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## Acclimation to low light intensity in photosynthesis and growth of *Pseudo-nitzschia multiseries* Hasle, a neurotoxic diatom

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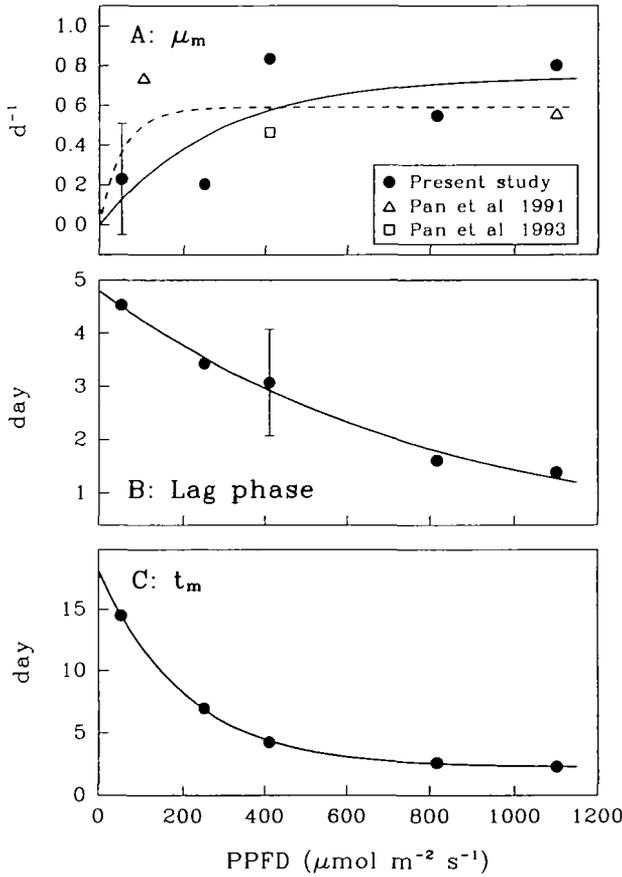
**Abstract.** *Pseudo-nitzschia multiseries*, a neurotoxic diatom, was grown in batch culture at light intensities between 53 and 1100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Cellular contents of carbon, nitrogen and chlorophyll *a*, and the relationship between photosynthesis and light levels, were studied during exponential (day 4) and stationary phases (day 12). In the stationary phase at low light, there was an increase in cellular chlorophyll *a* and the initial slope of *P-I* curves ( $\alpha^B$ ), which permitted a photosynthetic assimilation of energy equivalent to that of cells grown at high light. In past incidents of domoic acid poisoning, this may have facilitated domoic acid production at low light intensities.

### Introduction

In most toxic algae, photosynthesis is not only the essential process in primary metabolism, but is also required for toxin production. For example, the yield of saxitoxins per cell in *Alexandrium catenella*, a dinoflagellate implicated in paralytic shellfish poisoning, was proportional to hours of light per day (Proctor *et al.*, 1975). In stationary phase, domoic acid production by the diatom *Pseudo-nitzschia multiseries* ceased during periods of darkness, but resumed soon after the transition from dark to light (Bates *et al.*, 1991), the same as in the haptophyte *Prymnesium parvum* (Shilo, 1971). Moreover, domoic acid production was inhibited by addition of the photosynthetic inhibitor DCMU (Bates *et al.*, 1991).

Our research on domoic acid production by *P. multiseries* (Pan *et al.*, 1996) and the work of Shilo (1971) and Bates *et al.* (1991), suggest that biogenic energy through photosynthesis is essential to toxin production. Nevertheless, variation in light intensity seems to have a relatively small effect on toxin production (Ogata *et al.*, 1987, 1989; Boyer *et al.*, 1985; Bates *et al.*, 1991) even though it affects carbon assimilation (Pan *et al.*, 1991). Differences in the initial rates of domoic acid production at low and high light intensities were not significant (Bates *et al.*, 1991).

Chlorophyll *a*, essential to photosynthesis, is positively correlated with domoic acid production in *P. multiseries* (Pan *et al.*, 1996). A higher content of chlorophyll *a* is associated with the cells grown at low light (Falkowski, 1981; Prézélin and Matlick, 1983; Pan *et al.*, 1991). It seems possible that the reduced potential of energy assimilation at low light may be compensated for by an increase in chlorophyll *a* concentration. In this study, we investigated variations in photosynthesis, growth and cellular content of chlorophyll *a*, carbon and nitrogen under various



**Fig. 1.** Variations with light levels in (A) the maximal growth [the solid curve is fitted by equation (1) to the five filled points only and the broken line is fitted to all eight points], (B) the duration of the lag phase and (C) the time when maximal growth is reached [fitted by equation (2)]. The vertical bar indicates SD of curve fitting (others contain SD smaller than the size of the points).

light intensities. We also made a comparison with the results of our earlier studies (Pan *et al.*, 1991, 1993).

## Method

Non-axenic *P. multiseriis* (strain NPBIO) was grown as batch culture in medium FE (Subba Rao *et al.*, 1988) at 10°C. The stock culture was grown at five photosynthetic photon flux densities (PPFD) of 53, 250, 410, 815 and 1100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  under continuous cool white fluorescent light. After an acclimation period of 10 days (two subculturings) at exponential growing condition, 200 ml of a 4-day-old culture were inoculated into a 3 l Fernbach flask with 1800 ml FE medium. Samples were taken during the exponential phase (day 4) and stationary phase (day 12) for measurement of cell concentration, chlorophyll *a*, particulate carbon and nitrogen, dissolved nitrate, phosphate and silicate. However, the culture at 53

$\mu\text{mol m}^{-2} \text{s}^{-1}$  was still in lag phase on day 4 and in late exponential phase on day 12. More frequent samples for cell concentrations were collected to facilitate the quantification of growth characteristics.

Experiments on the relationship of photosynthesis and PPFD ( $P-I$ ) were conducted on day 4 and day 12. The methods for the measurement of carbon assimilation ( $^{14}\text{C}$  method) remained the same as in Pan *et al.* (1991).  $\text{NaH}^{14}\text{CO}_3$  was added to a culture sample and 1 ml aliquots were incubated in 48 clean glass vials for 30 min under various PPFD levels ranging from 11 to 5000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at  $10^\circ\text{C}$ . The relationship was described using a modified photoinhibition model (Platt *et al.*, 1980; Pan *et al.*, 1991). Maximal growth rates and the duration of the lag phase were determined by fitting the Gompertz model [ $\ln(N/N_0) = a \exp(-\exp(b-ct))$ ], see Zwietering *et al.*, 1990] to the cell concentration data (days 0, 1, 4, 7, 10, 12). Methods for chemical analyses (Strickland and Parsons, 1972) of chlorophyll *a* (fluorometric), carbon and nitrogen (combustion), and dissolved nutrients (autoanalyzer) remained the same as described in Pan *et al.* (1991).

## Results

### Growth

The cultures grew at different rates with various durations of lag phases under different PPFD levels (Figure 1). Upon inoculation into a fresh medium, *P. multiseries* usually experienced a lag phase with a duration of 0–5 days, the growth rate gradually attained a maximum and decreased afterwards. Generally, cultures entered exponential phase earlier and grew faster at higher PPFD. For example, the culture at 1100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  approached stationary phase on day 4, while the culture at 53  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was still in the exponential phase on day 12.

Maximal growth rates ( $\mu_m$ ) ranged from 0.21 to 0.84  $\text{day}^{-1}$  and their relationship with PPFD levels can be described by equation 1 (Figure 1A):

$$\mu_m = \mu_{m(m)} [1 - \exp(-\alpha_g I)] \quad (1)$$

where,  $\mu_{m(m)}$  is the maximal growth rate at optimal growth PPFD,  $\alpha_g$  is the initial slope of the  $\mu_m-I$  curve ( $\text{day}^{-1} [\mu\text{mol m}^{-2} \text{s}^{-1}]^{-1}$ ), representing the enhancement effect of increasing PPFD on growth when  $I \rightarrow 0$ . In fitting the Gompertz model to the data from the culture at 410  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , it was recognized that there were insufficient points to clearly define the change from lag phase to exponential phase. This resulted in a large degree of uncertainty in the duration of the lag phase (Figure 1B), and an abnormal high value of  $\mu_m$  at 410  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Figure 1A).

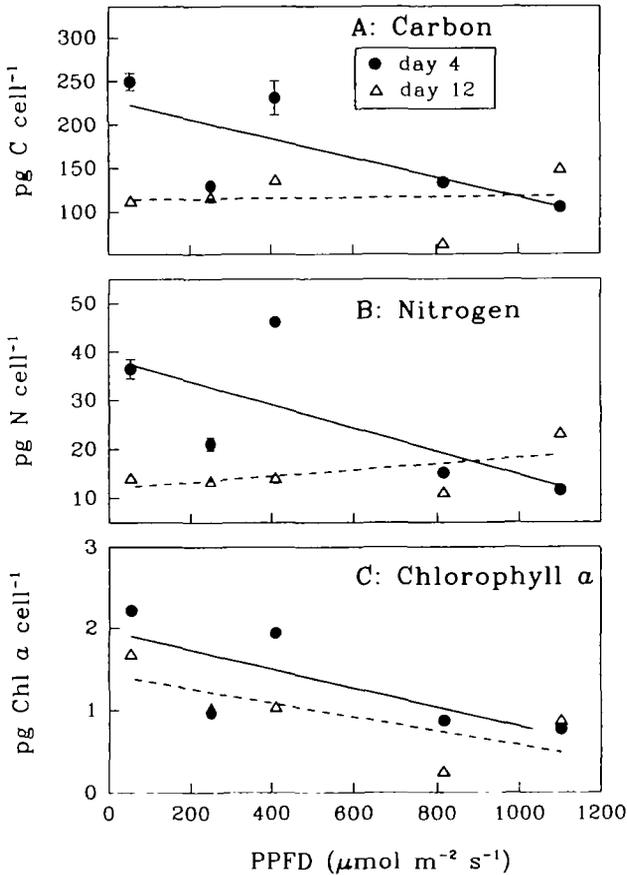
Replacing  $a$ ,  $b$  and  $c$  in the Gompertz model with the fitted values, the time ( $t_m$ , in days) when the culture reached  $\mu_m$  was determined. The relationship between  $t_m$  and PPFD was well described by equation 2 (Figure 1C):

$$t_m = (t_d - g) \exp(-fI) + g \quad (2)$$

where  $t_d$  (same units as  $t_m$ ) is the maximum  $t_m$  when the culture is in the dark,  $f$  ( $\text{day} [\mu\text{mol m}^{-2} \text{s}^{-1}]^{-1}$ ) is the negative initial slope at  $I \rightarrow 0$ , and  $g$  describes the

**Table I.** Values of parameters in equation (2)

Parameter	Value	SD
$t_d$ (day)	18.16	0.21
$f$ (day [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ] $^{-1}$ )	0.004905	0.000143
$g$ (day)	2.24	0.096



**Fig. 2.** Variations with light levels in the cellular (A) carbon, (B) nitrogen and (C) chlorophyll *a*. The lines are linear regressions.

asymptote ( $t_m$  at saturating  $I$ ), representing the potential minimum time needed for the diatom to reach maximal growth. Based on the data of  $t_m$  (Figure 1C), fitted values are in Table I.

*Cell chemical composition*

As PPFD increased, cellular carbon and nitrogen generally decreased on day 4, but were relatively constant on day 12 (Figure 2); cellular chlorophyll *a* decreased

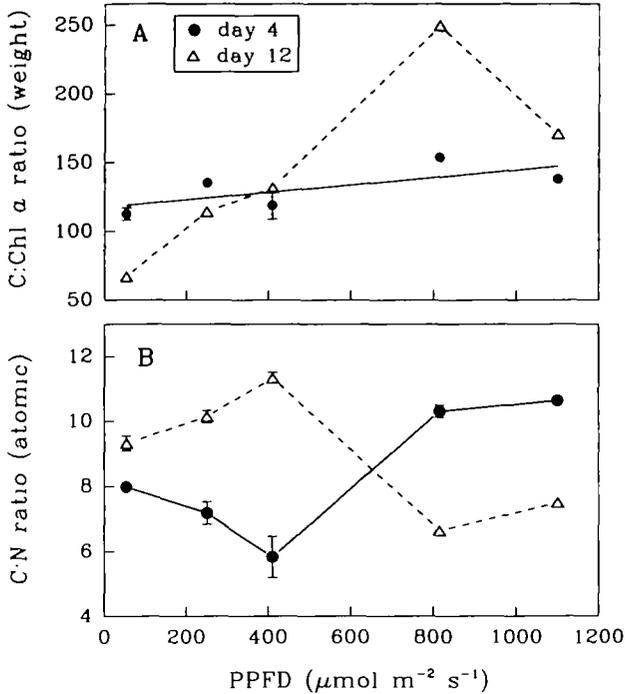


Fig. 3. Variations with light levels in the ratios of (A) carbon to chlorophyll *a* and (B) carbon to nitrogen.

on both day 4 and day 12. Ratios of C:Chl *a* in relation to PPFD were not significantly different on day 4 (Figure 3A), but increased markedly on day 12 from 67 at  $53 \mu\text{mol m}^{-2} \text{s}^{-1}$  to 249 at  $815 \mu\text{mol m}^{-2} \text{s}^{-1}$ . At higher PPFD ( $>815 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), C:Chl *a* ratio decreased, suggesting photoinhibition. The ratio of carbon to nitrogen was variable between 5.84 and 11.33. On day 4, the C:N ratio decreased from 7.98 to 5.84 when PPFD increased from 53 to  $410 \mu\text{mol m}^{-2} \text{s}^{-1}$  and then increased as PPFD further increased (Figure 3B). In contrast, the C:N ratio on day 12 was a mirror image of that on day 4.

### Photosynthesis

Photosynthetic characteristics varied with changing levels of PPFD. The initial slope ( $\alpha^B$ ) of the *P-I* curve ranged from 0.40 to  $6.22 \text{ ng C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1} [\mu\text{mol m}^{-2} \text{ s}^{-1}]^{-1}$  (Figure 4A) while  $P^B_m$  ranged from 0.10 to  $1.16 \mu\text{g C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1}$  (Figure 5A). Regardless of the biomass indices used for normalization, as PPFD increased,  $\alpha^B$  increased on day 4, but decreased on day 12 (Figure 4). These variations were more pronounced on day 12 than on day 4. In the exponential phase (day 4),  $P^B_m$  (normalized to chlorophyll *a*, Figure 5A) increased as PPFD increased from 53 to  $250 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , and remained approximately constant between 250 and  $410 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . At higher PPFD,  $P^B_m$  decreased slightly,

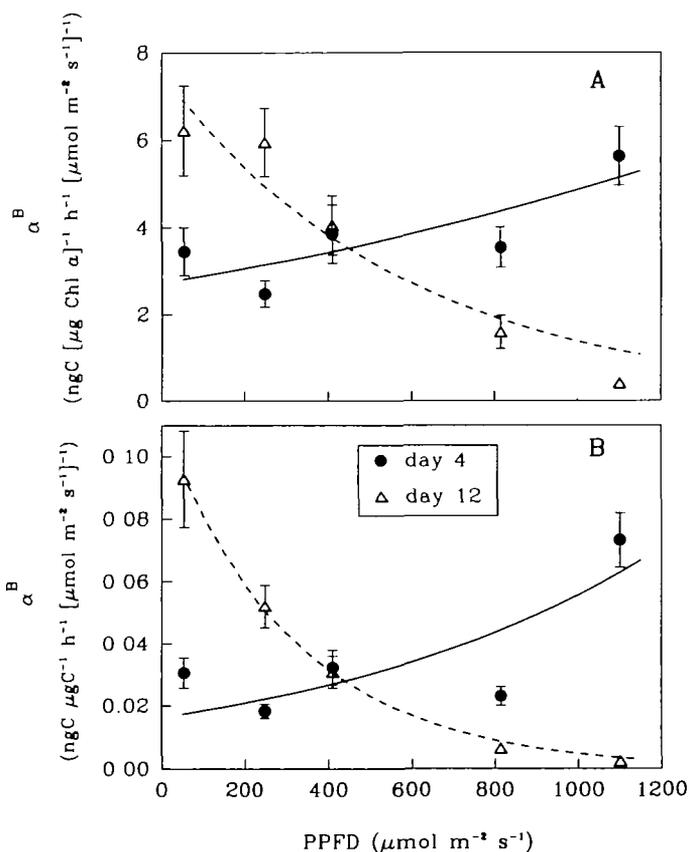


Fig. 4. Variations of  $\alpha^B$  with growth light levels. (A) Normalized to chlorophyll *a*, (B) normalized to carbon. The curves are fitted by exponential functions.

showing photoinhibition. In the stationary phase (day 12), on the other hand,  $P_m^B$  generally decreased at higher PPFDs. Cultures grown at higher PPFD generally had higher  $I_m$  values (Table II). For individual cultures, however, the  $I_m$  value was higher on day 4 than day 12. Nevertheless, the other two photoadaptive parameters,  $I_k$  and  $I_s$ , did not follow the variation of PPFD.

Table II. Changes in photoadaptive parameters  $I_m$ ,  $I_k$  and  $I_s$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) during growth at different PPFDs. Definitions of  $I_m$ ,  $I_k$  and  $I_s$  are the same as described by Platt *et al.* (1980)

PPFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Day 4			Day 12		
	$I_m$	$I_k$	$I_s$	$I_m$	$I_k$	$I_s$
1100	1320	198	217	900	218	222
815	1430	231	249	560	186	155
410	730	278	1029	430	112	120
250	900	431	4132	780	201	209
53	500	175	212	430	93	99

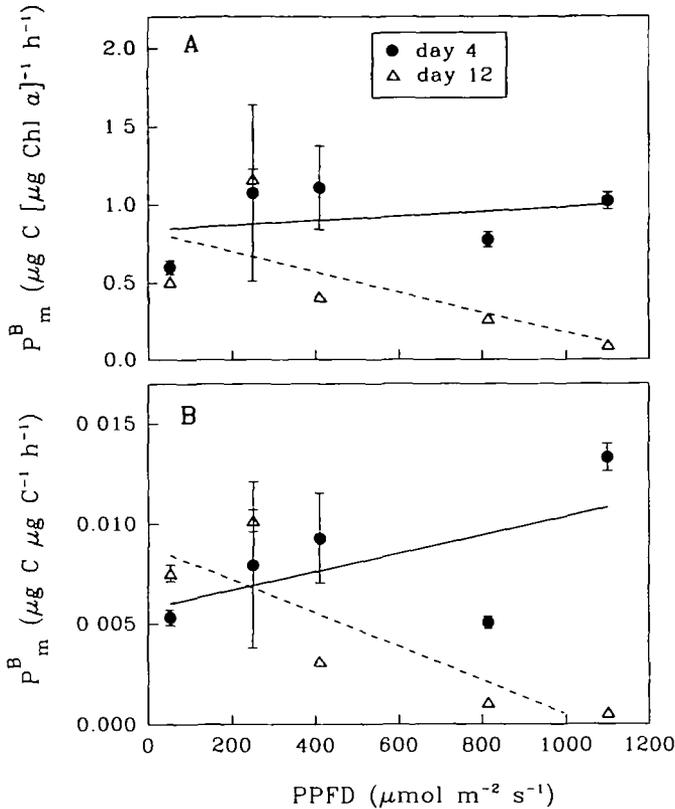


Fig. 5. Variations of  $P_m^B$  with growth light levels. (A) Normalized to chlorophyll *a*, (B) normalized to carbon. The lines are linear regressions.

## Discussion

### Growth

A variety of cell functions changed as the batch culture population aged. Faster growth due to higher light accelerated aging of *P. multiseriis*, even before the cells reached the stationary phase (Pan *et al.*, 1991). Cultures had a shorter lag period, grew faster and reached stationary phase earlier under higher light (Figure 1), similar to the observations of Bates *et al.* (1991). The signs of delay in aging were found in the cultures at low light, which had higher cell contents of carbon, nitrogen and chlorophyll *a* on day 4 (Figure 2) compared to those in high light, similar to the dinoflagellate *Gonyaulax polyedra* (Prézelin, 1982).

Extrapolating the exponential curve in Figure 1C to the point  $I = 0$  [equation (2)], a maximal value of  $t_m (=t_d)$  was obtained. A negative value of compensation light ( $I_c$ ) and a positive intercept on the *y*-axis was also obtained by supplying an additional parameter of  $I_c$  to the  $\mu$ - $I$  model [equation (1)]. Although a negative  $I_c$  is physiologically meaningless, a positive intercept on the *y*-axis with a  $t_d$  value of

18.16 days suggested that *P. multiseriis* might be able to grow in the dark in the presence of some energy source other than light. Observations of D.V.Subba Rao and G.D.Wohlgeschaffen (unpublished data) showed growth of *P. multiseriis* in the dark in the presence of a variety of organic substrates. This suggests that *P. multiseriis* may be both photo- and heterotrophic. Heterotrophic growth has been found in other diatoms such as *Nitzschia laevis* (Lewin and Hellebust, 1978) and *Phaeodactylum tricornutum* (Flynn and Syrett, 1986). However, caution should be exercised in the interpretation of these data since the  $t_m$  value (14.50 days) of the culture at  $53 \mu\text{mol m}^{-2} \text{s}^{-1}$  was beyond the termination (day 12) of the experiment. A slightly different value is possible, but a positive value of  $t_d$  is certainly expected.

The light level for the optimal growth of *P. multiseriis* was  $410\text{--}1100 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Figure 1A), which was consistent with the variation of  $P^B_m$  (Figure 5A). Similar saturation functions between growth and incident light exist in most other diatoms and dinoflagellates (Kiefer and Mitchell, 1983; Falkowski *et al.*, 1985; Langdon, 1987). For example, in the diatoms *Ditylum brightwellii* and *Pseudonitzschia turgidula* (Paasche, 1968), and dinoflagellate *G. polyedra* (Rivkin *et al.*, 1982a), growth rates increased as a saturation function of light. Langdon (1987) found an  $\alpha_g$  of  $17 \times 10^{-3} \text{ day}^{-1} [\mu\text{mol m}^{-2} \text{s}^{-1}]^{-1}$  for *Skeletonema costatum* grown at  $20^\circ\text{C}$ . In the present study on *P. multiseriis*,  $\alpha_g$  was  $2.7 (\pm 1.9) \times 10^{-3} \text{ day}^{-1} [\mu\text{mol m}^{-2} \text{s}^{-1}]^{-1}$  which was generally low compared with other diatoms, but similar to dinoflagellates (Table III). However, because of the scarcity and scattering of data points in Figure 1A, caution should be exercised when comparing these data with the literature values. For example, after introducing the three points from our earlier studies (Pan *et al.*, 1991, 1993), the  $\alpha_g$  value increased to  $18 (\pm 16) \times 10^{-3} \text{ day}^{-1} [\mu\text{mol m}^{-2} \text{s}^{-1}]^{-1}$  (broken line in Figure 1A), which was comparable to other diatoms.

**Table III.** Initial slopes of  $\mu$ -I curves in selected diatoms and dinoflagellates.  $\alpha_g = 10^{-3} \text{ day}^{-1} [\mu\text{mol m}^{-2} \text{s}^{-1}]^{-1}$

Taxon	$T^\circ\text{C}$	$\alpha_g$	References
<b>Diatoms</b>			
<i>P. multiseriis</i>	10	2.7–18	Present study
<i>Skeletonema costatum</i>	15	17.00	Langdon, 1987
<i>Thalassiosira weissflogii</i>	20	20.10	Laws and Bannister, 1980
	18	9.29	Falkowski <i>et al.</i> , 1985
<i>Leptocylindrus danicus</i>	10	11.78	Verity, 1982
<i>Chaetoceros protuberans</i>	19	7.07	Morel <i>et al.</i> , 1987
<i>Phaeodactylum tricornutum</i>	22–24	25.00	Geider <i>et al.</i> , 1985
<b>Dinoflagellates</b>			
<i>Gonyaulax polyedra</i>	23	2.63	Rivkin <i>et al.</i> , 1982a
<i>Alexandrium tamarense</i>	15	3.40	Langdon, 1987
<i>Prorocentrum micans</i>	18	0.35	Falkowski <i>et al.</i> , 1985
<i>Gyrodinium cf. aureolum</i>	20	17.33	Garcia and Purdie, 1992
<i>Pyrocystis noctiluca</i>	23	1.73	Rivkin <i>et al.</i> , 1982b

### Chemical composition

Growth rate is related to chemical composition. On day 4, the growth rate increased as light increased and attained a plateau, but cellular carbon and nitrogen appeared to decrease (Figure 1A and B). Similarly, the highest cellular carbon and nitrogen occurred at 0°C when the growth of *P. multiseries* was restricted by low temperature (Pan *et al.*, 1993). In *Thalassiosira weissflogii*, on the other hand, a high content of carbohydrates and protein was usually associated with a high growth rate under higher light levels (Post *et al.*, 1985). A similar trend was found in *Skeletonema costatum* (Smith *et al.*, 1992).

Cellular chlorophyll *a* was positively correlated with growth rate and maximum photosynthetic rate when light levels remained unchanged (Pan, 1994). When light increased, however, growth rate increased while cellular chlorophyll *a* decreased. Systematic differences in cellular chlorophyll *a* existed in the cultures under different light levels no matter how fast the cells grew (Pan *et al.*, 1991), due to the photoadaptation mechanism (Falkowski, 1981).

### Photosynthesis

Earlier, we found that cultures grown at higher light had higher  $P^B_m$  than those grown at low light (Pan *et al.*, 1991). This phenomenon was tested further in the present study. As light intensity increased,  $P^B_m$  (normalized to carbon, Figure 5B) increased on day 4 but decreased on day 12 (Figure 5B). The physiological states of cultures under various light levels were different on day 4 as well as on day 12. On day 4, for example, the cultures at 250 and 410  $\mu\text{mol m}^{-2} \text{s}^{-1}$  were approaching or in the period of maximal growth, but the culture at 53  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was still in the lag phase and those at 810 and 1100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  had already passed the period of maximal growth (Figure 1). Similarly, as light increased,  $\alpha^B$  increased on day 4, but decreased on day 12. Generally, in the exponential phase,  $P^B_m$  was larger for the cultures under higher light; in the stationary phase, on the other hand, the cultures grown at low light had higher  $P^B_m$  and  $\alpha^B$ . This suggests cells survive longer under low light.

### Overview

Photosynthesis and growth were well coupled (Figure 6). After integrating all the data from earlier work and the present study, a regression analysis (Model II, Laws and Archie, 1981) showed that the specific rates of growth ( $\mu$ ) and photosynthesis ( $P^B_m$ ) were positively correlated [equation (3)].

$$\mu = 1.263 P^B_m + 0.047 \quad (n = 28, P < 0.001) \quad (3)$$

A positive intercept of  $\mu$  when  $P^B_m$  is zero further suggests that *P. multiseries* might be able to grow heterotrophically, as discussed earlier.

Photosynthesis and growth declined in low light. However, as an adaptive strategy to low light, algae employ complementary pigmentation (by increasing

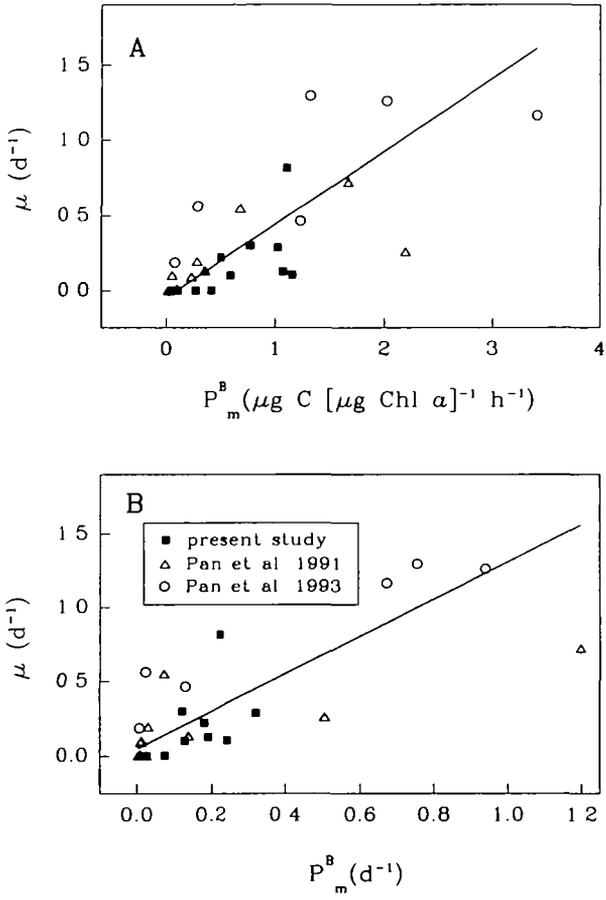


Fig. 6. Relationship between growth rate ( $\mu$ ) and photosynthetic rate ( $P_m^B$ ). (A)  $P_m^B$  is normalized to chlorophyll *a* and (B)  $P_m^B$  is normalized to carbon. The lines are linear regressions.

chlorophyll *a* and other photosynthetic pigments) and maximize absorption efficiency (Robinson *et al.*, 1995). Domoic acid production seems to depend on the photosynthesized energy and to be related to chlorophyll *a* concentration (Pan *et al.*, 1996), but the initial production rate did not differ significantly under various light levels (Bates *et al.*, 1991). During the winter in Cardigan Bay, where the episodes of the toxigenic bloom of *P. multiseri*s occurred, light levels were low (on a bright sunny day in November, the maximum light level was  $\sim 850 \mu mol m^{-2} s^{-1}$  on the surface and  $200 \mu mol m^{-2} s^{-1}$  at 2 m). The present study showed that in the stationary phase, cells grown at low light had higher values of  $\alpha^B$  and higher chlorophyll *a* concentration. These high values permitted a photosynthetic assimilation of energy equivalent to that of cells grown at high light. Unlike other diatoms, *P. multiseri*s was able to grow at very low rates ( $\leq 0.1 day^{-1}$ ) under nutrient stresses for a prolonged duration and produced toxin (Pan *et al.*, 1994). Similarly, cells of *P. multiseri*s with less metabolic cost at low light will survive longer.

This may explain in part the persistence of the toxigenic bloom of *P. multiseriis* in Cardigan Bay (for 3 months) during the winter of 1987.

### Acknowledgements

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