TEMPERATURE ADAPTATION IN *PHAEODACTYLM TRICORNUTUM* Bohlin: PHOTOSYNTHETIC RATE COMPENSATION AND CAPACITY

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Abstract: Two views of temperature adaptation in marine phytoplankton have been proposed. The first emphasizes that adaptation is manifest as a compensation of photosynthetic rate (some time after the initial chemical kinetic response to temperature shift) so that eventually cells are able to perform more or less at the same level over a wide temperature range. The second emphasizes that adaptation is manifest as a change in the maximum photosynthetic ability. In order to examine these two concepts concurrently, short-term light saturated photosynthetic rates per cell (*P*ₘ) were examined as a function of temperature for *Phaeodactylum tricornutum* Bohlin grown at various temperatures in turbidostat cultures. Within the range from 10 to 25°C, when cells grown at a high temperature were transferred to a low temperature, *P*ₘ decreased according to Arrhenius-type kinetics in the short term. However, prolonged exposure at the lower temperature led eventually to compensation which partly restored *P*ₘ to its original value. Within the same temperature range, growth at lower temperatures resulted in higher maximum photosynthetic abilities (i.e. *P*ₘ measured at the optimal assay temperature) which were positively correlated with in vitro activities of ribulose-1,5-bisphosphate carboxylase (RuBPCase). Within this temperature range, the amount of total protein per cell did not change but there was an increase in the amount of chlorophyll a per cell with temperature. It is suggested that the regulation of RuBPCase activity may be associated with the two manifestations of temperature adaptation exhibited by *P. tricornutum*: partial compensation of *P*ₘ and change in maximum photosynthetic ability.

INTRODUCTION

Unicellular microorganisms are ectotherms; that is, their source of heat is from the environment and they cannot maintain an internal temperature different from the ambient temperature. Changes in environmental temperature rapidly lead to changes in the rates of cellular chemical reactions which comprise metabolism and growth. In order that these rates do not exceed viable limits, many ectotherms apparently invalidate the fundamental laws that describe temperature effects on chemical reaction kinetics (Hochachka & Somero, 1973). This widely held concept of temperature adaptation describes the events following a temperature change as follows (Christophersen, 1973): there is first a change in metabolic rate which closely obeys the rules of chemical kinetics (e.g. the Arrhenius formulation), but,
after a while, there may be a compensation which results in a rate at the new temperature which is close to that at the original temperature. The compensation (or adaptation) may result in a rate exactly equal to the original rate (ideal compensation) or in a rate intermediate between the original rate and that attained immediately following temperature shift as demanded by chemical kinetics (partial compensation). In some cases, there may be an overcompensation, no compensation at all or even an inverse compensation (Christophersen, 1973).

There is another view of adaptation which has arisen mainly from studies of marine phytoplankton (Morris & Glover, 1974; Beardall & Morris, 1976). The results of these studies suggested that phytoplankton grown at sub-optimal conditions adapt to these conditions by losing their ability to utilize optimal conditions, perhaps by a regulated disappearance of specific enzymes.

These two views of adaptation are not incompatible. The first emphasizes a comparison of rate responses at various growth temperatures, whereas the second emphasizes a comparison of rate responses at the temperature which optimizes the metabolic function under consideration. It is possible to consider both views of adaptation together if the rate response of phytoplankton grown at various temperatures is measured over an assay temperature range wide enough to cover both the growth temperature and the temperature which optimizes the metabolic rate.

In this study, we wish to show that as the temperature of growth changes, the diatom *Phaeodactylum tricornutum* Bohlin does indeed exhibit partial compensation (cf. Christophersen, 1973) in photosynthetic rate and that there also are changes in the optimal photosynthetic rate which are correlated with changes in the in vitro activity of ribulose-1,5-bisphosphate carboxylase (RuBPCase).

In order to obtain a clear assessment of the effects of temperature without complications of interactions due to nutrient limitation (Kiel, 1976), *P. tricornutum* was grown in a nutrient-sufficient turbidostat at various temperatures. Thus, although growth temperature was varied, cells were always in the same relative physiological state (Goldman, 1980) of $\mu : \mu_m = 1$ where $\mu$ is the specific growth rate (day$^{-1}$) and $\mu_m$ is the maximum value of $\mu$.

**MATERIALS AND METHODS**

**GROWTH IN CONTINUOUS CULTURE**

Axenic batch stock cultures of *P. tricornutum* (Cambridge Culture Collection) maintained on f/2 enriched (Guillard & Ryther, 1962) natural sea water were used as inocula for the turbidostat cultures. The continuous culture vessel was a 4-l Pyrex reaction kettle with an outflow arm maintaining the culture volume at 3.5 l. The Pyrex lid had openings plugged with silicone stoppers through which were passed attachments for nutrient inflow, culture sampling, and a cooling finger. The vessel,
including its lid and attachments, was autoclaved empty. Sterile f/2 medium was supplied to the vessel from a reservoir by peristaltic pump (Orion 375A). Temperature was maintained at desired levels by the immersed cooling finger with water circulating from a temperature-controlled bath (Forma 2095). Culture temperature was monitored by a thermometer placed in a finger (glass test-tube) immersed in the culture. The culture was mixed by a circular magnetic spinner. Continuous irradiation was supplied by cool-white fluorescent lights. Irradiance measured by a quantum sensor (LI-170) was \( \approx 250 \, \mu \text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) at the surface of the vessel closest to the lights. The amount of irradiance received at the opposite side of the vessel (i.e. irradiance transmitted after passing through the culture) during steady state was \( \approx 180 \, \mu \text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \). Growth rate versus irradiance curves for \( P. \) tricornutum at 9 and 25 °C (unpubl. results) and at 18 °C (Beardall & Morris, 1976) indicate that the \( I_c \) (Talling, 1957) for growth is not greater than \( \approx 100 \, \mu \text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) under these conditions. It is therefore reasonable to assume that cells in our turbidostat were growing at light-saturated rates. Culture turbidity was monitored by the selenium photocell and relay system used by Beardall & Morris (1976). Cells were sampled by sterile disposable syringes and counted in a hemocytometer (Spencer-Bright line).

After inoculation, the culture was allowed to proceed in a batch mode until its turbidity reached the balance value previously set for the photocell-relay system whereupon the culture was automatically operated in a continuous mode. Cell counts were determined throughout the batch and continuous phases. Although no sterility checks were made for bacterial contamination, microscopic observations did not appear to indicate any gross contamination. Specific growth rates during the batch phase (\( \mu_m, \text{ day}^{-1} \)) were taken as the slopes of the linear portions of \( \ln \) (cells \cdot ml\(^{-1} \)) versus time plots. Slopes were estimated by least-squares linear regression analyses. Correlation coefficients for these analyses ranged from 0.9838 \((n = 6)\) to 0.9983 \((n = 4)\). Cell concentrations during steady state \((10^5 \text{ cells} \cdot \text{ml}^{-1} \pm \text{SD})\) at 5, 10, 15, and 25 °C were respectively 6.27 ± 0.82 \( (n = 27) \), 6.64 ± 0.40 \( (n = 24) \), 7.02 ± 0.68 \( (n = 29) \), and 5.06 ± 0.76 \( (n = 28) \).

Outflows from the cultures were collected in storage bottles for measurement of flow rates. Flow rates were taken as the slopes of the linear regressions of effluent volume on time. Correlation coefficients for these analyses ranged from 0.9971 \((n = 12)\) to 0.9980 \((n = 13)\). Dilution rates were calculated by dividing culture volume into flow rates. At steady state, the dilution rate was taken to be equal to \( \mu_m \).

**MEASUREMENT OF PHOTOSYNTHETIC CARBON ASSIMILATION**

When the continuous culture had reached steady state, a sample was removed from the growth vessel and NaH\(^{14}\)CO\(_3\) (New England Nuclear Corp.) added to give a final concentration of 4.75 mM and a specific activity of 0.4 \( \mu \text{Ci} \cdot \mu \text{mol}^{-1} \). This sample was divided into 10-ml aliquots which were placed in Teflon-lined screw-capped culture tubes \((16 \times 100 \text{ mm})\). Tubes were immersed horizontally in
water baths set at different temperatures and illuminated from above. Incident irradiance was varied for individual tubes by covering with neutral density screening. One-ml samples were removed at various intervals over 4 hr and processed for total \(^{14}\)C incorporation measurements as described elsewhere (Li & Goldman, 1981). Cellular incorporation of \(^{14}\)C was linear over 4 hr. Particulate carbon was analysed on precombusted glass fibre filters (Gelman A/E) with a Hewlett Packard 185B CHN analyser. Five replicate samples were analysed for cells at each temperature. The coefficient of variation for carbon analyses ranged from 1.23 to 4.60%. Over all temperatures considered, the coefficient of variation had a mean and SD of 2.60 ± 1.48%. Chlorophyll \(a\) was determined spectrophotometrically (Strickland & Parsons, 1972) employing the equation of Jeffrey & Humphrey (1975). Three replicate samples were analysed for cells at each temperature. The coefficient of variation for chlorophyll analyses ranged from 0.66 to 5.55%. Over all temperatures considered, the coefficient of variation had a mean and SD of 2.20 ± 2.26%.

ESTIMATE OF PROTEIN SYNTHESIS RATE

The cellular rate of protein synthesis (\(\rho\), pg protein \cdot cell\(^{-1}\) \cdot day\(^{-1}\)) for an asynchronously growing cell population in balanced steady-state condition is calculated as the product of the specific growth rate (\(\mu\), day\(^{-1}\)) and the cellular protein content (\(Q\), pg protein \cdot cell\(^{-1}\)) (Li, 1980). Protein content of cells collected on glass fibre filters were determined as follows. Filters were placed in a homogenizing tube cooled by ice and ground for 90 s in 0.5 ml 0.1 N NaOH using a Teflon-coated pestle. The pestle was rinsed with a further 0.5 ml of 0.1 N NaOH which was then pooled with the cell homogenate. The protein content of the homogenate was determined on a 0.5-ml aliquot using the Lowry method (Lowry et al., 1951). Filter and cell debris were sedimented from the homogenate by centrifugation prior to absorbance measurement at 750 nm. Bovine serum albumin was used as standard. Five replicate samples were analysed for cells at each temperature. The coefficient of variation for protein analyses ranged from 2.67 to 6.55%. Over all temperatures considered, the coefficient of variation had a mean and SD of 4.72 ± 1.63%.

An apparent rate of protein synthesis (\(\rho'\), pg protein \cdot cell\(^{-1}\) \cdot day\(^{-1}\)) was also determined independently as follows: a 4-ml aliquot of the cell suspension used for the measurement of \(^{14}\)C assimilation (vide supra) was removed after 4 hr incubation and filtered onto a glass fibre filter (Gelman A/E). The cellular fractionation procedure previously described (Li et al., 1980) was used to determine the amount of protein carbon labelled per cell per hour. The cellular rate of protein labelling was calculated using a protein carbon: protein ratio of 0.54 (w:w). This ratio was determined using the amino acid composition of \(P.\ tricornutum\) as reported by Chuecas & Riley (1969) and a protein: total unhydrolyzed amino acid residues ratio of 0.86 (w:w). The rate of protein synthesis thus determined using radio-labelling is to be regarded as an apparent rate for the reasons discussed by O'Kane & Jones (1978).
MEASUREMENT OF CARBOXYLASE ACTIVITIES

Activities of ribulose-1,5-bisphosphate carboxylase (RuBPCase) and phosphoenolpyruvate carboxylase (PEPCase) were determined from 5-ml culture aliquots each, using assays previously described (Glover & Morris, 1979). Temperature profiles of RuBPCase and PEPCase activities from crude cell-free extracts of *P. tricornutum* indicate optima at 40 and 35 °C respectively and activation energies of 12.9 kcal·mol⁻¹ and 13.0 kcal·mol⁻¹ respectively (unpubl. results). Based on these values, enzyme activities measured at 25 °C were converted to activities expected at their respective temperature optima. Three replicate samples per enzyme were analysed for cells at each temperature. The coefficient of variation for enzyme activity assays ranged from 0.76 to 16.16% for RuBPCase and 2.46 to 21.15% for PEPCase. Over all temperatures considered, the coefficient of variation had a mean and SD of 11.08 ± 7.03% for RuBPCase and 12.25 ± 9.52% for PEPCase.

RESULTS

GROWTH RATES

The temperature optimum for light-saturated growth rate ($\mu_m$) of *P. tricornutum* was 20 °C (Fig. 1). No growth was observed at 30 °C. At temperatures between 5

![Graph showing growth rate ($\mu_m$) as a function of temperature ($T$) for Phaeodactylum tricornutum.](image)

Fig. 1. Specific growth rate of *Phaeodactylum tricornutum* at light saturation ($\mu_m$) as a function of temperature: ●, turbidostat steady-state dilution rates; ○, specific rates of cell number increase in batch culture.
and 15 °C, the relationship between \( \mu_m \) and temperature \( (T, \ °K) \) was well described by the Arrenhius equation: \[ \log(\mu_m) = a - bT^{-1} \]. Least-squares linear regression of \( \log(\mu_m) \) on \( T^{-1} \) \( (r = -0.98, n = 7) \) yielded estimates with 95\% confidence intervals of 26.3 ± 6.8 for \( a \) and 10,465 ± 1931 °K for \( b \).

At a given temperature, \( \mu_m \) estimated from turbidostat dilution rate at steady state did not differ from \( \mu_m \) calculated from cell number increase during exponential batch growth (Fig. 1).

PHOTOSYNTHESIS–IRRADIANCE CURVES

A set of photosynthesis versus irradiance \( (P–I) \) curves such as that shown in Fig. 2 describes short-term temperature and light response characteristics for \( P \).

![Graph](image)

**Fig. 2.** Rate of photosynthesis per cell as a function of incident irradiance at different experimental temperatures: *Phaeodactylum tricornutum* cells were cultured in a turbidostat at 10 °C receiving 250 \( \mu E \cdot m^{-2} \cdot s^{-1} \) incident irradiance.

*tricornutum* cultured under one temperature–light combination. Fig. 2 shows the response of cells grown in a turbidostat at 10 °C and receiving irradiance of 250 \( \mu E \cdot m^{-2} \cdot s^{-1} \). Based on the low level of discrimination afforded by data points at low irradiances, there was apparently no effect of temperature insofar as the rate of light-limited photosynthesis and compensation intensity were concerned, except at supraoptimal temperatures (30 and 35 °C). However, the adjustments of light-saturated rates of photosynthesis \( (P_m) \) in response to temperature changes in these short-term radiolabelling incubations were considerable.
$P_m$—TEMPERATURE CURVES

The short-term effect of assay temperature on $P_m$ (Fig. 3a) is seen clearly by plotting values of $P_m$ taken from Fig. 2 against assay temperatures. Also included in Fig. 3a are $P_m$—$T$ curves for $P. tricornutum$ grown in turbidostats at 5, 15, and

![Graph a](image)

![Graph b](image)

Fig. 3. a, rate of photosynthesis per cell at light saturation as a function of experimental temperature (abscissa); response curves for $Phaeodactylum tricornutum$ cultured at 5 °C (∇), 10 °C (●), 15 °C (Δ) and 25 °C (□) are shown; heavy line indicates photosynthetic rate extant in growth vessel; b, data of assimilation numbers.

25 °C ($I = 250 \mu E \cdot m^{-2} \cdot s^{-1}$). In the same manner that 10 °C cells had been assayed over wide temperature and irradiance ranges (Fig. 2), so too were cells grown at the latter three temperatures. The sets of $P$—$I$ curves corresponding to Fig. 2 for cells grown at 5, 15, and 25 °C are not shown.
In comparing $P_m - T$ curves derived from cells grown at different temperatures (Fig. 3a), several features may be noted: (1) $Q_{10}$ values (Hochachka & Somero, 1973) for $P_m$ did not change significantly with growth temperature. The average value of $Q_{10}$ over all four growth temperatures was $2.6 \pm 0.3$ (SD) in the assay temperature range from 5 to 15°C. (2) Both the assay temperature for which $P_m$ was optimal and the assay temperature above which $P_m = 0$ were higher for cells grown at higher temperatures. (3) $P_m$ values assayed at the temperature corresponding to that at which the cells had been grown (heavy line in Fig. 3a) increased as growth temperature increased. (4) Optimal values of $P_m$ achieved by the cells increased as growth temperature changed from 5 to 10°C, but decreased as this temperature changed from 10 to 15 to 25°C.

![Graph](image)

**Fig. 4.** Specific growth rate (●), $P_{\text{max}}$ measured at the growth temperature (○) and $P_{\text{max}}$ measured at the optimum experimental temperature (□) as functions of growth temperature: units for photosynthetic measurements are pg C incorporated · pg cell C$^{-1}$ · day$^{-1}$ (= day$^{-1}$).
The latter two observations are illustrated more clearly in Fig. 4 in which growth temperature (rather than assay temperature as in Fig. 3) is shown as the independent variable on the abscissa. In this figure, photosynthesis is indicated by the specific carbon incorporation rate (day$^{-1}$), i.e. $P_m$ divided by cell carbon content (pg C · cell$^{-1}$). In these units, apparent photosynthesis rates may be directly compared with growth rates.

ASSIMILATION NUMBER–TEMPERATURE CURVES

The assimilation number (pg C · pg Chl a$^{-1}$ · h$^{-1}$), i.e. $P_m$ divided by chlorophyll a content (pg Chl a · cell$^{-1}$) is shown as a function of assay temperature for cells grown at various temperatures in Fig. 3b. Because there was an increase in cellular Chl a content as cells were grown at higher temperatures, the assimilation number assayed at the temperature corresponding to that of growth (heavy line in Fig. 3b) remained fairly constant at $\approx 3.8$ pg C · pg Chl a$^{-1}$ · h$^{-1}$ for growth temperatures from 10 to 25°C. *P. tricornutum* grown at 5, 10, 15, and 25°C contained 0.120, 0.176, 0.205 and 0.256 pg Chl a · cell$^{-1}$ respectively.

ENZYME ACTIVITIES

Activities of RuBPCase and PEPCase (normalized to cell carbon content) are shown as a function of growth temperature in Fig. 5. The ratio of RuBPCase: PEPCase activities was 12.2, 4.0, 3.6, and 1.6 for cells grown at 5, 10, 15, and 25°C respectively. Both RuBPCase activity and the sum of activities from the two carboxylases ($\Sigma$Case) exhibited a peak at a growth temperature of 10°C (Fig. 5).

PROTEIN SYNTHESIS RATE

Both $\rho$ and $\rho'$ increased with growth temperature (Table I). Between 10 and 25°C, the relative temperature dependencies of $\mu_m$ and $\rho$ were similar, as indicated by the constancy of protein content within this temperature range. The fidelity of $\rho'$ as a measure of $\rho$, as indicated by $\rho' : \rho$, increased as growth temperature (and therefore also $\mu_m$) increased.

**Table I**

<table>
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<th>$T$</th>
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<th>$Q$</th>
<th>$\rho$</th>
<th>$\rho'$</th>
<th>$\rho' : \rho$</th>
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<tr>
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</table>

Net and apparent cellular rates of protein synthesis for *Phaeodactylum tricornutum* grown at various temperatures: $\mu_m$, specific growth rate (day$^{-1}$); $Q$, cellular protein content (pg protein · cell$^{-1}$); $\rho$, net rate of protein synthesis (pg protein · cell$^{-1}$ · day$^{-1}$); $\rho'$, apparent rate of protein synthesis (pg protein · cell$^{-1}$ · day$^{-1}$); $T$, growth temperature (°C).
Fig. 5. Variations of RuBPCase activity (○), PEPCase activity (△) and their sum (□) with growth temperature: units for enzyme activities are pg C incorporated pg cell C$^{-1}$ day$^{-1}$ (= day$^{-1}$).

**DISCUSSION**

The ability of cells to exhibit short-term and long-term physiological changes to variations in environmental conditions clearly indicates the need for an accurate description of the history of cells in studies of such variations. The rate of growth provides a standard for such a description as it represents the net result of subcellular responses.

Much of the evidence regarding temperature adaptation in phytoplankton is based on short-term photosynthetic rate measurements performed on cells harvested from batch cultures (Aruga, 1965; Jørgensen & Steemann-Nielsen, 1965; Jørgensen, 1968; Steemann-Nielsen & Jørgensen, 1968a, b; Morris & Farrell, 1971; Morris &
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Glover, 1974; Sheridan & Ulik, 1976; Tomas, 1980; other references cited in Li, 1980). However, batch-cultured algae undergo very large physiological changes even during the period when cell numbers are increasing at a constant exponential rate (Griffiths, 1973; Morris & Glover, 1974; Bates, 1981). Wilson & Levedahl (1968) emphasized that “the attainment of steady state kinetics during cell multiplication in batch culture does not guarantee the maintenance of constant cell composition”. More recently, the need for using algal cells adapted in continuous cultures for assessments of temperature effects has been recognized in physiological ecology studies (Goldman, 1977a,b, 1979; Ahlgren, 1978; Goldman & Mann, 1980; Rhee & Gotham, 1981).

In the present study, the effects of temperature were assessed on cells for which both \( \mu \) and \( \mu_m \) were well defined, the latter being the same at all temperatures examined. We wished to evaluate for *P. tricornutum* the two concepts of adaptation presented earlier: first, is there compensation of metabolic rate some time after a temperature shift so that eventually, cells are able to perform more or less at the same level over a wide temperature range; second, is the potential available for maximum metabolic activity dependent on growth temperature.

In short-term assays of \( P_m \) as a function of temperature, the response of *P. tricornutum* grown at 5, 10, 15, or 25°C was similar insofar as an Arrhenius-type of activation was observed in each case (Fig. 3a). The value of \( Q_{10} \) exhibited by *P. tricornutum* in short-term photosynthetic assays (2.6) is typical of that shown by many other plants (Rabinowitch, 1956).

Comparison of the four short-term \( P_m-T \) curves at the assay temperature corresponding to growth temperature (heavy line in Fig. 3a) indicates partial compensation (cf. Christophersen, 1973) of photosynthesis between 10 and 25°C. For example, a temperature shift from 25 to 15°C decreased \( P_m \) from 1.00 pg C·cell\(^{-1}\)·h\(^{-1}\) to 0.37 pg C·cell\(^{-1}\)·h\(^{-1}\) in the short term. However, when cells were cultured at 15°C, \( P_m \) at 15°C was 0.81 pg C·cell\(^{-1}\)·h\(^{-1}\). Likewise, a temperature shift from 15 to 10°C decreased \( P_m \) from 0.81 pg C·cell\(^{-1}\)·h\(^{-1}\) to 0.48 pg C·cell\(^{-1}\)·h\(^{-1}\) in the short term. However, when cells were cultured at 10°C, \( P_m \) at 10°C was 0.62 pg C·cell\(^{-1}\)·h\(^{-1}\). Interestingly, there was inverse compensation (cf. Christophersen, 1973) between 5 and 10°C. That is, a temperature shift from 10 to 5°C decreased \( P_m \) from 0.62 pg C·cell\(^{-1}\)·h\(^{-1}\) to 0.33 pg C·cell\(^{-1}\)·h\(^{-1}\) in the short term. However, when cells were cultured at 5°C, \( P_m \) at 5°C was 0.26 pg C·cell\(^{-1}\)·h\(^{-1}\) – a value even lower than the value of \( P_m \) reached immediately after shifting from 10 to 5°C.

When assimilation numbers were considered in the same manner (Fig. 3b), there was almost perfect compensation between 10 and 25°C but none at all from 5 to 10°C. Similar patterns of temperature adaptation in assimilation numbers have been observed in other algal cultures and natural phytoplankton populations (Li, 1980).

A recent study by Rhee & Gotham (1981) indicated that *Scenedesmus* sp. grown
in turbidostats exhibited perfect compensation in carbon fixation rate per cell from 9 to 20 °C. In contrast to the effect of temperature on Chl a content of continuously cultured \textit{P. tricornutum} (this study) and of several batch-cultured algae studies by others (summarized in Li, 1980), Rhee & Gotham found that \textit{Scenedesmus} contained less Chl a at higher temperatures. Thus, carbon fixation rate per unit Chl a increased with growth temperature. It appears that although photosynthetic compensation does indeed occur over a range of temperatures, at least for \textit{P. tricornutum} and \textit{Scenedesmus}, the extent of such compensation may vary among species.

The ability of algae to alter respiratory rate in order to compensate for changes in growth temperature also has been examined (Ryther & Guillard, 1962; Jones, 1977; Harris & Piccinin, 1977). Depending on the species, the response of respiration to changes in growth temperature ranged from overcompensation to inverse compensation (cf. Christophersen, 1973). It should be noted that these results refer to comparisons made among populations for which \( \mu : \mu_m \) was not specified.

Adaptation in marine phytoplankton also has been considered from the viewpoint of changes in the level of maximum metabolic ability. Originally arising from results of batch culture experiments on temperature effects (Morris & Glover, 1974), this concept has been expanded in discussions of the effects of light (Beardall & Morris, 1976) and nutrients (Dugdale, 1977; McCarthy & Goldman, 1979; Glibert & Goldman, 1981). Our results (Figs. 3, 4) show that as growth temperature decreased from 25 to 10 °C, that is, over the range where \( P_m \) exhibited partial compensation, there was an increase in the value of \( P_m \) measured at the optimum assay temperature, i.e. maximum photosynthetic ability. As growth temperature further decreased from 10 to 5 °C and \textit{P. tricornutum} exhibited inverse compensation, there was a decrease in this capability (Fig. 3, 4). Concomitant with these changes in maximum photosynthetic ability were parallel changes in the in vitro activity of RuBPCase with growth temperature (Fig. 5). It is interesting to suggest that the ability of \textit{P. tricornutum} to regulate cellular carboxylase activities may perhaps be associated with its ability to compensate against large \( P_m \) changes in the face of temperature variations.

Jørgensen (1968) proposed the hypothesis that compensation of photosynthetic rate to low temperatures is achieved by an increase in the concentration of enzymes involved with carbon dioxide assimilation. Indirect support for this hypothesis was that cells of \textit{Skeletonema costatum} grown at 7 °C had greater amounts of protein than cells grown at 20 °C. Further support for this hypothesis was presented by Morris & Farrell (1971) who found that growth at low temperatures also led to greater protein content in \textit{Dunaliella tertiolecta} and \textit{P. tricornutum} as well as higher RuBPCase activity in the former. Morris & Glover (1974) have since questioned the validity of using these results in support of the hypothesis because of possible batch culture artifacts.

It is generally held that the basic problem of rate compensation to temperature changes is one of enzyme activity regulation (Hochachka & Somero, 1973). A
change in the concentration of pre-existing enzymes is only one of several ways of achieving this. Other strategies, though not mutually exclusive, include utilizing different enzyme variants (isozymes) at different temperatures and modulating the activities of pre-existing enzymes. Our measurements of carboxylase activities do not allow a discrimination among these possible strategies. Measurements of total protein content (Table I) do not provide an unequivocal test of the increased carboxylating enzyme concentration hypothesis. Rabinowitz et al. (1975) found that RuBPCase accounted for $\approx 9\%$ of the total protein in autotrophic *Euglena gracilis*.

The relative constancy of protein content over a wide temperature range for *P. tricornutum* in the present study (Table I) agrees with observations made by others that temperature does not greatly influence cellular composition of microorganisms as long as the cells maintain the same $\mu$; $\mu_m$ as temperature varies (Tempest, 1976; Goldman, 1980). This contrasts with recent observations made by Rhee & Gotham (1981) on *Scenedesmus* sp. grown in turbidostats. Measurements of cell protein and RNA showed that concentrations of these macromolecules increased with decreasing temperature. The reason for these differences is not clear.

In balanced steady-state growth, a cell population whose protein content remains relatively invariant as temperature increases must have a net rate of protein synthesis that increases with temperature in order that overall population growth rate also increases with temperature ($\rho = \mu_m \cdot Q$). This was confirmed qualitatively by measurements of $\rho'$ (Table I). Quantitative differences between $\rho'$ and $\rho$ may be due to a variety of causes (O’Kane & Jones, 1978) and appear to be related either to temperature or growth rate in our study (Table I). When the reasons for these differences are more clearly understood, it may be possible to use $\rho': Q$ as an estimate of absolute growth rate ($\mu$) of natural phytoplankton populations.

In a similar approach, Bates (1981) has recently used the rate of $^{35}$S incorporation into protein to estimate $\rho'$. However, he pointed out that since phytoplankton protein may contain between 0.9 to 3.3% sulfur by weight (Chuecas & Riley, 1969), there is an almost four-fold range of uncertainty in estimates based on the sulfur method.

**CONCLUSIONS**

In the most general sense, we suggest that an organism adapts to a given environmental condition when its response to that condition depends on the state of the condition prevailing during its growth. Thus, for *Phaeodactylum tricornutum*, the photosynthetic response to temperature ($P_m-T$ curve) depends on the temperature experienced by the cells during their growth. It may seem that “true” adaptation should be considered only when growth rate is the same at various temperatures. However, for any given species of unicellular alga, $\mu_m$ is not independent of temperature (Eppley, 1972; Li, 1980). Nevertheless, it seems that algae
do in fact have the ability for metabolic rate compensation; what we observe as \( \mu_m \) at a given temperature is the net outcome of cellular regulation which has partially attenuated the effect of temperature on a complex series of chemical reactions.

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