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Effects of silicate limitation on production of domoic acid, a neurotoxin, by the diatom *Pseudo-nitzschia multiseries*. I. Batch culture studies

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ABSTRACT: Domoic acid (DA) production by *Pseudo-nitzschia multiseries* (Hasle) was studied at various silicate concentrations and under silicate perturbation. Both slowly dividing and non-dividing populations produced DA, and the production rates were inversely correlated with the ambient silicate concentrations. Production of DA was significantly enhanced when overall cell metabolism (i.e. growth rate) declined as a result of silicate stress. Following silicate starvation, cultures supplemented with silicate registered uptake, but suspended DA production. Results suggest that luxury uptake of Si by *P. multiseries* may happen only in physiologically active populations, i.e. the exponential phase, but not in the stationary phase. There were 2 stages of DA production. The first stage corresponded to a decline in growth caused by moderately low levels of remaining silicate in the medium, while the second stage was caused by severe silicate limitation. The production rate during the second stage (13.67 to 30.20 fg DA $cell^{-1}$ d^{-1}) was about an order of magnitude higher than during the first stage (0.97 to 4.98 fg DA $cell^{-1}$ d^{-1}). Increases and decreases in cellular DA content corresponded to decreases and increases in growth rates.

KEY WORDS: Domoic acid · *Pseudo-nitzschia multiseries* · Silicate limitation · Batch culture

INTRODUCTION

Pseudo-nitzschia multiseries, formerly known as *Pseudonitzschia pungens* f. *multiseries* (Hasle, 1995), produces domoic acid (DA), which has caused amnesic shellfish poisoning (ASP) in Atlantic Canada (Addison & Stewart 1989). The ASP problem has now been observed on both the Atlantic and Pacific coasts of North America (Fritz et al. 1992, Garrison et al. 1992). Studies on DA production have shown it to occur only during the stationary phase, coinciding with low levels of silicate in the medium (Subba Rao et al. 1990, Bates et al. 1991, Pan et al. 1991). These observations suggest a possible relationship between silicate limitation and DA production. However, direct linkage between DA production and silicon depletion is unlikely, as silicate is neither a component of DA nor apparently involved in its synthesis.

The magnitude of a diatom bloom is often directly related to the availability of silicon in sea water. Silicon regulates the growth and frustule formation of diatoms. Decreases in silicate concentrations to low or undetectable levels in marine and freshwater habitats during diatom blooms have been well documented (Paasche 1973a, Sommer & Stabel 1983, Egge & Aksnes 1992, Harrison et al. 1993).

In cultures, silicate concentrations in the medium may regulate the yield of diatom cells (Taguchi et al. 1987). Cessation of cell division, which may be due to cessation of DNA synthesis (Darley & Volcani 1969, Sullivan & Volcani 1973), was found to accompany depletion of silicon in the culture medium (Lewin 1955, Lewin & Chen 1968, Vaulot et al. 1987, Brzezinski et al. 1990).

Bates et al. (1991) first showed a connection between silicate limitation and DA production. Two of their 5 treatments with low silicate concentration produced about 35% more DA than those with normal levels of

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silicate. These authors suggested that the higher DA values were the result of an extended stationary phase. They did not report the rate of DA production, nor its temporal variation. This study attempts to quantify responses both to varying levels of silicate depletion and to sharply increased silicate concentration.

MATERIALS AND METHODS

Pseudo-nitzschia multiseries (non-axenic clone NPBIO) was cultured in 1000 ml polycarbonate Erlenmeyer flasks at 15 (±1)°C under 410 (±80) µmol m^{-2} s^{-1} continuous cool-white fluorescent light. The stock culture grown in medium FE (Subba Rao et al. 1988b) was transferred to medium F (Guillard & Ryther 1962) 15 d before the experiment started. This stock culture was sub-cultured twice in medium F before the experiment.

Our earlier study in medium FE (Pan et al. 1991) showed that *Pseudo-nitzschia multiseries* was limited by dissolved inorganic silicate (DISi) when it entered the stationary phase. However, medium FE contains soil extract, which has various concentrations of Si depending on the origin of the soil. So, medium F was used in this study.

There were 4 treatments, designated A, B, C and D, each performed in triplicate. Five-day-old culture (110 ml) was added to 1000 ml polycarbonate flasks containing 760 ml of medium F. DISi concentration was adjusted for these 4 treatments. Unmodified medium F was used in the control, designated as Treatment A. After inoculation, the measured DISi in the control was 95.3 µM. Treatments B, C and D had initial DISi of 190.5, 60.9 and 60.9 µM respectively. Treatment C was spiked with additional DISi (64 µM) during early stationary phase (Day 14), whereas Treatment D was spiked with additional DISi (122 µM) during late stationary phase (Day 25).

Culture aliquots (10 ml) were collected for cell concentration determinations. Duplicate aliquots (20 ml in the early exponential phase or 10 ml in later phases) were collected for chlorophyll *a* (chl *a*) measurement. Growth was described by the Gompertz model. Detailed methods for measurements of cell concentration, chl *a* and growth rates were the same as those described in Pan et al. (1991).

Duplicate aliquots (10 ml) were collected for particulate phosphorus and silicon (PSi). The samples were filtered through Nuclepore filters (pore size 1.0 µm, 25 mm diameter). Cells on the filter were rinsed twice with isotonic saline (0.5 to 1.0 ml) immediately upon completion of filtration. The samples were stored frozen (–20°C) in plastic petri dishes pending analysis. Samples were oxidized using alkaline persulphate by autoclaving for 60 min (Koroleff 1983a, b), then allowed to stand overnight (~12 h) at room temperature. Standard solutions of Na_2SiO_3 and NaH_2PO_4 were treated simultaneously for calibration. The silicate and phosphate concentrations were measured using a Phillips Model PU8625 UV/VIS spectrophotometer.

For DA determination, duplicate samples (20 ml) were filtered onto Nuclepore filters (25 mm, pore size 1.0 µm). The filters were stored frozen in plastic petri dishes until analyzed. Filtrate was also saved for measurement of DA in the medium. DA was determined by the 9-fluorenyl-methoxycarboxyl (FMOC) method using high performance liquid chromatography (HPLC) (Pocklington et al. 1990).

RESULTS

During the culture periods, phosphate was never below 25.4 µM. The dissolved nitrogen in our medium would never have been below 1000 µM, as suggested by our earlier data on cellular nitrogen in the post mid-exponential phase culture of the same species in the nitrogen rich medium (<30 pg N $cell^{-1}$; Pan et al. 1991) and the initial concentration of nitrate in the fresh F medium (1765 µM). All other nutrients were also in excess. Thus, the dynamics of our culture populations were controlled by silicate concentration.

Cell concentrations increased exponentially until the onset of the stationary phase, 9 to 12 d following inoculation, depending on DISi regime (Table 1; Figs. 1 to 4). The onset of the stationary phase in all DISi regimes coincided with a low level of DISi (<21.2 µM) in the spent medium. The initial DISi concentration in the medium had no significant effect on growth rate except for in Treatment B ([Si] = 190.5 µM), which had an increase in growth rate of approximately 20%. Cell yields (× 10^7 l^{-1}) in the stationary phase were 14.0, 18.7, 8.7 and 9.3 corresponding to 95.3, 190.5, 60.9 and 60.9 µM initial DISi (Table 1). Silicon and DA concentrations in cells were also dependent on the initial DISi.

Treatment A (control, [Si] = 95.3 µM)

Two stages of DA production could be recognized. The first stage occurred during the mid-late exponential phase and corresponded to a decline in growth rate of the culture, resulting from a decrease in DISi from 70.9 µM on Day 3, to 30.1 µM on Day 6 (Fig. 1). DA production (1.16 to 4.98 fg DA $cell^{-1}$ d^{-1}) in the first stage was low compared to the high DA production rates (13.67 to 28.27 fg DA $cell^{-1}$ d^{-1}) in the second stage. Initiation of the second stage coincided with the lowest DISi level (3.2 µM) on Day 30. This low DISi was insuf-

Table 1. *Pseudo-nitzschia multiseries*. Maximum values of growth rates (μ_{max}), cell concentration, cellular chlorophyll *a* (chl *a*), particulate silicon (PSi) and domoic acid (DA) for the 4 dissolved inorganic silicate (DISi) treatments. Values in parentheses give the ages (day) of the culture when these measurements were made. Exp.. Exponential phase; Stat.: Stationary phase

Treatment	DISi supplied (µM)	μ_{max} (d^{-1})	Cell conc. (10^7 cells l^{-1})	Chl *a* (pg cell ^{1})	PSi (pg $cell^{-1}$) Exp.	Stat.	Domoic acid (fg $cell^{-1}$)
A (control)	95.3	0.20	14.0 (35)	0.82 (6)	65.7 (1)	31.2 (15)	431.8 (50)
B	190.5	0.25	18.7 (35)	0.66 (6)	214.4 (1)	63.5 (15)	177.2 (41)
C	60.9	0.21	8.7 (14)	0.78 (6)	33.3 (6)		
	+64		14.1 (30)			30.0 (50)	296.6 (50)
D	60.9	0.21	9.3 (15)	0.78 (9)	35.3 (9)	27.7 (15)	
	+122		15.0 (30)			28.4 (50)	143.8 (35)

ficient to support any further frustule formation, but the toxin levels increased to a maximum of 431.76 fg DA $cell^{-1}$. DA concentration probably increased further as the production rate remained at approximately 25 fg DA $cell^{-1}$ d^{-1} between Days 41 and 50. The initiation of DA production in each stage did not coincide with the onset of the stationary phase, which was on Day 12 when DISi was 19.2 (±1.94) µM, but it did coincide with a drastic decrease in DISi before Day 6 and on Day 30. Depletion of DISi enhanced DA production markedly during the second stage.

Treatment B (high-Si medium, [Si] = 190.5 µM)

That DA production proceeded in 2 stages was also evident in this treatment. Although DISi was lowest (3.46 µM) on Day 12, the PSi content per cell remained similar to that at Day 9 (Fig. 2). The initiation of the first stage of DA production coincided with the onset of stationary phase on Day 9 and a low level of DISi, but the production rate was very low (0.97 to 1.74 fg DA $cell^{-1}$ d^{-1}). DA production remained low until Days 25 to 30 when cellular silicon decreased from 47.8 pg $cell^{-1}$ on Day 25 to 31.8 pg $cell^{-1}$ on Day 30 (Fig. 2D, E). The concentration of DA reached a maximum of 177.0 fg DA $cell^{-1}$ on Day 41 and then decreased to 148.2 fg DA $cell^{-1}$, coinciding with an increase in cellular silicon from 28.5 pg Si $cell^{-1}$ on Day 41 to 39.2 pg Si $cell^{-1}$ on Day 50.

Treatments C and D (low-Si medium, [Si] = 60.9 µM)

At low initial DISi, cultures were characterized by lower cell concentrations and lower PSi (Table 1). Cellular silicon was 14 to 35 pg Si $cell^{-1}$, which was ⅔ that of Treatment A. DISi slightly increased during the first day, but decreased later (Figs. 3 & 4). Initiation of DA production (1.16 to 2.49 fg DA $cell^{-1}$ d^{-1}) was 3 d earlier than in the control and followed a drastic decrease of

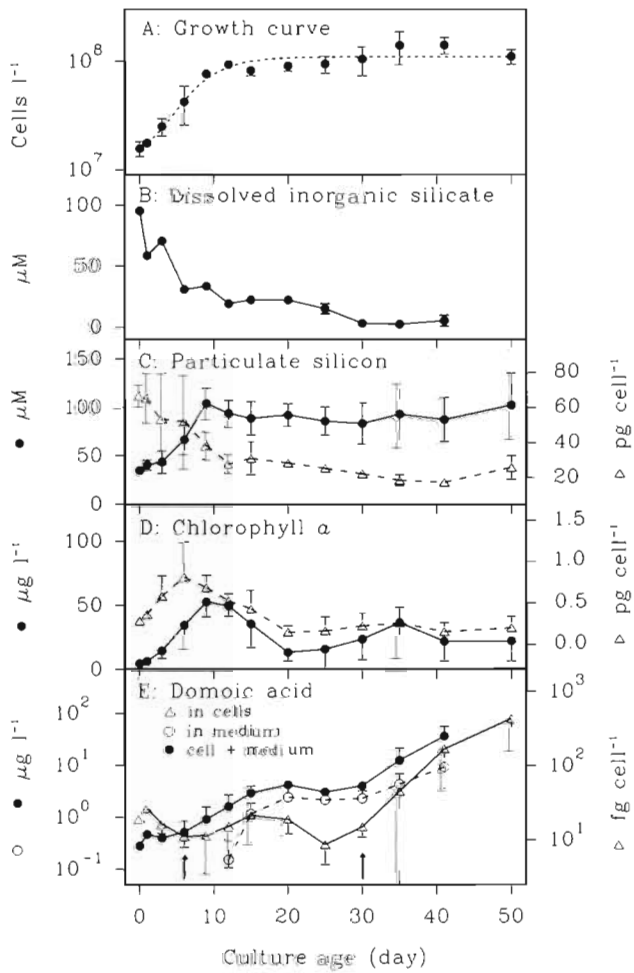

Fig. 1 *Pseudo-nitzschia multiseries*. Treatment A. Variations in cell concentration, dissolved inorganic silicate (DISi), particulate silicon (PSi), chlorophyll *a* (chl *a*), and domoic acid (DA) during the growth cycle. Error bar = 1 SD. Absence of error bar means the SD is smaller than the symbol. The curve in A is fitted by Gompertz model (see Pan et al. 1991). Arrows in E indicate the initiation of first and second stage of DA production

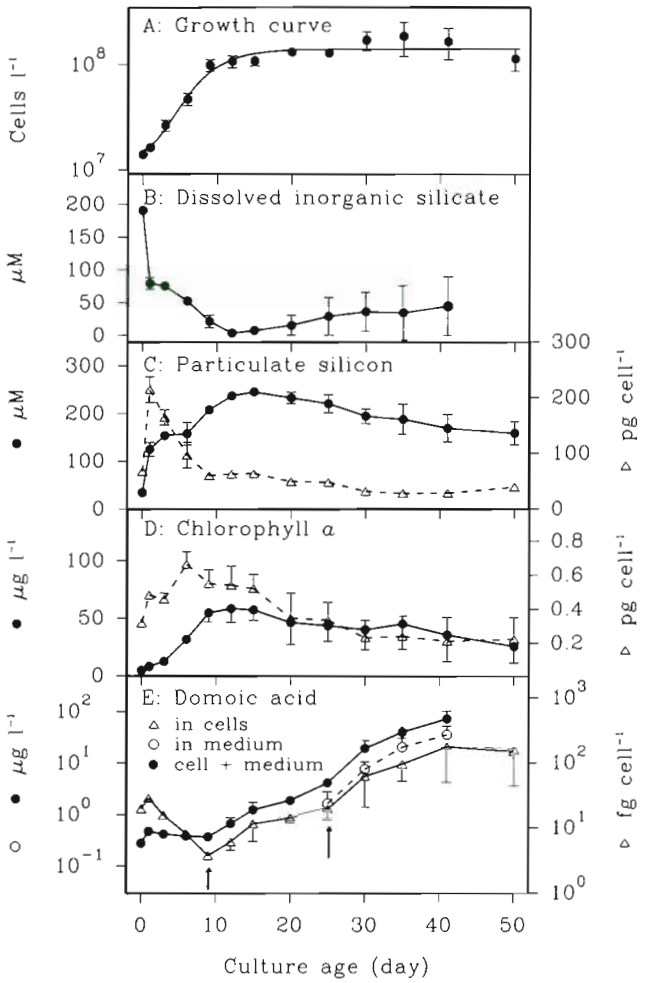

Fig. 2. *Pseudo-nitzschia multiseries*. Treatment B. Variations in cell concentration, DISi, PSi, chl *a*, and DA during the growth cycle. Growth curve, arrows in E, and error bars as in Fig. 1

DISi (from 66.6 µM on Day 1 to 22.7 µM on Day 3). The second stage of DA production immediately succeeded the first, reflecting the onset of the stationary phase as a result of further decrease in DISi. The production rates in the second stage were 5.14 to 24.86 fg DA cell^{-1} d^{-1}.

Silicate perturbation

After cultures were spiked with DISi (64 µM, Day 14, Treatment C; Fig. 3) in the early stationary phase, cell concentration and PSi increased to levels similar to those in the control (Table 1). Maximum chl *a* concentration was less than that in the control at the same period. Importantly, soon after more DISi was added on Day 14, DISi and PSi increased, but the second stage of DA production was interrupted. Cellular DA concentration dropped from 52.53 to 32.16 fg DA cell^{-1} and then increased when a further silicate limitation occurred on Day 35. Following that, cells continued to produce DA at rates of 4.67 to 30.20 fg DA cell^{-1} d^{-1} until a peak of 296.6 fg DA cell^{-1} was reached at the end of the experiment.

In Treatment D (Fig. 4), after addition of DISi (122 µM) on Day 25, the cell concentration increased from 10.5 to 15.0 × 10^7 cells l^{-1}, chl *a* from 24.2 to 66.5 µg l^{-1}, and PSi from 83.5 to 97.6 µM. However, DA production was suspended for 5 d but resumed afterwards.

In Treatments C and D, cells started accumulating DA only when the growth rate declined (Fig. 5) as a result of partial or total depletion of DISi (Figs. 3B & 4B). When DISi was elevated through perturbation in the early and late stationary phases it caused cultures

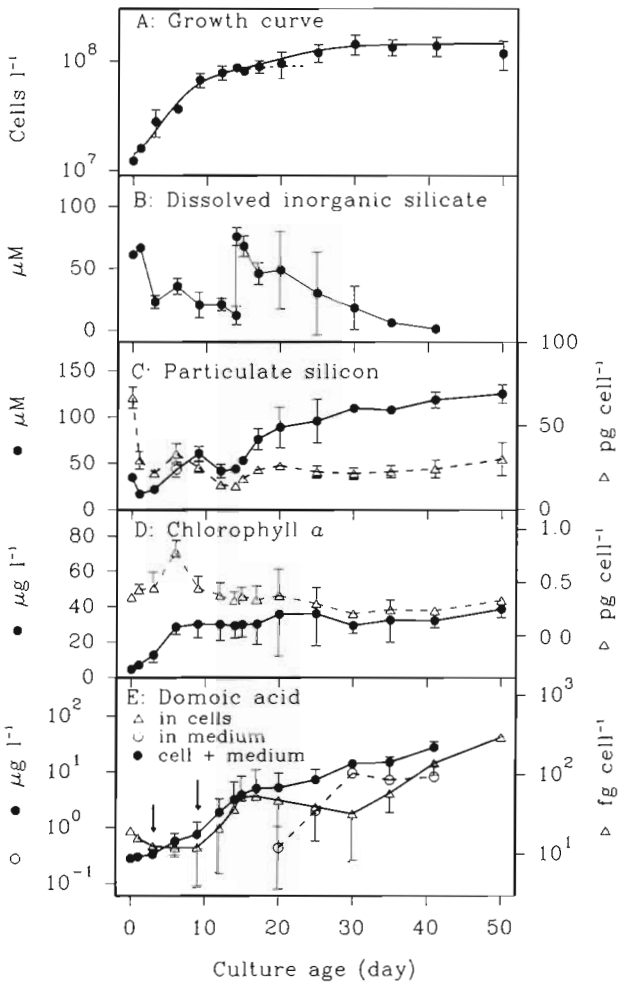

Fig. 3. *Pseudo-nitzschia multiseries*. Treatment C (64 µm silicate added on Day 14). Variations in cell concentration, DISi, PSi, chl *a*, and DA during the growth cycle. Growth curve, arrows in E, and error bars as in Fig. 1

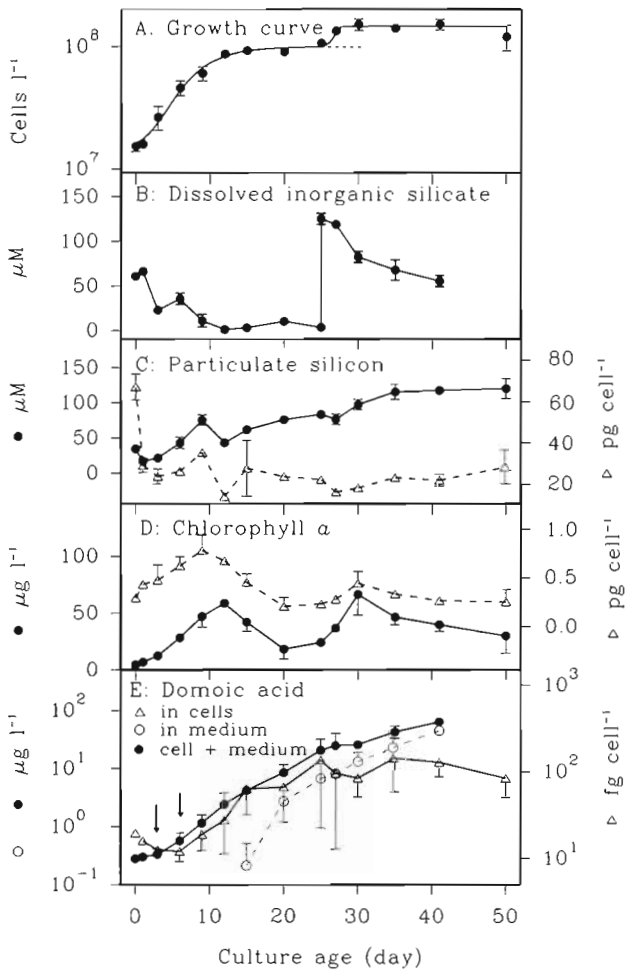

Fig. 4. *Pseudo-nitzschia multiseries*. Treatment D (122 μm silicate added on Day 25). Variations in cell concentration, DISi, PSi, chl *a*, and DA during the growth cycle. Growth curve, arrows in E, and error bars in Fig. 1

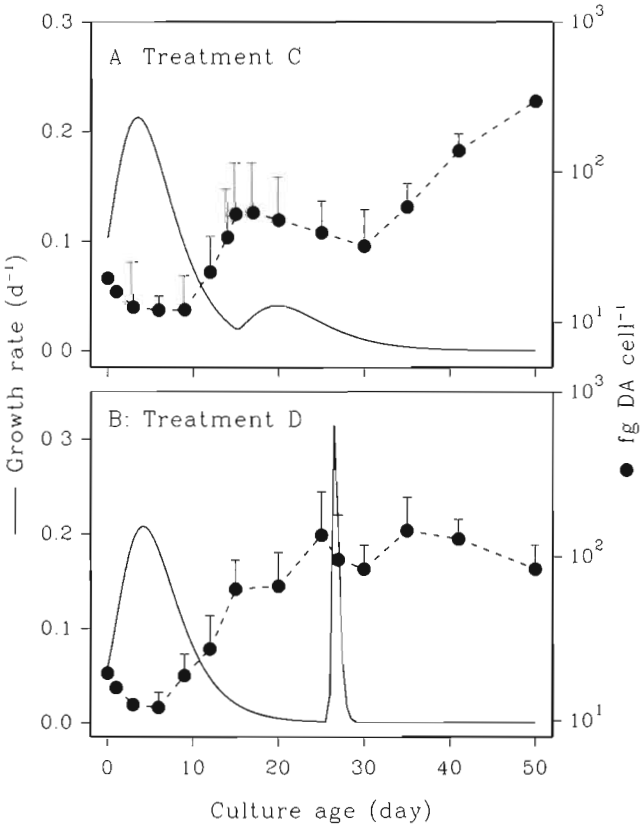

Fig. 5. *Pseudo-nitzschia multiseries*. Relationship between cellular DA and growth rate during DISi perturbation experiments. Solid curves are the slopes of curves in Figs. 3A (A) & 4A (B) respectively. Error bar = 1 SD. Absence of error bar means it is smaller than the symbol

to resume growth; DA production stopped and did not resume until the growth rate decreased.

In all 4 treatments, the first stage of DA production was characterized by an increase of total DA concentration in the culture (cells + medium), with very little increase in cellular DA and low production. In the second stage, however, DA increased both in the culture and in cells, population growth was arrested by depletion of DISi and production was high (Table 2).

DA was not found in the medium until the culture populations entered the stationary phase, after which it gradually increased (Figs. 1E, 2E, 3E & 4E). The proportion of DA in the culture medium accounted for as much as 70% of the total in the later stationary phase (Day 41, Treatment D).

Table 2. *Pseudo-nitzschia multiseries*. Characteristics of the 2 stages in DA production

Characteristic	First stage	Second stage
Population growth	Declined or stopped	Stopped
Silicate in medium	Moderately low	Depleted
DA in culture	Increased	Increased
DA in cells	No or very little increase	Increased
DA production (fg cell^{-1} d^{-1})	Low (0.97–4.98)	High (13.67–30.20)

DISCUSSION

Production of DA in relation to silicate limitation

The 2-stage DA production phenomenon was obvious in Treatments A (control) and B ([Si] = 190.5 μM) but was not clear in Treatments C and D ([Si] = 60.9 μM). At higher DISi (Treatments A and B), the depletion of DISi did not coincide with the onset of the stationary phase. After initiation of the first produc-

tion stage, which occurred in the later exponential phase because of a minor physiological stress, the cells probably adjusted themselves to the new low silicate habitat with a new uptake kinetic strategy for further growth using the residual silicate. When silicate was depleted in the medium, cells were not able to adjust to permit further growth. The cells may have been arrested at a certain phase(s) in the cell cycle, as has been found for *Thalassiosira weissflogii* (Brzezinski 1992). This may be a cue for DA synthesis. At lower DISi (Treatments C and D), on the other hand, cells were not able to manage any further growth after they entered the stationary phase due to depletion of silicate. Therefore, the second production stage immediately followed the first. This gave an image of a continuous linear increase of DA production rate. DA production was vigorous when cells were not able to grow further due to depletion of DISi and the intracellular silicate pool.

A noteworthy feature in the experiments was the temporal shift in the initiation of first stage DA production. The production started earlier as a result of a decrease in the initial DISi. When the initial DISi was highest (190.5 µM, Treatment B), first stage DA production started on Day 9 at the onset of the stationary phase, when the population growth had almost stopped (Fig. 2). When the initial DISi was 95.3 µM (control), first stage DA production was initiated on Day 6 in the late exponential phase, when the population growth was declining (Fig. 1). At lower initial DISi (60.9 µM), first stage DA production was initiated on Day 3 at the mid-exponential phase, when growth peaked or had just started to decline (Fig. 5). This suggests that DA production is not necessarily associated with cessation of cell division, but triggered by physiological stress such as silicate limitation. There was a negative significant correlation ($r = 0.84$, $p < 0.05$) between the maximum cellular DA and the supply of silicate in the culture medium (Fig. 6). A similar relationship was reported by Bates et al. (1991).

Substantial amounts of extracellular DA were found in the culture medium only in stationary phase cultures (Fig. 1E, 2E, 3E & 4E), and not in the exponential phase. This result is similar to that of Bates et al. (1991) and suggests that when the cells are physiologically active, DA may be restrained in cells. On the other hand, in the physiologically impaired cells, such as those in the late stationary phase, DA tends to be released into the culture medium. This may be due to damage in the structure of the cell wall and the cell membrane (Pan et al. 1996 — this issue).

Subba Rao et al. (1990) and Bates et al. (1991) concluded that DA production occurs only after cell division has stopped. The initial DISi in medium FE used by Subba Rao et al. (1990) was higher than that in medium F because medium FE contains soil extract. The DISi level of FE in their study may have been comparable to that of Treatment B in the present study, i.e. initiation of the first DA production stage coincided with onset of the stationary phase. On the other hand, the initial DISi in medium F/2 used by Bates et al. (1991) was comparable to that in Treatments C and D in the present study. In their silicate experiment, the silicate levels ranged from 5 to 105 µM; DA production before the onset of the stationary phase was not reported. The production during the first stage in their cultures might have been too low to be noticed.

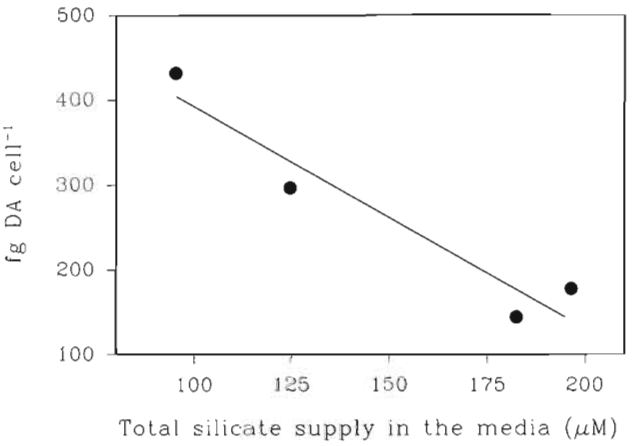

Fig. 6. *Pseudo-nitzschia multiseries*. Relationship of maximum cellular DA concentrations with silicate supply. Line is a linear regression

Addition of silicate to silicate-depleted cultures facilitated further growth and interruption of DA production (Figs. 3 & 4). But DA production resumed when population growth declined as a result of further silicate stress (Fig. 5). This suggested that (1) other nutrients were not limiting and (2) the initiation of DA production was attributable to silicate stress.

Role of silicon in cell metabolism

Cell yields of *Pseudo-nitzschia multiseries* were proportional to the supply of silicate, but the growth rates were not. Cultures reached the stationary phase when low levels of silicate were present in the medium. Addition of silicate to silicate-limited cultures facilitated further growth. These results were consistent with the work of Taguchi et al. (1987) on *Chaetoceros gracilis*, *Hantzschia* sp. and *Cyclotella* sp. Cells continued to accumulate silicon even after cultures entered the stationary phase, which supported further growth of the population (Figs. 1 & 2); this was consistent with observations made on *Navicula pelliculosa* (Lewin 1957).

Diatoms require silicon not only for cell frustule formation, but also for other metabolic processes, such as DNA replication. Addition of silicate to silicon-starved *Cylindrotheca fusiformis* cells caused initiation of DNA synthesis and an increased synthesis of nuclear DNA polymerases (Okita & Volcani 1978). Therefore, silicon availability affects the cell cycle progression in diatoms. Brzezinski et al. (1990) showed that silicon deprivation halted progression of the cell cycle by arresting the cells in G_1, G_2 and M phases in 6 of the 7 diatom species they examined. Silicon starvation of diatoms leads to an accumulation of cells at the G_1/S boundary and in G_2 and/or M phase (Vaulot et al. 1987, Brzezinski et al. 1990).

The effect of silicate availability on the cell cycle and on DNA synthesis suggests that silicate stress may indirectly regulate production of toxin by restraining regular cell metabolism. The relationship between DA production and the cell cycle may parallel the observed relationship between saxitoxin production by *Alexandrium* spp. and its cell cycle. Production of saxitoxins by *Alexandrium tamarense* occurred during the first half of the S phase but then stopped for more than 10 h as cells went through mitosis and divided (Anderson 1990). Anderson (1990) concluded that toxin synthesis was not continuous throughout the cell cycle, but was temporally linked to a pulse of DNA synthesis.

Uptake of silicon by diatoms seems to be confined to the G_2 phase of the cell cycle (Brzezinski 1992). Immediately after addition of silicate to the medium, the duration of G_2 shortens and the cell division rate increases. In contrast, soon after the depletion of Si in the medium or when Si-replete cells are suspended in low Si medium, G_2 lengthens and the generation time increases. This may be related to the pause in DA synthesis, which is parallel to the pause in saxitoxin synthesis by *Alexandrium* spp. in certain phase(s) of the cell cycle. In the first stage of DA production when growth rate declines, the generation time increases, and the rate of DA production also increases. This strongly suggests that DA synthesis may be linked to certain cell cycle phase(s), such as G_2, or adjacent periods of G_2. This merits further investigation.

The data on DISi and cellular silicon demonstrated uncoupling between uptake of silicate by *Pseudo-nitzschia multiseries* and its growth when the ambient DISi was high. In Treatment B (Fig. 2), for example, when initial DISi was high at 190.5 µM, 59% was taken up by the diatom population on the first day, although there was very little increase in cell concentration (16%). Cellular silicon increased from 67.1 to 214.4 pg Si cell^{-1} However, addition of a substantial quantity of DISi in the stationary phase populations did not result in a similar magnitude of cellular Si increase. Also, early stationary phase cells responded to the addition faster (1 d) than did those in the later phase (>2 d) and the ability to incorporate Si was greater (2.7 to 4.4 pg Si cell^{-1} d^{-1} compared with 0.7 to 1.0 pg Si cell^{-1} d^{-1}) regardless of the quantity added. This suggested that luxury uptake of Si by *P. multiseries* can only happen at the time when cells are physiologically active.

There have been different views expressed on the existence of luxury uptake of silicate by diatoms. For example, Sullivan & Volcani (1981) suggested the existence of an intracellular silicate pool, a surplus of silicate needed for cell metabolism. In contrast, Brzezinski (1992) demonstrated that the increased uptake rate of silicate by diatoms resulted from a shortening of the G_2 phase of the cell cycle. This also caused temporal imbalance between population growth and silicate uptake. We believe that there may be some differences among species; although Brzezinski (1992) observed shortening of the G_2 phase, he did not reject the existence of such an Si pool. We found a little reactive silicate in cells of *Pseudo-nitzschia multiseries* (unpubl. data), especially when cells were newly transferred to a fresh Si-rich medium. Nevertheless, this imbalance between Si uptake and population growth is more likely to happen during the exponential phase when cells are healthy.

The general pattern of cellular silicon was that cells in the lag and early exponential phases had higher silicon content than did cells in the later phases. However, the silicon content in stationary phase cells of Treatment B (28.2 to 63.5 pg Si cell^{-1}) was higher than that of cells in the exponential phase of Treatments C and D (21.8 to 35.3 pg Si cell^{-1}). At these silicon levels, the former stopped dividing while the latter were ready to divide. Addition of DISi during the stationary phase of Treatments C and D did not raise cellular silicon to a level comparable to that found in the cells of Treatment B. Different magnitudes of perturbation did not result in different levels of cellular silicon (Table 1). This suggests that the cellular silicon level was primarily determined by the initial DISi in the fresh medium. Immediately after the seeding of a population in a fresh medium, the cells probably adjust their requirement for silicate according the available resources. Perturbation had less effect on the intracellular silicon level but promoted growth of the population.

Cellular silicon varied from 14 to 214.4 pg Si cell^{-1}, which is not common among other diatoms (Table 3). The ratios of maximum to minimum cellular silicon (Q_{max}:Q_{min}) were hardly over 5 for most other diatoms, but were 15 for *Pseudo-nitzschia multiseries*. Such a high ratio for *P. multiseries* probably suggests that this diatom has the ability to respond to a wide range of silicate levels and may explain its ubiquitous distribution

Table 3. Variation in the cellular silicon content in selected diatom species. Q_{max}: maximum cellular silicon; Q_{min}: minimum cellular silicon

Taxa	Cell size (µm)		Q_{max} (pg Si cell^{-1})	Q_{min}	Q_{max}:Q_{min}	Reference
Pennate	Width	Length				
Pseudo-nitzschia multiseries	5–10	50–70	214	14	15.3	Present study
Nitzschia palea			62	14	4.4	Jørgensen (1955)
Navicula pelliculosa			3.3	0.36	9.2	Lewin (1957)
Centric	Diameter	Length				
Skeletonema costatum	4–6		7	3.8	1.8	Paasche (1973b)
	4–5	8–13	2.16	0.84	2.6	Harrison et al. (1976, 1977)
Thalassiosira pseudonana	3–6		1.81	0.6	3.0	Paasche (1973a, b)
Thalassiosira decipiens	17–28		330	150	2.2	Paasche (1973b)
Thalassiosira nana			430	89	4.8	Jørgensen (1955)
Thalassiosira gravida	7–9	14–24	56	30.24	1.9	Harrison et al. (1977)
Licmophora sp.	14–40		210	80	2.6	Paasche (1973b)
Ditylum brightwellii	13–52		900	200	4.5	Paasche (1973b)
Coscinodiscus granii	95–190		6412	728	8.8	Taylor (1985)
Chaetoceros debilis	16–20	6–9	8.46	3.11	2.7	Harrison et al. (1977)
Chaetoceros affinis	7–27		42	33	1.3	Paasche (1980)
Cerataulina pelagica	36–56	70–120	97	88	1.1	Paasche (1980)
Rhizosolenia fragilissima	12–20	42–67	51	28	1.8	Paasche (1980)

(Pan 1994). However, caution should be taken when interpreting these data. The highest value of 214.4 pg Si cell^{-1} seems to have resulted from luxury uptake by physiologically active cells, when ambient silicate concentration was high. For the physiologically inactive cells, the luxury uptake may be absent, or the cells may need a recovery period.

Concluding remarks

DA was produced when population growth of *Pseudo-nitzschia multiseries* declined. The production rate reached its maximum when the population was severely stressed by depletion of silicon. Interestingly, in Cardigan Bay (PEI, Canada) where the first ASP episode occurred, the peak of DA production was 10 d after the peak of a *P. multiseries* bloom (Smith et al. 1990, Silvert & Subba Rao 1992), consistent with the present study. At the peak of the bloom, DISi in the sea water was depleted (Subba Rao et al. 1988a), which probably stressed the bloom population and in turn enhanced DA production as demonstrated in the present study. Based on our laboratory results, an enrichment of DISi due to land run-off or tidal mixing could have resulted in a resumption of population growth and DA production would have been suspended until further silicate depletion occurred. This probably explains the persistence of the toxic bloom for 3 mo in the fall and winter of 1987.

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