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Saturated uptake kinetics: transient response of the marine diatom *Thalassiosira pseudonana* to ammonium, nitrate, silicate or phosphate starvation

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Abstract

Changes in the saturated uptake kinetics of the limiting nutrient were followed as *Thalassiosira pseudonana* (Clone 3 H) batch cultures entered ammonium, nitrate, silicate and phosphate starvation. Cultures starved of ammonium or phosphate developed very high specific uptake capacities over a 24 to 48 h starvation period, due to both decreases in cell quota and increases in uptake rates per cell. In particular, the cell phosphorus quota decreased ca. 8-fold during phosphate starvation and specific uptake rates exceeded 100 d^{-1}. In contrast, cultures entering nitrate or silicate starvation underwent little or no further cell division, and the uptake capacity declined during starvation. After 24 to 48 h starvation, an induction requirement for uptake of nitrate or silicate was apparent. These responses are consistent with adaptation to the pattern of supply of these nutrients in the field.

Introduction

This study examines the development of enhanced uptake capacities for the limiting nutrient in batch cultures of *Thalassiosira pseudonana* starved of nitrate, phosphate or silicate. It represents an extension of a previous study of changes in ammonium uptake capacities during ammonium-limited growth transients using the same organism (Parslow *et al.*, 1984b). The rationale for such studies was developed in some detail in the earlier paper. Briefly, the study of phytoplankton nutrient limitation could be said to be in the middle of a paradigm shift. Recent studies have emphasized the importance to phytoplankton dynamics of fluctuations in nutrient supply (e.g. McCarthy and Goldman, 1979; Turpin and Harrison, 1979; Legendre, 1981; Parsons *et al.*, 1983). However, our present models of nutrient-limited uptake and growth dynamics (e.g. Droop, 1977; Burmaster, 1979) are based primarily on results from steady-state chemostat cultures (Rhee, 1980). In order to develop a theory which can be applied under fluctuating conditions, the assumptions and predictions of these models must be tested under transient conditions (e.g. Cunningham and Maas, 1978).

Uptake rates have played a key role in this theory. When saturating levels of the limiting nutrient are added to steady-state chemostat cultures, the specific uptake rate exceeds the maximum growth rate, in some cases by a wide margin (Caperon and Meyer 1972b; Eppley and Renger, 1974; Conway *et al.*, 1976; Davis, 1976; Laws and Caperon, 1976; Rhee, 1978; McCarthy and Goldman, 1979; Gotham and Rhee, 1981a, b; Goldman and Glibert, 1982). These rates may also decline quickly, on time scales of minutes (Conway *et al.*, 1976; McCarthy and Goldman, 1979) or even seconds (Goldman and Glibert, 1982) following the nutrient addition. This behaviour has been interpreted as an adaptation to fluctuating nutrient levels in the environment. However, if natural populations are exposed to fluctuating nutrient levels, their physiological states may not be comparable to those of steady-state chemostat cultures. Studies of the development of uptake capacities in cells exposed to changing nutrient conditions are required.

The study of transients is intrinsically more complicated than the study of steady states, because so many kinds of transients are possible. A simple way to generate transients is to allow batch cultures in exponential growth to exhaust the ambient nutrient and starve. A number of studies have contrasted uptake kinetics in batch cultures under nutrient-replete and nutrient-starved conditions (e.g. Conway and Harrison, 1977; Dortch *et al.*, 1982). If kinetic information is to be obtained, the time course of starvation must be followed. For some species and nutrients, this time course may involve a range of cell nutrient quotas comparable to the range observed in steady state at all dilution rates (e.g. Brown and Harris, 1978; Collos, 1980; Dortch *et al.*, 1984).

The present study follows transient responses in uptake capacity for different limiting nutrients using similar tech-

niques and time scales. It was intended to allow comparison across nutrients, as well as to provide new information for each nutrient. One might expect *a priori* that differences among nutrients in the pattern of supply, and/or the biochemistry of uptake and assimilation, would have produced differences in phytoplankton responses; however, comparisons among nutrients have hitherto been complicated by differences among studies in the techniques used and/or the organisms studied.

Materials and methods

Culture

Thalassiosira pseudonana (Clone 3H) from the North-East Pacific Culture Collection, Department of Oceanography, University of British Columbia, was grown under constant blue light at an irradiance of 80 μE m^{-2} s^{-1} and at a temperature of 18 °C in an artificial seawater medium (ESAW; Harrison *et al.*, 1980). Further details of culture conditions and modifications to the medium can be found in Parslow *et al.* (1984b). The nitrate enrichment was reduced from 550 to 100 μM for nitrate-limited cultures. Both silicate- and phosphate-limited cultures received the full 550 μM nitrate addition, while silicate and phosphate additions were respectively reduced to 4.7 and 1.8 μM. The cultures were maintained in exponential growth, with the limiting nutrient above 50% of the enrichment level, for at least 5 d preceding any starvation time course.

Biomass was monitored as *in vivo* fluorescence, on a Turner Model 10 fluorometer, and as cells l^{-1} with a Coulter Counter® Model TAII. The difference between the ambient concentration of the limiting nutrient and its enrichment level was assumed to have been incorporated into phytoplankton biomass. This difference was used in calculating cell quotas and specific rates from disappearance traces.

Uptake rates

Uptake rates were measured as disappearance of the limiting nutrient, using a self-cleaning filter technique described previously (Parslow *et al.*, 1984a). In the case of ammonium and phosphate limitation, where uptake rates were generally enhanced and changed rapidly over the first few minutes following the saturating nutrient addition, the ambient concentration was measured continuously. For both nutrients, a short-term, 2 μM perturbation was used to obtain continuous estimates of saturated uptake rates over the first 10 min, while a long-term 5 μM perturbation yielded continuous estimates of saturated uptake rates from 10 to 40 min after the nutrient addition. Only the average uptake rates over the periods from 0 to 1 min, 1 to 5 min, and 10 to 30 min after the nutrient addition, are presented here. Both saturated uptake rates per cell (ϱ_m^{0-1}, ϱ_m^{1-5} and ϱ_m^{10-30}) and saturated specific-uptake rates (V_m^{0-1}, V_m^{1-5} and V_m^{10-30}) were calculated, and are presented here as a function of starvation period.

In the case of nitrate and silicate, uptake rates were much lower and changed much more slowly following the nutrient perturbation. Subsamples were perturbed to 5 μM and the ambient concentration measured at 18-min intervals over a 2 h period. Average uptake rates were calculated over time intervals chosen to demonstrate increases or decreases in uptake rate during the experiment. Uptake rates are again presented on a per cell basis (e.g. ϱ_m^{0-18}) and specific to cell nutrient (e.g. V_m^{0-18}).

Results

Nitrate limitation

The transient response to nitrate starvation was complicated by the release of nitrite by *Thalassiosira pseudonana* during growth on nitrate. When frequent monitoring of the ambient nitrogen concentration commenced, there was 8 to 10 μM nitrate and 3 to 6 μM nitrite present (Fig. 1 A). Nitrate was subsequently taken up preferentially, with the uptake of nitrite accelerating as the ambient nitrate concentration approached zero. The nitrite concentration reached zero approximately 4 h after the nitrate disappeared, and this time was designated, somewhat arbitrarily, as zero-hours starvation. However, changes in growth rate and presumably in cell nitrogen status occurred well before the depletion of inorganic nitrogen. The growth rate in exponential phase was 1.7 d^{-1}, but this declined to an average rate of 0.9 d^{-1} over the 4 h preceding nitrate depletion. At 0 h of starvation, the cell quota was 7.8 × 10^{-2} pg-at. N cell^{-1} (Fig. 1 B), similar to cell nitrogen

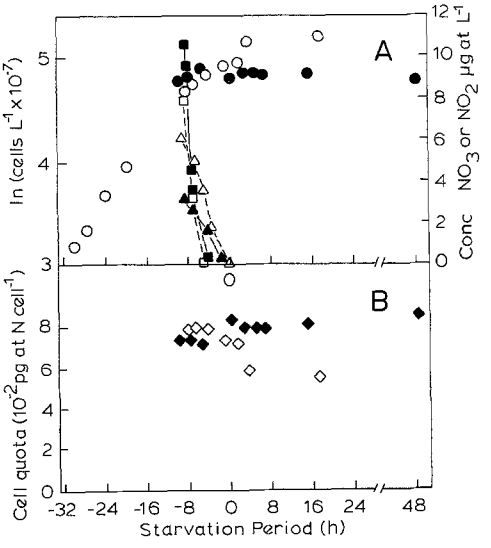

Fig. 1. *Thalassiosira pseudonana.* (A) Changes in cell density (circles), ambient nitrate (squares) and ambient nitrite (triangles) in two replicate nitrate-starved batch cultures; (B) Changes in cell nitrogen quota in same two replicate nitrate-starved batch cultures shown in (A)

quotas in ammonium-grown cultures at corresponding times (Parslow *et al.*, 1984b). However, of the two replicate nitrate-grown cultures, only one showed a modest increase in cell number (Fig. 1A) and consequent decline in cell quota (Fig. 1B) as starvation continued.

The observed perturbation uptake rates also indicated a decline in activity preceding nitrate and nitrite exhaustion. Some 6 to 8 h before the disappearance of external nitrite, the specific uptake rate for nitrate, obtained on perturbing the remaining 5 to 10 μM NO_3 with a 5 μM NO_3 addition, had declined to between 0.5 and 0.7 d^{-1}. This rate is well below the maximum specific growth rate, μ_{max}, but similar to the cell division rate at that time. As nitrogen starvation continued, the uptake rate during the first hour of perturbation experiments declined rapidly, approaching zero after 24 h of starvation (Fig. 2A, B). The uptake rate during the second hour of the perturbation experiments declined more slowly (Fig. 2C, D), so that an induction of uptake became more pronounced with increasing starvation period. However, in 48 h-starved cultures, the specific uptake rate recovered only slightly, to 0.1 to 0.4 d^{-1}, after 2 h exposure to 5 μM NO_3. An induction period considerably longer than 2 h must be required for specific uptake rates in 24 or 48 h-starved cultures to recover to the level of μ_{max}.

Ammonium uptake rates following ammonium additions to nitrate-starved batch cultures were also measured. The initial uptake rate, V_m^{0-1}, was already considerably enhanced, at 9 d^{-1}, 6 h prior to the 0 h starvation point (Fig. 3). This rate increased further, reaching a level of 18 d^{-1} after 4 h starvation. The "long-term" rate, V_m^{5-10}, which is close to V_m^{10-30} for ammonium uptake (Parslow *et al.*, 1984b) was close to μ_{max} when first measured, and increased relatively little, to 3.2 d^{-1}, after 5 h starvation.

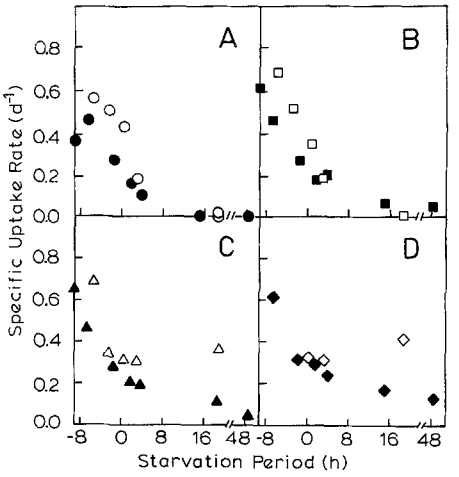

Fig. 2. *Thalassiosira pseudonana.* Changes in nitrogen-specific nitrate uptake rates (V_m) for two replicate nitrate-starved batch cultures. (A) V_m^{0-18}, (B) V_m^{18-54}, (C) V_m^{54-96}, and (D) V_m^{96-132}

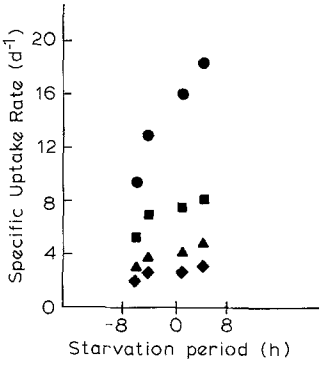

Fig. 3. *Thalassiosira pseudonana.* Changes in nitrogen-specific ammonium uptake rates in a nitrate-starved batch culture: ● = V_m^{0-1}, ■ = V_m^{1-2}, ▲ = V_m^{2-5}, and ◆ = V_m^{5-10}

Silicate limitation

Observations from a total of six replicate cultures showed little or no increase in cell numbers following the depletion of external silicate (Fig. 4). An apparent decline in cell numbers after 48 h of starvation was attributed to cell clumping and the onset of sexual reproduction. Total cell volume remained approximately constant up to and including 48 h starvation. The average cell number from 0 to 24 h starvation was 1.28×10^8 cells l^{-1}, yielding a Si quota of 3.7×10^{-2} pg-at. $cell^{-1}$.

Following silicate perturbation, the uptake rate of silicate tended to decline during the (2 h) perturbation uptake experiments conducted early in the starvation time course. Averaging over experiments conducted during the first 12 h of silicate starvation, the specific uptake rate declined from 7.7 d^{-1} during the first 18 min, to 5.6 d^{-1} during the next 36 min and 4.2 d^{-1} during the last 54 min. After 24 and 48 h of starvation, the decline in uptake rate during the first hour following a silicate addition was reversed in the second hour. This reversal may reflect a partial recovery from the physiological stress of silicate

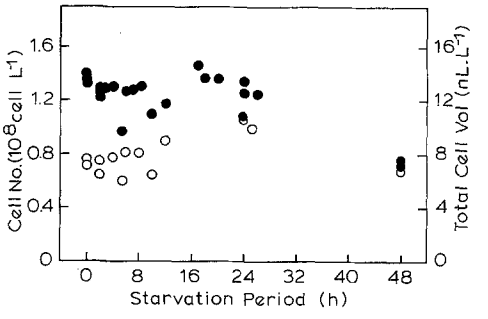

Fig. 4. *Thalassiosira pseudonana.* Changes in cell density (●) and total cell volume (○) in several replicate silicate-starved batch cultures

starvation which was indicated by cell-clumping in 24 and 48 h-starved cultures. Overall, uptake rates declined steadily with increasing starvation period, except for an apparent increase over the first 2 to 4 h of starvation (Fig. 5). The maximum specific uptake rate observed was 11 d^{-1}, or abour six times μ_{max}.

Phosphate limitation

Approximately three cell divisions occurred after the depletion of external phosphate. The cell quota dropped correspondingly from 1.62×10^{-2} pg-at. cell^{-1} to 0.22×10^{-2} pg-at. cell^{-1} (Fig. 6). The cultures maintained a growth rate equal to μ_{max} (1.7 d^{-1}) during the first 12 h of starvation. The growth rate slowed to an average 1.25 d^{-1} from 12 to 24 h of starvation and again to an average 0.45 d^{-1} from 24 to 48 h of starvation. Cell division ceased after that time.

The specific uptake rates measured in perturbation experiments showed a dramatic increase with starvation period (Fig. 7). The initial rate increased most quickly with starvation. At zero hours, V_m^{1-5} was already 5.3 d^{-1}, or three times μ_{max}. After 4 h of starvation, V_m^{1-5} had reached 24 d^{-1}. The first measurements of V_m^{0-1}, at 4 h of starvation, averaged 46 d^{-1}, and V_m^{0-1} increased to a maximum of 125 d^{-1} after 24 h. The long-term rate, V_m^{10-30}, increased more slowly with phosphate starvation, being less than half V_m^{0-1} after 24 h of starvation. However, V_m^{10-30} continued to increase up to 48 h, by which time the distinction between initial and long-term uptake had almost disappeared. The maximum value observed for V_m^{10-30} was 101 d^{-1}, or about sixty times μ_{max}.

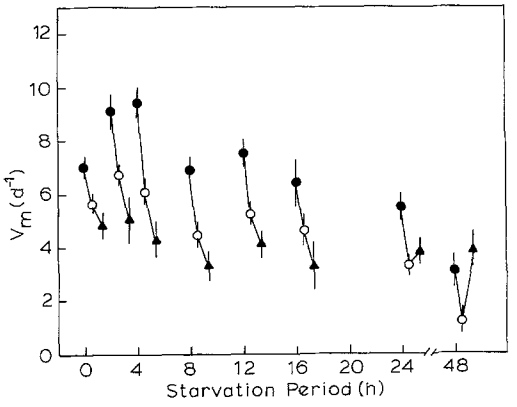

Fig. 5. *Thalassiosira pseudonana.* Changes in silica-specific silicate uptake rates in replicate silicate-starved batch cultures. ● = V_m^{0-18}, ○ = V_m^{18-54}, and ▲ = V_m^{54-108}. Vertical bars indicate ±1 SE

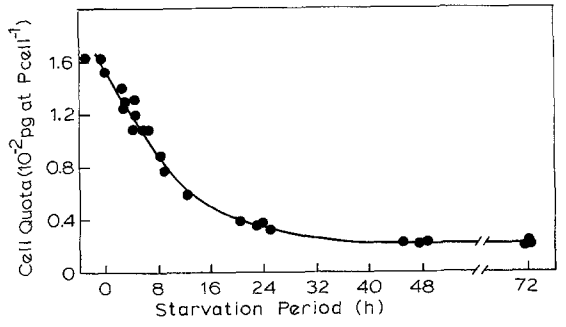

Fig. 6. *Thalassiosira pseudonana.* Changes in cell phosphorus quotas in three replicate phosphate-starved batch cultures

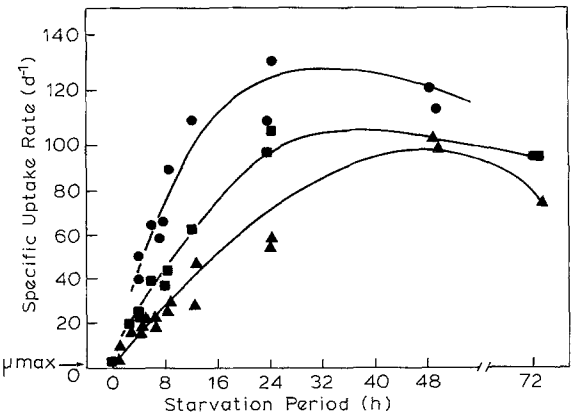

Fig. 7. *Thalassiosira pseudonana.* Changes in phosphorus-specific phosphate uptake rates in replicate phosphate-starved batch cultures. ● = V_m^{0-1}, ■ = V_m^{1-5}, and ▲ = V_m^{10-30}. μ_{max}: maximum specific growth rate

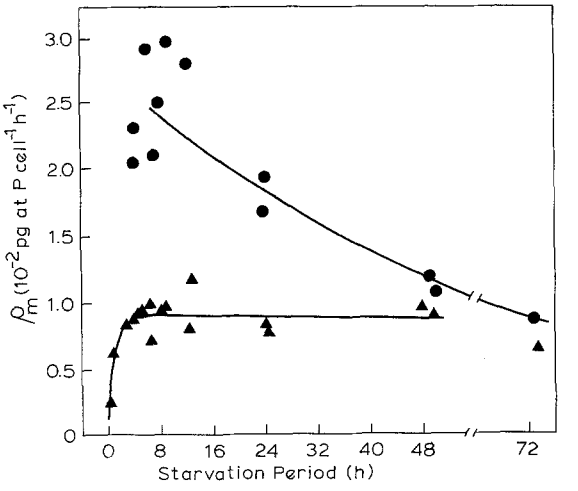

Fig. 8. *Thalassiosira pseudonana.* Changes in phosphate uptake rates (ϱ_m) per cell in replicate phosphate-starved batch cultures. ● = ϱ_m^{0-1}, and ▲ = ϱ_m^{10-30}

The expression of uptake rates on a per cell basis gave a rather different pattern (Fig. 8). The long-term rate, ϱ_m^{10-30}, increased about 4-fold during the first 4 h of starvation, but then remained constant up to 48 h. The increase in V_m^{10-30} after 4 h of starvation can therefore be attributed to the decline in cell quota. The initial uptake rate per cell, ϱ_m^{0-1}, was high when first measured (at 4 h starvation), but declined continuously after 8 h starvation, approaching ϱ_m^{10-30} after 48 and 72 h of starvation.

Discussion

Nitrate

The response of batch cultures of *Thalassiosira pseudonana* to nitrate starvation contrasted strongly with the response to ammonium starvation (Parslow *et al.*, 1984 b). Both the

cell division rate and the perturbation uptake rate declined while ambient levels of nitrate and nitrite were relatively high (5 to 10 μM). This decline continued during nitrogen starvation, and initial uptake rates following nitrate additions approached zero after 20 h starvation. When this pattern was first observed, it seemed possible that the culture had become light-limited through self-shading, or limited by some other nutrient. However, a test culture grown on medium containing 200 μM nitrate maintained a growth rate close to μ_{max} until external nitrate levels again fell to ca. 10 μM.

A marked feature of the nitrate-grown cultures was the release of nitrite into the medium, and its subsequent utilization following nitrate depletion. The excretion of nitrite by phytoplankton growing on nitrate has been reported under a variety of conditions. Olson *et al.* (1980) reported the excretion of nitrite by *Thalassiosira pseudonana* in continuous culture under nitrate limitation. In that study, the excretion rate of nitrite varied, reaching a maximum of 5% of the rate of nitrate reduction. The cyanophyte *Oscillatoria rubescens* was observed to excrete nitrite in batch culture, although the initial nitrate enrichment was very large at 10 mM (Ohmori, 1978). Other studies have reported the transient excretion of nitrite following nitrate perturbations to continuous cultures of diatoms (Collos, 1982 a, b), to starved batch cultures (Serra *et al.*, 1978) and to natural populations (Harrison and Davis, 1977).

The early decline in saturated nitrate uptake rates may have been caused by a switch to nitrite uptake. However, nitrate-starved batch cultures have typically shown low perturbation uptake rates. Estimates of V_m in the range 0.2 to 1.2 d^{-1} were reported for the diatoms *Skeletonema costatum*, *Asterionella japonica* and *Nitzschia longissima* (Romeo and Fisher, 1982). *Phaeodactylum tricornutum* showed a small increase in V_m from 1.3 d^{-1} at 0 h starvation to 2.5 d^{-1} at 48 h starvation (Collos, 1980). However, the enhanced uptake rate was only obtained after several hours exposure to high nitrate levels. The development of an induction requirement for nitrate uptake following nitrate starvation has also been noted previously (Serra *et al.*, 1978).

In contrast to nitrate-starved cultures, nitrate-limited chemostat cultures have consistently shown enhanced specific uptake rates (3 to 7 d^{-1}) in perturbation uptake experiments (Caperon and Meyer, 1972 b; Laws and Caperon, 1976; Laws and Wong, 1978; Rhee, 1978; Collos and Slawyk, 1979; Collos, 1980; Gotham and Rhee, 1981 b; Romeo and Fisher, 1982; Terry, 1982). Part of this difference in the response to starvation and steady state limitation can be associated with differences in cell quota. Cell quotas remained high during nitrate starvation of *Thalassiosira pseudonana* in this study. In contrast, cell quotas in nitrate-limited chemostats are significantly lower at lower dilution rates (e.g. Caperon and Meyer, 1972 a). Uptake rates per cell also declined during nitrate starvation in this study. This could be due to the loss of an active uptake system (Falkowski, 1975) or to the inactivation of the nitrate reductase system (Syrett, 1981). However, inactivation of the nitrate reductase system alone need not prevent initial nitrate uptake, since other marine diatoms have been observed to form internal nitrate pools (Dortch, 1982).

In the present study, nitrate-starved batch cultures of *Thalassiosira pseudonana* did develop strongly enhanced initial uptake rates ($V_m^{0\,-1}$) for ammonium. These results agree with previous reports of the development of enhanced uptake rates for reduced forms of nitrogen by cells grown on oxidized forms (Horrigan and McCarthy, 1982). The long-term specific uptake rates ($V_m^{5\,-10}$) for ammonium in nitrate-starved cultures were low compared with long-term rates in ammonium-starved cultures (Parslow *et al.*, 1984 b). Again, this may be explained by the relatively large cell quotas in nitrate-starved cells, since much of the increase in specific uptake rates in ammonium-starved cells was associated with a decrease in cell quota.

Silicate

Following the depletion of external silicate by *Thalassiosira pseudonana*, there was an abrupt cessation of cell division, and little subsequent increase in total cell volume. It is generally accepted that Si starvation of diatoms results in the cessation of cell division (Werner, 1977), as frustules cannot be completed. The maximum specific uptake rates for silicate observed in this study (ca. 7 d^{-1} or four times μ_{max}) were comparable to or higher than rates reported for silicate-limited continuous cultures of marine diatoms (Conway *et al.*, 1976; Davis, 1976; Conway and Harrison, 1977). The observation of a decline in uptake rate on a relatively long time scale of the order of an hour following the silicate addition is also consistent with results reported previously for *Skeletonema costatum* (Conway *et al.*, 1976).

Si-specific uptake rates by *Thalassiosira pseudonana* in this study declined with increasing starvation period up to 48 h starvation. In an earlier study of *T. pseudonana*, cultures starved of silicate for 48 h showed a relatively low uptake rate, ca. 1.5 d^{-1} (Nelson *et al.*, 1976), over 4 h incubations. In this study, after 48 h of starvation, the uptake rates over the first hour of the silicate perturbation experiment declined to 1.0 d^{-1}, but this rate then increased to about 3 d^{-1} in the second hour. An induction requirement for Si uptake after long-term (72 h) starvation has been reported for *Skeletonema costatum* and *Chaetoceros debilis* (Conway and Harrison, 1977).

The results shown in Figs. 4 and 5 were unexpected in that the cultures showed characteristics one might associate with severe Si stress (cessation of cell division, maximal enhancement of uptake rates) at zero-hours starvation. This is probably due to the relatively low initial silicate enrichment in the medium. Half-saturation constants for silicate uptake and growth have typically fallen in the range 0.7 to 2.3 μM (Paasche, 1973; Conway *et al.*, 1976; Davis, 1976; Nelson *et al.*, 1976). Even if a specific uptake rate close to μ_{max} had been maintained by the batch culture down to very low silicate concentrations, it would

have taken approximately 8 h to remove the last 2 μM external silicate. Thus, cells are likely to have experienced a degree of Si limitation for some hours prior to the nominal zero-hours starvation point, and an enhanced uptake capacity may have developed during this period. The cell quota observed in this study at 0 h starvation, 3.7×10^{-2} pg-at. cell^{-1}, was intermediate between the minimum cell quota of 2.1×10^{-2} pg-at. cell^{-1} and the maximum cell quota of 5.7×10^{-2} pg-at. cell^{-1}, reported by Paasche (1973) in Si-limited chemostat studies of *Thalassiosira pseudonana* (Clone 3H).

Phosphate

The response of *Thalassiosira pseudonana* to phosphate starvation can be characterized as an exaggerated version of the ammonium response reported by Parslow *et al.* (1984b). Cell division continued for approximately 24 h of ammonium starvation, resulting in a 2-fold decrease in cell nitrogen quota (Fig. 2 of Parslow *et al.*, 1984b). Under phosphate starvation, cell division continued for a longer period, and resulted in an eight-fold decrease in phosphate cell quota. The minimum cell quota (q_0) of phosphate observed in this study (2.2×10^{-3} pg-at. cell^{-1}) exceeded values of q_0 reported in the literature for this species (0.9 fg-at. cell^{-1}, Fuhs, 1969; 0.68 fg-at. cell^{-1}, Perry, 1976).. It is not clear whether cell division stopped before the quota dropped to q_0 in starved cultures, or whether the cultures studied here had a larger q_0. The ratio of maximum to minimum quotas observed here during the starvation transient is similar to that observed by Fuhs (1969) in steady state cultures over a range of dilution rates.

Previous studies of perturbation uptake rates using a variety of species in P-limited steady state culture have shown one of three patterns of uptake rate vs dilution rate (Gotham and Rhee, 1981a). Some authors have found uptake rates per cell (ϱ_m) to be independent of dilution rate (e.g. Burmaster and Chisholm, 1979). Others have found ϱ_m to be constant, except for a sudden decline as the dilution rate approaches μ_{max} and steady state ambient phosphate levels become detectable (Fuhs *et al.*, 1972; Healey and Hendzel, 1975; Nyholm, 1977). The third pattern involves a smooth decline in ϱ_m with increasing dilution rate (Gotham and Rhee, 1981a). The data obtained for long-term uptake per cell (ϱ_m^{10-30}) in this study were consistent with the second pattern. A rapid increase in ϱ_m^{10-30} accompanied a small decrease in cell quota following the depletion of external phosphate. This uptake rate then remained constant over a wide range of decreasing cell quotas. Similar results were reported for P-starved batch cultures of the cyanophyte *Nostoc* sp. (Brown and Harris, 1978). In the present study the initial uptake rate, ϱ_m^{0-1}, did not behave according to any of the above patterns, but declined with cell quota after the first 8 h of starvation. At 4 h starvation, ϱ_m^{0-1} greatly exceeded the level required for balanced growth, V_m^{0-1} being almost thirty times μ_{max}. Presumably ϱ_m^{0-1} underwent a rapid increase as the external phosphate was depleted. In this respect, *Thalassiosira pseudonana* behaved similarly to *Scenedesmus quadricauda* (Healey and Hendzel, 1975).

The ratio $\varrho_m^{0-1}:\varrho_m^{10-30}$ changed with starvation period, being highest at 4 to 8 h starvation, and declining to near unity after 48 h of starvation. Many studies of perturbation uptake rates by P-limited or P-starved cultures have not reported rapid transients in uptake rate. This may be due to the condition of the cultures or to inadequate time resolution in perturbation uptake studies. At a low dilution rate (0.17 d^{-1}), a chemostat culture of *Thalassiosira pseudonana* (Perry, 1976) yielded values of ϱ_m close to the maximum values of ϱ_m^{0-1} observed in this study. The earlier study did not report the duration of uptake experiments nor the existence of rapid uptake transients. It may be that continuous cultures can maintain high uptake rates per cell at lower cell quotas and for longer periods than batch cultures, resulting in a superior capacity for P-specific uptake.

Under ammonium starvation, the specific uptake rates exhibited by *Thalassiosira pseudonana* immediately following saturating ammonium additions increased to about 20 d^{-1} after 24 h (Fig. 9), although long-term uptake rates were lower at about 8 d^{-1}. The specific uptake rates for phosphate observed in this study were much higher. After 48 h of starvation, even the long-term rate, V_m^{10-30}, reached ca. 4 h^{-1} or 100 d^{-1}, sixty times μ_{max}. These high rates were the result of an early increase in uptake rate per cell and a continuing decline in cell phosphate quotas, which are of course much lower than cell nitrogen quotas. It is intriguing, though possibly coincidental, that the maximum long-term uptake rates of ammonium and phosphate, expressed on a per cell basis, are similar (ca. 10^{-2} pg-at. cell^{-1} h^{-1}).

Comparison among nutrients

The responses of *Thalassiosira pseudonana* to starvation by the limiting nutrients ammonium, nitrate, silicate and phosphate can be grouped into two categories (Fig. 10). The response to ammonium or phosphate starvation in-

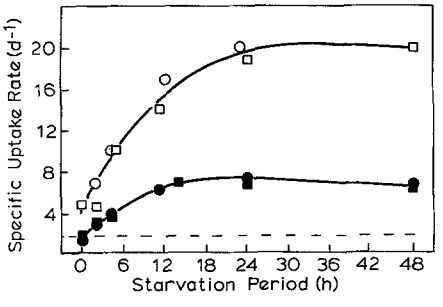

Fig. 9. *Thalassiosira pseudonana.* Changes in nitrogen-specific ammonium uptake rates in ammonium-starved batch cultures. Open symbols denote short-term rates (V_m^{0-1}) and filled symbols denote long-term rates (V_m^{10-30}) (from Parslow *et al.*, 1984b)

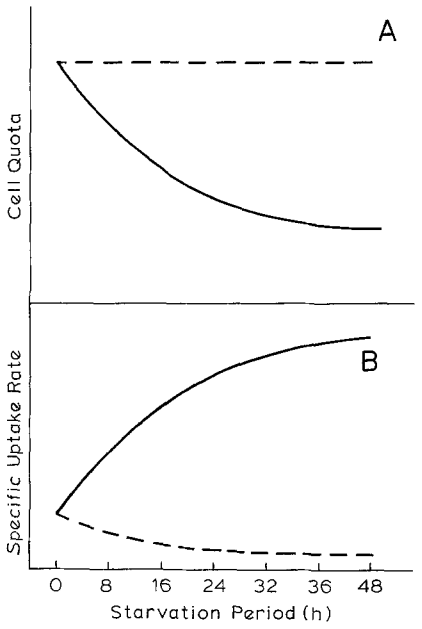

Fig. 10. *Thalassiosira pseudonana.* Idealized responses to starvation of cell quota (A) and specific uptake rate (B) for two classes of nutrient characterized by ammonium and phosphate (continuous lines) and by nitrate and silicate (dashed lines)

volves the continuation of cell division for 1 to 2 d following external nutrient depletion and the development of strongly enhanced uptake rates. These rates decline rapidly over the first minutes following limiting nutrient additions, except in the later stages of phosphate starvation. The response to nitrate or silicate starvation involves a cessation of cell division when the external nutrient is depleted, a decline in uptake rates with starvation period and the development of an induction requirement for uptake.

This study and others indicate that cultures of *Thalassiosira pseudonana* starved of ammonium and phosphate develop uptake capacities for these nutrients which are generally comparable to those of continuous cultures with similar cell quotas (Perry, 1976; Parslow *et al.*, 1984b). For these nutrients, one may be able to extrapolate from observations of transients on one time scale, such as the long-term starvation transients studied here, to transients on different time scales. Nitrate- and silicate-starved batch cultures develop very different uptake capacities from nitrate- and silicate-limited continuous cultures, so that extrapolation to other time scales is more difficult. For example, it is not clear whether the elevated uptake rates observed in nitrate-limited chemostat cultures would be maintained in semi-continuous cultures pulsed at intervals of hours or days. The development of comprehensive models of nitrate- and silicate-limited growth and uptake transients will require studies on a wide range of time scales.

As Dortch *et al.* (1982) have pointed out previously in a comparison of ammonium and nitrate starvation, these patterns of response seem to correspond in a general way to patterns of nutrient availability in the field. Ammonium and phosphate are both rapidly recycled and, in oligotrophic environments, form pools with high turnover rates. Nitrate and silicate are recycled on much longer time scales and are supplied to the euphotic zone primarily by physical events such as upwelling, wind-mixing and tidal mixing, at intervals of days, weeks or longer.

The assignment of specific ecological roles to the uptake capacities reported here is in some cases still controversial. The transient elevated uptake rates for ammonium or phosphate have been interpreted as adaptations to microscale nutrient patches generated by zooplankton (McCarthy and Goldman, 1979; Lehman and Scavia, 1982). Following this interpretation, we could point out, for example, that *Thalassiosira pseudonana* could, on the basis of our observations, double its cell P quota each day if exposed to saturating phosphate concentrations for just 12 min each day. However, the contribution of microscale patches in the field has been seriously challenged on the grounds that these patches disperse very rapidly due to diffusion (Jackson, 1980; Williams and Muir, 1981). As we have pointed out previously (Parslow *et al.*, 1984b), high saturated uptake rates may also be an adaptation to increase growth rates at very low uniform concentrations. At steady state, the specific uptake rate must equal the growth rate μ, so one can write:

$$\mu = V_m S^* (K_s + S^*)^{-1},$$

where K_s is the half-saturation constant, and S^* is the steady state concentration of the limiting nutrient. For example, assuming that the uptake kinetics observed in starvation transients ($V_m = 100\ d^{-1}$, $K_s = 0.6\ \mu M$) can be applied to continuous cultures with similar cell quotas, the predicted concentration of phosphate at steady state for $\mu = 0.5\ \mu_{max}$ is just 5 nM.

Elevated ammonium and phosphate uptake rates could also represent adaptations to temporal fluctuations in nutrient concentration, caused for example by zooplankton diurnal migration. The starvation responses for these nutrients would increase average growth rates in systems dominated by ammonium or phosphate inputs at even longer intervals, of several days duration, but it is difficult to imagine mechanisms which would produce nutrient distributions of this type.

It is generally accepted that mechanisms such as episodic wind-mixing or upwelling and neap-spring cycles in tidal mixing produce communities dominated by nitrate inputs on time scales of days to weeks. However, *Thalassiosira pseudonana* in these experiments showed no adaptation to such pulses, neither maintaining cell division after external nitrate was depleted, nor developing elevated uptake capacities in readiness for the next pulse. There are several factors which may explain the absence of these adaptations. Their contribution to average growth rates decreases as the interval between patches increases. The physical mechanisms of nitrate supply also dilute phytoplankton concentrations in the euphotic zone, thereby decreasing the demand for nutrient, increasing the lifetime

of the nitrate pulse and consequently reducing the benefits of transient elevated uptake rates. Finally, the intervals between nitrate pulses may be characterized not by nitrogen starvation but by limitation by recycled ammonium. (The development of high ammonium uptakes by nitrate-starved *T. pseudonana* could be an adaptation to this pattern.) Given that any or all of these factors are operating, any metabolic costs of maintaining high uptake capacities for nitrate between pulses may outweigh the benefits.

In these experiments, cells entered nutrient starvation by depleting the limiting nutrient during batch growth. In other studies, starvation has been imposed suddenly by collecting nutrient-replete cells and resuspending them in nutrient-free medium. These two approaches may produce different transient responses in uptake rate, because batch cultures must undergo a period of sub-saturating nutrient concentrations before the latter approach zero. The duration of this period will clearly vary among nutrients, depending in particular on the biomass of the culture and the K_s for uptake or growth. It appears in our experiments to have most strongly affected the silicate-starved cultures, which involved low particulate Si concentrations and a high half-saturation constant for uptake. Since bloom populations in the field enter nutrient starvation in the same way, variations in pre-bloom nutrient levels and half-saturation constants will strongly affect the types of nutrient transients encountered.

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