation of the weekly averages of biomass yield and irradiance in the heated ponds cannot explain the relatively large fluctuations observed in the daily output rates of algal mass (Fig. 5), which naturally results in a much lower correlation of output and irradiance on a daily basis as compared to a weekly average basis. Particularly in summer, these daily fluctuations were not directly related to irradiance or temperature, which in summer, do not, essentially, vary under our conditions. It was concluded that the large daily fluctuations in output indicated that in summer, when the genetically inherent capacity of *Spirulina* to grow and reproduce could be manifested most acutely, other limitations of growth than sheer quantities of temperature and irradiance become expressive, exerting deceisive
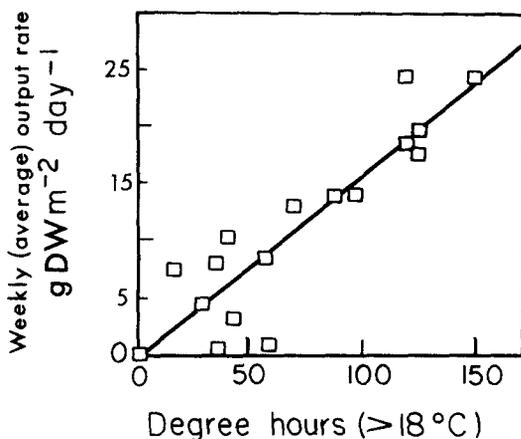
effects on growth and productivity. Significant improvement in increasing the output rate of mass cultures thus seems to require a better comprehension of growth limitations other than environmental.

## Conclusions

In a subtropical climate with little cloudiness such as exist in much of the Meditteraniean basin, it should be possible to attain an annual output rate of *Spirulina* of some 60 to 70 t dry matter ha$^{-1}$ yr$^{-1}$ provided the optimal temperature for the growth of *Spirulina* is maintained all along day light, photoinhibition is curtailed or altogether elliminated, harvesting in summer performed in the evening, and night loss of dry mass as well as contamination by foreign microorganisms would be substantially reduced. Under such circumstances, our observations indicate it would be possible to obtain sustained output rates of some 25 to 27 g DW m$^{-2}$ d$^{-1}$ for 6 months (May to October) and some 12 to 14 g m$^{-2}$ d$^{-1}$ for November through April. Further improvement in productivity necessiates identification of physiological limitations to growth that are not directly related to the environment.

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*Fig. 6.* Weekly average of output of dry mass correlated with the temperature (see Methods) throughout the year.

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