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The Concept of Light Intensity Adaptation in Marine Phytoplankton: Some Experiments with *Phaeodactylum tricornutum*

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Abstract

The historical background on adaptation of algae to various light intensities is analysed. It is argued that there is little evidence to suggest that previous growth at low light intensities enhances the ability of an alga to utilize these low light levels. Rather, the published evidence suggests that the most general response to growth at sub-optimal light intensities is a reduced ability to utilize saturating levels. The present experiments with *Phaeodactylum tricornutum* Bohlin have tested this concept of light intensity adaptation. Changing photosynthetic abilities during batch growth depended on the light intensity used for growth and these changes affected interpretations of the data. When measurements were made at a single time towards the end of the growth curve, cells grown at lower light intensities appeared to photosynthesize (at all intensities) better than did those grown at higher light levels. When the changes during batch growth were taken into account, or when the alga was grown in turbidostat cultures, a different picture was obtained. Growth at reduced light intensities was accompanied by (a) increased chlorophyll content, (b) decreased rate of light-saturated photosynthesis expressed on a chlorophyll, cell number or cell protein basis, and (c) decreased activity of RuDP carboxylase. One result suggested that growth at a sub-optimal light intensity did enhance the ability to utilize lower light levels. The light-saturation curve of cells grown in batch culture at 0.7 klux showed higher slopes at the low light intensities than did those grown at 12 klux. This was most marked when photosynthesis was expressed per cell, but was also apparent when it was put on a per chlorophyll basis.

Introduction

In 1966, Yentsch and Lee cited the remark of D.M.S. Watson (addressing the British Association in 1929): "There is no branch of zoology in which assumption has played a greater part or evidence a lesser part than in the study of adaptation." Almost half a century later, the same sentiment can be expressed over continued attempts to define the ways in which phytoplankton are "adapted" to the dominant environmental factors such as light, temperature and nutrient concentration.

Recent work from our laboratory (Morris and Glover, 1974) questioned the generally accepted hypothesis for temperature adaptation. Data from a number of different marine algae suggested that previous growth at sub-optimal temperatures does not enhance their ability to utilize such lower temperatures and that the earlier evidence indicating that algae do gain such an ability resulted from changing photosynthetic abilities during growth in batch culture. Rather, it appears that the general response of algae to growth at lower temperatures is a loss of ability to utilize the higher optimal temperatures.

In this paper, we present studies of the response of *Phaeodactylum tricornutum* to growth at various light intensities suggesting that "adaptation" to light intensities be considered as comparable to the recently modified view of temperature adaptation. That is, the data suggest that a major response of P. tricornutum to growth at sub-optimal light intensities is a loss of ability to use saturating levels. However, some evi-

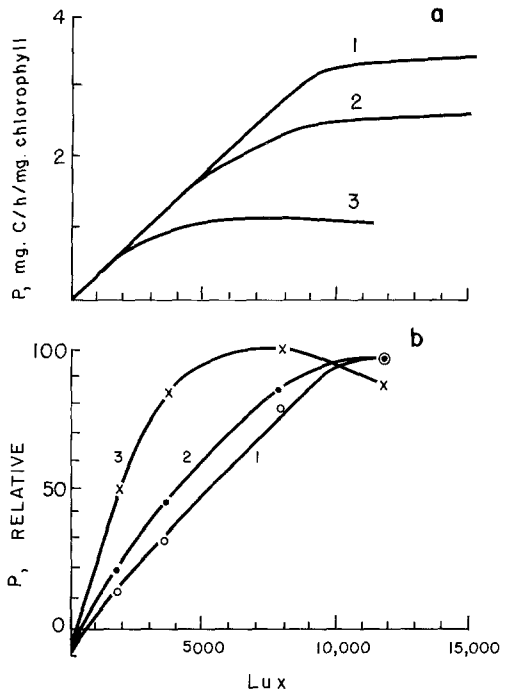


Fig. 1 Light saturation curves for photosynthesis of Arctic summer plankton from three depths. 1: Surface; 2: 27 m; 3: 50 m. (a) Gross photosynthesis per unit chlorophyll; (b) normalized curves (P_{max} is put at 100). (From Steeman Nielsen and Hansen, 1959)

dence suggests an improved utilization of low light intensities by cells previously grown at reduced light levels.

An appreciation of the possible general relevance of these observations depends on an understanding of the somewhat confused literature which has appeared on light adaptation of phytoplankton. For this reason we present the history of the subject in more detail than is usual. In 1957, Talling introduced the concept of I_k, the light intensity at which photosynthesis reaches saturation. In 1959, Steeman Nielsen and Hansen emphasized this parameter, I_k, in their interpretation of light intensity/photosynthesis curves for natural populations of phytoplankton. The central point which Steeman Nielsen and Hansen made can be illustrated by Fig. 1 (taken from Figs. 1 and 2 of Steeman Nielsen and Hansen, 1959). By normalizing the data (P_{max} is put at 100) from Fig. 1a, emphasis is placed on the intensity at which photosynthesis saturates, and this is considered to be the dominant influence of the previous light history - i.e., the depth from which the phytoplankton sample is taken. Superficially, the normalized curve (Fig. 1b) suggests that phytoplankton from deeper water are better able to utilize low light intensities. A closer examination of the original data (Fig. 1a) illustrates the point that the reduction in I_k results simply from lower rates of light-saturated photosynthesis (P_{max}) and not from any increased ability at lower light intensities. Comparable data have been presented by Ichimura *et al.* (1962) and Aruga and Ichimura (1968).

Later, Yentsch and Lee (1966) re-examined the concept of light intensity adaptation amongst phytoplankton, and emphasized the fact that reduced I_k values brought about by decreased values of P_{max} did not mean an enhanced ability to utilize lower light intensities. These workers also suggested that such changes in P_{max} and I_k could be induced by methods other than growth at reduced light intensity; that they were best thought of as responses to an "inferior environment" and not as specific adaptations to sub-optimal light intensities.

Steeman Nielsen and Jørgensen (1968) carried the discussion further by reporting the results of some experiments with *Chlorella pyrenoidosa*. These workers emphasized the difference between expressing photosynthetic rates per cell and per chlorophyll. In this alga, it was argued, adaptation to reduced light intensity was achieved mainly through increasing the cellular content of chlorophyll. As a result of this, the rate of photosynthesis per cell was greater in cells previously grown at lower light intensities. That is, growth at sub-optimal light intensities did not enhance the efficiency with which chlorophyll utilized reduced intensities, but it did increase the rate of photosynthesis on a cellular basis by increasing the chlorophyll content. This point is summarized in Fig. 2, taken from Fig. 2 of Steeman Nielsen and Jørgensen (1968).

These observations of Steeman Nielsen and Jørgensen (1968) were the first ones suggesting that so-called "adaptation" to reduced light intensity involved an increased ability to use such low light levels. The same authors recognized, however, that this phenomenon was not universal amongst algae pointing particularly to observations which suggested that "adaptation" of *Cyclotella meneghiniana* to low light intensities was not caused by changing chlorophyll content. Rather, in this diatom the major change was in the rate of light-saturated photosynthesis per cell, suggesting a change in the level of enzymes involved in the dark reactions of photosynthesis.

There are a number of uncertainties in the interpretations of the data of Steeman Nielsen and Jørgensen (1968).

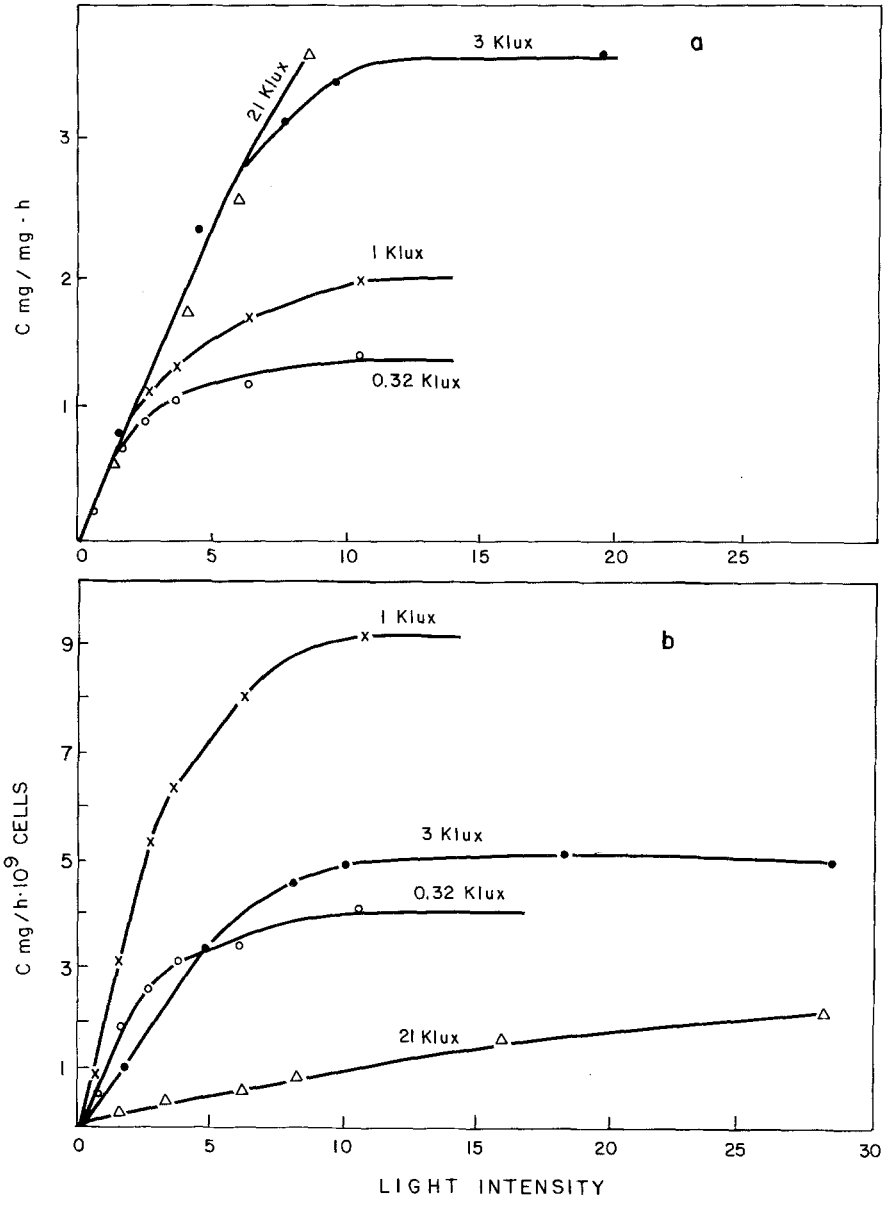


Fig. 2. *Chlorella pyrenoidosa*. Light saturation curves for photosynthesis by algae grown at 4 different light intensities. Photosynthesis expressed on per unit chlorophyll (a) and cell-number (b) basis. (From Steeman-Nielsen and Jørgensen, 1968)

Firstly, the difference between *Chlorella pyrenoidosa* and *Cyclotella meneghiniana* might not be as marked as the workers suggest. For example, cells of *Chlorella pyrenoidosa* showed most significant increases in chlorophyll levels only when growth occurred at the lowest light intensities (less than 3 klux) whereas the experiments with *Cyclotella menighiniana* did not consider light intensities less than 3 klux. Also, the increased chlorophyll content induced by growth of *Chlorella pyrenoidosa* at low light intensities was not the only change; other changes resulting in an increased cell size occurred at the lowest light intensities. Finally, it is not clear whether the enhanced chlorophyll content was an "adaptation" in the sense that it increased the growth rate of *Chlorella pyrenoidosa* at low light intensities.

One further observation of Steeman Nielsen and Jørgensen (1968) is worth comment. When expressed per unit cell number, growth at low intensities (except the lowest, 0.32 klux) not only increased photosynthesis at low light levels, it also increased P_{max} (Fig. 2b). In that sense, therefore, the changes brought about by growth at sub-optimal

light intensities were not specific to the light reaction of photosynthesis (i.e., to the utilization of low light levels), but were general for the entire photosynthetic machinery.

In this paper we question whether growth of *Phaeodactylum tricornutum* at reduced light intensities improves the photosynthetic capacity at any or all light intensities. In doing this, we have recognized the need to consider changes which occur during growth of the alga in batch culture, to utilize continuous cultures operating as light-limited turbidostats, and to measure activities of photosynthetic enzymes as well as whole-cell rates of photosynthesis.

Materials and Methods

Growth of Phaeodactylum tricornutum in Batch Culture

Phaeodactylum tricornutum Bohlin (obtained from the Cambridge Culture Collection) was grown in the "f-1" medium of Guillard and Ryther (1962) under continuous illumination as described by Glover *et al.* (1975). Light intensity was varied by covering the culture vessels with layers of muslin. For all experiments, cultures were inoculated from suspensions maintained at the appropriate light intensity for 2 to 3 days. Growth was followed by measuring cell numbers (with a haemacytometer) twice daily.

Growth of Phaeodactylum tricornutum in Turbidostat Culture

Turbidostat continuous-flow cultures of *Phaeodactylum tricornutum* were maintained in a 10 l glass vessel with a working volume of 8 l, stirred with a stainless-steel baffle at 200 rpm. The growth vessel was illuminated by two banks of 5 "warm white" fluorescent tubes giving a light intensity at the surface of 11 klux.

A selenium photocell attached to the surface of the growth vessel measured light transmitted through the culture from the growth lights. The voltage generated was proportional to the cell density of the culture and the amplified signal operated a relay controlling a peristaltic pump. At pre-selected cell densities the pump was operated and fresh medium transferred from a reservoir to the growth vessel. Constant volume was achieved by means of an overflow system. For turbidostat cultures, "f-1" medium containing nitrate as the sole source of nitrogen was used.

Steady states were maintained for at least 3 generation times before harvesting the culture.

Measurement of Photosynthetic Carbon Dioxide Assimilation

Cells were harvested by centrifugation at 6000 rpm and suspended in fresh "f-1" medium at a density of 2 x 10^5 cells/ml. For turbidostat grown cultures, medium containing nitrate only was used. Photosynthesis was then measured by the method described by Beardall and Morris (1975) under an air atmosphere. Light intensity was varied by adjusting the distance between light source and the cell suspension and/or covering the vessel with muslin.

Measurement of Enzyme Acitivities in Cell-Free Extracts

Cell extracts were prepared and RuDP carboxylase [E.C. 4. 1.1. 39; 3-phospho-D-glycerate carboxylase (dimerising)] measured as described by Beardall and Morris (1975) Malate dehydrogenase (E.C. 1.1.1. 37 C l-malate: NAD oxidoreductase) was assayed by the method of Morris and Syrett (1965) in a Unicam S P 1800 spectrophotometer using a system containing: 0.6 ml 0.1 M Tris-HCl buffer, pH 8.6, 0.1 ml of 2.0 mM NADH, 0.1 ml of 0.1 M $MgCl_2$ and 0.1 ml of extract. The blank cuvette contained Tris-HCl buffer in place of NADH. 0.1 ml of 10 mM oxaloacetate was added to each cuvette and the rate of change in absorbance at 340 nm determined.

Measurement of Chlorophyll a and Carotenoids

Chlorophyll *a* was determined by the method of Lorenzen (1967). Between 50 and 100 ml of cell suspension were filtered through glass-fiber discs, moistened with a drop of saturated magnesium carbonate solution. The cells were homogenized for 1 min in 3 ml 90% (v/v) acetone. After washing with 1 ml 90% acetone, the broken suspension was centrifuged and the absorption of the supernatant measured at 665 nm with a Unicam S P 1800, before and after adding a drop of 1 N HCl. The concentration of chlorophyll *a* was then calculated using the equation of Lorenzen (1967). Carotenoid concentrations were determined using the same supernatant before acid treatment. The absorbance at 480 and 750 nm was

measured, and concentration calculated using the equations of Parsons and Strickland (1963).

Results

Effect of Light Intensity on Growth Rate

It is important that the response of photosynthesis to light intensity be related to growth. That is, it is necessary to specify the relationship between any "adaptation" identified in photosynthesis measurements and growth responses. For this reason we describe the effects of light intensity on the growth of *Phaeodactylum tricornutum* in batch culture (Fig. 3). Comparable results (with lower absolute growth rates) were observed in our turbidostat cultures. For subsequent photosynthesis experiments with batch cultures the alga was grown at 12 klux, 5 klux (both giving maximum growth rate) and 0.7 klux (29.7% of maximum growth rate).

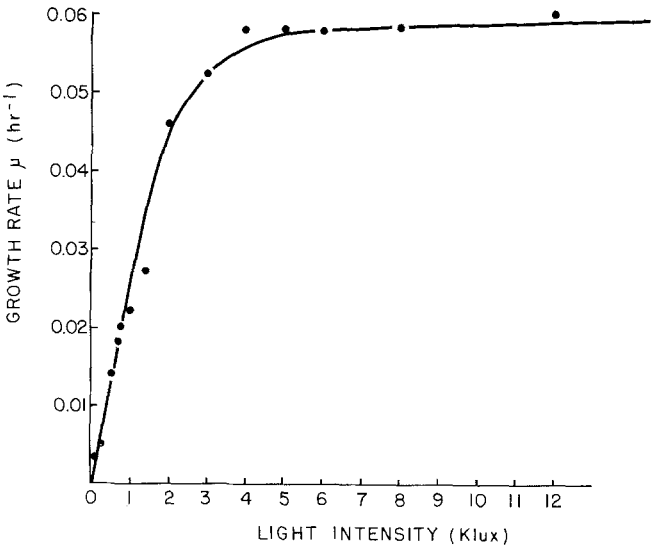


Fig. 3. *Phaeodactylum tricornutum*. Relationship between light intensity and growth rate for phytoplankton grown in batch culture

Changing Photosynthetic Abilities During Growth in Batch Culture

Our earlier experiments (Morris and Glover, 1974) suggested that the changes in photosynthetic rates during batch growth were significant; that these changes depended on the conditions under which the alga was grown; and that they should be taken into consideration in any attempts to understand so-called "adaptation". Fig. 4 shows the changing rates of P_{max} (light-saturated photosynthesis) by cultures grown at 12 and 0.7 klux. The maximum rate of photosynthesis occurred later (4 days) in the cultures grown at the lower light intensity. This means that, if the comparison is made after 7 days, as in Steeman Nielsen and Jørgensen's work (1968) with *Chlorella pyrenoidosa*, cells grown at low light intensities show higher P_{max} values than those grown at higher light intensities. This is comparable to the results of Steeman Nielsen and Jørgensen (1968) presented in Fig. 2b of this paper. However, it can be seen that this conclusion is not valid if the earlier maximum rate of photosynthesis in cultures at 12 klux is considered. Fig. 5 shows the changing chlorophyll content during batch growth (a) and the changing P_{max} (b) expressed on a chlorophyll basis. Again, a comparison between the two cultures is valid only when early peaks of photosynthesis are considered; comparisons at a single time towards the end of batch growth give a misleading view.

Characteristics of Photosynthesis in Cultures Grown at Various Light Intensities

Fig. 6 shows light saturation curves for photosynthesis by cultures of *Phaeodactylum tricornutum* grown at 3 light intensities. These experiments were performed at those times which gave maximum rates of light-saturated photosynthesis in each culture. When expressed on a chlorophyll basis, the P_{max} was greater for cultures grown at higher light intensities. When expressed per cell, the values for P_{max} were approximately the same. The most interesting observation from these experiments was the response of the various cultures to low light intensities. Cells grown at the lowest intensity were able to utilize intensities below saturation levels more efficiently than those grown at saturating intensities. Only part of the explanation lay in the higher chlorophyll content, since when expressed on a chlorophyll basis, cells grown at the lowest light intensity utilized low light levels more efficiently than did the others. The difference between cultures grown at 5 and 12 klux was less marked. Although, therefore, there appeared to be adaptation of the photosynthetic mechanism to low light intensities, it is worth emphasizing that the growth rate at these intensities was less than at saturating levels (Fig. 3).

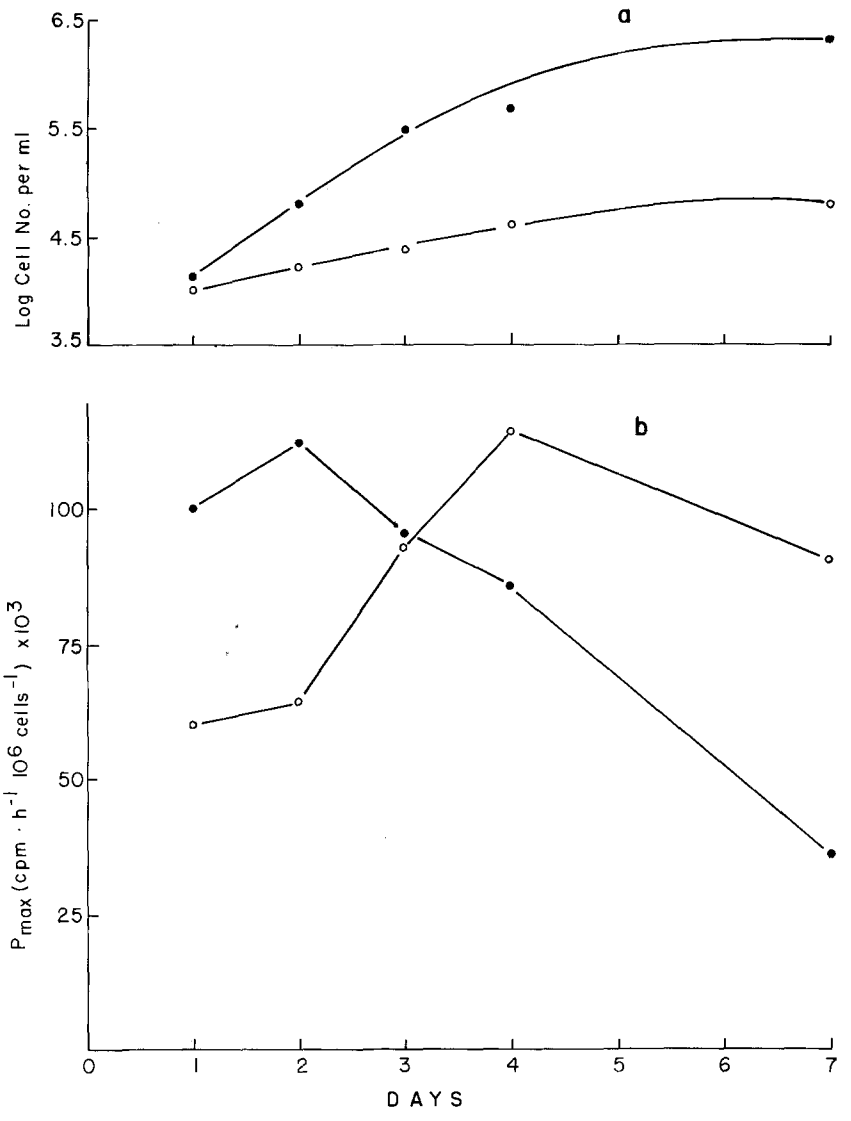
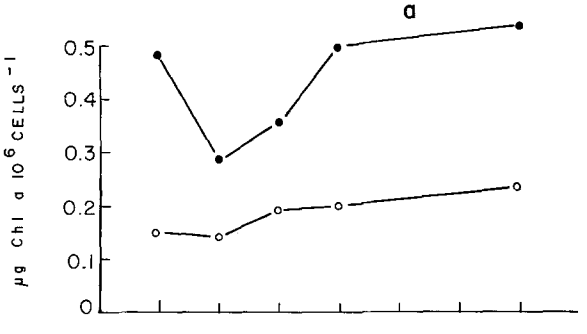


Fig. 4. *Phaeodactylum tricornutum*. Changes in cell density (a) and rates of light-saturated photosynthesis (P_{max}) per cell (b) for phytoplankton grown at 12 klux (closed circles) and 0.7 klux (open circles) in batch culture. cpm: Counts per min



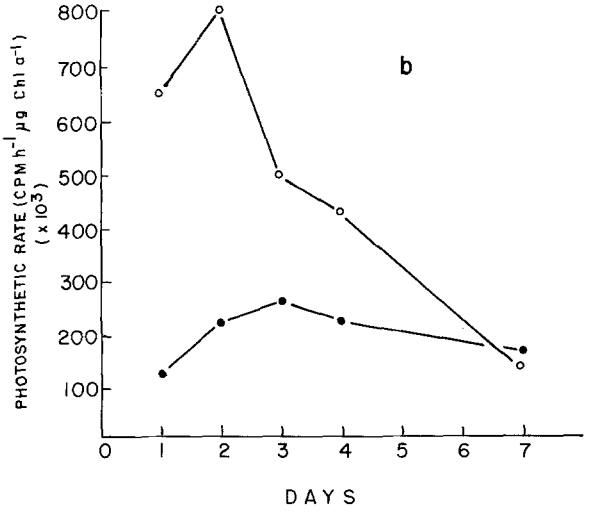


Fig. 5. *Phaeodactylum tricornutum*. Changes in chlorophyll *a* content (a) and P_{max} expressed on chlorophyll basis (b) for phytoplankton grown in batch culture at 12 klux (closed circles) and 0.7 klux (open circles)

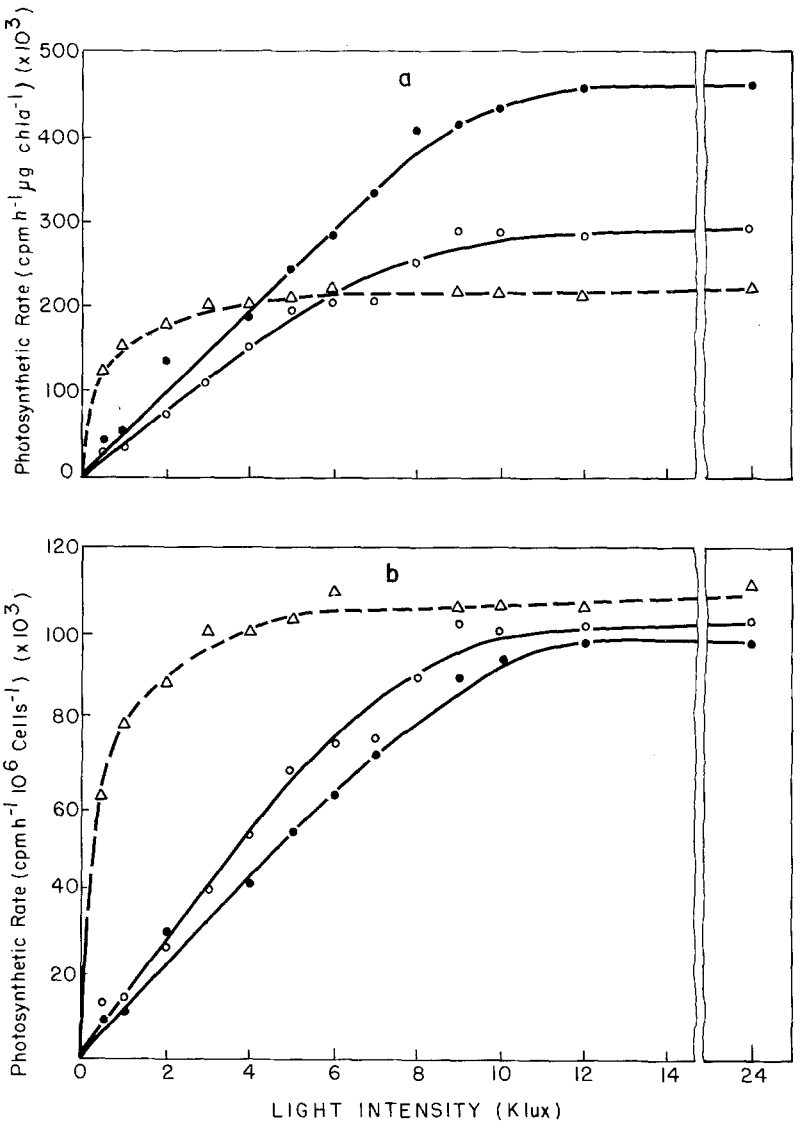


Fig. 6. *Phaeodactylum tricornutum*. Light saturation curves for photosynthesis of phytoplankton grown in batch culture at 12 klux (closed circles), 5 klux (open circles) or 0.7 klux (triangles). Photosynthesis is expressed on per unit chlorophyll (a) and cell-number (b) basis

Experiments with Turbidostat Cultures

It is apparent that changes during batch growth can obscure the response of algae to various light intensities. These problems can be overcome by using continuous cultures operating as turbidostats with growth limited by light intensity. In our turbidostat experiments we have emphasized rates of light-saturated photosynthesis rather than the response of the alga to low light levels. Our main aim has been to put the changing rates of photosynthesis on an enzymatic basis and to describe the way in which the various components of the photosynthetic machinery are influenced by the light intensity used for growth. Figs. 7, 8 and 9 summarize these data: Fig. 7 shows levels of photosynthetic pigments and bulk cell protein, Fig. 8 compares the rates of light-saturated photosynthesis with the activities of RuDP carboxylase (expressing both rates on a chlorophyll and cell number basis) and Fig. 9 compares P_{max}, RuDP carboxylase activity and malate dehydrogenase activity, expressing all on a protein basis.

The main points to emphasize from Figs. 7, 8 and 9 are the following:

(a) Growth at low light intensities produced higher cellular contents of both chlorophyll *a* and carotenoids. There was little change in the protein content. (Measurements with batch cultures also showed a slight decrease in mean cell volume with growth at the lower light intensity.)

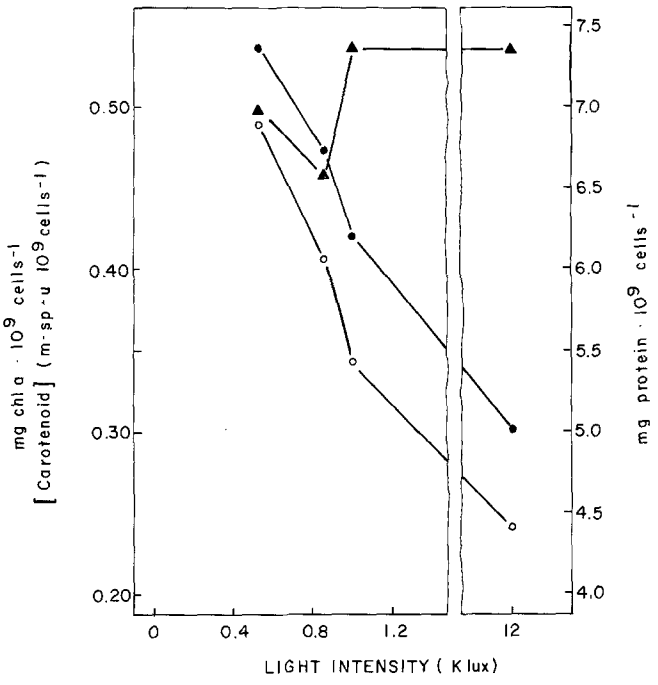


Fig. 7. *Phaeodactylum tricornutum*. Changes in cellular content of chlorophyll *a* (closed circles), carotenoid (open circles) and protein (triangles) in cells grown at different light intensities in turbidostat cultures. m-sp-u: Millispecific plant pigment unit

(b) The changing P_{max} values were paralleled by altered activities of RuDP carboxylase. This was not part of general changes in enzymes since the activity of malate dehydrogenase did not correlate as closely with P_{max} as did the carboxylase.

(c) Between 12 and 1 klux the major change was a 39% increase in the cellular chlorophyll content. This was the cause of the observed reductions (at the lower intensity) of P_{max} and RuDP carboxylase when expressed on a chlorophyll basis. At the two lower intensities (0.86 and 0.53 klux), however, there was a change in P_{max} and RuDP carboxylase activity per cell. That is, at these low intensities the increased concentration of chlorophyll in the cell was accompanied by decreased levels of the enzymatic machinery responsible for the dark reactions of photosynthesis.

Time Required for "Adaptive" Changes

We agree with Steeman Nielsen and Jørgensen (1968) in emphasizing the distinction between genotypic and phenotypic adaptation. When organisms are grown under different environmental conditions

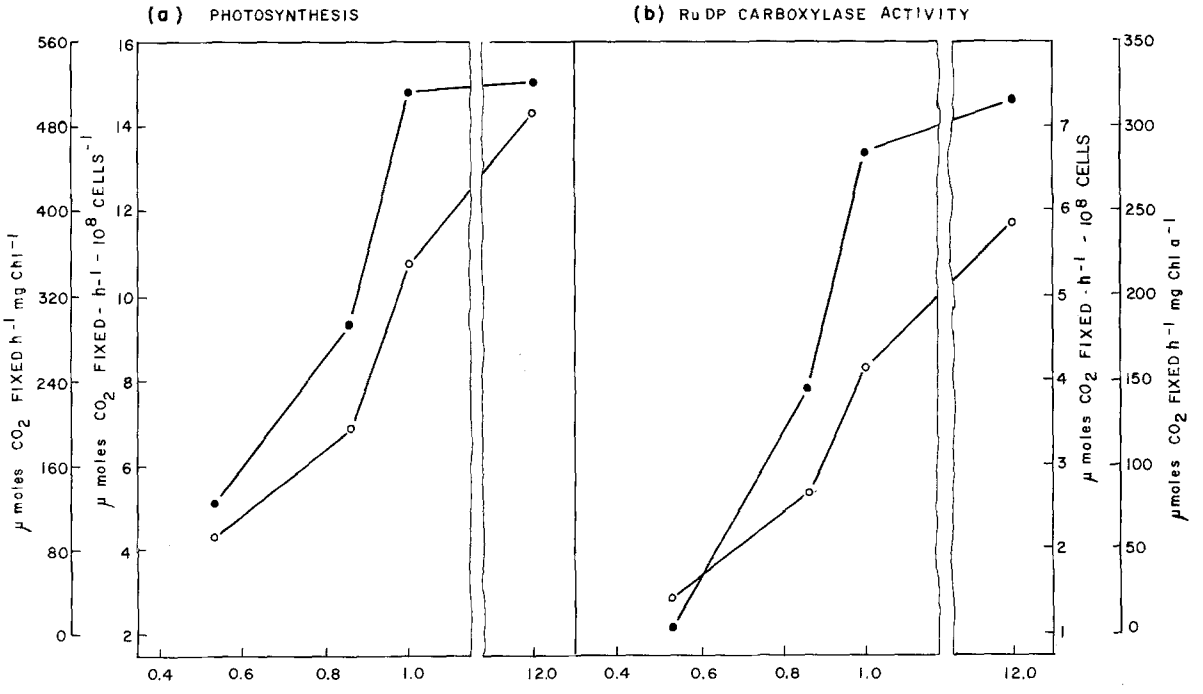


Fig. 8. *Phaeodactylum tricornutum*. Changes in (a) rate of light-saturated photosynthesis and (b) RuDP carboxylase activity in cells grown at different light intensities in turbidostat cultures. Data are expressed on chlorophyll basis (open circles) and per unit number of cells (closed circles)

for several generations, strain selection – as opposed to physiological changes to the entire population – can complicate interpretation of the data. In our experiments we have made no critical attempts to distinguish between these two types of responses. However, the observations presented in Fig. 10 suggest a physiological response. That is, the time taken for changes in chlorophyll content and in rate of light-saturated photosynthesis per chlorophyll occurred over a time period equal to (or less then) a generation time.

Discussion and Conclusions

The conclusions to be drawn from the investigation presented here fall into 4 main categories.

Firstly, analysis of the published literature on light-intensity adaptation suggests that too great an emphasis has been placed on I_k, the light intensity at which photosynthesis saturates and too little on the changes in P_{max}. Because of this, it has been implied that adaptation to sub-optimal light intensities involves some kind of enhanced

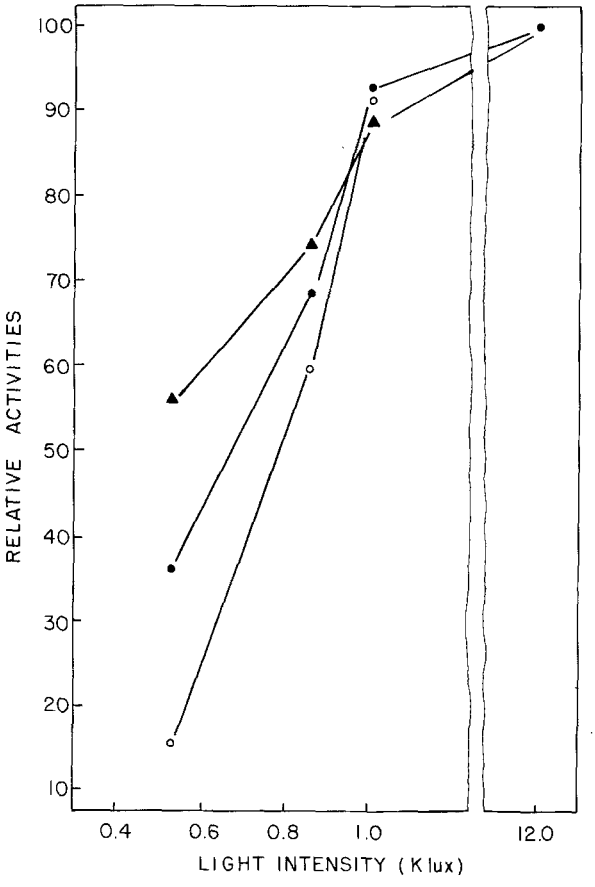


Fig. 9. *Phaeodactylum tricornutum*. Changing rates of photosynthesis (closed circles), RuDP carboxylase activities (open circles) and malate dehydrogenase activity (triangles) in cells grown at different light intensities in turbidostat cultures. All activities are expressed per unit amount of cell protein and are related to a value of "100" given for cultures grown at 12 klux

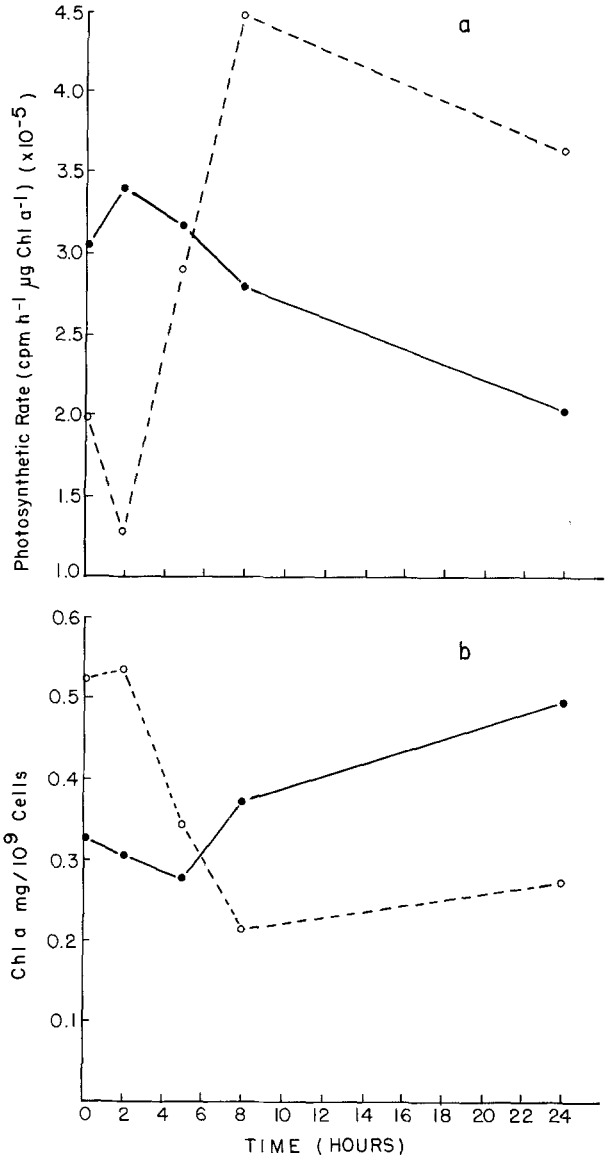


Fig. 10. *Phaeodactylum tricornutum*. Changing rates of light-saturated photosynthesis (a) and chlorophyll content (b) during 24 h following transfer of cells grown at 0.7 klux to 12 klux (open circles) and following transfer from 12 klux to 0.7 klux (closed circles)

ability to use the low light levels, whereas it appears that a reduced ability to utilize saturating light intensities is the dominant characteristic of algae grown at low intensities.

Secondly, it is argued that the data of Steeman Nielsen and Jørgensen (1968) for *Chlorella pyrenoidosa* might be difficult to interpret because of changing photosynthetic abilities during growth in batch culture. These workers observed increased chlorophyll levels in cells grown at low light intensities and reported enhanced rates of photosynthesis at all light intensities per cell (but not per chlorophyll) by such algae from low intensities. We obtained comparable results when the measurements were made at a single time towards the end of the growth curve. However, when the changing photosynthetic abilities during batch growth were taken into account, a different result was obtained.

Thirdly, when photosynthesis measurements are made at the time of maximum photosynthetic ability during batch growth, adaptation to light intensity through enhanced utilization of low light levels can be observed. This is most marked when photosynthesis is expressed per cell number, but is also apparent when expressed on a chlorophyll basis. In our view, this is the first published evidence suggesting that growth of an alga at reduced light levels enhances its ability to photosynthesize at sub-optimal intensities and reduces its ability to utilize saturating levels. It is worth emphasizing, however, that this enhanced photosynthetic ability does not result in increased growth rates; growth at 0.7 klux being 29.7% of the maximum rate.

Fourthly, it has been possible to relate changing photosynthetic abilities to changing activities of enzymes responsible for the dark reactions of photosynthesis. Over a wide intensity range (12 to 1 klux) the RuDP carboxylase level per cell remained more or less constant. However, over this same range the ratio of carboxylase:chlorophyll decreased because of an increase in chlorophyll content. At lower intensities, there was a reduced activity of RuDP carboxylase per cell and per unit amount of cell protein. Comparable reductions in RuDP carboxylase levels have been observed in shade-adapted species of higher plants (Bjorkmann, 1968).

This decrease in the level of the enzymatic machinery required for maximum utilization of saturating light intensities can be seen as an adaptive response to growth at limiting light levels, i.e., "adaptation" should not be considered only in terms of "gaining an ability". A regulated disappearance of specific enzymes under growth conditions not utilizing maximum activities is a physiological adaptation which enables the cellular metabolism of an organism to respond to changes in the environment. Our previous paper (Morris and Glover, 1974) on temperature adaptation and the present data argue for this modified view of adaptation in phytoplankton. Hitherto, the approach has generally been concerned with the way in which organisms adapt to sub-optimal conditions. A shift of emphasis such as is suggested here makes one ask questions about the biochemical changes required of phytoplankton when they move into conditions allowing maximum growth; saturating light intensities, optimum temperatures, and possibly saturating nutrient concentrations. It is interesting that Dugdale (personal communication) is asking comparable questions concerning the "shift-up" processes which may occur in phytoplankton in response to higher nutrient levels and which may be basic to an understanding of the initiation of phytoplankton blooms.

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