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RELATIONSHIPS BETWEEN NITRATE REDUCTASE ACTIVITY AND RATES OF GROWTH AND NITRATE INCORPORATION UNDER STEADY-STATE LIGHT OR NITRATE LIMITATION IN THE MARINE DIATOM *THALASSIOSIRA PSEUDONANA* (BACILLARIOPHYCEAE)[1]

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ABSTRACT

Although activity of the enzyme nitrate reductase (NR) can potentially be used to predict the rate of nitrate incorporation in field assemblages of marine phytoplankton, application of this index has met with little success because the relationship between the two rates is not well established under steady-state conditions. To provide a basis for using NR activity measurements, the relationships among NR activity, growth rate, cell composition, and nitrate incorporation rate were examined in cultures of Thalassiosira pseudonana *(Hustedt) Hasle and Heimdal, growing a) under steady-state light limitation, b) during transitions between low and high irradiance (15 or 90 μmol quanta·$m^{-2}\cdot s^{-1}$), and c) under steady-state nitrate limitation. Using a modified assay for NR involving additions of bovine serum albumin to stabilize enzyme activity, NR activity in light-limited cultures was positively and quantitatively related to calculated rates of nitrate incorporation, even in cultures that were apparently starved of selenium. During transitions in irradiance, growth rates acclimated to new conditions within 1 day; through the transition, the relationship between NR activity and nitrate incorporation rate re-*

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mained quantitative. In nitrate-limited chemostat cultures, NR activity was positively correlated with growth rate and with nitrate incorporation rates, but the relationship was not quantitative. NR activity exceeded nitrate incorporation rates at lower growth rates (<25% of nutrient-replete growth rates), but chemostats operating at such low dilution rates may not represent ecologically relevant conditions for marine diatoms. The strong relationship between NR activity and nitrate incorporation provides support for the idea that NR is rate-limiting for nitrate incorporation or is closely coupled to the rate-limiting step. In an effort to determine a suitable variable for scaling NR activity, relationships between different cell components and growth rate were examined. These relationships differed depending on the limiting factor. For example, under light limitation, cell volume and cell carbon content increased significantly with increased growth rate, while under nitrate limitation cell volume and carbon content decreased as growth rates increased. Despite the differences found between cell composition and growth rate under light and nitrate limitation, the relationships between NR activity scaled to different compositional variables and growth rate did not differ between the limitations. In field situations where cell numbers are not easily determined, scaling NR activity to particulate nitrogen content may be the best alternative. These results establish a strong basis for pursuing NR activity measurements as indices of nitrate incorporation in the field.

Key index words: Bacillariophyceae; cell composition; enzyme activity; light limitation; marine phytoplankton; nitrate incorporation rate; nitrate reductase; nutrient limitation; Thalassiosira pseudonana

Accurate measurements of rates of nitrate metabolism in marine phytoplankton are important in marine ecology because nitrogen often limits marine primary productivity (Ryther and Dunstan 1971) and because nitrate supports new production (Platt et al. 1992). Because of high variability in spatial and temporal distributions of phytoplankton and problems associated with commonly used incubation techniques (see e.g. Leftley et al. 1983), researchers have sought instantaneous biochemical measurements of rates of nitrogen metabolism.

One promising index of rates of nitrate incorporation is the activity of the enzyme nitrate reductase (NR) (e.g. Eppley et al. 1969, Blasco et al. 1984). However, the majority of work with NR suggests that the enzyme is not satisfactory as an index of nitrogen incorporation rates (see Eppley et al. 1969, Collos and Slawyk 1977, Dortch et al. 1979, Blasco et al. 1984). Reasons for these findings may relate to the inadequacy of earlier NR assays or to problems in interpreting the relationship between nitrate incorporation and NR activity, but it is difficult to distinguish between these possibilities because the majority of studies have examined transient states including nitrate exhaustion, additions of nitrogen sources other than nitrate, or variations in irradiance and nitrate concentration (e.g. Dortch et al. 1979, Slawyk and Rodier 1986, Smith et al. 1992). Steady-state relationships are necessary in order to validate the use of NR activity as an index of nitrate incorporation, but they have been largely neglected. Recently, an improved assay for NR was developed and validated in different species of marine phytoplankton (Berges and Harrison 1995). Using this improved NR assay, we have reexamined the relationship between NR activity and incorporation rates under different steady-state conditions.

If NR is to be useful in predicting nitrate incorporation, the two rates must be quantitatively related; ideally, NR would be rate-limiting (see Newsholme and Crabtree 1986, but see also Berges and Harrison 1993 for a discussion of the potential usefulness of non-rate-limiting enzymes). Experimentally, it can be difficult to establish a rate-limiting role for an enzyme, because a given enzyme may only be limiting in a reaction sequence under a particular set of conditions; more than one enzyme may be involved in rate control as conditions change. This means that it is important to examine NR activity and nitrate incorporation when different factors limit phytoplankton growth.

For our experiments, we selected two different limiting conditions. Light limitation restricts the rate at which photosynthesis can provide the cell with energy in the forms of ATP and reductant (NAD(P)H), and fixed carbon. A cell can make a variety of acclimations to changing irradiance, including changes in cell composition (Falkowski and LaRoche 1991). The situation under nutrient limitation is potentially more complex. Synthesis of structural components of the cell may be limited in a straightforward way, but for elements such as nitrogen, where the products are functional proteins involved in energy collection and transduction, these processes will also be affected (Cullen et al. 1992). It is also important to distinguish between "limitation" (the restricted supply of a nutrient) and "starvation" (the removal of a nutrient). A nutrient-limited cell may be able to make a range of acclimations to low nutrients, including changes in composition and photosynthetic parameters (Herzig and Falkowski 1989), but these strategies may not be available to a starved cell. The case of starvation is distinct and will be reserved for consideration in a future study.

For unialgal cultures in the laboratory, NR activity can be meaningfully scaled to cell numbers, but this becomes problematic in the field where organisms occur in multispecies assemblages. Thus, it is important to determine a variable suitable for scaling NR activity. The variable to which enzyme activity is scaled can affect the interpretation of the results (see Berges and Harrison 1993). For example, Dortch et al. (1979) grew *Skeletonema costatum* at two nitrate-limited growth rates (0.8 and 1.6 d^{-1}) and compared NR activities. When NR activity was scaled to cell volume, activity was higher in the lower growth rate culture, but when NR activity was scaled

to chlorophyll *a* (chl *a*), the high growth rate culture showed higher NR activity. Scaling NR activity to cell nitrogen or protein quotas resulted in no difference in NR activity between the cultures. The issue of scaling NR activity becomes especially important in comparing light and nutrient limitation; different growth rates affect the composition of cells, and evidence indicates that these effects differ between light- and nutrient-limiting conditions (see Rhee 1979, Morris 1981).

In this study, we have examined NR activity under two different steady-state limitations and one transient state. The principle questions addressed were a) how does NR activity vary with rates of growth and nitrate incorporation, b) is there evidence indicating that NR is rate-limiting for (and thus a good index of) nitrate incorporation, and c) considering how cell composition varies under light or nitrate limitation, to what parameter should NR activity be scaled when cell numbers cannot be determined.

MATERIALS AND METHODS

Cultures of *Thalassiosira pseudonana* (Hustedt) Hasle and Heimdal (3H clone) were obtained from the North East Pacific Culture Collection (NEPCC #58) and maintained on artificial medium (ESAW), based on Harrison et al. (1980) and modified as noted in Berges and Harrison (1993), at 17.5° C, under continuous light.

Steady-state light-limited experiments. Light-limited experiments were performed as described in Berges and Harrison (1993) using semicontinuous batch cultures and measuring specific growth rates (μ) by *in vivo* fluorescence or cell counts. Cell volumes were determined using a TAII Coulter Counter equipped with a 70-μm aperture and a population accessory. Cell carbon and nitrogen were measured in samples filtered onto precombusted 13-mm Gelman A/E filters using a Carlo Erba CNS analyzer with sulfanilamide as a standard. Samples for protein were collected on 25-mm Whatman GF/F filters and measured with a Coomassie Blue dye-binding assay as described in Berges et al. (1993) using bovine serum albumin (BSA) as a standard. Cell constituents were plotted against specific growth rate and analyzed by linear regression analyses using SYSTAT MGLH routines (Wilkinson 1990).

NR activity was measured using an assay based on Eppley (1978) but improved by the addition of BSA and Triton X-100 (Berges and Harrison 1995). Samples were always analyzed immediately after collection. NR was extracted in 200 mM phosphate buffer, pH 7.9, containing 5 mM EDTA, 0.1% (v/v) Triton X-100, 0.03% (w/v) dithiothreitol, 0.3% (w/v) polyvinyl pyrrolidone, and 3% (w/v) BSA. Assays were conducted in 200 mM phosphate buffer, pH 7.9, containing 0.2 mM NADH. Reactions were initiated by the addition of 10 mM KNO_3 and monitored spectrophotometrically following the oxidation of NADH at 340 nm in a temperature-controlled LKB Ultrospec II spectrophotometer interfaced to a personal computer (Berges and Virtanen 1993). Absorbance changes over time were converted to enzyme activity using a millimolar extinction coefficient of 6.22. This method was verified against the rate of nitrite production as previously described (Berges and Harrison 1995). NR activities were plotted against specific growth rates or calculated nitrate incorporation rates. Under these steady-state conditions, we define nitrate as being incorporated when it is retained in filtered samples and detectable by carbon-nitrogen analysis. Incorporation rates were calculated as the product of cell nitrogen content and cell specific growth rate.

Light transition experiments. Transition experiments were conducted as described in Berges and Harrison (1993). Six 1-L cultures of *T. pseudonana* were established, three at low light (15 μmol quanta$\cdot$m$^{-2}\cdot$s^{-1}) and three at high light (90 μmol quanta$\cdot$m$^{-2}\cdot$s^{-1}). The onset of light saturation (I_K) under these conditions was approximately 40 μmol quanta$\cdot$m$^{-2}\cdot$s^{-1} (see Berges and Harrison 1993). These cultures were acclimated for a minimum of eight generations, sampled at 0 and 24 h, and then transposed (i.e. high to low irradiance, H $\rightarrow$ L, or low to high irradiance, L $\rightarrow$ H) and sampled again at approximately 24-h intervals for 3 more d. At each sampling, cell volumes and numbers, cell carbon, nitrogen and protein, and NR activities were determined as before. Cultures were maintained in logarithmic growth phase, diluting with fresh medium as necessary. Nitrate incorporation rates were also calculated as in steady-state light-limited experiments. Within each time interval, differences in cell constituents, growth rates, NR activities, and nitrate incorporation rates were tested using paired *t*-tests (see Berges and Harrison 1993).

Steady-state nitrate-limited experiments. For three separate experiments with *T. pseudonana,* six nitrate-limited chemostats were set up in 1-L flasks that were mixed with Teflon-coated stir bars and magnetic stirrers and provided with an inlet for medium, an overflow for excess culture medium, and a sampling port. Chemostats were run by positive pressure; new medium was pumped into the culture using a multichannel peristaltic pump (Manostat model 10A), and excess culture was forced out the outflow by pressure. Growth rate was controlled by adjusting the rate of the peristaltic pump or the diameter of the tubing. Medium (ESAW) prepared for these experiments contained 40 μM nitrate and 4 mM bicarbonate. In each experiment, medium for six cultures was provided from a common 20-L reservoir. Cultures were judged to have reached steady state when the cell fluorescence and the concentration of phosphate (a nonlimiting nutrient) of the outflow remained constant over 2 d. At this point, cultures were sampled for cell volume and numbers, cell carbon, nitrogen, protein quotas, and NR activities, as described previously. In these experiments, chl *a* was also measured by fluorometric methods after extraction in 90% acetone (Parsons et al. 1984). Cell constituents were plotted against specific growth rate (calculated from dilution rate and culture volume, Rhee 1979) and analyzed by linear regression as before. NR activities were plotted against specific growth rates or calculated nitrate incorporation rates.

Scaling of NR activity. For *T. pseudonana* light- and nitrate-limited experiments, NR activity was scaled to cell volume, cell carbon, nitrogen, chl *a,* or protein quotas. These activities were plotted against specific growth rates and analyzed by linear regression analyses. In each case, regressions were performed for light-limited cultures alone, nitrate-limited cultures alone, and both sets of cultures together. Regression slopes and intercepts were compared following Steel and Torrie (1980).

RESULTS

Steady-state light-limited experiments. Significant and positive relationships with growth rate were seen only for cell volume (Fig. 1A, Table 1) and carbon (Fig. 1B, Table 1), indicating that cells growing faster have larger volumes and carbon contents. No statistically significant relationships were found for cell nitrogen (Fig. 1C), for cell protein (Fig. 1D), or in the C:N ratio (Fig. 1E) ($P > 0.4$ in all cases). In one replicate experiment of three cultures that used a separate batch of seawater medium, the cultures showed unusual behavior; cells became elongated and considerably greater in volume. This appeared to correspond to limitation by selenium, as previously described by Price et al. (1987). These cultures were excluded from regression analyses but are shown as open symbols in Figure 1.

NR activity was positively related to growth rate

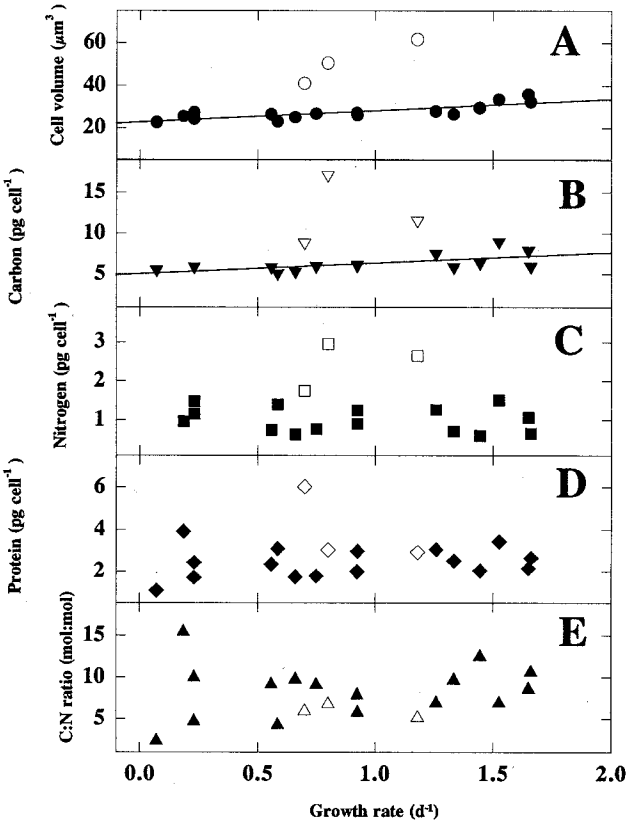

FIG. 1. Cell composition versus light-limited specific growth rate for *Thalassiosira pseudonana* grown between 6 and 120 μmol quanta·m^{-2}·s^{-1}. A) cell volume, B) cell carbon, C) cell nitrogen, D) cell protein, and E) cell C:N ratio. Each data point represents the mean of duplicate determinations from a single culture. Open symbols represent three cultures where selenium limitation may have occurred and were not used in the regressions. Lines represent statistically significant least-squares regressions. Regression parameters are given in Table 1.

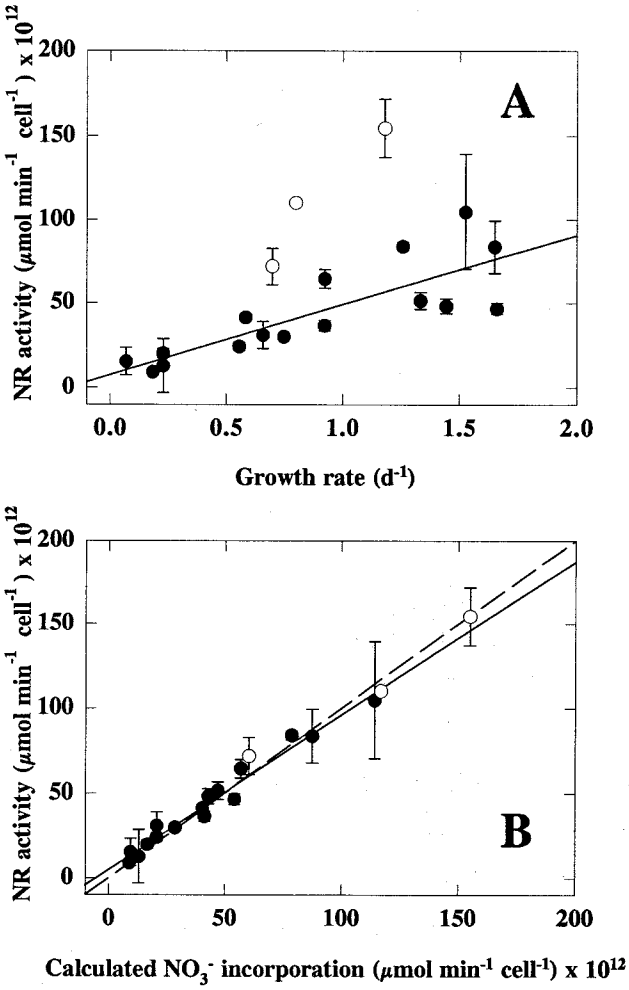

FIG. 2. NR activity versus light-limited growth rate in *Thalassiosira pseudonana*. A) NR activity versus specific growth rate, and B) NR versus calculated rate of nitrate incorporation. Each point represents the mean NR activity in a single culture. Error bars represent standard errors of the mean of two NR assays. Open symbols represent cultures where selenium limitation may have occurred and were not used in the regressions. Solid lines represent least-squares regressions. Dashed line represents the 1:1 relationship.

(NR = 7.46 + 41.6 (±7.86) μ, $R^2 = 0.67$, $P < 0.002$; Fig. 2A). As with the composition data, the cultures that were potentially selenium-limited were omitted from the analysis. The relationship between NR activity and the calculated rate of nitrate incorporation was not different from the 1:1 relationship (NR = 4.49 + 0.98 (±0.03) nitrate incorporation rate, $R^2 = 0.98$, $P < 0.001$; Fig. 2B). Selenium-limited cultures were indistinguishable in the regression analysis.

Light transition experiments. Composition data from the transition experiments did not follow all of the trends seen in the steady-state experiments. Cell volumes were no different between H → L and L → H cultures at any point during the experiment, although H → L cells were significantly smaller after the transition (Fig. 3A, $P < 0.01$). Some significant differences in carbon were seen. In agreement with steady-state experiments, H → L cells had higher carbon quotas than L → H cells before the transition, but changes in carbon quotas followed no pattern during the transition (Fig. 3B). In contrast to the steady-state data, L → H cells were significantly higher in nitrogen than H → L cells before the transition. By the end of the experiment, the L → H cells had lower nitrogen quotas and H → L cells had significantly higher nitrogen quotas (Fig. 3C). Largely because of differences in nitrogen, a similar pattern was seen in the C:N ratio (Fig. 3D).

Growth rates changed when cultures were transposed (Fig. 4A). There was some indication that when cells were moved from low to high light they actually increased their growth rate above that which the high light-grown cells had shown, but this difference disappeared by the end of the experiment. Throughout the transition experiment, the NR activity matched the nitrate incorporation rate almost perfectly (Fig. 4B).

TABLE 1. *First-order linear regression parameters for statistically significant composition versus growth rate relationships in light- or nitrogen-limited cultures of* Thalassiosira pseudonana. *P-values represent the probability that the slope is equal to zero.*

Limitation	Parameter	Slope	Intercept	R^2	*P*-value
Light	Cell volume	5.48	22.7	0.67	<0.001
	Carbon	1.30	5.06	0.41	<0.02
Nitrate	Cell volume	−10.8	40.2	0.46	<0.002
	Carbon	−5.80	11.9	0.41	<0.005
	Chl *a*	0.049	0.033	0.73	<0.001
	C:N ratio	−4.84	11.8	0.37	<0.008
	C:chl *a* ratio	−215	299	0.54	<0.001

Steady-state nitrate-limited experiments. No nitrate or nitrite was detected in chemostat outflows except in the case of the two highest dilution rates in each experiment where nitrate was between 0.4 and 2 μM and nitrite was approximately 0.2 μM.

Relationships between growth rate and cell composition were quite different during nitrate limitation than for steady-state light-limited experiments.

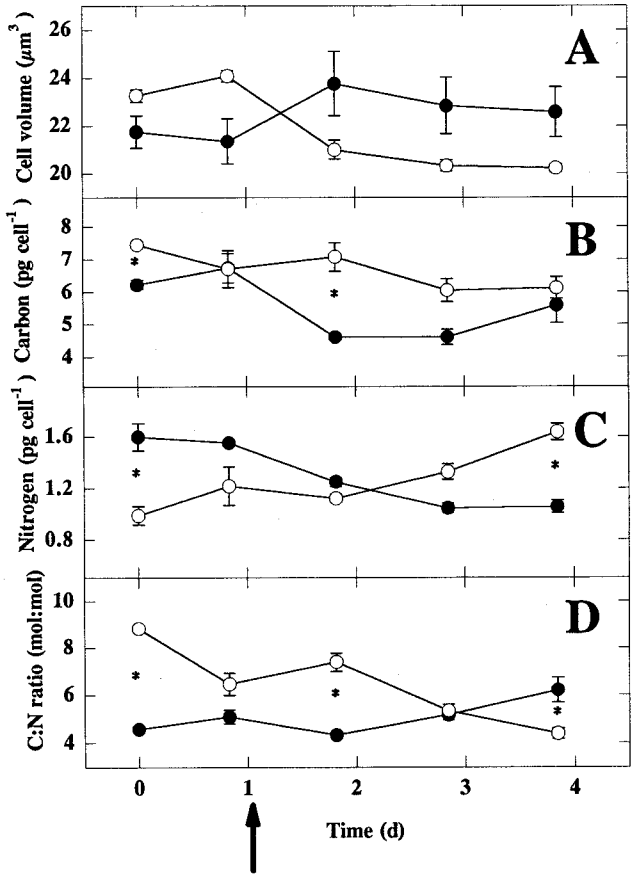

FIG. 3. Changes in cell composition on transition from low light (15 μmol quanta·m⁻²·s⁻¹) to high light (90 μmol quanta·m⁻²·s⁻¹) (●) or high light to low light (○) in *Thalassiosira pseudonana*. A) cell volume, B) cell carbon, C) cell nitrogen, and D) cell C:N ratio. Transitions were made at the point indicated by the arrow. Each point represents the mean and standard error of three separate cultures. Asterisks (*) indicate significant differences at $P < 0.05$.

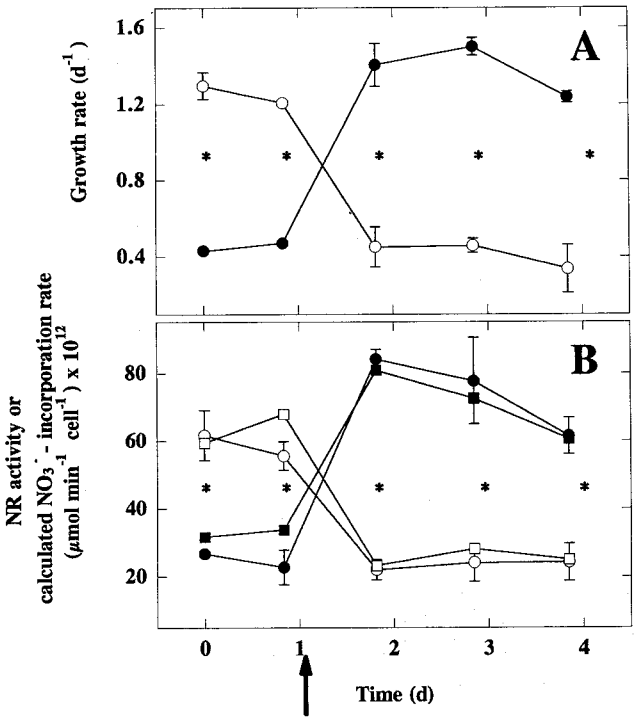

Fig. 4. Effects of transitions from high to low light (open symbols) or low light to high light (closed symbols) on A) growth rate and B) nitrate reductase activity (●, ○) or calculated nitrate incorporation rates (■, □) in *Thalassiosira pseudonana*. Transitions were made at the point indicated by the arrow. Each point represents the mean and standard error of three separate cultures. Asterisks (*) indicate significant differences at $P < 0.05$.

Significant negative relationships were found for cell volume and carbon versus growth rate (Fig. 5A, B, Table 1) in contrast to the positive relationships for light-limited cultures. Again, nitrogen and protein showed no significant relationships with growth rate (Fig. 5C, E; $P > 0.7$ in both cases). C:N ratios significantly declined as growth rate increased (Fig. 5F; Table 1). Chlorophyll *a* content of cells decreased as growth rates increased (Fig. 5D; Table 1), which, combined with carbon decreases, led to significant decreases in the C:chl *a* ratio with increasing growth rate (Fig. 5G; Table 1).

NR activities in chemostat cultures were positively correlated with growth rate (NR = 22.7 + 23.9 (±6.95) μ, $R^2 = 0.43$, $P < 0.004$; Fig. 6A) and positively related to calculated rates of nitrate incorporation (NR = 24.2 + 0.485 (±0.125) nitrate incorporation rate, $R^2 = 0.48$, $P < 0.002$; Fig. 6B). However, in contrast to light-limited experiments, the slope of the relationship was less than 1:1; NR activity was significantly higher than the calculated nitrate incorporation rate at low growth rates (i.e. $\mu < 0.4$ d^{-1}, or <25% of nutrient-replete growth rates).

Scaling of NR activity. NR activity scaled to cell volume (Fig. 7A), carbon quota (Fig. 7B), nitrogen quota (Fig. 7C), or protein (Fig. 7E) were all signif-

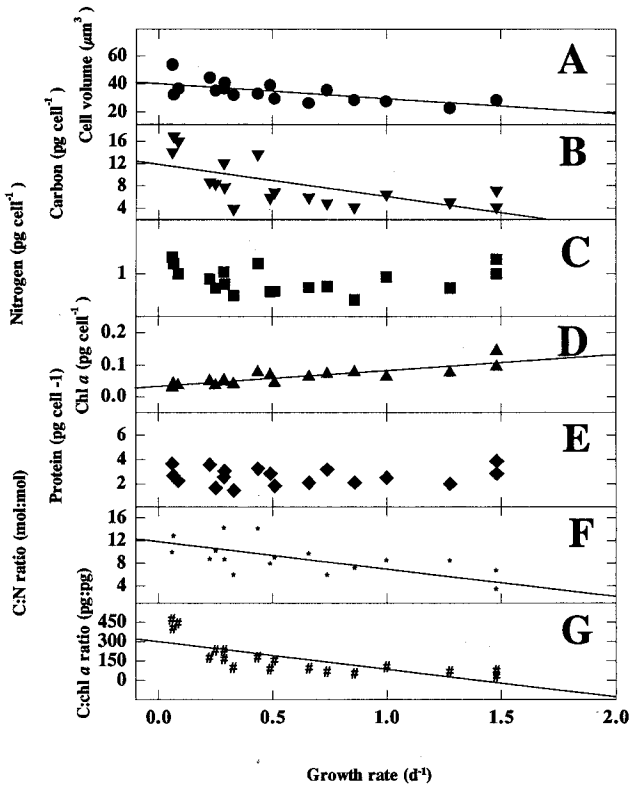

FIG. 5. Cell composition with growth rate in nitrate-limited chemostat cultures of *Thalassiosira pseudonana*. A) cell volume, B) cell carbon, C) cell nitrogen, D) cell chl *a*, E) cell protein, F) cell C:N ratio, and G) cell C: chl *a* ratio. Each point represents the mean of two determinations from a single culture. Lines represent statistically significant least-squares regressions. Parameters are given in Table 1.

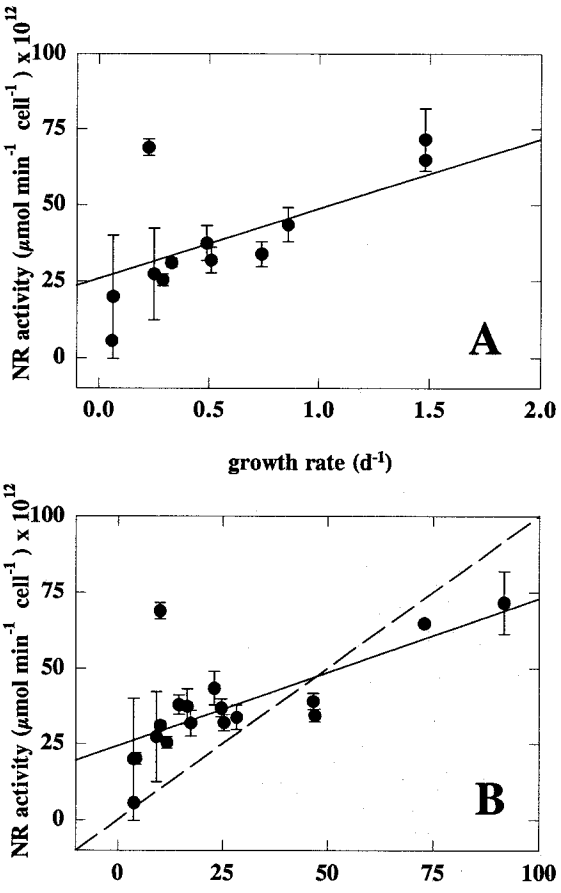

FIG. 6. Relationship between NR activity and A) specific growth rate or B) calculated rate of nitrate incorporation in nitrate-limited chemostats of *Thalassiosira pseudonana*. Each point represents the mean NR activity in a single culture. Error bars represent standard errors of the mean of two NR assays from a single chemostat. Solid lines represent least-squares regressions. Dashed line represents the 1:1 relationship.

icantly related to growth rate (Table 2), but this was not true for NR activity scaled to chl *a* (Fig. 7D). Where regressions were significant, no significant differences were found between relationships for light-limited versus nitrate-limited cultures ($P > 0.5$ in all cases).

DISCUSSION

Variation in NR activity with growth rate. NR activity per cell under light limitation was positively correlated with growth rate and quantitatively related to nitrogen incorporation rate. Thus, the NR activity was much more strongly related to growth than to factors such as cell size or cell composition. There are virtually no systematic laboratory studies of variation in NR activity with growth rate; most authors have chosen to investigate simple presence or absence of NR activity (e.g. Everest et al. 1986), NR activity in field situations (e.g. Packard et al. 1971, Blasco et al. 1984), or NR activity in cultures in transient states (e.g. Dortch et al. 1979, Clayton 1986, Smith et al. 1992). Data from studies in which nitrate incorporation rates and NR activity were compared are summarized in Table 3. Few other studies have found strong relationships between NR and nitrogen incorporation rates. In fact, only Morris and Syrett (1965) and Kristiansen (1987) found NR activity sufficient to account for observed nitrate incorporation rates, and relatively few studies compared cultures at different light-limited growth rates. The good correlations found in the present study are likely the result of improvement to the NR assay.

A good agreement between NR activity and growth rate under light limitation might not have been anticipated. If cultures were light-limited, this would be evident in a limitation of energy and a decreased ability to fix carbon, but this would not necessarily affect nitrogen uptake or incorporation. However, extensive evidence indicates that nitrogen and carbon metabolism are very closely connected. As Turpin (1991) pointed out, because protein content is high in algae, over 50% of all algal carbon is integrally coupled with nitrogen metabolism. Pace et al. (1990) and Kaiser and Brendle-Behnisch (1991) have also shown that there is a close coupling be-

TABLE 2. *Comparison of first-order linear regression parameters for nitrate reductase activity scaled to different parameters versus growth rate in* Thalassiosira pseudonana *in light-limited batch cultures (L), nitrate-limited chemostats (N), or both types of cultures together (both). P-values represent the probability that the slope is equal to zero.*

Parameter	Data sets	Slope	Intercept	R^2	*P*-value
Cell volume	L	1.25	0.81	0.48	<0.006
	N	1.12	0.50	0.75	<0.001
	Both	1.33	0.53	0.63	<0.001
Carbon	L	6.34	2.96	0.44	<0.02
	N	6.57	1.91	0.59	<0.001
	Both	6.78	2.12	0.56	<0.001
Nitrogen	L	49.6	5.18	0.69	<0.001
	N	23.9	30.3	0.30	<0.02
	Both	34.0	22.0	0.49	<0.001
Chl *a*	N				>0.6
Protein	L	13.9	11.0	0.32	<0.05
	N	7.33	10.2	0.34	<0.02
	Both	12.3	9.51	0.37	<0.001

tween photosynthesis and nitrate reduction in higher plants.

Transition experiments showed that the changes in NR activity in response to new photosynthetic regimes and growth rates occurred in less than 1 d. There is evidence indicating that it took up to 3 d after the transition in irradiance before a new steady state was reached (note that the L → H cultures temporarily increased growth rates above those of the H → L cultures before the transition), but throughout this period NR and nitrate incorporation rates were closely coupled. Most previous work on transitions has involved spike additions of nitrate or nitrogen starvation (Clayton 1986), and frequently it is not clear whether or not cells were initially in a steady state. Under these conditions, any time-dependent measurements (i.e. 3 h nitrate uptake) might not be expected to correlate well with an instantaneous enzyme measurement.

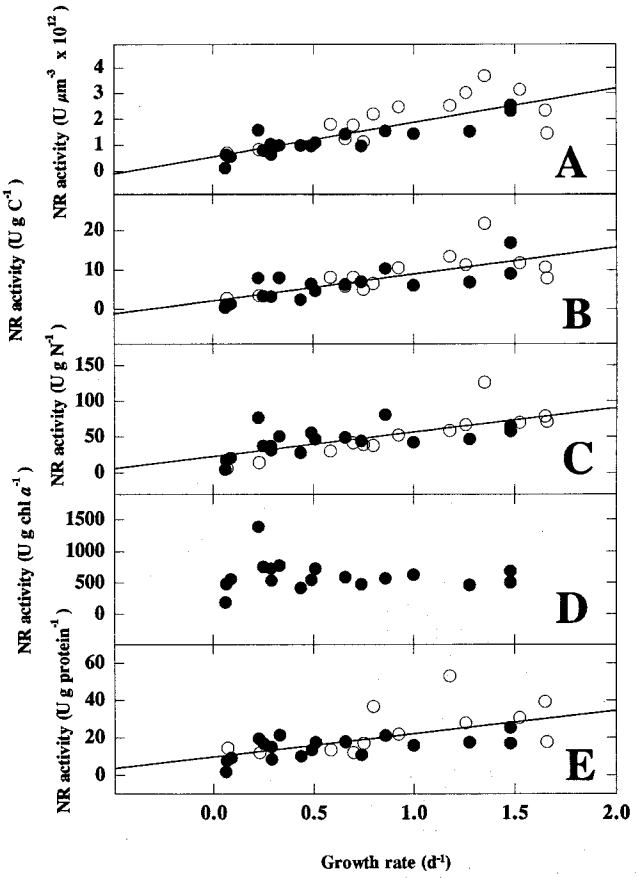

FIG. 7. NR activity scaled to different parameters versus growth rate of *Thalassiosira pseudonana* in light-limited batch (●) or nitrate-limited chemostat (○) cultures. A) cell volume, B) carbon, C) nitrogen, D) chl *a*, and E) protein. Each point represents the mean of two assays from a single culture. Lines represent statistically significant least-squares regressions. Regression parameters are given in Table 2.

TABLE 3. *Relationship of nitrate reductase activity with increasing growth rate and percentage of nitrate incorporation accounted for by NR (% NR/N) in various species under different limitations. Light is continuous and chemostats are nitrate-limited unless otherwise noted.*

Species	Conditions	Relationship	% NR/N	References
Light-limited				
Chaetoceros affinis	Nitrate spike	—	50	Slawyk and Rodier 1986
Skeletonema costatum	Nitrate spike	—	50	Smith et al. 1992
Skeletonema costatum	Steady state	—	<80	Clayton 1986
Skeletonema costatum	12:12 h LD	—	10–80	Kristiansen 1987
Skeletonema costatum	Steady state	Positive	100–200	Berges and Harrison 1995
Ditylum brightwellii	Steady state	—	25	Eppley et al. 1969
Thalassiosira pseudonana	Steady state	Positive	100	Present study
Chlorella vulgaris	Steady state	—	>100	Morris and Syrett 1965
Gonyaulax polyedra	12:12 h LD	Positive	50	Harrison 1976
Amphidinium carterae	Steady state	Positive	20	Berges and Harrison 1995
Nitrate-limited				
Chaetoceros affinis	Chemostat	—	338	Slawyk and Rodier 1986
Thalassiosira pseudonana	Chemostat	Positive	83–190	Present study
Thalassiosira oceanica	Chemostat	Negative	—	Eppley and Renger 1974
Skeletonema costatum	Chemostat	Positive	87–176	Dortch et al. 1979
Chlorella stigmatophora	Chemostat	Negative		Everest et al. 1986

As was the case under light limitation, NR in nitrate-limited chemostat cultures was well correlated with growth rate. However, the relationship between NR activity and calculated nitrate incorporation rates was not quantitative; NR at low dilution rates was much higher than the calculated rates, while NR at higher dilution rates was slightly lower than the calculated rate. There was always some nitrate and nitrite detected in the outflow of the chemostats run at the highest dilution rates. This could have occurred if the dilution rate was very close to the growth rate; minor fluctuations in either the pump rate or the growth rate of the cells may have resulted in periods when dilution was greater than growth and so growth rates calculated from dilution rates would be overestimates.

In the literature, trends in NR activity found in nitrogen-limited cultures vary widely (Table 3). The chemostat experiments of Eppley and Renger (1974) and Everest et al. (1986) both showed negative relationships; NR increased as growth rate decreased. Results of Dortch et al. (1979) Slawyk and Rodier (1986) are more similar to the present study. Although results from these two studies are based on only three cultures in total, they indicated that NR activity at low dilution rates exceeded calculated incorporation rates (174 and 338%), while at the high dilution rates NR was closer to the calculated rate (87%).

NR activity in the present study exceeded that needed to account for observed rates of nitrate incorporation at low nitrate-limited growth rates. Nitrogen-limited cells develop the ability to rapidly take up limiting nutrients (so-called "surge uptake," see Conway et al. 1976). It is possible that cells at low growth rates maintain NR at higher levels than needed in anticipation of periods of rapid uptake (see Slawyk and Rodier 1986). Alternatively, Ingemarsson (1987) suggested that at low growth rates in the duckweed, *Lemna,* it is the flux of nitrate (i.e. the transport step) and not the NR activity that is limiting. This agrees with data from Dortch et al. (1979) showing a correlation between NR activity and internal nitrate concentration, but not with those of Collos and Slawyk (1977), where this relationship was not seen.

For diatoms, long periods of nutrient limitation may not be commonly experienced in the field. Typically, diatoms are first in successional patterns; they dominate at high nutrient concentrations due to their rapid division rates (see Guillard and Kilham 1977). Later, as nutrients are exhausted, other species such as flagellates replace the diatom community, which often sinks to the pycnocline. Thus, nutrient-limited chemostats run at low dilution rates may have little relevance for natural populations of diatoms (see also Rhee 1979, Zevenboom 1986), and this may explain some of the unusual features of cell composition and NR activity at low growth rates.

NR and the control of nitrate incorporation. The strong relationship between NR activity and nitrate incorporation provides support for the idea that NR is rate-limiting for this process. In biochemical terms, NR fits many of the characteristics of a rate-limiting enzyme; that is, NR has a complex structure (Solomonson and Barber 1990), it catalyzes a nonequilibrium reaction (K_{eq} is on the order of 10^{25}–10^{40}, and $\Delta G^{o\prime}$ is very large and negative, Solomonson and Barber 1990), and there is also evidence indicating that NR, like many regulatory enzymes, is regulated by phosphorylation (Huber et al. 1992). Furthermore, there are data showing that nitrate pools accumulate within tissues and cells (Dortch et al. 1979, Dortch 1982, Campbell 1988). In some cases, intracellular nitrate can represent up to 55% of nitrogen in cells of *S. costatum,* although it is more normally only a few percent (e.g. in nitrate-sufficient *T. pseudonana,* nitrate pools were 2.8% of particulate nitrogen). Furthermore, in higher plants NR activities are low relative to other enzymes in the pathway of nitrate incorporation (e.g. NiR, GS) (see Guererro et al. 1981). For marine phytoplankton, this may also be true for many species, but there are very few data (see review by Collos and Slawyk 1980).

On the other hand, there is evidence indicating that, in specific cases, NR may not be rate-limiting. Both Ingemarsson (1987) in *Lemna* and Watt et al. (1992) in *Chlamydomonas reinhardtii* present evidence that nitrate uptake is limiting, particularly at low nitrate supply. Because NiR and NR are coinduced in higher plants and apparently closely coregulated (Redinbaugh and Campbell 1991), NiR could also be limiting, but virtually nothing is known about the activity or regulation of NiR in marine phytoplankton. Regulation at the ammonium assimilation step may also occur. Seguineau et al. (1989) proposed that GS played a key role in nitrate incorporation and nitrogen metabolism in general in *Dunaliella primolecta,* based largely on the very high degree of regulation of the enzyme. Alternatively, it may be that the entire pathway adapts to the prevailing nitrate incorporation rate; Stewart and Rhodes (1977) have shown that NR and GS activities closely parallel each other in higher plants.

Cell composition and scaling of NR activity. The reason for the observed relationships between various cell constituents and growth rate are based on complex physiological mechanisms that are beyond the scope of the present study. However, they are relevant in deciding which variables are likely to be useful in scaling NR activity in the field. Results of the present study suggest that relationships between composition and growth rate are likely to differ, depending on the specific limiting factor. Under light limitation, for example, cell volume and carbon increased with increasing growth rate. These results agree with the majority of previous studies (see Thompson et al. 1991). In contrast, nitrate limita-

tion resulted in lower cell volumes and carbon content as growth rate increased. Volume changes in the opposite direction were reported for nitrate-limited diatoms (Eppley and Sloan 1966, Caperon and Meyer 1972), but similar trends in reduced carbon content with increased growth rate have also been seen (Caperon and Meyer 1972, Rhee 1979). These variations suggest that volume and carbon content may be responding to uncontrolled variables. Furthermore, the absolute values of these constituents vary by a factor of greater than two between studies, even when the same clonal isolate is used (i.e. comparing results of the present study with those of Caperon and Meyer 1972 and Thompson et al. 1991). The precise reasons for these differences is unknown, but such variability over time in the size of clonal isolates is well documented (Armbrust and Chisholm 1992). No changes in cell nitrogen or protein were noted under either limitation. This is consistent with results from studies where cells were light-limited (e.g. Geider et al. 1985, Sakshaug and Andresen 1986, Claustre and Gostan 1987) but is at odds with nitrate-limitation experiments that frequently show increased nitrogen contents with growth rate (e.g. Caperon and Meyer 1972, Zevenboom 1986, Herzig and Falkowski 1989). This discrepancy might be explained by the fact that the present study includes cells grown at unusually low dilution rates compared to the majority of those in the literature. If nitrate-limited cultures growing at less than 0.4 d^{-1} are eliminated, there is a significant, positive relationship between cell nitrogen and growth rate ($P < 0.05$).

Given the range of differences in cell composition and the different responses of cell constituents to different limitations, it might have been anticipated that NR activity scaled to a given biomass variable would correlate poorly with growth rate. In fact, this is not so; in all cases except chl *a*, scaled NR activity was significantly and positively related to growth rates and the light-limited cultures were no different from the nitrate-limited chemostat cultures. The variability was high; however, typically only 50–60% of the variance in scaled NR activity was explained by growth rate. As discussed in Berges and Harrison (1993), this may be the result of the variation in the biomass measurement increasing variability in the scaled enzyme data. The scaling problem is not an issue in the laboratory, but it becomes critical in the field. Scaling of NR to carbon is problematic because of the large amounts of detrital carbon found in marine waters, but while nitrogen potentially suffers the same problem, Dugdale and Wilkerson (1991) found that nitrogen could be used as a scaling factor for nitrogen uptake without apparent interference from nonphytoplankton sources. Scaling enzyme activity to protein is convenient and is frequently done, but, as discussed in Berges et al. (1993), it is uncertain what commonly used spectrophotometric assays for protein actually measure. The relationship between NR scaled to protein and growth rate was also one of the poorer relationships found. Cell volume may offer an alternative, but this would require tedious microscopic measurements, which have large errors associated with them (Montagnes et al. 1994). As is the case for nitrate uptake rates, scaling NR activity to particulate nitrogen seems to be the most practical course.

In summary, in this study, strong relationships between NR and growth rates and rates of nitrate incorporation were demonstrated under steady-state culture conditions. The relationship in *Thalassiosira pseudonana* is better under light limitation than nitrate limitation, where NR activity tends to exceed nitrate incorporation rates at low growth rates. Low nitrate-limited growth may not be a common situation for marine diatoms and thus may not be ecologically relevant and of less importance to the use of NR activity in the field. These findings suggest that the control of nitrate reduction may be at the level of the enzyme under steady-state conditions. The 1:1 relationship between NR activity and nitrate incorporation (particularly under light limitation) implies that NR activity can indeed be used to quantitatively predict metabolic rates *in vivo*. Enzyme scaling to biomass parameters is somewhat problematic because cell composition changes with growth rate are different depending on the specific limiting factor. However, this appears to be severe only in the case of chl *a*. We suggest that NR be scaled to particulate nitrogen, based on the problems found in accurately measuring alternatives such as carbon, cell volume, or protein.

It is important to note that the conclusions of the present study apply only under steady states or, for light transitions, between steady states. Some nonsteady states invoke special regulatory features of NR (see Solomonson and Barber 1990) that may lead to an uncoupling between *in vitro* and *in vivo* activity of the enzyme; in a future study, we consider relationships between NR activity and nitrate incorporation rate under diel periodicity in irradiance and nitrate exhaustion and in the presence of ammonium.

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