

# Effects of temperature on growth rate, cell composition and nitrogen metabolism in the marine diatom *Thalassiosira pseudonana* (Bacillariophyceae)

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**ABSTRACT:** Although temperature effects on phytoplankton growth and photosynthesis can be clearly demonstrated in the laboratory, their relevance in the field is much harder to establish. Recently, however, it has been recognized that temperature has a significant influence on nitrogen uptake. In particular, temperate marine diatom species may be limited by their ability to acquire nitrate at temperatures above approximately 16°C. In order to explore this idea, we grew the diatom *Thalassiosira pseudonana* at 8, 17 and 25°C, and compared cell composition, and rates of growth ( $\mu$ ),  $^{15}\text{N}$  incorporation, calculated nitrate incorporation (the product of  $\mu$  and cell N content), and the activity of nitrate reductase (NR), a key enzyme involved in nitrate incorporation. Cell N content, protein and volume remained relatively constant across different temperatures, but cell C, chlorophyll *a* (chl *a*), and C:N ratio increased with increasing temperature, suggesting that C metabolism was affected more strongly than N metabolism. Classical temperature models suggested that growth and various indices of nitrate metabolism all responded to temperature, with  $Q_{10}$  values of close to 2 over the whole temperature range. However,  $Q_{10}$  values over the interval from 8 to 17°C were higher than 2 and much lower than 2 between 17 and 25°C. Limitations to the  $Q_{10}$  concept are considered. Temperature effects on different measures of nitrate metabolism were very similar, supporting the hypothesis that the effects of temperature on diatom nitrate metabolism are mediated at the level of NR activity. Recent biochemical data for NR also supports this idea.

**KEY WORDS:** Nitrate reductase · Enzyme activity ·  $^{15}\text{N}$  uptake · Phytoplankton ecology · Chemical composition · Temperature adaptation

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## INTRODUCTION

Although there can be little doubt that temperature exerts a strong influence on algal growth and photosynthesis (e.g. Raven & Geider 1988, Davison 1991), biological oceanographers have tended to concur with Eppley's

(1972) observation that 'temperature does not seem to be very important in the production of phytoplankton in the sea'. Indeed, temperature does not even appear as a variable in a recent review of phytoplankton primary productivity models (Behrenfeld & Falkowski 1997). Reasons for this apparent contradiction, include the fact that effects of temperature can be overshadowed by factors such as irradiance (e.g. Gibson & Foy 1989), and the considerable ability of phytoplankton to acclimate or adapt to growth at different temperatures (e.g. Li 1980, Smith et al. 1994, Suzuki & Takahashi 1995).

Temperature effects on algal metabolism extend beyond growth and photosynthesis, however. There is

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a large body of literature showing that temperature affects cell composition, short-term nutrient uptake and, in particular, nitrogen metabolism (see Morris et al. 1974, Yoder 1979, Terry 1983, Raimbault 1984, Whalen & Alexander 1984, Thompson et al. 1992, Reay et al. 1999, Sakamoto & Bryant 1999). These effects, while more subtle than those on growth and photosynthesis, can have enormous implications at the level of the ecosystem. Recently, for example, Lomas & Glibert (1999a,b) proposed that diatoms and diatom nitrate uptake are strongly limited by temperature; this has important implications for species succession and the biogeochemistry of nitrogen in marine environments.

In order to investigate such effects, it is necessary to examine both cellular and subcellular processes. The effects of temperature are usually considered to have their basis in altering enzyme-mediated biochemical processes. The relationship between temperature and a given biological rate can be modelled in several different ways (see Ratkowsky et al. 1983, Ahlgren 1987), but the temperature coefficient  $Q_{10}$  (the factor by which a biological rate is increased by a 10°C rise in temperature) has been most commonly used. The use of  $Q_{10}$  values assumes an Arrhenius-type relationship between rates and temperature and relies on chemical kinetics controlling the observed rate (Ahlgren 1987). Under such conditions, biochemical processes are expected to have a  $Q_{10}$  near 2. There are relatively few examples where the effects of temperature on physiological and biochemical processes have actually been examined simultaneously.

Previous work considering the effects of temperature on nitrogen metabolism is somewhat disjointed. We know, for example, that nitrogen uptake can show a pattern of increase, optimum and rapid decline as temperature is increased (Rhee & Gotham 1981, Terry 1983, Whalen & Alexander 1984), that there can be a pronounced uncoupling between transient uptake and assimilation of nitrogen at lower temperatures (Raimbault 1984), and that the activity of nitrogen-reducing enzymes such as nitrate reductase (NR) varies strongly with temperature (Kristiansen 1983). Recent work by Gao et al. (2000) provided some evidence of the biochemical basis for the observations of Lomas & Glibert (1999a,b); NR from the diatom *Skeletonema costatum* shows optimal activity at a relatively low temperature and is unstable above ~16°C.

In the present study, we examined the effects of temperature on cell composition, nitrate uptake and incorporation, nitrate reduction (catalyzed by NR) and ultimately on rates of growth, represented by the rate of cell division rate, in near-steady-state cultures of the marine diatom *Thalassiosira pseudonana*. This represents one of the first attempts to compare ecological, physiological and biochemical rates to deduce at what

level temperature is acting. Our hypothesis was that observed changes in nitrate incorporation can be explained by changes at the biochemical level, i.e. in NR activity.

## MATERIALS AND METHODS

**Culture conditions.** Cultures of the marine diatom *Thalassiosira pseudonana* (Clone 3H) were obtained from the Northeast Pacific Culture Collection and grown in 1 l semi-continuous batch cultures on artificial medium, under continuous light (150  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ), as previously described (Berges & Harrison 1993). All nutrients were in excess, and the sole nitrogen source (nitrate) was maintained at >20  $\mu\text{M}$  at all times. Cultures were stirred and bubbled gently with air. Triplicate cultures were grown at either  $8 \pm 1^\circ\text{C}$ ,  $17 \pm 1^\circ\text{C}$  or  $25 \pm 1^\circ\text{C}$  using a combination of a circulating cooled water bath and immersion heaters. The semi-continuous cultures were acclimated for a minimum of 8 generations by diluting to one-sixth their original density as they neared the end of the logarithmic phase of growth. Growth rates were monitored by *in vivo* fluorescence measured in a Model 10-AU fluorometer (Turner Designs, Sunnyvale, CA), or by cell counts (see next subsection). All sampling was performed on mid-log-phase cultures. Under these conditions, growth can be maintained at a constant rate virtually indefinitely, and cell composition is essentially invariant over time (e.g. Berges & Harrison 1995a,b).

**Cell composition measurements.** Cell counts and cell volume determinations were performed on living cells using a Model TALL counter (Beckmann Coulter, Brea, CA) equipped with a population accessory. A 70  $\mu\text{m}$  aperture was used, and the instrument was calibrated with 5  $\mu\text{m}$  latex microspheres. Chl *a* was measured fluorometrically in samples collected by filtration onto 25 mm GF/F glass-fibre filters, and extracted in 90% acetone (Parsons et al. 1984). Samples taken on precombusted 13 mm Gelman A/E filters were analyzed for nitrogen and carbon content using a CNS analyzer (Carlo Erba, Milan, Italy), with sulfanilamide as a standard. Protein was determined using a modified Bradford method (described in Berges et al. 1993), homogenizing samples filtered onto 25 mm GF/F filters in TCA, and resolubilizing proteins in 1 N NaOH.

**Nitrogen metabolism.** Nitrate uptake rates were measured using the stable isotope  $^{15}\text{N}$ . Duplicate samples from each culture were inoculated with 10  $\mu\text{M}$   $^{15}\text{NO}_3^-$  and incubated at 8, 17 or 25°C alongside the original cultures in 500 ml polycarbonate bottles for 3 to 4 h. After incubation, samples for  $^{15}\text{N}$  analysis were collected on precombusted GF/F filters and analyzed using the micro-Dumas dry-combustion technique, as

described by La Roche (1983) and Harrison (1983), and a Model N-150 (Jasco International, Tokyo, Japan) emission spectrometer (Fiedler & Proksch 1975). Nitrate uptake rates were calculated following Dugdale & Wilkerson (1986).

*In vitro* assays for nitrate reductase (NR) were performed as described in Berges & Harrison (1995a), monitoring production of nitrite colourimetrically. Duplicate samples from each culture were collected on GF/F filters and homogenized. Subsamples of each homogenate were assayed at all temperature, i.e. 8, 17 and 25°C.

Rates of nitrate incorporation in cultures at the temperature of acclimation were also calculated as the product of specific growth rate ( $\mu$ ) and particulate nitrogen per cell (PN).

**Analyses.** For rates of growth,  $^{15}\text{N}$  uptake, NR activity and calculated nitrate incorporation, values of  $Q_{10}$  and activation energy ( $E_a$ ) were calculated across temperature intervals as:

$$\ln Q_{10} = \frac{10(\ln V_2 - \ln V_1)}{(T_2 - T_1)}$$

and

$$\ln E_a = \frac{R(\ln V_2 - \ln V_1)}{(T_1 - T_2)}$$

where  $V_1$  and  $V_2$  are rates of reaction at temperatures  $T_1$  and  $T_2$  (in °K), and  $R$  is the gas constant.

Comparisons of growth rates, cell composition and rates of nitrogen metabolism at different temperatures were made using 1-way ANOVA designs followed by Tukey multiple comparisons, using SigmaStat Version 1.0 (Jandel Scientific, San Rafael, CA); all tests were made at the 95% confidence level.

## RESULTS

In terms of cell composition, there were no significant differences in particulate nitrogen (Fig. 1C;  $p > 0.1$ ), protein (Fig. 1B;  $p > 0.2$ ) or cell volume (Fig. 1F;  $p > 0.4$ ) among different temperatures. Particulate carbon per cell increased with increasing temperature (Fig. 1A;  $p < 0.05$ ), as did the C:N ratio (Fig. 1E;  $p < 0.01$ ) and chl *a* per cell (Fig. 1D;  $p < 0.01$ ); the magnitude of increases between 17 and 25°C was greater than those between 8 and 17°C.

For rate measurements,  $Q_{10}$  values differed substantially depending on the temperature interval used for the calculation. In general, rates calculated over the whole range from 8 to 25°C tended to be near 2, while those from 8 to 17°C were greater than 2 (Table 1). Between 17 and 25°C, some rates ( $^{15}\text{N}$  uptake and NR activity) actually declined, resulting in  $Q_{10}$  values of  $< 1.0$ . Growth rate increased between 8 and 17°C with a  $Q_{10}$  of  $> 3$ , but there was a substantially smaller change be-

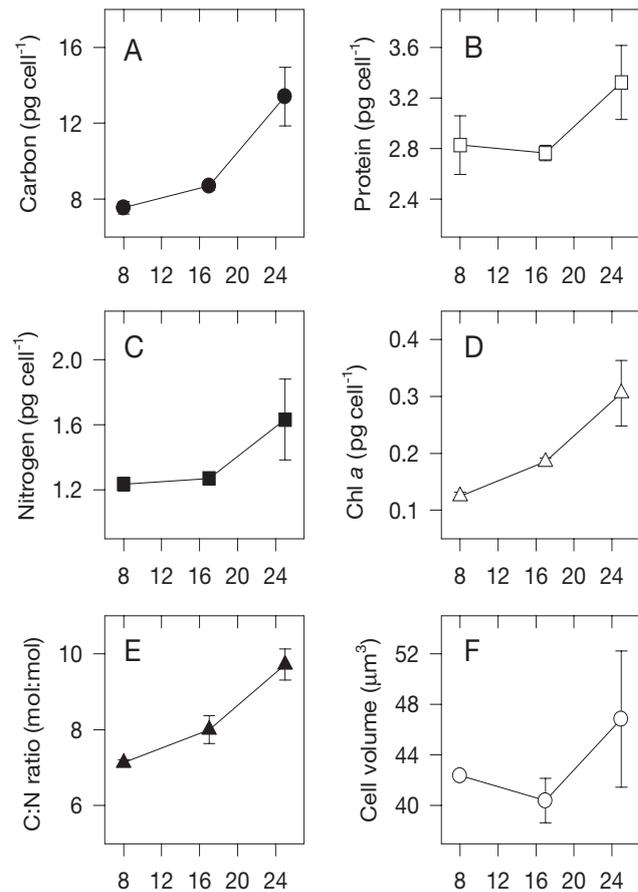


Fig. 1. *Thalassiosira pseudonana*. Cell constituents in cultures grown under continuous saturating irradiance at different temperatures. (A) particulate carbon; (B) protein; (C) particulate nitrogen; (D) chl *a*; (E) carbon:nitrogen molar ratio; (F) cell volume. Cells were grown in semi-continuous batch cultures and acclimated to each temperature for a minimum of 8 generations before sampling; all cultures were in mid-logarithmic phase when sampled. Each point represents the mean ( $\pm$ SE) of 3 replicate cultures; where error bars are not visible, they are smaller than the symbol

Table 1. *Thalassiosira pseudonana*. Values of  $Q_{10}$  and apparent activation energy ( $E_a$ , kJ mol<sup>-1</sup>, in parentheses) for growth rate ( $\mu$ ),  $^{15}\text{N}$  uptake, and NR activity and calculated nitrate incorporation rate (the product of  $\mu$  and particulate nitrogen per cell, PN) over different temperature ranges, for diatom cultures. Variables were measured in triplicate log-phase cultures acclimated to the temperature of measurement for a minimum of 8 generations.  $Q_{10}$  and  $E_a$  were calculated as described in last subsection of 'Materials and methods'

Variables	8 to 17°C	17 to 25°C	8 to 25°C
$\mu$	3.09 (76.3)	1.19 (12.6)	1.97 (46.0)
$^{15}\text{N}$ uptake	2.88 (71.6)	0.98 (-0.95)	1.65 (33.9)
NR activity	2.71 (67.3)	0.95 (-3.9)	1.74 (37.5)
$\mu$ PN	3.18 (78.2)	1.63 (35.1)	2.32 (57.0)

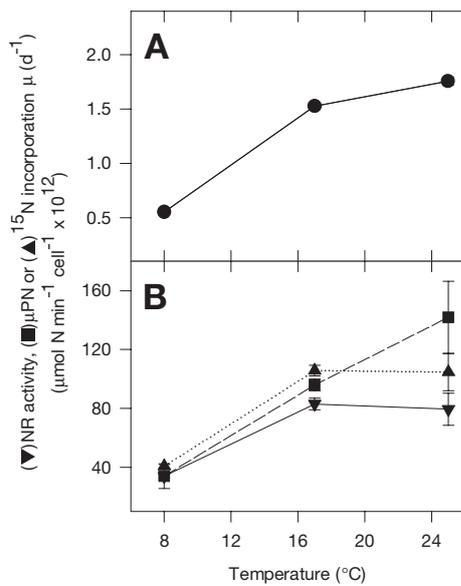


Fig. 2. *Thalassiosira pseudonana*. Rates of growth (A) and indices of nitrogen incorporation (B) in cultures grown under continuous saturating irradiance at different temperatures. Growth was calculated from changes in cell number. Nitrogen incorporation was estimated as nitrate reductase (NR) activity ( $\nabla$ ), rate of nitrogen incorporation as the product of specific growth rate ( $\mu$ ), and cell nitrogen content (PN,  $\blacksquare$ ), or rate of  $^{15}\text{N}$ -nitrate uptake ( $\blacktriangle$ ). Culture conditions and sampling as in Fig. 1

tween 17 and 25°C (Fig. 2A, Table 1). NR activity,  $^{15}\text{N}$  uptake and calculated rates of nitrate incorporation ( $\mu\text{PN}$ ) were not significantly different from each other at any temperature (Fig. 2B;  $p > 0.05$  in all cases). In terms of rate constants, the  $Q_{10}$  and  $E_a$  for NR activity,  $^{15}\text{N}$  uptake and calculated rates of nitrate incorporation were very close to those for growth rate between 8 and

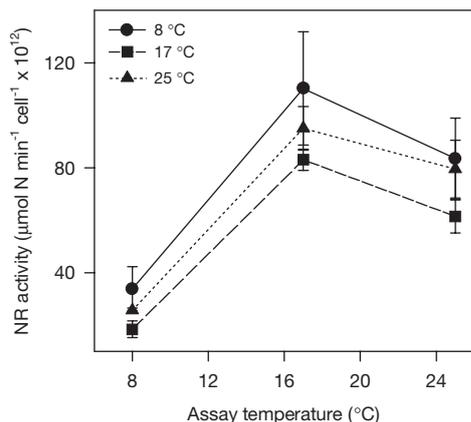


Fig. 3. *Thalassiosira pseudonana*. Nitrate reductase (NR) activity in cultures grown at 8, 17 or 25°C and assayed at different temperatures. Culture conditions, sampling and symbols as in Fig. 1

17°C (Table 1). Between 17 and 25°C,  $Q_{10}$  and  $E_a$  for NR activity and  $^{15}\text{N}$  uptake rates were no different from each other ( $p > 0.1$ ); although rate constants for  $\mu\text{PN}$  appeared to be higher than for  $^{15}\text{N}$  uptake rates or NR activities (Table 1), these differences were not statistically significant ( $p > 0.05$ ).

When NR activity was plotted against assay temperature, trends were similar for cultures grown at different temperatures (Fig. 3). A 2-way ANOVA examining the effects of growth temperature and assay temperature showed no significant differences between NR activity of cultures that were grown at different temperatures and assayed at a common temperature ( $p > 0.1$ ), and no interactions between growth temperature and assay temperature ( $p > 0.1$ ). Significant differences were found between assay temperatures ( $p < 0.05$ ), regardless of the temperature at which cultures had been grown; NR activity was lower at 8°C and not significantly different between 17 and 25°C.

## DISCUSSION

### Temperature and cell composition

The results for cell composition are largely consistent with previous findings: increasing C content and chl a content with increasing temperature have been noted many times before for algae in general (see Thompson et al. 1992), and diatoms in particular (e.g. Lomas & Glibert 1999a, Gao et al. 2000). There is more variability in the results for cell volume. In some cases, increasing volume with increasing temperature has been noted by some investigators (e.g. Lomas & Glibert 1999a), while others have found no changes (e.g. Gao et al. 2000). This may be related to the light regime: cell volume increases tend to occur in studies using a light:dark cycle (e.g. Lomas & Glibert 1999a), as opposed to continuous light (as in the present study).

The finding that C:N ratio increases with increasing temperature is generally supported by other data (e.g. Thompson et al. 1992), but this may also depend on the light regime, since Lomas & Glibert (1999a) noted no changes in C:N ratio with temperature in diatoms and dinoflagellates grown on light:dark cycles. In the present study, the increase in C:N ratio was largely the result of increased carbon content without changes in nitrogen. Yoder (1979) found that in the diatom *Skeletonema costatum*, N content was largely independent of temperature under light limitation, and this seems also to be the case where light is not limiting. The mechanism of this change remains unclear. Raven & Geider (1988) hypothesized that algae grown at low temperature could commit a larger fraction of cell carbon to catalysts than algae at higher temperatures. If

so, assuming that catalysts are enzyme proteins, we might expect that low-temperature-acclimated cells would have relatively more carbon tied up in protein than those at higher temperatures, and thus the C:N ratio would be lower at low temperatures. However, were this the case, we might also expect to see changes in protein, which were not observed. Similarly, Gao et al. (2000) did not detect any changes in cell protein in *S. costatum* grown at temperatures between 5 and 25°C. Alternatively, temperature could affect rates of carbon and nitrogen incorporation differentially.

### The $Q_{10}$ concept

Before comparing the results of the various measurements of rates of growth and nitrogen metabolism, it is necessary to evaluate the  $Q_{10}$  and  $E_a$  calculations. Both measurements varied considerably, depending on the temperature interval chosen. Correct use of a  $Q_{10}$  value implies that the data follows an Arrhenius-type relationship (linearity of a log vs inverse absolute temperature). Several authors have argued against such functions, advocating Belehradek or empirical square-root relationships instead (see Ratkowsy et al. 1983, Ahlgren 1987). One of the important failings of the  $Q_{10}$  concept is that it is almost certain that different processes become limiting at different temperature (see Jumars et al. 1993). For example, for photosynthesis, photosynthetic electron transport is probably limiting at lower temperatures, while processes related to transport and fixation of carbon are probably limiting at higher temperatures (see Davison 1991).

However, rather than simply abandoning the use of  $Q_{10}$  as a means to describe and predict temperature effects on metabolic rates, we suggest that  $Q_{10}$  could be used in a more fundamental way to provide information about the temperatures to which organisms are adapted. Such an idea is, in fact, quite common in the zoological literature; for example, Brown (1989) defined the ranges of temperature in which fishes experienced thermal stress as the ranges of temperature in which oxygen consumption changed by a  $Q_{10} > 4$ . Such a region could be defined for algae based on growth rate: Jitts et al. (1964) examined cell division of 5 species of microalgae and was able to identify ranges of 6 to 10°C, outside of which the  $Q_{10}$  for growth varied considerably around 2. Such a region could be defined for enzyme activities as well; Burke (1995) identified regions of temperature stability based on enzyme kinetics that he termed a 'thermal kinetic window' (TKW), i.e. the range in which the effective  $K_m$  for an enzyme was within 200% of the minimum observed. For higher plants, this was usually in the 5 to 8°C ranges (and was thus much less than the range of tem-

perature in which plants were capable of growing). Such a concept has not been applied to phytoplankton to our knowledge.

### Temperature effects on growth

Considerable data are available on the effects of temperature on algal growth; however, results are usually based either on changes in the rate of cell division (i.e.  $\mu$ , largely in laboratory studies), or on rates of carbon fixation (largely in fieldwork). In order to compare our results across these data sets, we make the assumption that growth and photosynthesis are closely linked and that cell composition is relatively constant. While such assumptions are almost certainly false in particular cases (see preceding subsection), it nonetheless appears that both growth and photosynthetic rates respond to temperature with an apparent  $Q_{10}$  near 2 (Eppley 1972, Raven & Geider 1988, Davison 1991); a more precise value of 1.88 is often quoted (see Raven & Geider 1988). If the whole temperature range of growth rate data is considered in the present study, a quite similar value of  $Q_{10}$  (1.97) is found, but this differs considerably depending on the temperature interval chosen. In general, diatoms do not appear to be exceptional to the general rule for algae. Lomas & Glibert (1999a) found a  $Q_{10}$  of 2.46 for *Thalassiosira weissflogii* grown between 10 and 20°C, and Smith et al. (1994) and Suzuki & Takahashi (1995) quote values in the neighbourhood of 1.9 for a variety of other species. As in the present study, Thompson et al. (1992) noted for *T. pseudonana* that values of  $Q_{10}$  varied between 1.8 and 3.1, depending on the particular range of temperatures selected. Interestingly, Suzuki & Takahashi noted, for 8 Arctic diatom species, that the temperatures at which maximal growth rates were found were all very near the upper limit for growth and generally higher than the temperature from which the species were isolated. This suggests that temperature could be an important factor for these species and that the range of growth itself is less important ecologically than some smaller subset of this range (see preceding subsection).

It has been noted for photosynthesis that the stability of enzymes associated with carbon fixation (e.g. ribulose biphosphate carboxylase/oxygenase: RuBisCO) generally exceeds that of whole-plant photosynthesis, i.e. enzymes are less temperature-sensitive than the integrated process in which they participate (see Davison 1991). Moreover, Devos et al. (1998) showed that, in a number of *Chloromonas* species (both psychrophiles and species adapted to higher temperatures), growth rates, rates of photosynthesis and activities of Rubisco have similar thermal optima and Rubisco

enzymes from different isolates have similar thermostability. Such findings contrast with the case for NR (see next subsection).

### Temperature effects on nitrate metabolism

Effects of temperature on NR activities and other indices of nitrate incorporation appear to be consistent with each other, within the errors of the measurements. While such correlations cannot prove causation, the results do support the hypothesis that temperature effects on nitrate metabolism are mediated at the enzyme level. Of particular interest is the lack of change in nitrate metabolism between 17 and 25°C; directly measured rates of  $^{15}\text{N}$  uptake and NR activity are virtually unchanged, and although rates calculated from N content and cell division rates do appear to increase, they are not statistically different. This is evidence that the processes of N acquisition are more sensitive to temperature than are cell division and photosynthesis in diatoms, and fits very nicely with Lomas & Glibert's (1999a,b) hypothesis. More recently, Lomas & Glibert (2000) have provided additional evidence that the critical step is that catalyzed by NR and not nitrite reductase (NiR). Their work has also highlighted differences between diatoms and flagellate species that can be related to differing temperature optima for NR.

In reviewing the algal literature, it appears that temperature optima and general characteristics of nitrate uptake and NR activities tend to be very similar in a variety of species including cyanobacteria (e.g. Shukla & Kashyap 1999) and dinoflagellates (Kristiansen 1983, Witt et al. 1999). Temperature responses of NR are also similar: Witt et al. (1999) found  $E_a$  values for NR from *Peridinium gatunense* of 23 to 29 kJ mol $^{-1}$ ; Kristiansen (1983), derived an  $E_a$  of 38 kJ mol $^{-1}$  for NR from *Heterocapsa triquetra*; and Gao et al. (1993) calculated an  $E_a$  of 36.5 kJ mol $^{-1}$  for NR from *Skeletonema costatum*, all very similar to the overall value found in the present study.

NR activity from a wide number of marine species from lineages containing chl *c* show thermal optima in the range of 10 to 20°C (e.g. Kristiansen 1983, Davison & Davison 1987, Gao et al. 1993). Interestingly, all the available data for diatom NR seem to suggest very similar thermal optima for the enzyme, near 16°C. This temperature is not well related to the temperature from which the phytoplankton have been isolated (see Packard et al. 1971, Kristiansen 1983, Gao et al. 1993), and this remains something of a puzzle. Such an optimum is much lower than that found for green algae and higher plants (e.g. Solomonson & Vennesland 1972, Solomonson & Barber 1990, Loppes et al. 1996). This

difference has been discussed in an evolutionary context (Gao et al. 2000). Intriguingly, Kudo et al. (2000) have recently demonstrated that NR from *Phaeodactylum tricornutum* shows very little variation in activity with changing temperature. This result contrasts with many other studies in diatoms (e.g. Kristiansen 1983 and Gao et al. 2000), and may indicate fundamental differences between *P. tricornutum* and other species.

### Thermal regulation of NR activity

How is the effect of temperature on diatom NR mediated? Based on previous work, NR activity in diatoms such as *Thalassiosira weissflogii* appears to be regulated largely by synthesis (e.g. Gao et al. 2000); to a first approximation, a continuous background degradation accomplishes its turnover (see Vergara et al. 1998). In contrast to NR, which is rather labile in diatoms (e.g. Kristiansen 1983, Gao et al. 1993), proteolytic enzymes in phytoplankton are remarkably thermostable (Berges & Falkowski 1996). We can hypothesize that, as temperature increases, the activities of the proteases that normally degrade NR also increase. Thus, without any more complicated cellular regulation, increased temperature should lead to greater degradation of NR and not necessarily to greater synthesis; lower NR activity could simply be a consequence of these features of the enzyme's regulation.

In reality, regulation of NR may be much more complicated. Simply changing the quantity (and thus activity) of enzymes is a relatively poor strategy for dealing with temperature changes; altering enzyme kinetic constants (e.g.  $K_m$ ) through post-translational modification, or through temperature-specific expression of isozymes are more efficient and often-used strategies (see Graham & Patterson 1982, Burke 1995). With respect to algal nitrogen metabolism, nothing is yet known about such strategies.

As mentioned above, Gao et al. (2000) hypothesised that there are significant evolutionary differences between NR from green algae and higher plants and NR from chromophytes. One way in which this manifests itself is in the light regulation of NR: higher plants show a phosphorylation mechanism (Huber et al. 1992), while diatoms do not (Berges 1997, Vergara et al. 1998). Curiously, earlier work (Nussaume et al. 1995) demonstrated that NR from higher plants carries an N-terminal domain that is not conserved in other organisms. When the N-terminal domain is removed from tobacco NR, not only is post-translational light regulation abolished, but the thermal optimum of the enzyme shifts from 30 down to near 15°C. We have no diatom NR sequences at present but, when they are available, it will be interesting to compare N-terminal sequences.

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