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The interactive effects of temperature and silicon limitation on the psychrophilic ice diatom *Pseudonitzschia seriata*

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Abstract *Pseudonitzschia seriata*, isolated from sea ice in the Canadian Arctic, was grown in silicon-limited batch and semi-continuous culture to determine the effects of temperature on its nutrient utilization. Resource-saturated growth rate (μ_{\max}) increased significantly with temperature from -1.5 to 6°C with a Q_{10} of 1.63. The efficiency of the algae in using exogenous silicic acid for growth had no significant monotonic relationship with temperature but was significantly ($P < 0.05$) greater in cultures at $> 0^{\circ}\text{C}$ than in those at lower temperatures. Silicic acid uptake kinetics did not differ between high and low temperatures. Silicon cell quotas were significantly higher at temperatures $\leq 0^{\circ}\text{C}$, indicating greater silicon demand at low temperatures. *P. seriata* should not suffer silicon limitation in its natural ice habitat based on the observed kinetics, but its behaviour provides some support for the suggestion that temperatures $\leq 0^{\circ}\text{C}$ are associated with diminished efficiency of nutrient utilization by cold ocean microalgae.

Introduction

Environmental temperature sets an upper limit to the growth and productivity of microalgae (Eppley 1972). Interspecific comparisons show that optimal resource-saturated growth rates increase non-linearly with temperature, with a Q_{10} of 1.8–2.0 (e.g. Eppley 1972; Ahlgren 1987; Raven and Geider 1988). A similar variation of resource-saturated growth rates with temperature is often seen within species over a substantial part of their natural temperature range (Ahlgren 1987). The

effects of temperature when growth is resource limited are less consistent. The relatively few experimental studies of temperature-nutrient interactions show a variable influence of temperature on nutrient-limited growth (e.g. Rhee and Gotham 1981; Mechling and Kilham 1982; VanDonk and Kilham 1990). Ahlgren (1987) concluded that the half-saturation concentration for growth, despite its variation with temperature in some instances, was best regarded as generally invariant with temperature.

The influence of temperature on nutrient utilization by microalgae is of particular interest in polar oceans, where some observations suggest that very low temperatures may lead to inefficient utilization of major nutrients by microalgae. Unusually high half-saturation concentrations for uptake and growth of major nutrients have been reported for Southern Ocean phytoplankton (Jacques 1983; Sommer 1986), while Arctic ice algae have shown evidence of nutrient limitation at concentrations that would normally be thought saturating to growth (Maestrini et al. 1986; Gosselin et al. 1990). Related physiological processes, such as photosynthesis and respiration, are often highly temperature sensitive ($Q_{10} > 4$) in field studies conducted over small temperature intervals near 0°C (Tilzer et al. 1986; Tilzer and Dubinsky 1987; Kottmeier and Sullivan 1988). There are as yet few data on the nutrient-limited growth and uptake kinetics of algae from polar oceans, and no experimental studies of temperature-nutrient interactions over the range of low temperatures at which such algae live.

Pseudonitzschia seriata (formerly *Nitzschia seriata*) is a common member of bottom sea ice communities in the Canadian Arctic. It is psychrophilic, like many of the cold ocean algae studied to date, with an upper lethal limit of 12 – 15°C and an optimum for resource-saturated growth and photosynthesis between 6 and 12°C (Smith et al. 1994). Bottom-ice communities are limited in their growth largely by light and temperature (Welch and Bergmann 1989; Smith et al. 1988) but in

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the later stages of their bloom period they may show signs of nutrient limitation (Maestrini et al. 1986; Cota and Horne 1989; Gosselin et al. 1990). Silicon seems the most likely of the major nutrient elements to limit growth of ice algae in the Canadian Arctic (Cota et al. 1990) and the Weddell Sea (Dieckmann et al. 1991), and of phytoplankton in some waters in the Antarctic (Nelson and Tréguer 1992; McMinn et al. 1995). Concentrations of soluble silicic acid are nonetheless high enough in bottom ice, even when algal biomass is large, that they would not normally be expected to limit algal growth (Cota et al. 1990).

A major goal of the current study was to determine whether sub-zero temperatures, typical of the habitat occupied by *P. seriata* and other polar marine microalgae, would significantly reduce its ability to use silicic acid, as reflected in growth, uptake kinetics and cell quotas. Previous investigations suggest that cell quotas are often minimal at temperatures close to the optimum for growth (Rhee and Gotham 1981; Kilham 1984; VanDonk and Kilham 1990), while uptake kinetics observed in the field are sometimes (Jacques 1983) but not always (Nelson and Tréguer 1992) suggestive of relatively inefficient transport of external nutrients into the cell at low temperatures. Neither uptake kinetics nor cell quotas for major nutrients have previously been determined under controlled conditions for ice algae.

Materials and methods

Pseudonitzschia seriata (formerly (*Nitzschia seriata*) was isolated from samples of bottom ice taken in late April 1989 near Cornwallis Island in the Canadian Arctic Archipelago (71°41'N, 95°50'W) and was maintained subsequently in the synthetic seawater medium AQUIL (Morel et al. 1979) modified by elevated concentrations of the major nutrients (Smith et al. 1994). Light was supplied continuously at a saturating (Smith et al. 1994) irradiance of 30 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from cool white fluorescent bulbs. Cultures were grown in polycarbonate tubes (30-ml volume) and gently mixed by rocking mixers throughout the nutrient-limited growth and uptake experiments.

Growth kinetics

Growth kinetics were measured in batch cultures at seven different temperatures (Table 1) maintained in controlled environment chambers. Periodic measurements confirmed that culture temperatures were within 1°C of the chamber temperature. Algae were acclimated to each experimental temperature and silicic acid concentration by the semi-continuous batch method of Brand and Guillard (1981), which involved transfer of inocula well before depletion of soluble silicic acid in each treatment. The cultures were transferred when at least four successive *in vivo* chlorophyll fluorescence readings (Turner Designs Model AU) formed a linear slope on the semi-log plot of fluorescence vs time. Acclimation was judged to be complete when the slopes of successive replicate cultures were not significantly different ($P > 0.05$ by *t*-test). Two transfers (approximately 2 weeks of growth under the given treatment) proved sufficient to fulfil the criterion for acclimation.

Table 1 The fitted values of the Monod model parameters for Si-limited growth of *Pseudonitzschia seriata* in batch culture. The 95% confidence intervals are given in parentheses

Temperature °C	μ_{max} day ⁻¹	K_s μM	$\mu_{\text{max}}:K_s$ l· $\mu\text{mol}^{-1}\cdot\text{day}^{-1}$
-1.5	0.30 (0.21–0.38)	0.69 (0.11–1.27)	0.43 (0.08–1.04)
-1	0.32 (0.27–0.37)	0.66 (0.21–1.11)	0.48 (0.16–0.80)
0	0.35 (0.28–0.42)	1.03 (0.45–1.61)	0.34 (0.12–0.56)
2	0.37 (0.31–0.42)	0.37 (0.15–0.60)	1.00 (0.40–1.88)
4	0.35 (0.31–0.38)	0.33 (0.12–0.54)	1.06 (0.35–1.59)
6	0.49 (0.42–0.57)	0.57 (0.27–0.87)	0.86 (0.38–1.43)
10	0.46 (0.39–0.53)	0.53 (0.13–0.93)	0.94 (0.22–1.66)
All ≤ 0	0.32 (0.29–0.35)	0.79 (0.56–1.03)	0.42 (0.33–0.50)
All > 0	0.42 (0.36–0.48)	0.45 (0.33–0.57)	0.95 (0.85–1.05)

Growth rates reported here are the specific growth rate, μ , of population size, N :

$$N(t) = N(0) \cdot e^{\mu t} \quad (1)$$

Growth rates were determined by linear regression of $\ln(\text{fluorescence})$ vs time, with fluorescence readings collected over 3–5 days. Cell counts on samples taken at 3-day intervals and preserved with Lugol's iodine were performed using an inverted microscope at $\times 200$ magnification (minimum 100 cells per count). Systematic comparison between cell counts and *in vivo* fluorescence indicated that fluorescent yield did not vary significantly with time when acclimation was complete, so fluorescence should be a good measure of growth under the conditions used here.

Growth rates were measured in three to four replicate tubes at each of six nominal silicic acid concentrations, from 0.1 to 10 μM . Actual concentrations were determined at time of inoculation and transfer of each culture, by analysis of soluble reactive silicic acid, measured colorimetrically by the method of Strickland and Parsons (1972) as modified for our smaller samples (5 ml). Growth rates were fitted by iterative non-linear regression in Systat (Wilkinson 1989) to the Monod model:

$$\mu = \mu_{\text{max}} \cdot S / (K_s + S) \quad (2)$$

where μ_{max} is the silicic acid-saturated growth rate (day^{-1}), S is the silicic acid concentration (μM), and K_s is the half-saturation constant for growth (μM). The ratio of $\mu_{\text{max}}:K_s$, equivalent to the initial slope of the Monod curve, was calculated to provide an index of nutrient utilization efficiency at low nutrient concentrations (Healey 1980). Confidence intervals for μ_{max} and K_s were provided by Systat, while those for $\mu_{\text{max}}:K_s$ were calculated by error propagation from the variance of μ_{max} and K_s , assuming zero covariance (Bevington 1969).

Cell quotas and uptake kinetics

Cell quotas were measured in triplicate on samples of 10 ml from the batch cultures grown at the higher silicic acid concentrations (5–10 μM). Samples were filtered on 0.2- μm -pore-size polycarbonate membrane filters. Particulate silicon on the filters was then hydrolysed in sodium carbonate (Paasche 1980) and soluble reactive silicic acid measured as previously described. Parallel samples were taken for cell counts.

Measurements of uptake kinetics were performed on samples from semi-continuous cultures of 300 ml grown in 500-ml polycarbonate flasks mounted on a rocking mixer. The purpose of the semi-continuous cultures was to provide relatively large quantities of culture volume and biomass in growing but nutrient-limited condition. Dilution was performed daily at a standard time, removing 30 ml of culture and replacing with an equal volume of fresh, sterile medium. The *in vivo* fluorescence was measured daily and steady state was judged to have been attained when fluorescence varied by less than 2% over 3 days. Systematic sampling at four times of day in a pilot culture revealed no periodicity of fluorescence or cell counts.

Uptake kinetics were measured as soon as steady state was achieved. The steady-state culture was dispensed in 25-ml aliquots to 50-ml polycarbonate flasks. Silicic acid was then added from a stock made in synthetic seawater to make nominal concentrations of 0.5, 1, 2.5, 5, 10 and 20 μM , with duplicate flasks at each concentration. Flasks were incubated, with mixing, under the experimental light and temperature regime. Soluble reactive silicic acid was measured on 0.2- μm filtrate at 0, 2, 4 and 6 h elapsed time (0, 3, 4 and 6 h at 6°C). Uptake rates were calculated from the disappearance of silicic acid and normalized to cell concentration using cell counts on samples taken at the outset of the uptake experiment. The uptake rates (V , $\mu\text{mol}\cdot\text{cell}^{-1}\cdot\text{h}^{-1}$) were fitted to the Michaelis-Menten model:

$$V = V_{\max} \cdot S / (K_m + S) \quad (3)$$

where V_{\max} ($\mu\text{mol}\cdot\text{cell}^{-1}\cdot\text{h}^{-1}$) is the substrate-saturated uptake rate, and S and K_m (both in μM) are the silicic acid concentration and the half-saturation constant respectively, using Systat as described above.

The Arrhenius model (Ahlgren 1987) is frequently used to describe the temperature response of algal growth and other physiological processes, and was fitted to data in the present study by linear regression of $\ln(Y)$ on $[1/T]$, where Y is the process of interest and T is absolute temperature ($^{\circ}\text{K}$).

Results

Batch cultures grown at the lower added silicic acid concentrations often had longer lag phases than those at higher concentrations, but in all cases a well-defined log phase of growth existed. Growth rates determined over the log phase increased as a saturating function of external silicic acid concentration (Fig. 1). Within the limited precision of the data, the results were described reasonably well ($R^2 = 71\text{--}89\%$) by the Monod model (Fig. 1). At all temperatures, there were at least three different experimental concentrations within the nutrient-limited response region, and at least two at, or very near saturation, so the data should be well-suited to estimation of the Monod parameters.

Resource-saturated growth rates (μ_{\max}) ranged from 0.3 to 0.49 day^{-1} . The highest values were at temperatures $> 0^{\circ}\text{C}$, with the maximum at 6°C (Table 1). The

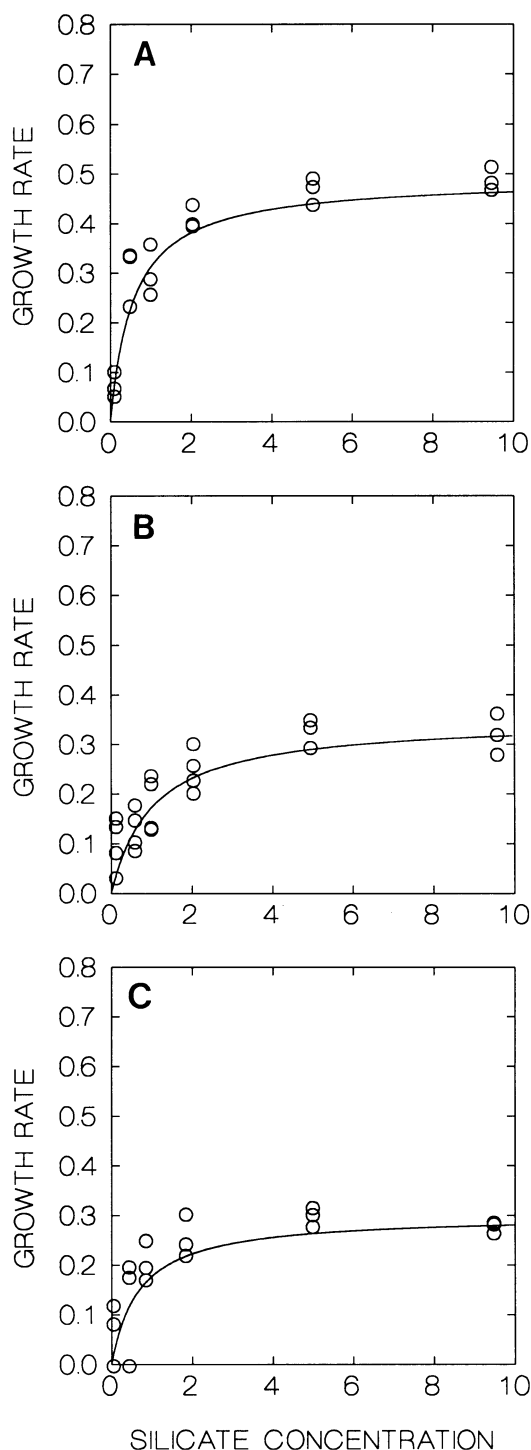


Fig. 1A–C Examples of the functional relationship between instantaneous growth rate (day^{-1}) and exogenous silicic acid concentration (μM) for *Pseudonitzschia seriata* grown in batch culture at 6°C **A**, 0°C **B** and -1.5°C **C**, with fitted values of the Monod model shown as solid lines

Arrhenius model fitted the μ_{\max} vs temperature relationship significantly ($P < 0.05$) from -1.5 to 6°C , with an R^2 of 77%. K_s , by contrast, displayed its highest values at temperatures of 0°C and less, with the

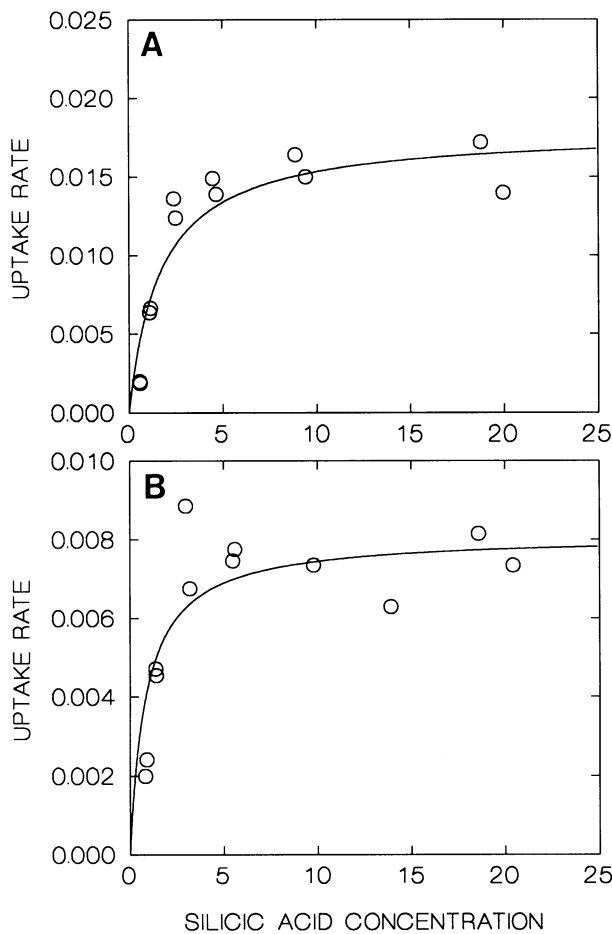


Fig. 2A, B Examples of silicate uptake rate ($\text{pmol}\cdot\text{cell}^{-1}\cdot\text{h}^{-1}$) as a function of exogenous silicic acid concentration (μM) for *Pseudonitzschia seriata* grown in semi-continuous culture at -1°C **A** and -1.5°C **B**, with fitted values of the Michaelis-Menten model shown as solid lines

maximum at 0°C (Table 1). Neither K_s nor the ratio of $\mu_{\max}:K_s$ showed orderly trends with temperature, and regression analysis indicated no significant agreement ($P > 0.17$) with either the Arrhenius model or a power model of temperature response. The efficiency of nutrient use at low exogenous concentrations, as indexed by the ratio $\mu_{\max}:K_s$, was largest at temperatures $> 0^\circ\text{C}$ (Table 1). The confidence intervals on the estimates of K_s and $\mu_{\max}:K_s$ at each experimental temperature were very wide and overlapped between sub-zero and plus-zero temperature ranges. The average value of $\mu_{\max}:K_s$ for temperatures $> 0^\circ\text{C}$ was, however, significantly ($P < 0.05$) greater than the average value for lower temperatures according to *t*-test (Table 1). The average value of K_s was marginally ($0.05 < P < 0.1$) smaller for the higher temperatures compared to the lower, while the reverse was true for μ_{\max} (Table 1).

Based on the growth kinetics observed in batch culture, the semi-continuous cultures, with a dilution rate of 0.1 day^{-1} , were operating at 18–33% of μ_{\max} . Thus, the semi-continuous cultures were quite strongly Si-

Table 2 The cell quotas (silicon-saturated, Q_{\max}) and fitted values of the Michaelis-Menten model parameters for Si uptake of *Pseudonitzschia seriata*. The 95% confidence intervals are given in parentheses

Temperature $^\circ\text{C}$	Q_{\max} $\text{pmol}\cdot\text{cell}^{-1}$	V_{\max} $\text{pmol}\cdot\text{cell}^{-1}\cdot\text{h}^{-1}$	K_m μM
-1.5	0.802 (0.624–0.098)	0.0081 (0.0063–0.0099)	0.87 (0.45–1.29)
-1	0.456 (0.386–0.526)	0.0163 (0.0127–0.0199)	1.69 (1.06–2.32)
0	0.418 (0.304–0.532)	ND	ND
2	0.207 (0.187–0.227)	0.0111 (0.0083–0.0139)	1.07 (0.54–1.60)
4	0.298 (0.0212–0.384)	ND	ND
6	0.287 (0.197–0.377)	0.0126 (0.0108–0.0144)	1.46 (1.07–1.85)
10	0.338 (0.284–0.392)	ND	ND
All ≤ 0	0.559 (0.359–0.758)	0.0122	1.28
All > 0	0.282 (0.228–0.337)	0.0118	1.26

limited. Silicic acid uptake rates measured on samples from the semi-continuous cultures increased with exogenous silicic acid concentration in a saturating function that was adequately described by the Michaelis-Menten model. Figure 2 illustrates the best and worst fits to the model ($R^2 = 66\%$ at -1.5°C and 84% at -1°C). In some cases the data suggested the existence of a non-zero threshold concentration for uptake (e.g. Fig. 2B), but no threshold significantly greater than zero could be quantified by fitting a suitably modified form of equation 3. The uptake parameters V_{\max} and K_m showed no regular or significant variation with temperature (Table 2).

Cell quotas measured in silicic acid-saturated batch cultures ($5\text{--}10\ \mu\text{M}$ silicic acid) provided estimates of Q_{\max} (Table 2). Q_{\max} displayed a broadly U-shaped response to temperature, with a minimum at 2°C and maxima at -1.5 and 10°C . Q_{\max} increased rapidly at temperatures $< 2^\circ\text{C}$ compared to the more gradual and limited increase at temperatures $> 2^\circ\text{C}$ (Table 2). The average Q_{\max} was significantly ($P < 0.05$) larger for temperatures $\leq 0^\circ\text{C}$ than for higher temperatures (Table 2).

Discussion

The few previous studies on nutrient-limited growth of cold ocean algae have suggested that the psychrophilic algae characteristic of such habitats are relatively inefficient in their nutrient utilization, at the prevailing low

temperatures, compared to mesophilic microalgae of temperate habitats (Maestrini et al. 1986; Jacques 1983; Sommer 1986). *Pseudonitzschia seriata* is psychrophilic and a common member of bottom ice communities in the Arctic (Smith et al. 1994), although its distribution includes more temperate waters. The Arctic isolate of *P. seriata* studied here revealed silicon-limited growth kinetics that were reasonably efficient compared to typical values for mesophiles. A comprehensive review of published values for kinetic constants of silicon-limited growth of microalgae (mostly mesophilic; Stapleford 1994) showed that we would expect μ_{\max} of 0.33–0.57 day⁻¹, K_s of 0.37–0.88 μM and $\mu_{\max}:K_s$ of 0.89–0.65 l· μmol^{-1} ·d⁻¹ over the temperature range 0–10°C. Considering the uncertainties in the estimates, both experimental and from the literature, there is little reason to think that *P. seriata* is less efficient than expected from the average behaviour of mesophiles. Psychrophilic diatoms are therefore not necessarily inefficient in their silicon utilization.

The efficiency of *P. seriata* is such that we would not expect it to suffer significant silicic acid limitation in its natural bottom ice habitat. Silicic acid ranges from 4 to 15 μM in melted samples of bottom ice from the Canadian Arctic (Cota et al. 1990). Although we cannot be sure that such concentrations apply to the micro-environment of the algae in situ, they are considerably higher than the K_s of *P. seriata* at any of the temperatures studied here. Other species of bottom ice algae may be less efficient, however. Sommer (1986) found a wide range of nutrient utilization efficiency among Southern Ocean diatoms, and ice algae may vary similarly.

Resource-saturated growth of *P. seriata* was temperature dependent over most of the range studied here, although the Q_{10} value of 1.63 was somewhat lower than the value of 1.9 reported previously (Smith et al. 1994). By contrast, nutrient-limited growth as indexed by $\mu_{\max}:K_s$ had no significant monotonic relationship with temperature. Previous studies of temperature-nutrient interactions in mesophilic algae have similarly revealed little consistent variation of nutrient utilization efficiency with temperature (e.g. Rhee and Gotham 1981; Mechling and Kilham 1982; VanDonk and Kilham 1990). The nutrient utilization efficiency of *P. seriata* was, however, significantly lower at and below 0°C than at higher temperatures. Although modest in extent (an approximate 50% reduction in nutrient utilization efficiency at and below 0°C) the present findings provide the first direct experimental support for the idea that temperatures near and below 0°C are associated with diminished efficiency of nutrient utilization (Jacques 1983; Maestrini et al. 1986; Sommer 1986).

Saturated cell quotas, Q_{\max} , followed an asymmetrical but broadly U-shaped response to temperature from -1.5 to 10°C, consistent with the response shape previously reported for P-, N- and Si-limited freshwater

microalgae (Rhee and Gotham 1981; Kilham 1984; VanDonk and Kilham 1990). Cell quotas under limitation are generally expected to be lower than saturated quotas, but for silicon the difference is relatively small (Conley and Kilham 1989) and it may be assumed that silicon-limited quotas would follow a similar pattern with temperature. In absolute terms, the Q_{\max} of *P. seriata* fell closer to expectations for freshwater diatoms of its size (0.60 pmol·cell⁻¹ at 134 μm^3) than to values typical for marine diatoms (0.064 pmol·cell⁻¹), based on a comprehensive analysis of published Si quotas (Conley and Kilham 1989). Thus *P. seriata* seems relatively heavily silicified for a marine diatom, and might have proportionately low efficiency of utilization for growth unless its uptake kinetics are unusually efficient.

The uptake kinetics of *P. seriata* did in fact indicate a comparable or superior ability to acquire silicic acid from the environment, compared to mesophilic diatoms from marine and freshwaters (e.g. Conway and Harrison 1977; Tilman and Kilham 1976; Kilham et al. 1977; Harrison et al. 1989). Furthermore, the uptake kinetics were not impaired at the lower growth temperatures (-1.5 and -1°C). The significantly lower growth efficiency of the cultures at $\leq 0^\circ\text{C}$ was therefore due to elevated silicon demand (quota) rather than reduced uptake. Whether this is a general feature of temperature effects on silicon utilization by cold ocean algae can only be resolved by further work with additional species and/or natural populations.

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