Acclimation to low light in photosynthesis and growth of Pseudo-nitzschia multiseries (Hasle), a neurotoxigenic diatom

Pan, Youlian; Rao, D. V. Subba; Mann, K. H.

Publisher's version / Version de l'éditeur:
http://doi.org/10.1093/plankt/18.8.1427
Journal of Plankton Research, 18, 8, pp. 1427-1438, 1996-08

Journal of Plankton Research Vol.18 no.8 pp.1427–1438, 1996

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

Youlian Pan[1,2,3], D.V.Subba Rao[1] and K.H.Mann[1]

[1]*Habitat Ecology Section, Department of Fisheries and Oceans, Bedford Institute of Oceanography, PO Box 1006, Dartmouth, Nova Scotia, B2Y 4A2, Canada*
[2]*Department of Biology, Dalhousie University, Halifax, Nova Scotia, B3H 4J1, Canada*
[3]*To whom correspondence should be sent at: Institute for Marine Bioscience, NRC, 1411 Oxford Street, Halifax, Nova Scotia, B3H 3Z1, Canada*

Abstract. *Pseudo-nitzschia multiseries*, a neurotoxigenic diatom, was grown in batch culture at light intensities between 53 and 1100 μmol m^{-2} 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 (α^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 toxigenic 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; Prézelin 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

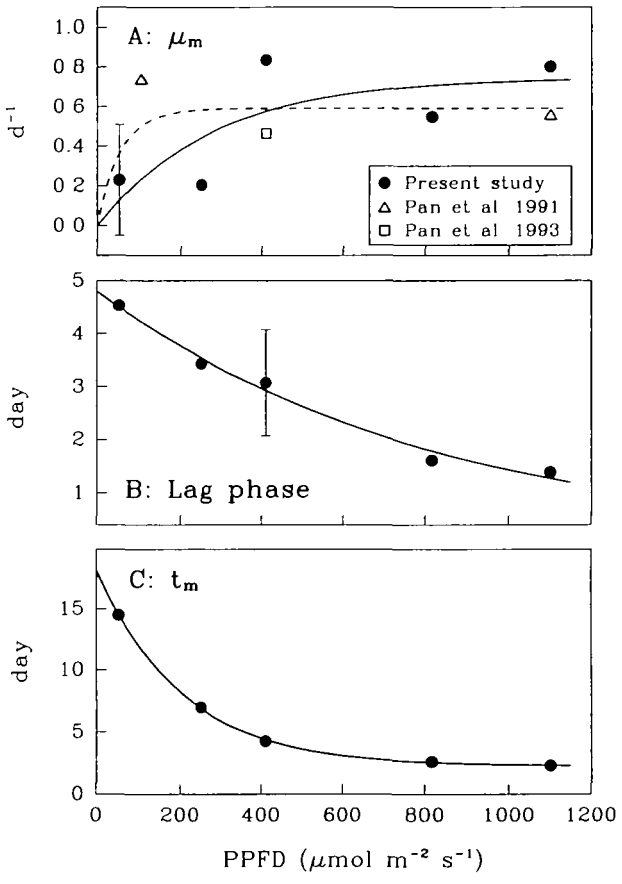

Fig. 1. Variations with light levels in (**A**) the maximal growth [the solid curve is fitted by equation (1) to the five filled points only and the broken line is fitted to all eight points], (**B**) the duration of the lag phase and (**C**) the time when maximal growth is reached [fitted by equation (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. multiseries* (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 μmol m^{-2} 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

μmol m^{-2} 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}C method) remained the same as in Pan *et al.* (1991). $NaH^{14}CO_3$ 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 μmol m^{-2} s^{-1} at 10°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 [$\ln(N/N_o) = a \exp(-\exp(b-ct))$, 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 μmol m^{-2} s^{-1} approached stationary phase on day 4, while the culture at 53 μmol m^{-2} s^{-1} was still in the exponential phase on day 12.

Maximal growth rates (μ_m) ranged from 0.21 to 0.84 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, α_g is the initial slope of the μ_m–*I* curve (day^{-1} [μmol m^{-2} 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 μmol m^{-2} 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 μ_m at 410 μmol m^{-2} 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 μ_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* (day [μmol m^{-2} 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 [μmol m^{-2} s^{-1}]$^{-1}$)	0.004905	0.000143
g (day)	2.24	0.096

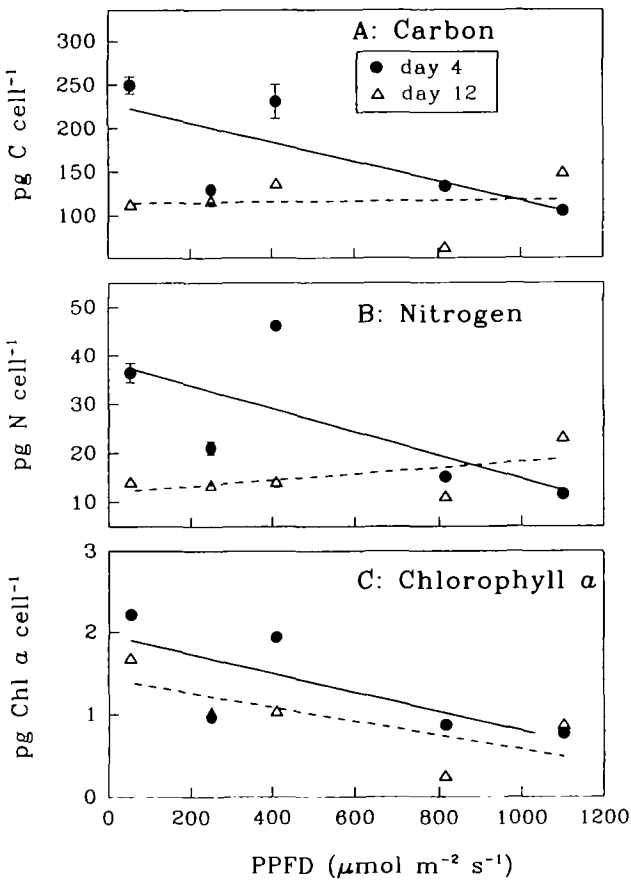

Fig. 2. Variations with light levels in the cellular (**A**) carbon, (**B**) nitrogen and (**C**) chlorophyll *a*. The lines are linear regressions.

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

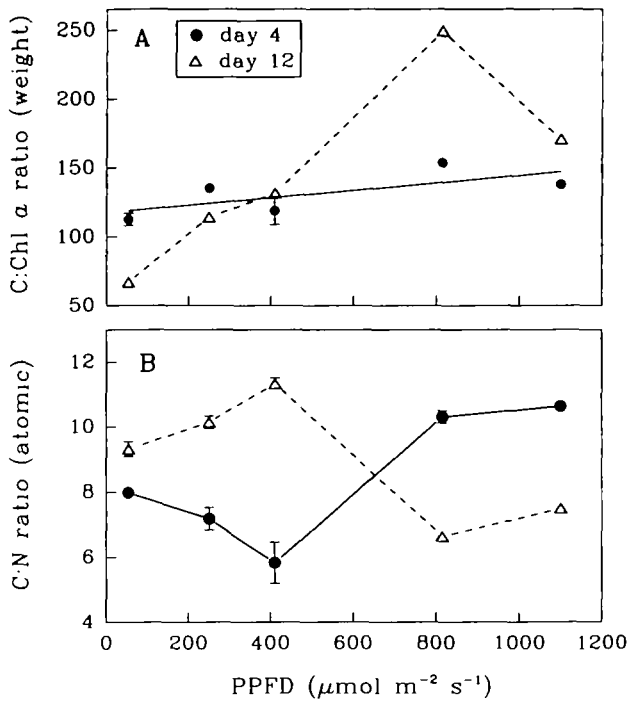

Fig. 3. Variations with light levels in the ratios of (**A**) carbon to chlorophyll *a* and (**B**) carbon to nitrogen.

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 μmol m^{-2} s^{-1} to 249 at 815 μmol m^{-2} s^{-1}. At higher PPFD (>815 μmol m^{-2} 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 μmol m^{-2} 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 (α^B) of the *P–I* curve ranged from 0.40 to 6.22 ng C [μg Chl *a*]$^{-1}$ h^{-1} [μmol m^{-2} s^{-1}]$^{-1}$ (Figure 4A) while P^B_m ranged from 0.10 to 1.16 μg C [μg Chl *a*]$^{-1}$ h^{-1} (Figure 5A). Regardless of the biomass indices used for normalization, as PPFD increased, α^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 μmol m^{-2} s^{-1}, and remained approximately constant between 250 and 410 μmol m^{-2} s^{-1}. At higher PPFD, P^B_m decreased slightly,

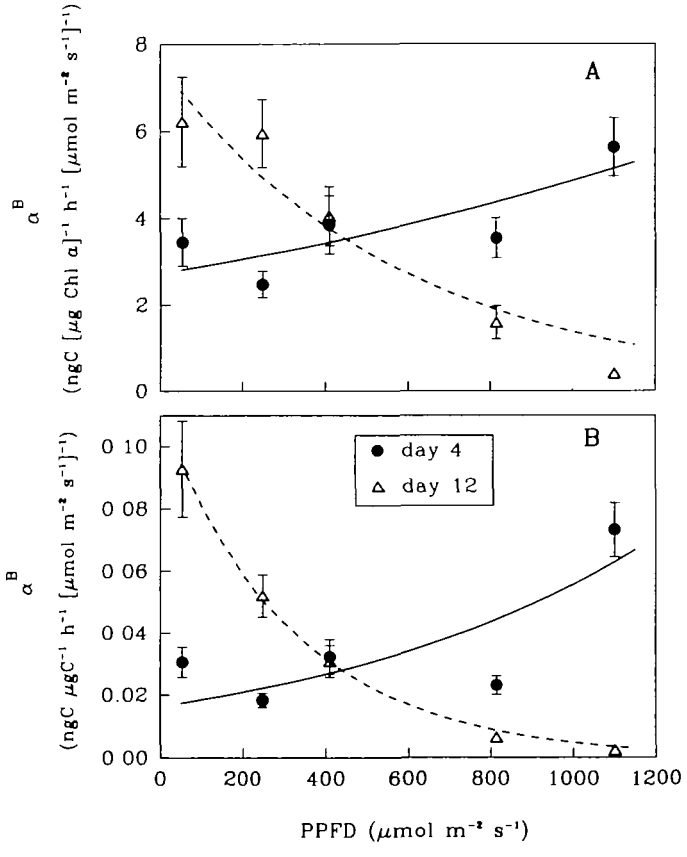

Fig. 4. Variations of α^B with growth light levels. (**A**) Normalized to chlorophyll *a*, (**B**) normalized to carbon. The curves are fitted by exponential functions.

showing photoinhibition. In the stationary phase (day 12), on the other hand, P^B_m 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 (μmol m^{-2} 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 (μmol m^{-2} 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

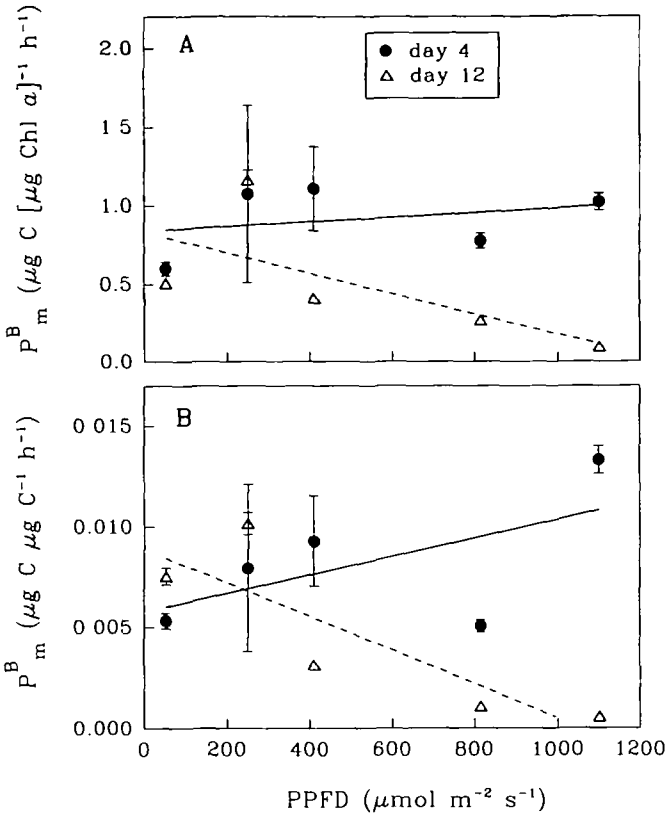

Fig. 5. Variations of P^B_m with growth light levels. (**A**) Normalized to chlorophyll *a*, (**B**) normalized to carbon. The lines are linear regressions.

Discussion

Growth

A variety of cell functions changed as the batch culture population aged. Faster growth due to higher light accelerated aging of *P. multiseries*, 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 μ–*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. multiseries* 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. multiseries* in the dark in the presence of a variety of organic substrates. This suggests that *P. multiseries* 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 μmol m^{-2} 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. multiseries* was 410–1100 μmol m^{-2} 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 α_g of 17×10^{-3} day^{-1} [μmol m^{-2} s^{-1}]$^{-1}$ for *Skeletonema costatum* grown at 20°C. In the present study on *P. multiseries*, α_g was 2.7 ($\pm$ 1.9) $\times 10^{-3}$ day^{-1} [μmol m^{-2} 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 α_g value increased to 18 ($\pm$16) $\times$ 10^{-3} day^{-1} [μmol m^{-2} s^{-1}]$^{-1}$ (broken line in Figure 1A), which was comparable to other diatoms.

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

Taxon	T°C	α_g	References
Diatoms			
P.multiseries	10	2.7–18	Present study
Skeletonema costatum	15	17.00	Langdon, 1987
Thalassiosira weissflogii	20	20.10	Laws and Bannister, 1980
	18	9.29	Falkowski *et al.*, 1985
Leptocylindrus danicus	10	11.78	Verity, 1982
Chaetoceros protuberans	19	7.07	Morel *et al.*, 1987
Phaeodactylum tricornutum	22–24	25.00	Geider *et al.*, 1985
Dinoflagellates			
Gonyaulax polyedra	23	2.63	Rivkin *et al.*, 1982a
Alexandrium tamarense	15	3.40	Langdon, 1987
Prorocentrum micans	18	0.35	Falkowski *et al.*, 1985
Gyrodinium cf. *aureolum*	20	17.33	Garcia and Purdie, 1992
Pyrocystis noctiluca	23	1.73	Rivkin *et al.*, 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 μmol m^{-2} s^{-1} were approaching or in the period of maximal growth, but the culture at 53 μmol m^{-2} s^{-1} was still in the lag phase and those at 810 and 1100 μmol m^{-2} s^{-1} had already passed the period of maximal growth (Figure 1). Similarly, as light increased, α^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 α^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 (μ) and photosynthesis (P^B_m) were positively correlated [equation (3)].

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

A positive intercept of μ 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

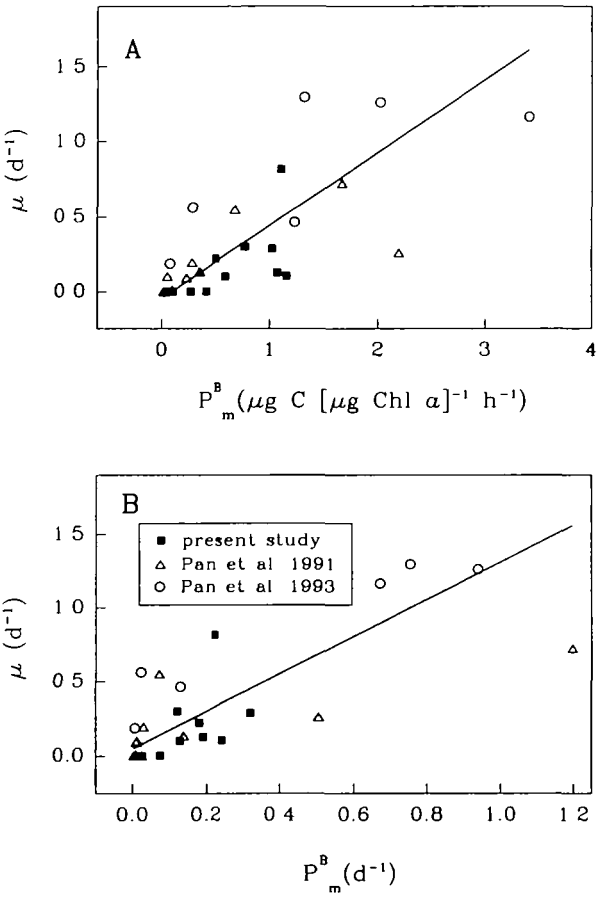

Fig. 6. Relationship between growth rate (μ) and photosynthetic rate (P^B_m). (**A**) P^B_m is normalized to chlorophyll *a* and (**B**) P^B_m is normalized to carbon. The lines are linear regressions.

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. multiseries* occurred, light levels were low (on a bright sunny day in November, the maximum light level was ~850 μmol m^{-2} s^{-1} on the surface and 200 μmol 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 α^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. multiseries* was able to grow at very low rates (≤0.1 day^{-1}) under nutrient stresses for a prolonged duration and produced toxin (Pan *et al.*, 1994). Similarly, cells of *P. multiseries* with less metabolic cost at low light will survive longer.

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

Acknowledgements

We are thankful to Drs W.G.Harrison, W.K.W.Li, B.B.Prézelin and the anonymous reviewer for constructive comments on the manuscript. This work has been funded by a research grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to K.H.M.

References

Bates,S.S., de Freitas,A.S.W., Milley,J.E., Pocklington,R., Quilliam,M.A., Smith,J.C. and Worms,J. (1991) Controls on domoic acid production by the diatom *Nitzschia pungens* f. *multiseries* in culture: nutrients and irradiance. *Can. J. Fish. Aquat. Sci.*, **48**, 1136–1144.

Boyer,G.L., Sullivan,J.J., Andersen,R.J., Harrison,P.J. and Taylor,F.J.R. (1985) Toxin production in three isolates of *Protogonyaulax* sp. In Anderson,D.M., White,A.W., Baden,D.G. (eds), *Toxic Dinoflagellates*. Elsevier, New York, pp. 281–286.

Falkowski,P.G. (1981) Light-shade adaptation and assimilation numbers. *J. Plankton Res.*, **2**, 203–216.

Falkowski,P.G., Dubinsky,Z. and Wyman,K. (1985) Growth-irradiance relationships in phytoplankton. *Limnol. Oceanogr.*, **30**, 311–321.

Flynn,K.J. and Syrett,P.J. (1986) Utilization of L-lysine and L-arginine by the diatom *Phaeodactylum tricornutum*. *Mar. Biol.*, **90**, 159–163.

Garcia,V.M.T. and Purdie,D.A. (1992) The influence of irradiance on growth, photosynthesis and respiration of *Gyrodinium* cf. *aureolum*. *J. Plankton Res.*, **14**, 1251–1265.

Geider,R.J., Osborne,B.A. and Raven,J.A. (1985) Light dependence of growth and photosynthesis in *Phaeodactylum tricornutum* (Bacillariophyceae). *J. Phycol.*, **21**, 609–619.

Kiefer,D.A. and Mitchell,B.G. (1983) A simple steady state description of phytoplankton growth rates based on absorption cross section and quantum efficiency. *Limnol. Oceanogr.*, **28**, 770–776.

Langdon,C. (1987) On the cause of the interspecific differences in the growth-irradiance relationship for phytoplankton. Part I. A comparative study of the growth-irradiance relationship of three marine phytoplankton species: *Skeletonema costatum*, *Olisthodiscus luteus* and *Gonyaulax tamarensis*. *J. Plankton Res.*, **9**, 459–482.

Laws,E.A. and Archie,J.W. (1981) Appropriate use of regression analysis in marine biology. *Mar. Biol.*, **65**, 13–16.

Laws,E. and Bannister,T.T. (1980) Nutrient- and light-limited growth of *Thalassiosira fluviatilis* in continuous culture, with implications for phytoplankton growth in the ocean. *Limnol. Oceanogr.*, **25**, 457–473.

Lewin,J. and Hellebust,J.A. (1978) Utilization of glutamate and glucose for heterotrophic growth by the marine pennate diatom *Nitzschia laevis*. *Mar. Biol.*, **47**, 1–7.

Morel,A., Lassara,L. and Gostan,J. (1987) Growth rate and quantum yield time response for a diatom to changing irradiance (energy and color). *Limnol. Oceanogr.*, **32**, 1066–1084.

Ogata,T., Ishimaru,T. and Kodama,M. (1987) Effect of water temperature and light intensity on growth rate and toxicity change in *Protogonyaulax tamarensis*. *Mar. Biol.*, **95**, 217–220.

Ogata,T., Kodama,M. and Ishimaru,T. (1989) Effect of water temperature and light intensity on growth rate and toxin production of toxic dinoflagellates. In Okaichi,T., Anderson,D.M. and Nemoto,T. (eds), *Red Tide: Biology, Environmental Sciences and Toxicology*. Elsevier, New York, pp. 423–426.

Paasche,E. (1968) Marine plankton algae grown with light-dark cycles. II. *Ditylum brightwelli* and *Nitzschia turgidula*. *Physiol. Plant.*, **21**, 66–77.

Pan,Y. (1994) Production of domoic acid, a neurotoxin, by the diatom *Pseudonitzschia pungens* f. *multiseries* Hasle under phosphate and silicate limitation. PhD Thesis Dalhousie University.

Pan,Y., Subba Rao,D.V. and Warnock,R.E. (1991) Photosynthesis and growth of *Nitzschia pungens* f. *multiseries* Hasle, a neurotoxin producing diatom. *J. Exp. Mar. Biol. Ecol.*, **154**, 77–96.

Pan,Y., Subba Rao,D.V. and Mann,K.H. (1996) Changes in domoic acid production and cellular chemical composition of the toxigenic diatom *Pseudo-nitzschia multiseries* under phosphate limitation. *J. Phycol.*, **32**, 371–381.

Pan,Y., Subba Rao,D.V., Mann,K.H., Li,W.K.W. and Warnock,R.E. (1993) Temperature dependence of

growth and carbon assimilation in *Nitzschia pungens* f. *multiseries*, a causative diatom of domoic acid poisoning. In Smayda,T.J. and Shimizu,Y. (eds), *Toxic Phytoplankton Blooms in the Sea*. Elsevier, Amsterdam, pp. 619–624.

Platt,T., Gallegos,C.L. and Harrison,W.G. (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *J. Mar. Res.*, **38**, 687–701.

Post,A.F., Dubinsky,Z., Wyman,K. and Falkowski,P.G. (1985) Physiological responses of a marine planktonic diatom to transitions in growth irradiance. *Mar. Ecol. Prog. Ser.*, **25**, 141–149.

Prézelin,B.B. (1982) Effects of light intensity on aging of the Dinoflagellate *Gonyaulax polyedra*. *Mar. Biol.*, **69**, 129–135.

Prézelin,B.B. and Matlick,H.A. (1983) Nutrient-dependent low-light adaptation in the dinoflagellate *Gonyaulax polyedra*. *Mar. Biol.*, **74**, 141–150.

Proctor,N.H., Chan,S.L. and Trevor,A.J. (1975) Production of saxitoxin by cultures of *Gonyaulax catenella*. *Toxicon*, **13**, 1–9.

Rivkin,R.B., Voytek,M.A. and Seliger,H.H. (1982a) Phytoplankton division rates in light-limited environments: two adaptations. *Science*, **215**, 1123–1125.

Rivkin,R.B., Seliger,H.H., Swift,E. and Biggley,W.H. (1982b) Light-shade adaptation by the oceanic dinoflagellates *Pyrocystis noctiluca* and *P. fusiformis*. *Mar. Biol.*, **68**, 181–191.

Robinson,D.H., Arrigo,K.R., Iturriaga,R. and Sullivan,C.W. (1995) Microalgal light-harvesting in extreme low-light environments in Mcmurdo Sound, Antarctica. *J. Phycol.*, **31**, 508–520.

Shilo,M. (1971) Toxins of crysophyceae. In Kadis,S., Ciegler,A. and Ajl,S.J. (eds), *Microbial Toxins 7. Algal and Fungal Toxins*. Academic Press, London. pp. 67–103.

Smith,G.J., Zimmerman,R.C. and Alberte,R.S. (1992) Molecular and physiological responses of diatoms to variable levels of irradiance and nitrogen availability: Growth of *Skeletonema costatum* in simulated upwelling conditions. *Limnol. Oceanogr.*, **37**, 989–1007.

Strickland,J.D.H. and Parsons,T.R. (1972) *A Practical Handbook of Seawater Analysis*. *Fish. Res. Board Can. Bull.*, **167**.

Subba Rao,D.V., Quilliam,M.A. and Pocklington,R. (1988) Domoic acid – a neurotoxic amino acid produced by the marine diatom *Nitzschia pungens* in culture. *Can. J. Fish. Aquat. Sci.*, **45**, 2076–2079.

Verity,P.G. (1982) Effects of temperature, irradiance, and daylength on the marine diatom *Leptocylindrus danicus* Cleve. IV. Growth. *J. Exp. Mar. Biol. Ecol.*, **60**, 209–222.

Zwietering,M.H., Jongenburger,I., Rombouts,F.M. and van't Riet,K. (1990) Modelling of the bacterial growth curve. *Appl. Environ. Microbiol.*, **56**, 1875–1881.

Received on November 16, 1995; accepted on March 19, 1996