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Physiological Adaptation of Nitrate Uptake by Phytoplankton Under Simulated Upwelling Conditions

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To study the physiological adaptation (shift-up) of phytoplankton under the simulated upwelling conditions, nitrate uptake capacity of *Dunaliella tertiolecta* batch culture was measured in the laboratory using the stable isotope ^{15}N-KNO_3. Contrary to the expected, there was no significant relationship between the maximum V_{NO3} (nitrogen specific nitrate uptake rate) and the initial nitrate concentration. However, there was a strong relationship between the maximum ρ_{NO3} (nitrate transport rate) and the initial nitrate concentration of <25 μM, which was also influenced by the physiological status of the culture. The increase in V_{NO3} was mainly due to the increase in PON (particulate organic nitrogen) concentration and partly due to the increase in V_{NO3}. When the phytoplankton population was severely shifted-down, the physiological adaptation of nitrate uptake was significantly inhibited at high initial nitrate concentrations.

The timing of the maximum V_{NO3} or ρ_{NO3} was related to the initial nitrate concentration. At higher initial nitrate concentrations, maxima in V_{NO3} and ρ_{NO3} occurred 1 or 2 days later than at lower nitrate concentrations. This relationship was the opposite to the prediction from the shift-up model of Zimmerman et al. (1987). The shift-up process is apparently controlled by an internal time sequence and the initial nitrate concentration, but the magnitude of V_{NO3} was affected little by changes in nitrate concentration.

Key words : *physiological adaptation, phytoplankton, upwelling, nitrate uptake*

Introduction

The upwelling systems contribute a larger portion of primary production than its area, especially for new production and vertical flux of particles due to input of new nutrients from below the thermocline (Dugdale and Goering, 1967; Koblents-Mishke et al., 1970). There are numerous studies from various upwelling areas of the world ocean (Barber, 1992; Dugdale, 1972; Dugdale and Wilkerson, 1989; Probyn, 1988). However, there are a few laboratory studies on the physiological adaptation of phytoplankton under the simulated upwelling conditions (Ishizaka et al., 1983; Smith et al., 1992).

Dugdale and Wilkerson (1991) have proposed that nitrate concentration may be a controlling factor for new production in upwelling systems. A plot of maximum surface V_{NO3} versus maximum surface [NO_3] from several coastal upwelling areas can be fitted by a straight line with a positive intercept of nitrate concentration around 7 μM, which they called the critical nitrate point (e.g., Fig. 1). A dependence of the rate of increase of V_{NO3} or acceleration (A), also appeared to be directly related to initial nitrate concentration in a series of field observations (Wilkerson and Dugdale, 1987). A similar relationship between acceleration in nitrate uptake and different initial nitrate concentrations was also observed in higher plants (MacKown and McClure, 1988). Dugdale et al. (1990) developed predictions of the realization of new production in upwelling areas based on initial nitrate concentration. The theoretical shape of the V_{NO3max} versus NO_{3max} was assumed to be linear but without a positive intercept or critical point on the nitrate axis. For this curve to be valid, nutrients (major nutrients and trace elements) other than nitrate in the water should not be limiting and growth conditions (e.g., temperature, light, and stability of water column) need to be favorable.

The purpose of this study is on the physiological adaptation (shift-up) of the phytoplankton *Dunaliella tertiolecta* under simulated upwelling conditions. To simulate upwelling conditions and examine the effect of initial nitrate concentration on shift-up, experiments were conducted using cultures with different concentrations of nitrate as a single limiting nutrient.

Hypotheses that were tested are as follows:

Hypothesis 1: Ho: There is no threshold concentration of nitrate for shift-up to occur.

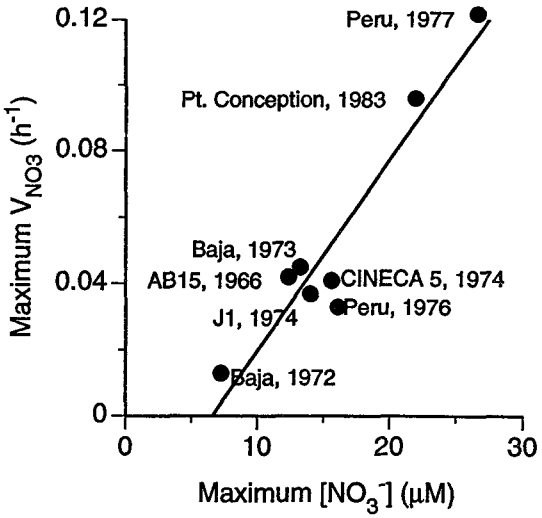

Fig. 1. The relationship between maximum nitrate concentration and maximum specific nitrate uptake rate at the surface from cruises in upwelling areas.

To test for the presence of shift-up and a threshold nitrate concentration, phytoplankton cultures were grown in media with ammonia as the only nitrogen source (nitrate free) and then transferred into media with different nitrate concentrations under nitrogen limitation. The rate of shift-up was determined from the increase in particulate organic nitrogen (PON) content and changes in ^{15}N nitrate uptake rate with time.

Hypothesis 2: Ho: The magnitude and timing of maximum V_{NO3} and ρ_{NO3} *do not depend on initial nitrate concentration.*

To test this hypothesis in the laboratory, experiments, basically the same as for Hypothesis 1, were performed to obtain the time series of V_{NO3} and ρ_{NO3} by phytoplankton provided with different initial nitrate concentrations. V_{NO3} (biomass specific nitrate uptake rate; h^{-1}) is in rate term, and is equivalent to the growth rate of phytoplankton based on nitrate nutrition, based on the assumption of exponential growth. ρ_{NO3} (nitrate transport rate; $\mu M\ h^{-1}$) indicates nitrate uptake rate per unit volume of the growth media (or seawater) and equivalent to new production.

Shift-up of the phytoplankton community

The term "shift-up" or physiological adaptation theory has stemmed from works of Schaechter (1968; see Zimmerman et al., 1987) on bacterial metabolism. Shift-up could be considered an induction mechanism in response to increased substrate concentration. Button and colleagues' work on marine bacteria (Button et al., 1973; Robertson and Button, 1989 and references therein) showed the kinetics of concentration dependent induction of substrate (toluene) uptake. Ishizaka et al. (1983) reported the effect of nitrate and temperature on the specific growth rate, lag period of growth, and maximum specific growth rate from laboratory experiments designed to simulate the upwelling conditions with different temperature and initial nitrate conditions. They reported high maximum V_{NO3} values at high initial nitrate concentrations.

The physiological basis for shift-up may be understood by examining higher plant literature. Laboratory experiments using higher plants show the acceleration of nitrate uptake with increased nitrate concentration (MacKown and McClure, 1988). Larsson and Ingemarsson (1989) reported that nitrate uptake kinetics may have more than one, and typically two saturable components, one with a high affinity and low V_{max}, the other with low affinity and high V_{max}. To these, a non-saturable component might be added. Two saturable components were observed in *Arabidopsis thaliana* (Doddema and Telkamp 1979) and in *Phaseolus vulgaris* (dwarf bean; Breteler and Nissen, 1982). The occurrence of several uptake phases may indicate the presence of a number of different systems or, alternatively, a single system that undergoes sharp changes in its mode of operation at different external concentrations (Nissen 1974; Epstein 1976; Borstlap 1981; Kochian and Lucas 1982).

Materials and Methods

Fig. 2 shows the summary diagram of the protocol for laboratory shift-up experiments. To simulate the upwelling conditions and examine the kinetics of shift-up, phytoplankton batch cultures of *Dunaliella tertiolecta* (green algae) were grown on modified f/2 media (Guillard and Ryther, 1962) with ammonia (40 μM) as the sole source of nitrogen until all the ammonia was depleted and reached the stationary phase. Cells were harvested by centrifuging (750×g at 5℃, for 15 minutes) in 250 mℓ polycarbonate centrifuge

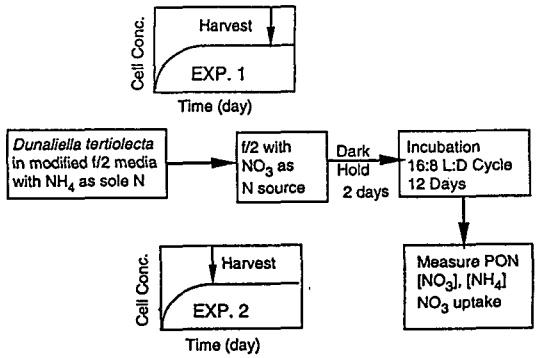

Fig. 2. Diagram showing the experimental procedure for laboratory shift-up experiments. Cells used in experiment 1 were harvested 5 days later than for experiment 2, after the culture reached a stationary phase.

bottles and resuspended in nitrogen free media. The cell suspension was dispensed into 250mℓ or 30mℓ (for nitrate-silicate interaction in experiment 2) polycarbonate bottles containing modified f/2 media with different concentrations of nitrate as the sole nitrogen source. Cells were kept in the dark for 3 days to simulate deep water shifted-down conditions and then exposed to light (white fluorescence lamps, General Electric, 150 $\mu E\ m^{-2}\ s^{-1}$) to simulate upwelling conditions. The light period used was a 16h : 8h L : D cycle. Nitrate and ammonia concentration, specific nitrate uptake (V_{NO3}), nitrate transport rate (ρ_{NO3}), growth rate based on V_{NO3} and PON, maximum yield (based on PON concentration), and maximum specific nitrate uptake rates were determined. ^{15}N nitrate uptake was measured every 24 or 48 hours for up to 12 days. The incubation for nitrate uptake measurements was started at hour 2 of the light cycle and lasted for 3 hours after inoculating with 10 μM ^{15}N nitrate. Before the start of ^{15}N nitrate uptake incubation, nutrient samples were placed in 20mℓ glass scintillation vials with plastic lining caps, and kept frozen at −20℃ until analyses. After the incubation, cells were filtered onto 24 mm GF/C glass fiber filters and dried at 60℃ until mass-spectrometer analyses (Europa Scientific Roboprep Tracermass) of ^{15}N atom % and PON content (Owens, 1988). Standards used were weighed $(NH_4)_2SO_4$ granules. Nutrients were measured with a Technicon AutoAnalyzer II according to Whitledge et al. (1987) for nitrate (+nitrite) and ammonia after thawing samples. No ammonia contamination was detected after storage. Because the samples were not filtered, nutrient concentrations may include both internal (reservoir in the phytoplankton cells) and external (in the growth media) fraction. Calculations of V_{NO3} and ρ_{NO3} were done according to Wilkerson and Dugdale (1987).

Experiment 1

Phytoplankton (*Dunaliella tertiolecta*) batch culture grown at 25℃ to the stationary phase (15 days in a modified f/2 medium with 40 μM NH_4Cl as a nitrogen source) were harvested and dispensed into 250mℓ polycarbonate bottles containing fresh modified f/2 media with different initial nitrate conditions. The media without additional NO_3 had 5.56 μM NO_3 (which was present in the aged seawater from which the medium was prepared) and ammonia was undetectable. Nitrate additions were 0, 1, 2, 3, 5, 7, 10, 15, 20, 25, 50, and 100 μM giving initial nitrate concentrations of 5.56, 6.56, 7.56, 8.56, 10.56, 12.56, 15.56, 20.56, 25.56, 30.56, 55.56, and 105.56 μM. Phosphate (PO4) additions were 20 μM for additions of 0 to 50 μM NO_3 and 40 μM PO_4 for 100 μM NO_3 addition. Silicate concentrations added were 40 μM for 0 to 25 μM NO_3 adition, 80 μM for 50 μM NO_3 addition, and 160 μM for 100 μM NO_3 addition. Judging from the Redfield ratio of 16 : 1 for N : P (Redfield, 1958), it is unlikely that phosphate will be limiting for the growth of *Dunaliella*. Silicate is not required for green algae, but media was prepared this way to simulate the conditions observed in the field and also for use in growing diatoms. Nitrate and ammonia were measured at the beginning of incubation to calculate ^{15}N nitrate uptake and nitrate disappearance rate.

Experiment 2

Dunaliella cells were grown at 18℃ and were harvested 5 days earlier (10 days in modified f/2 media with 40 μM NH_4Cl) than in experiment 1 and may have been under healthier conditions. Initial PON concentration was 3.5 μM [twice that of experiment 1 (1.7 μM)]. Initial NO_3 concentrations were 3.51, 4.79, 7.18, 9.45, 10.71, 13.40, 18.53, 26.43, 28.01, 45.92, and 81.60 μM.

Si-NO3 interaction

To test for the possible toxicity of high silicate concentrations on cell growth which occurred in experiment 1,

three high nitrate treatments in experiment 2 were divided into three subgroups (3×3=9 treatments); 14, 28, 56 μM Si(OH)4 for 28.01 μM NO_3, 23, 56, and 112 μM Si(OH)4 for 45.9 μM NO_3, and 41, 82, and 164 μM Si(OH)4 for 81.6 μM NO_3. ANOVA (Analysis of variance) statics were used to test for the interaction between silicate and nitrate. All the procedures were the same as in experiment 2, except incubations were performed in 30mℓ polycarbonate bottles instead of 250mℓ bottles.

Results

Experiment 1

For treatments with less than 20 μM NO_3 addition (the initial nitrate concentration of <25 μM), nitrate became depleted (<0.5 μM) in 4 days regardless of the initial nitrate concentration (Figs. 3 A1 to H1). At initial nitrate concentrations >30 μM, nitrate concentrations were maintained higher than 5 μM by Day 5 (Figs. 3 J1 to L1), and it took longer for the nitrate to be depleted as the initial nitrate concentration was increased (7 days for 25 μM and 8 days for 50 μM NO_3 addition). With additions of 100 μM NO_3, nitrate concentration stayed fairly constant (around 70 μM) after an initial slow decrease (Fig. 3 L1). Ammonia concentration showed a peak between Days 3 and 4 when nitrate concentration decreased rapidly to zero and remained undetectable for the rest of the experiment (Fig. 3 A1 to L1). The timing of the

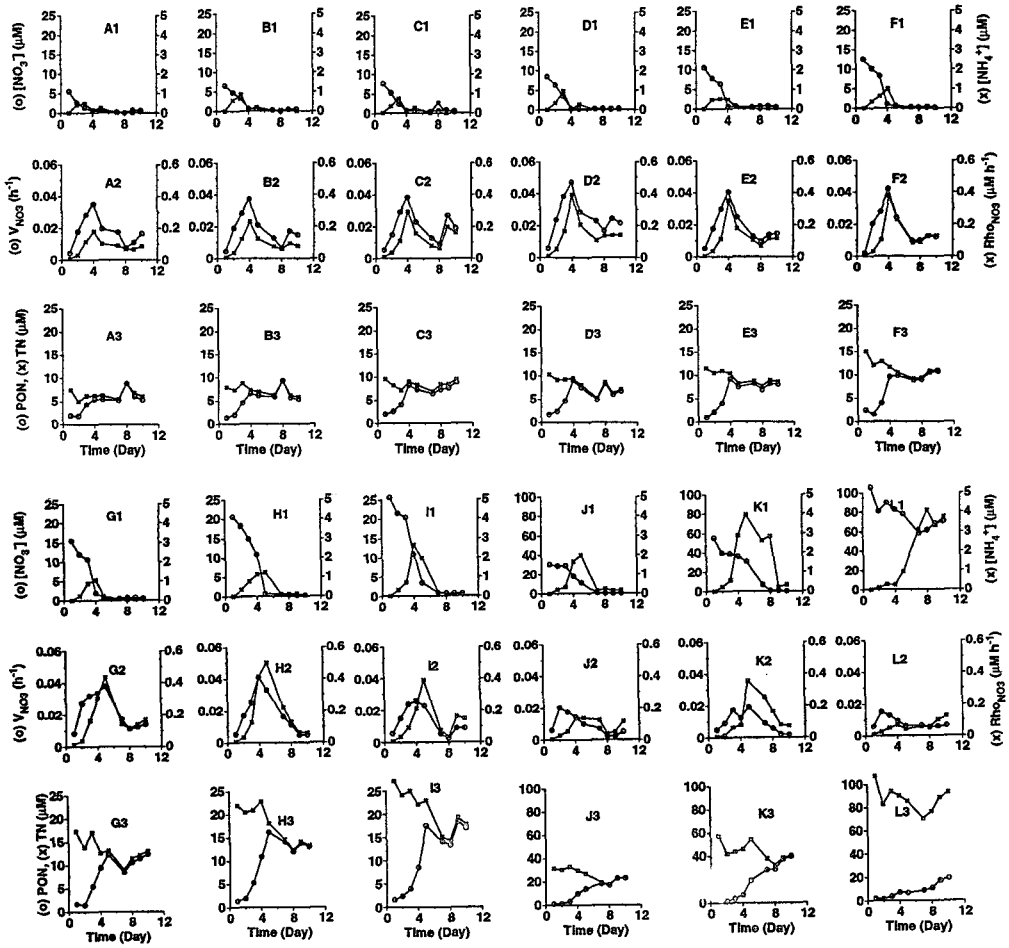

Fig. 3. Time series of (1) nitrate and ammonia, (2) biomass specific nitrate uptake rate (V_{NO3}), and nitrate transport rate (ρ_{NO3}), (3) PON and total N (PON+NO_3+NH_4) for shift-up experiment 1. Arranged in increasing order of initial nitrate concentrations (A to L).

Table 1. Summary of the shift-up experiment 1

No	[NO3]	A1-2	A2-3	A3-4	Tp	Vmax	Tp	ρmax	POX(I)	PONmax
1	5.56	0.568	0.436	0.271	4	0.0350	4	0.1811	1.869	8.843
2	6.56	0.605	0.382	0.370	4	0.0372	4	0.2304	1.366	9.271
3	7.56	0.405	0.590	0.377	4	0.0382	4	0.2942	1.874	8.809
4	8.56	0.735	0.609	0.367	4	0.0471	4	0.3946	1.706	9.012
5	10.5	60.519	0.512	0.442	4	0.0406	4	0.3510	0.911	9.196
6	12.5	60.766	0.313	0.592	4	0.0421	4	0.3769	2.442	10.700
7	15.5	60.790	0.191	0.078	5	0.0379	5	0.4371	1.689	12.304
8	20.5	60.483	0.348	0.656	4	0.0409	5	0.5022	1.376	16.100
9	25.5	60.398	0.378	0.077	4	0.0261	5	0.3911	1.624	18.434
10	30.5	60.598	−0.120	−0.134	2	0.0205	5	0.1392	1.128	22.758
11	55.5	60.179	0.336	−0.212	5	0.0194	5	0.3569	1.530	39.618
12	105.5	60.386	−0.094	−0.144	2	0.0151	10	0.1248	1.829	19.146

[NO3] : Initial nitrate concentration (μM).
A1-2 : Acceleration rate, A, between day 1 and 2 (h^{-2}×1000).
A2-3 : Acceleration rate, A, between day 2 and 3 (h^{-2}×1000).
A3-4 : Acceleration rate, A, between day 3 and 4 (h^{-2}×1000).
Tp : Time for the first peaks of V_{NO3} and ρ_{NO3} (d).
Vmax : The maximum V_{NO3} observed (h^{-1}).
ρmax : The maximum ρ_{NO3} observed (μM h^{-1}).
$PON_{(I)}$: Initial particulate organic nitrogen contont (μM).
PON_{max} : The maximum PON achiered (μM).

ammonia peak was delayed with the delay in nitrate decrease at higher initial nitrate concentrations, from Day 3 to Day 8. The magnitude of the ammonia peaks increased with initial nitrate concentration, from 0.5 μM at 5.56 μM initial nitrate to 4 μM at 105.56 μM initial nitrate concentration. The source of ammonia seems to be due to phytoplankton cell leakage and from internal storage pools. If it was due to bacterial decomposition, it would also show an increase at the end of the incubation when phytoplankton cells were deteriorating. However, there was no increase in ammonia concentration at the end of the incubation.

The pattern of changes in V_{NO3} with time was similar among treatments with different initial nitrate concentrations (Fig. 3 A2 to L2; Table 1). V_{NO3} increased up to Day 4 and then decreased, to a minimum after Day 8. Maximum V_{NO3}'s for different treatments were around 0.04 h^{-1} for initial nitrate concentration of up to 20 μM and decreased to approximately 0.02 h^{-1} at higher concentrations. This initial increase in V_{NO3} coincides with the decrease in nitrate concentration and also with the increase in ammonia concentration. No apparent inhibition of nitrate uptake by ammonia was observed. After an initial increase for 4 to 5 days, V_{NO3} decreased to a minimum (close to initial V_{NO3} values) around Day 8. The pattern of ρ_{NO3} change with time was similar to that of V_{NO3}, except changes in ρ_{NO3} with initial nitrate concentrations were significantly greater compared to V_{NO3} (Fig. 3 A2 to L2). Higher initial ρ_{NO3} concentrations (up to 20 μM) resulted in increased ρ_{NO3} (Fig. 5B1; Table 1). Maximum values ranged from 0.18 μM h^{-1} at 5.56 μM initial nitrate concentration to 0.51 μM h^{-1} at 20.56 μM initial nitrate concentration. However, the maximum ρ_{NO3} decreased when initial nitrate concentrations were >25 μM. At 100 μM nitrate addition, the first maximum was <0.1 μM h^{-1} and the second maximum (at Day 10) was <0.15 μM h^{-1} (Fig. 3L4). Changes in maximum ρ_{NO3} were mainly due to changes in PON concentration, because the maximum V_{NO3} stayed fairly constant with changes in the initial nitrate concentration.

The increase in PON content was more or less exponential up to Day 2 to 4 (Fig. 3 A3 to L3). After Day 4, the PON concentration stayed constant or increased further but at a slower rate depending on the external nit-

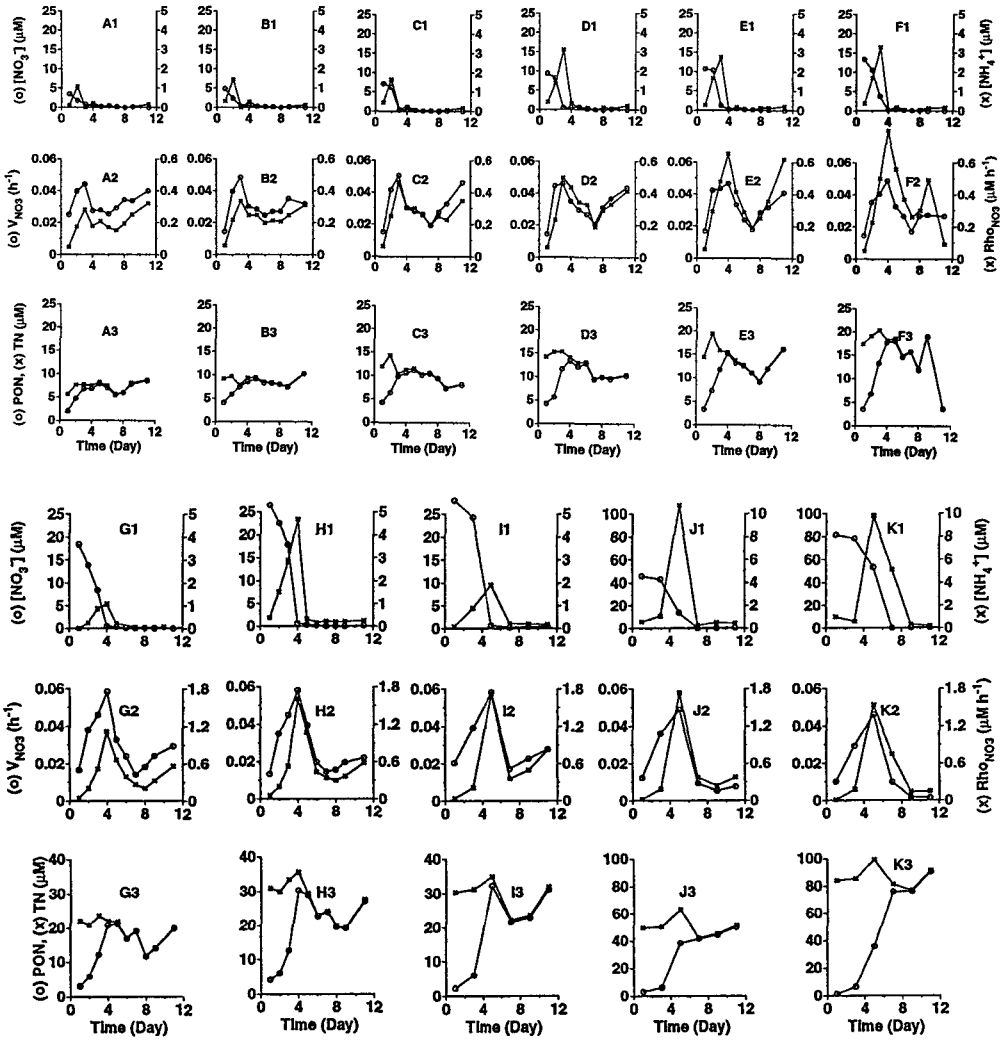

Fig. 4. Time series of (1) nitrate and ammonia, (2) biomass specific nitrate uptake rate (V_{NO3}), and nitrate transport rate (ρ_{NO3}), (3) PON and total N (PON+NO_3+NH_4) for shift-up experiment 2. Arranged in increasing order of initial nitrate concentrations (A to K).

rogen available for incorporation. Total nitrogen (nitrate +nitrite+ammonia+PON) stayed fairly constant but with some imbalance with the initial value. A decrease in PON content at high initial concentrations of nitrate was entirely unexpected. One possibility may be that silicate concentrations (80 μM and 160 μM) were too high for the cells to tolerate. This is tested in Si-NO_3 interaction experiment. Another possibility is that the phytoplankton, which were pre-conditioned to low nitrte conditions, could not adapt to high nitrate concentrations which do not occur normally in the natural environment.

Experiment 2

The decrease in nitrate concentration with time was much steeper than for experiment 1 (Fig. 4 A1 to L1), due to a combination of higher V_{NO3} and initial PON on Day 1 compared to experiment 1. Except for the treatment with the lowest initial nitrate concentration, all other experiments with <10 μM initial nitrate depleted nitrate below 0.5 μM within 3 days. At the lowest initial nitrate concentration (3.51 μM), it took one more day (4 days) to deplete the nitrate below 0.5 μM. With initial concentrations >10 μM, it took 4 days to deplete

Table 2. Summary of the shift-up experiment 2

No	[NO3]	A1-2	A2-3	A3-4	Tp	Vmax	Tp	ρmax	POX(I)	PONmax
1	3.51	0.617	0.17	−0.696	3	0.0441	3	0.3194	2.000	8.510
2	4.79	1.046	0.36	−0.370	3	0.0483	3	0.3351	4.045	10.184
3	7.18	1.092	0.37	−0.377	3	0.0508	3	0.4621	4.332	11.138
4	9.45	1.258	0.05	−0.367	3	0.0459	3	0.4961	4.260	13.216
5	10.71	1.063	0.05	0.125	4	0.0466	4	0.6192	3.298	13.085
6	13.40	0.858	0.22	0.346	4	0.0487	4	0.7992	3.556	18.900
7	18.53	0.888	0.32	0.521	4	0.0585	4	1.1156	3.104	21.222
8	26.43	0.900	0.40	0.558	4	0.0580	4	1.6079	4.122	30.323
9	28.01	0.390	n.d.	0.394	5	0.0582	5	1.7159	2.277	50.091
10	45.92	0.496	n.d.	0.267	5	0.0489	5	1.7351	3.181	50.091
11	81.60	0.402	n.d.	0.346	5	0.0460	5	1.5416	1.397	90.577

For experiments 9, 10 and 11, acceleration rates (A) are for days 1-3 and days 3-5.
The rest are the same as in Table 1.

nitrate below 0.5 μM. At initial nitrate concentration of >28 μM, the time to depletion increased to 5 days or longer. An ammonia peak appeared when there was a sharp decrease in nitrate concentration (Fig. 4 A1 to L1), in common with experiment 1. The magnitude of the ammonia peak was related to the rate of changes in nitrate concentration. When the slope of decrease in nitrate concentration was steeper, the ammonia peak was higher.

The maximum V_{NO3} for each treatment occurred between Day 3 and 5, depending on the initial nitrate (Fig. 4 A2 to L2; Table 2). At lower initial nitrate concentration, the maximum V_{NO3} appeared earlier than at higher initial nitrate concentrations. Compared to experiment 1, in which there was no prominent V_{NO3} peak at high initial concentrations, the V_{NO3} peak appeared throughout the entire initial nitrate range. The maximum V_{NO3} values were between 0.045 and 0.06 h^{-1}, a little higher than for experiment 1. The relationship between the magnitude of V_{NO3} and initial nitrate concentration was not linear over the concentration range tested, even though there was a positive relationship at initial nitrate concentrations <25 μM (Fig. 5 A2).

Maximum ρ_{NO3} values showed a positive linear relationship with initial nitrate concentrations <25 μM NO_3 (Fig. 5 B2). At nitrate concentration of >25 μM, ρ_{NO3} seems to level off. However, there was no severe depression in ρ_{NO3} at high initial nitrate concentrations in contrast to experiment 1. The magnitude of maximum ρ_{NO3} in experiment 2 was about 3 times higher than in experiment 1, with values ranging between 0.3 and 1.8 μM

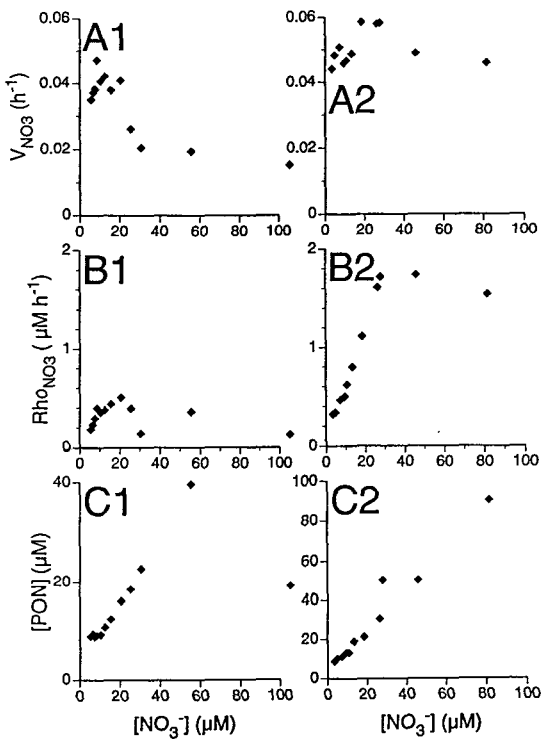

Fig. 5. The relationship between the initial nitrate concentration and (A) biomass specific nitrate uptake rate (V_{NO3}), (B) nitrate transport rate (ρ_{NO3}), and (C) particulate organic nitrogen (PON) for experiments 1 and 2.

h^{-1}. For initial nitrate of < 5 μM, PON did not show an exponential increase even though some increase was observed (Fig. 4A3 and B3). At nitrate concentration of > 5 μM, PON tended to show an exponential growth pattern for 2 to 4 days (Fig. 4 C3 to K3).

Si-NO3 interaction

A two factor ANOVA statistics table of the interaction between nitrate and silicate is shown in Table 3. Treatments with different concentrations of silicate did not show any significant difference among treatments. Also there was no significance in the interaction between nitrate and silicate concentration. Treatments with different nitrate concentrations showed significant differences among treatments. The suspicion that excessive silicate concentration may repress V_{NO3} can be excluded from this experiment. The major cause of the differences between experiment 1 and 2 appears to be the initial V_{NO3} of the stock *Dunaliella* culture, which reflects the physiological state of the culture. The culture used in experiment 1 may have been physiologically unhealthier (severely shifted-down) than the culture used for experiment 2 and may not have been able to adapt to high nitrate concentration during the experimental time span.

Table 3A. Two factor ANOVA (analysis of variance) for nitrate and silicate interaction on V_{NO3}

Source of variability	d.f.	Sum of Squares (S.S)	Mean Squares (M.S)	F-test	P value
A (NO_3)	2	0.00207	0.00103	3.26615	0.04740 (*)
B ($Si(OH)_4$)	2	0.00002	0.00001	0.03833	0.96240 (n.s.)
A×B	4	0.00029	0.00007	0.25462	0.92280 (n.s.)
Error	45	0.01425	0.00032		

* sigmificant at 95% confidence level.
NO_3-1; low NO_3 (24.01 μM).
NO_3-2; intermediate NO_3 (45.92 μM).
NO_3-3; high NO_3 (81.60 μM)
$Si(OH)_4$-1; low $Si(OH)_4$, one half nitrate concentration.
$Si(OH)_4$-2; intermediate $Si(OH)_4$, same as nitrate concentration.
$Si(OH)_4$-3; high $Si(OH)_4$, twice of nitrate concentration.

Table 3B. The A (NO3)×B ($Si(OH)_4$) incidence table on V_{NO3}

	NO_3-1	$Si(OH)_4$-2	$Si(OH)_4$-3	Totals
NO_3-1	32.68 (7.12)	28.28 (6.48)	32.16 (5.84)	31.04 (3.56)
NO_3-2	19.96 (7.25)	24.67 (9.54)	18.29 (6.78)	19.97 (4.39)
NO_3-3	15.07 (7.83)	16.09 (7.14)	18.45 (6.82)	16.54 (3.96)
Total	21.57 (4.45)	23.01 (4.42)	22.97 (3.86)	22.52

Numbers in parentheses are standard errors of the mean.
All values multiplied by 1000.

Discussion

Table 4 shows the summary of acceleration values from upwelling areas and from cultures using *Skeletonema costatum* and *Dunaliella tertiolecta*. Overall, acceleration rates from simulated upwelling experiments (7.4×10^{-4} h^{-2} for *Skeletonema costatum* and 12.6×10^{-4} h^{-2} for *Dunaliella tertiolecta*) were comparable to values from coastal upwelling areas (as high as 15.8×10^{-4} h^{-2}), while the equatorial Pacific was low (1.0×10^{-4} h^{-2}). This value was about one third of the maximum expected value calculated from nitrate concentration using the model from Zimmerman et al. (1987). During the Dunaliella tertiolecta culture experiments, when cells were depleted of nutrients (either ammonia or nitrate) for a prolonged period of time, it took longer for them to fully adapt to high nitrate concentrations or they were unable to adapt to high nitrate condition at all (Exp. 1).

The initial nitrate concentration had little effect on the magnitude of maximum V_{NO3} obtained. In contrast to the expected, the magnitude of V_{NO3} did not show any positive linear relationship with initial nitrate except at low nitrate concentrations (Fig. 5 A1 and A2). The timing of the maximum V_{NO3} and maximum PON were dependent on initial nitrate concentration, taking longer time to reach maximum V_{NO3} at higher initial nitrate concentrations (Table 1 and 2; Fig. 6), negating one of two hypotheses [The magnitude is dependent on the initial nitrate concentration] and accepting the other [The timing is dependent of the initial nitrate concentration]. However, the timing of the maximum was the opposite to what was expected from the model of Zimmerman et al. (1987). They predicted that maxima would occur earlier at highe initial nitrate concentrations than at lower initial nitrate concentrations. The relationship between initial nitrate concentration and ρ_{NO3} showed a highly linear relation-

Table 4. The maximum acceleration of nitrate uptake (A; h^{-2}) under upwelling and simulated upwelling situations

Location/ Organism	Acceleration Rate $h^{-2} \times 10^{-4}$	Comments	Refernce
15 °S Peru, 1977	5.5	Drogue I-2, 100% LPD	Dugdale et al., 1990
	3.2	Drogue I-2, 50% LPD	
	15.8	Drogue III-2, 50% LPD	
	12.4	Drogue III-3, 100% LPD	
Point Conception, 1983	3.8	Drifter S77, 100% LPD	Dugdale et al., 1990
	1.2	Drifter S239, 100% LPD	Wilkerson and
	12.0	Barrel 74B1	Dugdale, 1987
Skeletonema costatum	7.4	Laberatory culture	Smith et al., 1992
Equatorial Pacific, 1988	1.0	Holdlover Experiment	Yang, 1992
	2.3	Holdover with additional NO_3 enrichment	
Dunaliella tertiolecta	12.6	Laboratory culture	Yang, this study

Table taken from Dugdale and Wilkerson (1992) with data from this experiment.

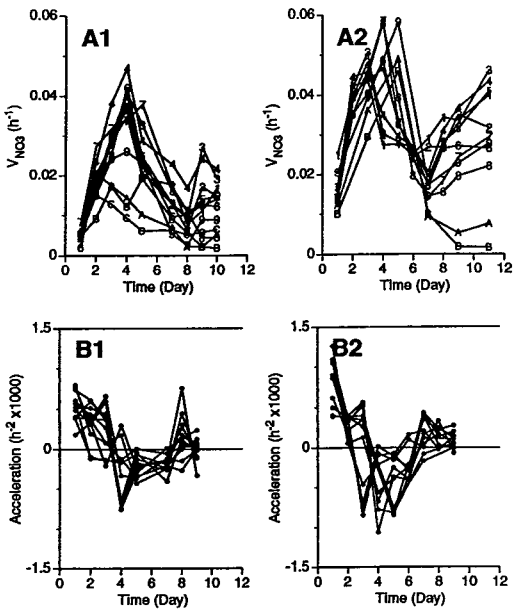

Fig. 6. Time series of (A) biomass specific nitrate uptake (V_{NO3}), and (B) acceleration rate (A; h^{-2}) for experiments 1 and 2.

ship at low initial nitrate concentrations (up to 25 μM) and stayed about the same at high nitrate concentrations for experiment 2 (Fig. 5 B2). However, in experiment 1 ρ_{NO3} was severely depressed at high nitrate concentrations (Fig. 5 B1). The maximum PON showed a linear relationship with initial nitrate concentration, except for the highest nitrate concentration of experiment 1 (Fig. 5 C1 and C2). The slope was lower for experiment 1 than experiment 2 which showed close to a 1 : 1 relation. Fig. 5 shows V_{NO3} and acceleration rate (A) for all shift-up experiments. It appears as if there is an internal time sequence for shift-up. The cycle of V_{NO3} change was quite similar at varying initial nitrate concentrations. Acceleration rates showed a maximum at Day 1 or 2 and decreased to a minimum around Day 4 and increased again when the internal reservoir of nitrogen (nitrate and ammonia) was depleted. The increase in ρ_{NO3} was more pronounced than that of V_{NO3} and was largely due to the exponential increase in PON at the beginning of the incubation. If the proportion of phytoplankton PON to total PON increases after the upwelling, this will lead to a greater increase in apparent V_{NO3} with little changes in true V_{NO3} which is due to phytoplankton PON only.

The intensity of light used for the culture experiment (150 μE m^{-2} s^{-1}) was about 10% of full surface sunlight and may not be saturating. Even though *Dunaliella tertiolecta* stock has been grown for generations under 80 μE

m^{-2} s^{-1} light intensity and could have been well adapted for low light, it is still possible that this low light intensity could limit maximum growth rate. However, the maximum V_{NO3}, which is comparable to hourly growth rate (h^{-1}), was 0.058 h^{-1}, equivalent to 1.4 d^{-1}, a value high enough to eliminate the possibility of light limitation in the shift-up experiments. The difference in temperature between experiment 1 (25℃) and experiment 2 (18℃) was not an important factor. The growth rate (indicated by V_{NO3}) was higher for experiment 2 than experiment 1. If temperature was important, then the result should have been reversed. The initial V_{NO3}, determined by the history of the cells and indicative of the physiological state of phytoplankton cells, remains as the most important factor controlling the V_{NO3} and ρ_{NO3} during the shift-up process. There was an indication of a secondary peak at the end of the incubation even though incubations were terminated before nitrate was depleted and full maxima were reached. This suggests that additional time may be required to synthesize the required enzymes for the low affinity and high V_{max} system to develop.

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모의 용승조건하에서 식물 플랑크톤 질산염 흡수기작의 생리적 적응

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식물 플랑크톤의 인위적인 용승조건 하에서 생리적 적응(shift-up)을 알아보기 위하여, 안정동위원소인 ^{15}N-KNO_3를 이용하여 실험실에서 *Dunaliella tertiolecta*의 질산염 흡수 능력을 측정하였다. 그 결과 예상과는 달리 최대 질소비 질산염 흡수 속도(V_{NO3})와 초기 질산염 농도 사이에는 유의성 있는 상관관계가 나타나지 않았다. 그러나 최대 질산염 운반 속도(ρ_{NO3})와 25 μM 이하의 초기 질산염 농도 사이에는 강한 상관성이 나타났으며, 이는 배양 세포의 생리적인 상태에 의한 영향에도 기인한다. ρ_{NO3}의 증가는 주로 입자성 유기 질소 농도의 증가와 함께 부분적으로는 V_{NO3}의 증가에 기인한다. 식물 플랑크톤 개체군이 심하게 shift-down되었을 경우 질산염 흡수의 생리적 적응은 높은 초기 질산염 농도에서 주목할 만큼 저해되었다.

최대 V_{NO3} 또는 ρ_{NO3}이 나타나는 시기는 초기 질산염 농도와 관련이 있다. 높은 초기 질산염 농도에서는 V_{NO3}와 ρ_{NO3}의 최대치가 낮은 초기 질산염 농도에서보다 1~2일 정도 늦게 나타났다. 이는 Zimmerman et al.(1987)의 shift-up 모델에서의 예측과 상반되는 결과이다. Shift-up 과정은 명백히 내부적인 시간순서와 초기 질산염 농도에 의하여 조절되지만, V_{NO3}의 크기는 질산염 농도의 변화에 거의 영향을 받지 않았다.