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Nitrate uptake kinetics by two marine diatoms using the radioactive tracer ^{13}N

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Abstract: The response of the nitrate uptake system to nitrate concentrations in the range 1 to 500 μM was examined with the radioactive isotope ^{13}N for two marine diatoms (*Skeletonema costatum* and *Thalassiosira weissflogii*) under various states of nitrogenous nutrition. Classical saturation kinetics were observed in the lower range of concentrations (1–40 μM), but a further increase in uptake was observed above 60 μM. Intracellular nitrate concentrations between 5 and 17 mM indicate that it is an active uptake system rather than a diffusion phenomenon which is responsible for uptake at those high external nitrate levels.

Key words: Eutrophic area; Nitrate uptake kinetics; ^{13}N label

Introduction

The kinetics of nitrate uptake by marine unicellular algae reported by Eppley et al. (1969) and MacIsaac & Dugdale (1969) have formed the basis for numerous models of nitrate utilization in the oceans (Coste & Slawyk, 1974; Dugdale, 1977; Falkowski, 1977). There remains, however, a number of problems with those earlier generalizations. First, the use of variable incubation times in the case of Eppley et al. (1969), for example, can lead to a distortion of kinetic curves (Colles, 1983; Harrison et al., 1989). Second, it was later shown that uptake parameters vary with the physiological state of the cells (Caperon & Meyer, 1972; Collos, 1980; Dortch et al., 1991a, b). Contradictory data also exist on this topic in the literature. For example, using the same species under practically the same conditions and the same technique as Eppley et al. (1969), Serra et al. (1978) found evidence for involvement of a diffusion phenomenon in nitrate uptake by *Skeletonema costatum* above 6 μM. More recently, the same value of nitrate concentration was identified as a possible threshold for increases in nitrate uptake by natural populations of marine phytoplankton (Dugdale & Wilkerson, 1990). Finally, the range of concentrations (0.5–10 μM) used by Eppley et al. (1969) may be too limited and not fully representative of natural situations. First, values up to 50 μM are observed

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in the euphotic zone of upwelling systems (Kokkinakis & Wheeler, 1987) and those concentrations are certainly exceeded in numerous coastal and estuarine waters (Sharp, 1983). Second, the nitrate concentrations reported above are determined in bulk samples, but much higher concentrations (up to 300 μM) have been reported in micropatches (Shanks & Trent, 1979). Those values may still be underestimates because sample volumes were too large to be considered as relevant to phytoplankton spatial scales (Allen, 1977; Harris, 1980).

It was therefore deemed appropriate to re-examine the kinetics of nitrate uptake by phytoplankton given the current importance of nitrate in estimating new production (sensu Dugdale & Goering, 1967) in the global carbon cycle. The radioactive isotope ^{13}N was used as a tracer of nitrate in the present investigation. This allowed the use of short incubations which are best to characterize kinetics of nutrient uptake (Clarkson, 1986; Harrison et al., 1989), as well as small amounts of material which are not appropriate for mass spectrometry (in the case of the ^{15}N isotope for example).

Methods

Cultures of marine phytoplankton

Two species were obtained from the Northeast Pacific Culture Collection (NEPCC), Department of Oceanography, University of British Columbia: *Skeletonema costatum* (NEPCC No. 18c) and *Thalassiosira weissflogii* (NEPCC No. 636). They were maintained in batch cultures in 2 l of autoclaved ESAW medium (Harrison et al., 1980) modified as in Price et al. (1987) in 3-l flat-bottomed flasks, under continuous light (at a photosynthetically available radiation level of 80 $\mu E \cdot m^{-2} \cdot s^{-1}$) in a water bath at 19 °C. Cultures were stirred mechanically twice each day. Cultures were either nitrogen-sufficient (initial nitrate 100–500 μM) or nitrogen-starved for 24 h.

Before incubation with ^{13}N-labelled nitrate, *Thalassiosira* was concentrated by reverse flow filtration on 10-μm mesh netting and resuspended in fresh ESAW medium without nitrate. This was done in order to rapidly reduce the nitrate concentration in the medium. Cell recovery was 80% and ^{13}N uptake did not change before and after concentration, indicating no physiological disturbance of the cells. Nitrate concentration after resuspension was between 4 and 12 μM. Such a procedure could not be used with *Skeletonema* as it led to a major reduction in nitrate uptake rate after concentrating the cells. Instead, for this species, the initial nitrate concentration in the medium was 100 μM and the culture was used in the experiment when the nitrate concentration was close to exhaustion (but still above 10 μM). Growth was monitored daily in batch cultures by in vivo fluorescence (calibrated vs. chlorophyll *a*) and cell counts. A range of growth rates was obtained by using cultures at different phases of the growth cycle of the batch culture. Nitrate was monitored daily by UV absorption (Collos et al., in prep.).

PRODUCTION AND PURIFICATION OF $^{13}NO_3$

The procedures were modified from Siddiqi et al. (1989) and Glass et al. (1990). Labelled nitrate was produced by proton irradiation of water on the TRIUMF cyclotron at the University of British Columbia. Contaminants such as ^{18}F were removed by passing the sample through a Sep-Pak Alumina N cartridge (Waters Associates) twice. $^{13}NO_2$ was removed by boiling with H_2SO_4 and H_2O_2. $^{13}NH_4$ was removed by passing through a cation exchange column. The acid was then neutralized with 0.4 g $CaCO_3$ and the sample filtered on a Whatman GF/F glass-fiber filter. 2 mg of catalase was added to the filtrate to remove the remaining H_2O_2, and the isotopic solution was used without further modification. The specific activity of the purified isotopic solution varied between 0.74 and 40.7 MBq·μmol·N^{-1}.

MEASUREMENT OF UPTAKE

Multiple substrate additions and short constant incubation times were used (Harrison et al., 1989). All incubations took place in 100-ml glass filtration funnels which were custom-made from stock 15-ml Millipore funnels, in a controlled environment room at 20 ± 2 °C and a photosynthetically available radiation level of 90 $\mu E \cdot m^{-2} \cdot s^{-1}$. The funnels were ready for subsequent filtration (i.e. with a filter in place). 50 ml aliquots of the culture were poured into the funnels. The nitrate concentration was first adjusted with cold sodium nitrate (50–300 μl of concentrated solutions: 1–500 μM

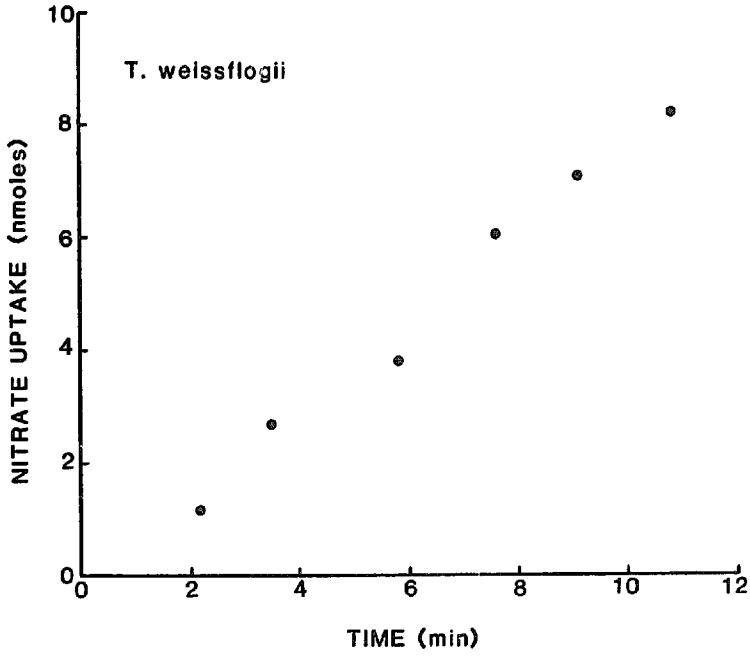

Fig. 1. Cumulative nitrate uptake by 50 ml aliquots of a culture of *Thalassiosira weissflogii* as a function of time. Biomass = 3.8 μg Chl $a \cdot l^{-1}$. Growth rate = 0.61 d^{-1}.

final concentrations), then the labelled nitrate was added (100–300 μl typically). Incubations lasted for about 10 min (measured exactly). Following filtration under reduced (10 cm Hg) vacuum, the filters were rinsed with nitrate-free ESAW medium, placed in scintillation vials and counted immediately in a γ-counter. After counting, the same filters were used for extraction of intracellular nitrate as in Collos (1982). The filters were put into standard 15-ml Millipore filtration manifolds and 25 ml of deionized distilled water was slowly passed through the filters. Nitrate concentrations in the medium and intracellular pools (after extraction) were measured by the method described in Grasshoff et al. (1983). Uptake was expressed on the basis of cell volume rather than particulate nitrogen or cell numbers because the former reflects differences in cell nitrogen content and the latter cannot be used to compare species of different sizes (Dortch, 1982).

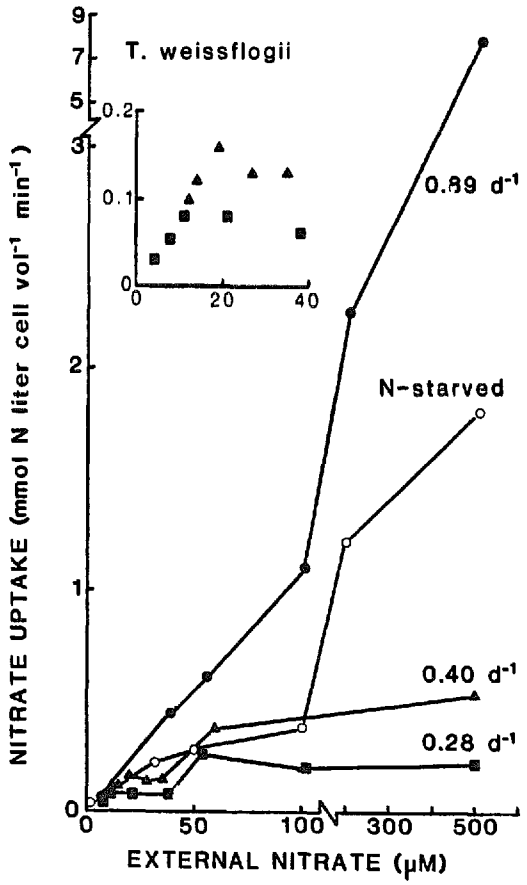

Fig. 2. Nitrate uptake by *Thalassiosira weissflogii* as a function of nitrate concentration and physiological state. Growth rates are indicated above each curve. N-starved cells were obtained from the batch culture that was growing at 0.89 d^{-1} one day earlier. Inset: nitrate uptake in the low concentration range.

RESULTS

Nitrate uptake was constant with time between 2 and 11 min of incubation (Fig. 1), indicating that, under the experimental conditions, any concurrent efflux of tracer was negligible relative to influx (Lee & Drew, 1986; Siddiqi et al., 1989).

Nitrate uptake by cells of *Thalassiosira weissflogii* as a function of increasing nitrate concentrations is shown in Fig. 2. When enough data points were obtained (e.g. cells growing at a rate of 0.28 and 0.40·d^{-1}), classical saturation kinetics were observed in the lower concentration range (10–40 μM; see inset). However, above these values, there was a dramatic increase in uptake which exhibited saturation at the lowest growth rate tested (0.28·d^{-1}), but not at higher growth rates. N-starved cells which were obtained from the batch culture growing at 0.89·d^{-1} a day earlier, showed a reduced capacity for uptake over the whole nitrate concentration range (2–4-fold difference).

The same patterns were exhibited by *Skeletonema costatum* (Fig. 3) with a marked increase in uptake above 100 μM for both N-sufficient and N-deficient cells. As in the other species, N starvation caused a decrease in uptake rate over the whole concentration range (2–14-fold difference). In both species, the nutritional state of the cells seemed to have an effect on nitrate uptake as values were reduced for N-starved cells.

Measurements of intracellular nitrate concentrations (Fig. 4) confirmed this general trend with a marked increase above 50 μM external nitrate in both species. In *Skeletonema costatum*, the values were similar for both N-sufficient and N-starved cells.

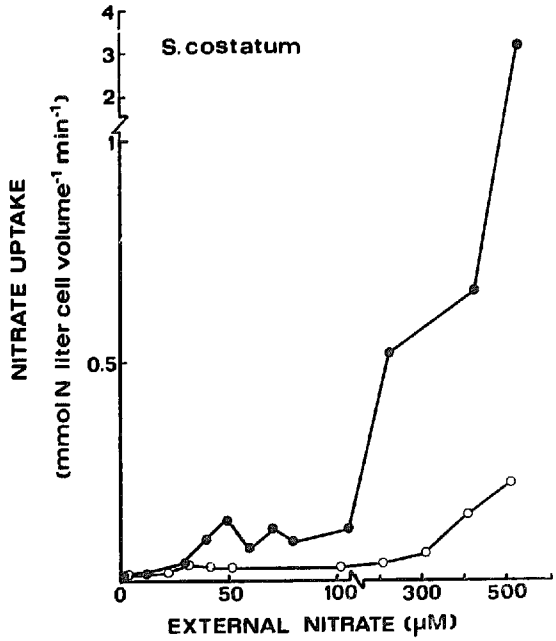

Fig. 3. Nitrate uptake by *Skeletonema costatum* as a function of nitrate concentration and physiological state. N-starved cells were deprived of nitrogen for 24 h. N-sufficient cells growth rate = 0.18 d^{-1}.

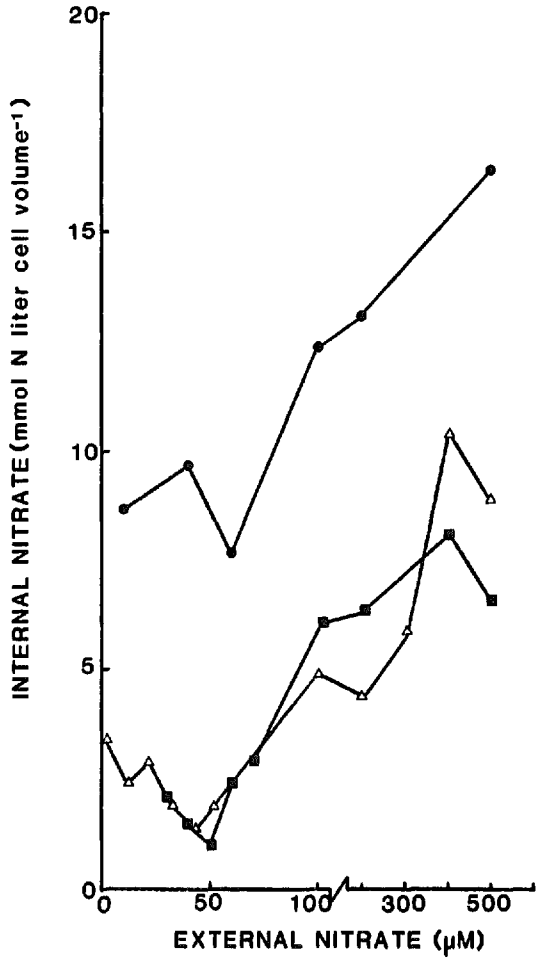

Fig. 4. Internal nitrate concentrations on a cell volume basis as a function of external nitrate concentrations after a 10 min incubation. *Thalassiosira weissflogii* (circles); *Skeletonema costatum* N-sufficient cells (squares) and N-starved cells (triangles).

In order to compare uptake and growth rates, nitrate uptake values for N-sufficient cells were normalized to particulate nitrogen (PN) estimated from chlorophyll *a* values using the 1 μg Chl *a*/1 μgat PN-N equivalence (Strickland, 1965). Table I shows that, at least for *Thalassiosira*, at low growth rates, the uptake rate at 500 μM nitrate is close to the growth rate, but at high growth rates, the uptake is an order of magnitude higher.

Discussion

The most striking feature of the above results is the increase in uptake above 100 μM in both species. This is similar to findings in higher plant studies (Pace & McClure,

TABLE I

Comparisons of growth rate and specific nitrate uptake at a substratum concentration of 500 μM. [a] Nitrate = 200 μM (same experiment as in Fig. 1).

Species	Growth rate (d^{-1})	Uptake rate (d^{-1})	Uptake/growth
Thalassiosira weissflogii	0.28	0.26	0.9
	0.40	0.49	1.2
	0.61[a]	0.92	1.5
	0.89	8.1	9.1
Skeletonema costatum	0.18	1.57	8.7

1986; Siddiqi et al., 1990). Such patterns are generally interpreted as evidence for either a second uptake system or a diffusion phenomenon. However, from considerations of the internal nitrate concentrations (5–17 mM, see Fig. 4), it is clear that uptake cannot possibly be diffusive. Note that those values are minimum estimates because they are based on total cell volume. We could not use the vacuole volume because the subcellular location of the internal nitrate pool is not known (Dortch, 1982). Evidence from higher plant research (Siddiqi et al., 1991) points to a $2H^+:1NO_3^-$ symport as has been proposed by Ullrich & Novacky (1981) and McClure et al. (1990).

Concerning *Skeletonema*, our results confirm those of Serra et al. (1978) rather than those of Eppley et al. (1969). For N-starved cells (Fig. 3), which are comparable to those of Serra et al. (1978) (24 h vs. about 60 h of starvation in their case), we observed an increase in uptake up to about 30 μM. Substrate saturation occurred until a nitrate concentration of about 100 μM was reached, and then uptake increased again. Data from Raimbault & Mingazzini (1987) on the same species tend to support this increase above 100 μM. Serra et al. (1978) used variable incubation times which can lead to distorted kinetic curves (Collos, 1983; Harrison et al., 1989). However, in their case, uptake was shown to be constant over the incubation period, so their data seem to be reliable.

In the lower concentration range (< 100 μM), nitrate uptake by N-sufficient cells of *S. costatum* was 2–4-times greater than N-deficient cells (Fig. 3), in agreement with findings by Dortch et al. (1982) on the same species. In the higher range, the same ratio ranged between 4 and 15.

Concerning intracellular nitrate, the present results confirm previous observations (Dortch, 1982) that external nitrate concentrations higher than 100 μM are necessary to saturate the internal nitrate pools of *S. costatum*. In *Thalassiosira nordenskioldii*, Dortch et al. (1984) found that a first plateau in intracellular nitrate was between 25 and 60 μM external nitrate, but a further (almost 2-fold) increase was noted between 60 and 100 μM external nitrate, which is consistent with our observations (Fig. 4).

There are large intergeneric differences in accumulation of nitrate pools in phytoplankton (Collos, 1982; Dortch et al., 1984). The species tested here belong to genera which appear to have large internal pools of nitrate (Dortch et al., 1984). Other gen-

era such as *Amphidinium*, *Chaetoceros* or *Dunaliella* accumulate very little nitrate (Collos, 1982; Dortch et al., 1984). It may therefore be an ecological advantage for those species capable of rapidly accumulating large amounts of nitrate in a variable environment, even if the high uptake rates do not necessarily translate instantly into growth.

There is considerable evidence for uncoupling between uptake and growth during nitrate assimilation in microalgae (Eppley & Thomas, 1969; Collos, 1982; Dortch, 1982; Dortch et al., 1984). The increase in such uncoupling with increasing growth rate (Table I) is similar to observations by Zehr et al. (1988) for *Dunaliella tertiolecta* and *Thalassiosira pseudonana*.

The significance of the high uptake rates described here becomes obvious in estuarine waters where 350 μM of nitrate can be found (Sharp, 1983) as well as in microzones of nitrification such as those described by Shanks & Trent (1979), where concentrations of nitrate can reach 300 μM. These features of the nitrate uptake system should be incorporated into future models of nitrate uptake in such areas. Present models consider that substrate saturation occurs near 10 μM, and thus they underestimate uptake of nitrate by some phytoplankton species under conditions where high concentrations occur in aquatic environments.

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