

## Nitrogen Uptake Characteristics of *Chordaria flagelliformis* (Phaeophyta) in Batch Mode and Continuous Mode Experiments

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### Abstract

The uptake of  $\text{NH}_4^+$  and urea by *Chordaria flagelliformis* was measured in a perturbed system (batch mode) and in an apparent steady-state system (continuous mode). The maximum uptake rates ( $V_{\text{max}}$ ) measured in short-term depletion experiments greatly exceeded those determined under a steady nutrient supply. *C. flagelliformis* briefly exposed to high nutrient concentrations has an impressive capacity for rapid uptake (high  $V_{\text{max}}$ ) which is uncoupled from growth. In contrast, under continuous, homogenous low N concentrations the alga appears well equipped (low half-saturation constant,  $K_m$ ) to take up the available N. The results show that previous work based on batch mode uptake experiments probably overestimated the N requirements for the growth of many seaweeds.

### Introduction

A number of studies have indicated that the inorganic nitrogen (N) content of the waters along the north-east coast of North America during the summer months is sufficiently low to limit the growth of macroalgae (Buggeln, 1974; Topinka and Robbins, 1976; Chapman and Craigie, 1977; Hanisak, 1979; Chapman and Lindley, 1980). The perennial kelp *Laminaria longicuris* is able to ameliorate this drawback by utilizing inorganic and organic N reserves accumulated during the winter months to sustain rapid growth rates for part of the summer (Chapman and Craigie, 1977). *Chordaria flagelliformis* is a summer annual seaweed whose macroscopic stage appears around the coast of Nova Scotia, Canada when ambient seawater inorganic N levels have already fallen to extremely low values (Probyn, 1981). The plant is consequently not able to draw on N stores accumulated during periods of N surfeit. These observations would suggest that *C. flagelliformis* is capable of taking up extremely low concentrations of nitrogenous nutrients from the environment with an efficiency similar to that of oceanic phytoplankton, and cer-

tainly better than published  $K_m$  values (Michaelis-Menten half saturation constant) for other macroalgae would suggest.

An alternative strategy, based on the ability of a species to exploit the spatial and temporal variability in nutrient concentration, as opposed to the efficient utilization of an integrated, low level nutrient resource, has recently been proposed for phytoplankton inhabiting N-depleted oceanic waters. It has been hypothesized that as a consequence of the uncoupling of growth and nutrient uptake over the short-term, N-limited phytoplankton cultures need only be exposed to patches of elevated N for periods that are short relative to their doubling time to satisfy their N requirement for that period (McCarthy and Goldman, 1979). By way of contrast with the efficient use of an homogenous N supply, the exploitation of a patchy N regime would favour species with a high  $V_{\text{max}}$  (maximal rate of uptake) rather than low  $K_m$  for uptake of the limiting nutrient in question (Turpin and Harrison, 1979).

The present study was undertaken to compare the short-term (< 1 h) uptake capabilities of *Chordaria flagelliformis* in a perturbed system with the long-term uptake (days) in a steady-state system. Urea and  $\text{NH}_4^+$  were employed as the N sources, as these are quantitatively the more important nitrogenous nutrients during the summer months (Probyn, 1981) and because they can be regenerated through animal excretion. It is proposed that the steady-state experiments reveal the efficiency with which the low, mean environmental concentrations of  $\text{NH}_4^+$  ( $\approx 0.3 \mu\text{g at l}^{-1}$ ) and urea ( $\approx 0.5 \mu\text{g at l}^{-1}$ ) are taken up, while the perturbation experiments show the seaweed's capacity to utilize N during short periods of elevated supply.

### Material and Methods

#### Perturbation Experiments (Batch Mode)

Healthy, darkly pigmented *Chordaria flagelliformis* thalli were collected the day before experimentation, weighed

and maintained in culture on a 15 hL:9 hD cycle at a temperature of 11 °C. This temperature was chosen as it approximated the prevailing mid-June ambient seawater temperature. During this pre-experimentation period freshly collected, low N seawater ( $\text{NH}_4^+ + \text{NO}_3^- + \text{urea} \approx 1 \mu\text{g at l}^{-1}$ ) was pumped through the culture dish on a continuous basis thereby maintaining the plant material close to the field adapted state. All experiments were done between 1500 and 1800 hrs at a quantum irradiance of  $180 \mu\text{E m}^{-2} \text{ s}^{-1}$  (400–700 nm), a level known to saturate growth (Probyn, 1981). Uptake experiments were commenced by transferring preweighed thalli to 2-l beakers containing filtered (0.45  $\mu\text{m}$ ) seawater enriched at different urea and  $\text{NH}_4^+$  concentration. The maximum starting concentrations were approximately  $100 \mu\text{g at l}^{-1}$  for urea and  $50 \mu\text{g at l}^{-1}$  for  $\text{NH}_4^+$ . Ratios of approximately 1 g for  $\text{NH}_4^+$  and 2 g for urea, fresh weight of plant tissue per litre of medium were used for all treatments. Mixing was provided by vigorous aeration and magnetic stirring. Water samples were removed at 15–20 min intervals for 60–90 min and the decrease in concentration, corrected for decrease in volume through sampling, plotted against time. Ammonium and urea concentrations were measured according to Grashoff (1976) modified for use on reduced (5 ml) sample volumes. Linear regression of the initial portion of a depletion curve after any lag period, was used to calculate uptake rate from the following equation:

$$V = \frac{\Delta n}{\Delta t \cdot \chi},$$

where  $V$  = uptake rate ( $\mu\text{g N} \cdot \text{g}^{-1} \text{ dry weight} \cdot \text{h}^{-1}$ );  $n$  = nitrogen content of the water in the uptake vessel ( $\mu\text{g N}$ );  $t$  = time (h), and  $\chi$  = dry weight (g), measured after 3 d at 60 °C. Appropriate controls revealed that volatilization of  $\text{NH}_4^+$  or removal of  $\text{NH}_4^+$  and urea by microorganisms present in the uptake medium was negligible over the time course of the experiment.

#### Steady Concentration Experiments (Continuous Mode)

Field collected *Chordaria flagelliformis* was maintained in flow through culture at 11 °C for a number of days until a steady residual nitrogenous nutrient concentration (concentration in reactor vessel) for each of the treatments was attained. With the present system this usually took 2–3 d.

Growth rate generally reached a maximum between the second and fourth day. We could not establish whether true steady-state conditions (i.e. balanced growth and uptake) prevailed during this period. To find out would have necessitated destructive sampling of experimental plants. Consequently experimental conditions can only be regarded as an “apparent” steady state. The plants were maintained under continuous illumination ( $180 \mu\text{E m}^{-2} \text{ s}^{-1}$ ) to avoid complications imposed by a photoperiodically-cued rhythm in uptake capacity (Table 1). Borosilicate desiccating dishes of 3-l capacity, equipped with overflow spouts, were used as culture vessels with mixing provided by vigorous aeration. Culture medium comprising freshly collected, filtered (0.45  $\mu\text{m}$ ) seawater enriched with  $\text{NH}_4^+$  or urea at six concentrations and  $3 \mu\text{M PO}_4^{3-}$ , was held in 20-l borosilicate glass carboys and supplied at a rate of  $175\text{--}180 \text{ ml} \cdot \text{h}^{-1}$  via a peristaltic pump. A ratio of approximately 1 g fresh weight per litre of culture vessel was maintained for all treatments.

As the plants were growing over the course of an experiment, a constant biomass for each treatment could not be achieved. However, once the ‘steady’ residual concentration was attained, it remained essentially unchanged for a further 24 h, indicating a negligible effect of biomass increment over this period (illustrated as a stable  $V_{\text{max}}$  in Table 1). A mean concentration for a 24-h period was used as the effective residual concentration. Uptake rate was calculated from (modified from Rhee, 1979):

$$V = \frac{f(S_1 - S)}{\chi},$$

where  $f$  = flow rate ( $\text{l} \cdot \text{h}^{-1}$ ) and  $S_1$  and  $S$  are the inflow and residual  $\text{NH}_4^+$  or urea concentrations ( $\mu\text{g N} \cdot \text{l}^{-1}$ ), respectively.

#### Analysis of Data

Uptake rates were assumed to follow the familiar Michaelis-Menten relationship (Dowd and Riggs, 1965) as a function of external residual nutrient concentration. Provisional estimates of  $K_m$  and  $V_{\text{max}}$  were derived from double reciprocal ( $1/S$  vs  $1/V$ ) or Hanes-Woolf ( $S$  vs  $S/V$ ) linear transformation (Dowd and Riggs, 1965). The values of these two uptake parameters were fine adjusted and standard errors fixed according to Wilkinson (1961).

**Table 1.** *Chordaria flagelliformis*. Diel variation in  $V_{\text{max}}$  for *C. flagelliformis* conditioned for 3 d under a 15 hL:9 hD cycle (LD) or in continuous light (L). Uptake rates (continuous mode), for both urea and  $\text{NH}_4^+$ , are expressed as a percentage of the maximum occurring over the 24-h period

	Dark period								
	1200 hrs	1500 hrs	1800 hrs	2100 hrs	2400 hrs	0300 hrs	0600 hrs	0900 hrs	1200 hrs
$\text{NH}_4^+$ - L	93	97	100	99	99	96	95	96	96
LD	99	100	100	87	63	78	93	94	95
Urea - L	99	99	99	99	99	99	100	100	100
LD	97	100	97	100	98	95	56	82	96

## Results

### Perturbation Experiments

Typical depletion curves for  $\text{NH}_4^+$  and urea exhibited a rapid initial uptake rate (15–30 min) tailing off with time (Fig. 1). This kind of response was usually more pronounced for  $\text{NH}_4^+$  than urea which frequently was removed at a constant rate over the time course of an experiment. The effect of initial concentration on short-term or transient uptake by *Chordaria flagelliformis* follows saturation kinetics (Fig. 2) though there appears to be a deviation at high  $\text{NH}_4^+$  concentrations ( $>20 \mu\text{g at l}^{-1}$ ) from the hyperbolic relationship expressed at lower concentrations. The double reciprocal plot, because of its inherent weighting of lower velocities, provided a better fit ( $r^2=0.95$ ) to this data set than the Hanes-Woolf transformation ( $r^2=0.90$ ) and was therefore used in the subsequent provisional estimation of  $K_m$  and  $V_{\max}$ . The  $K_m$  for transient uptake of  $\text{NH}_4^+$  was calculated to be  $10.6 \mu\text{g at l}^{-1}$  and the  $V_{\max}$   $2\,031 \mu\text{g N} \cdot \text{g}^{-1} \text{ dry weight} \cdot \text{h}^{-1}$ . For urea uptake the

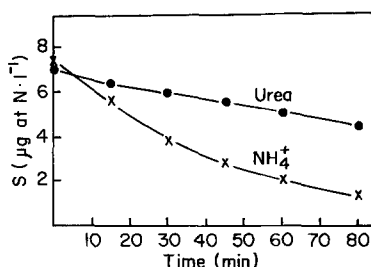


Fig. 1. *Chordaria flagelliformis*. Typical batch mode depletion curves showing changes in substrate concentration (S) over time as a result of uptake

Hanes-Woolf plot ( $r^2=0.98$ ) was used in calculating the  $K_m$  and  $V_{\max}$  values of  $22.5 \mu\text{g at l}^{-1}$  and  $932 \mu\text{g N} \cdot \text{g}^{-1} \text{ dry weight} \cdot \text{h}^{-1}$ , respectively.

It is evident that the derived  $V_{\max}$  values for both  $\text{NH}_4^+$  and urea are somewhat larger than their respective experimentally measured values (Fig. 2) suggesting that the Michaelis-Menten model does not rigorously apply to these short-term uptake data. In the absence of an alternative model, more accurate estimates of  $V_{\max}$  can be obtained graphically from the V vs S plots in Fig. 2 seeing that saturation was achieved in both cases. The  $V_{\max}$  values estimated in this manner are approximately  $1\,750 \mu\text{g N} \cdot \text{g}^{-1} \text{ dry weight} \cdot \text{h}^{-1}$  for  $\text{NH}_4^+$  and  $715 \mu\text{g N} \cdot \text{g}^{-1} \text{ dry weight} \cdot \text{h}^{-1}$  for urea.

### Steady Concentration Experiments

Table 1 shows that the relative uptake rates at steady residual N concentration measured over a 24-h period after establishing a steady residual nutrient concentration, remained almost unchanged in continuous light but appeared to be photoperiodically cued in a light/dark cycle.

Uptake rate under constant nutrient supply describe a saturatable response with increasing residual concentration (Fig. 3) as was the case for short-term perturbation experiments. The uptake parameters calculated for the two experimental modes are however, markedly different. The  $K_m$  and  $V_{\max}$  values for both  $\text{NH}_4^+$  (S vs S/V,  $r^2=0.99$ ) and urea (S vs S/V,  $r^2=0.99$ ) in steady-state are considerably lower than those estimated from batch experiments. For  $\text{NH}_4^+$  the  $K_m$  and  $V_{\max}$  were estimated at  $0.6 \mu\text{g at l}^{-1}$  and  $246 \mu\text{g N} \cdot \text{g}^{-1} \text{ dry weight} \cdot \text{h}^{-1}$ , respectively while the figures for urea were similar at  $0.52 \mu\text{g at l}^{-1}$  and  $188.25 \mu\text{g N} \cdot \text{g}^{-1} \text{ dry weight} \cdot \text{h}^{-1}$ , respectively.

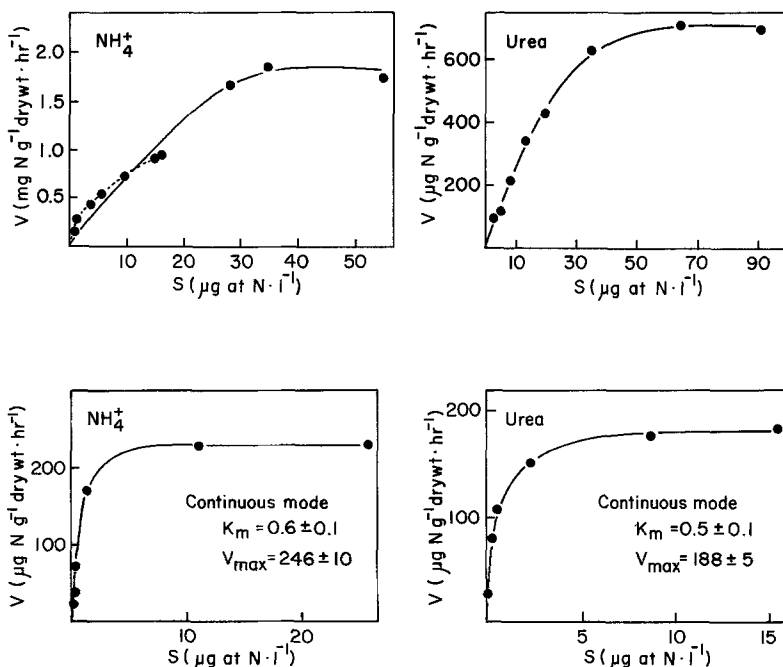


Fig. 2. *Chordaria flagelliformis*. Uptake rates (V) as a function of initial substrate concentration (S) for  $\text{NH}_4^+$  and urea in batch mode experiments. The uptake curve for  $\text{NH}_4^+$  is not truly hyperbolic and may be biphasic as indicated by the dashed line

Fig. 3. *Chordaria flagelliformis*. Uptake rates (V) as a function of residual substrate concentration (S) for  $\text{NH}_4^+$  and urea in steady-state flow-through culture

## Discussion

The batch mode and continuous mode uptake rates of *Chordaria flagelliformis* both appear to be a function of the external concentration of  $\text{NH}_4^+$  and urea. The relationships are generally hyperbolic though the batch mode  $\text{NH}_4^+$  uptake curve displays a tendency to be biphasic (Fig. 2), indicating the possible existence of more than one carrier system or a single multiphasic carrier. Such a dual system, if not merely an experimental artifact, would be advantageous in a non-homogenous environment where  $\text{NH}_4^+$  concentrations fluctuate frequently between the two systems. The overall effect would be a functional optimization, on the part of the seaweed, of a wide range of  $\text{NH}_4^+$  concentrations. Accurate estimates of  $K_m$  and  $V_{\max}$  can be obtained for the continuous mode experiments by fitting the data to the Michaelis-Menten expression. Uptake of both  $\text{NH}_4^+$  and urea in batch mode, however, is not adequately described by Michaelis-Menten kinetics. Consequently, the estimated  $K_m$  values derived for these experiments are unsuitable for comparison with values for other macroalgae. The experimentally measured  $V_{\max}$  values in batch mode indicate a much higher maximum potential for short-term uptake of  $\text{NH}_4^+$  as compared to urea. This  $V_{\max}$  value for  $\text{NH}_4^+$  is also markedly higher than found for other seaweeds with the exception of a number of green algae (Table 2). No information is available for comparison of urea uptake with other macroalgae.

One can calculate the potential doubling time of *Chordaria flagelliformis* from the  $V_{\max}$  values measured in these short-term experiments. Assuming these maximal uptake

rates for  $\text{NH}_4^+$  and urea could be sustained long enough, *C. flagelliformis* could assimilate enough N to account for a doubling time of 16 h on  $\text{NH}_4^+$  and 40 h on urea, assuming an initial N content of 28 mg N  $\text{g}^{-1}$  dry weight (unpublished results). Such high growth rates are far in excess of the maximum recorded in the field (unpublished results) and in culture (Probyn, 1981). This potential for extremely high short-term uptake uncoupled from growth has important considerations in the aquaculture of seaweeds. Ryther *et al.* (1981) have shown that *Gracilaria tikvahiae* need only be exposed to nutrient enriched seawater for 6 h every 2 wk to enable the plants to grow at maximal rates for those conditions. In an ecological context the advantages of possessing such impressive transient uptake kinetics are more likely to be displayed over the short-term (min), equipping the seaweed with a mechanism to rapidly absorb sporadic, local pulses of  $\text{NH}_4^+$  or urea caused by wind or tidal mixing, or excretion by epifauna (D'Elia and De Boer, 1978).

The low  $K_m$ , low  $V_{\max}$  uptake parameters measured at a steady residual N concentration are in marked contrast to those determined in batch mode. The half-saturation constant for  $\text{NH}_4^+$  uptake of 0.6  $\mu\text{g}$  at  $\text{l}^{-1}$  is the lowest reported to date for seaweeds (Table 2) and is close to those measured for oceanic phytoplankton (MacIsaac and Dugdale, 1969; Eppley *et al.*, 1969). The uptake parameters for urea at a steady N supply are comparable to those determined for  $\text{NH}_4^+$  (Fig. 3), a fact which has important implications for explaining the success of *Chordaria flagelliformis* during the summer. The  $K_m$  for urea uptake indicates an affinity comparable to or better than those reported for phytoplankton (McCarthy, 1972; Rees and Syrett, 1979).

**Table 2.** Kinetic parameters for  $\text{NH}_4^+$  uptake by a number of macroalgae

Species	Temp. °C	$K_m$ ( $\pm$ se) $\mu\text{g}$ at N $\cdot$ $\text{l}^{-1}$	$V_{\max}$ ( $\pm$ se) $\mu\text{gN} \cdot \text{g}^{-1}$ dry wt $\cdot$ $\text{h}^{-1}$	Reference
<b>Chlorophyceae:</b>				
<i>Codium fragile</i>	12	1.5 (0.3)	392 (16)	Hanisak and Harlin, 1978
<i>Cladophora</i> sp.	23	20.7 (11.4)	1820 (310)	Gordon <i>et al.</i> , 1981
<i>Codium decortatum</i>	20	12.0 (3.1)	188 (85)	Rosenberg and Paerl, 1981
<i>Ulva curvata</i>	20	— <sup>a</sup>	3500	Rosenberg, 1981
<i>Enteromorpha compressa</i>	—	24.0	515	Kautsky, 1982
<b>Rhodophyceae:</b>				
<i>Gracilaria foliifera</i>	20	1.6	333	D'Elia and De Boer, 1978
<i>Gracilaria foliifera</i>	20	— <sup>a</sup>	980	Rosenberg, 1981
<i>Neogardhiella baileyi</i>	20	2.3–4.9	79–420	D'Elia and De Boer, 1978
<i>Hypnea musciformis</i>	26	16.6 (1.8)	—	Haines and Wheeler, 1978
<b>Phaeophyceae:</b>				
<i>Fucus spiralis</i>	10	6.4 (4.9)	—	Topinka, 1978
<i>Macrocystis pyrifera</i> <sup>b</sup>	6–9	50	330 <sup>c</sup>	Wheeler, 1978
<i>M. pyrifera</i>	16	5.3 (1)	333 <sup>c</sup>	Haines and Wheeler, 1978
<i>Chordaria flagelliformis</i>	11	— <sup>a</sup>	1750	This study (batch)
	11	0.6 (0.1)	246 (10)	This study (continuous)

<sup>a</sup> Did not follow Michaelis–Menten kinetics

<sup>b</sup> Estimates using methylamine as an  $\text{NH}_4^+$  analog

<sup>c</sup> Estimated from a wet/dry weight ratio of 90%

*Chordaria flagelliformis* would appear to be suitably adapted to efficiently scavenge the low ambient  $\text{NH}_4^+$  ( $\sim 0.3 \mu\text{g}$  at  $l^{-1}$ ) and urea ( $\sim 0.5 \mu\text{g}$  at  $l^{-1}$ ) concentrations present during the summer months and also to rapidly exploit any temporal and spatial increase in N concentration. The relative importance of these two strategies in supporting the high growth rates of *C. flagelliformis* is difficult to estimate. The affinity of both the  $\text{NH}_4^+$  and urea uptake systems under steady nutrient supply suggests that growth could be maintained almost entirely by the efficient uptake of the prevailing mean nutrient concentrations with insignificant contributions from the utilization of patches of elevated N content. The typically highly wave-exposed character of areas colonized by *C. flagelliformis* would, through physical dispersion, promote spatial homogeneity of a nutrient resource thus limiting the effectiveness of pulses of  $\text{NH}_4^+$  or urea generated from point sources such as epifaunal excretion, in supporting transient elevated uptake rates. The high flushing rate of water over the plant surface would, however, have the advantage of maintaining a high rate of N-loading (N supply per unit time), even at very low concentrations.

Finally this study indicates that perhaps more caution should be exercised when attempting to explain the growth requirements of plants in the sea on the basis of uptake measurements in short-term batch cultures. These transient uptake rates can be far in excess of the growth requirement and probably represent the maximum capacity of the uptake system in question for a particular N status of the seaweed. Results of this kind can lead to gross overestimation of the uptake parameters  $K_m$  and  $V_{max}$ . Uptake kinetics measured at steady nutrient supply can be regarded as the truncation of the short-term response related presumably to some intracellular metabolite pool. Uptake regulated by internal factors is likely to be coupled closely with growth, providing more practical estimations of  $K_m$  and  $V_{max}$  for application to growth performance in the field.

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