

Nitrogen Uptake by *Gracilaria gracilis* (Rhodophyta): Adaptations to a Temporally Variable Nitrogen Environment

A. J. Smit

Botany Department, University of Cape Town, PO Box Rondebosch, 7700, Cape Town, South Africa, ajsmit@botzoo.uct.ac.za

The physiology of nitrogen acquisition was determined for *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine *et al.* Farnham in a series of perturbation experiments with the aim of examining uptake kinetics in response to transiently variable N. Experiments were designed to determine how variables such as history of exposure to nutrients, NO_3^- -N and NH_4^+ -N concentrations and interactions, temperature and water motion affect parameters of linear and Michaelis-Menten models. A third 'Michaelis-Menten parameter' (α) is introduced here and used to extract additional ecological relevant information from the model. Ammonium-nitrogen uptake was best described by a linear, rate-unsaturated response, with the slope increasing with N-limitation, indicating that *Gracilaria* is more efficient at acquiring nutrients when internally stored N pools were impoverished. Temperature also affected the slope of the linear regression in the case of N-replete material. Nitrate-nitrogen uptake was suppressed by approximately 38 % in the presence of NH_4^+ -N at concentrations above 5 μM , and the seaweed displayed a higher affinity for NH_4^+ -N than for NO_3^- -N at low temperatures. Nitrate-nitrogen uptake followed a rate-saturating mechanism best described by the Michaelis-Menten model. Increased temperature enhanced the affinity for NO_3^- -N only in N-limited thalli, while nutrient limitation enhanced affinity irrespective of temperature. The maximal velocity of uptake (V_{max}) and the half saturation constant (K_s) appeared to vary with experimental conditions, but these differences were not statistically significant. Water motion was shown to reduce 'diffusion transport limitation' experienced by the alga under conditions of low external dissolved inorganic nitrogen (DIN) concentrations, so that the rate of N uptake responds with a 4.5-fold increase under conditions of enhanced water motion. All results suggest that *Gracilaria gracilis* is well suited to remain productive in an upwelling environment dominated by the transient availability of DIN through the use of a high affinity system for NO_3^- -N and non-saturable uptake of NH_4^+ -N. Water motion interacts strongly with nutrient concentration, and may alleviate N limitation by reducing boundary-layer resistance to diffusion. Practical application of the results of this study is discussed in terms of significance to mariculture.

Introduction

Gracilaria gracilis (Stackhouse) Steentoft, Irvine *et al.* Farnham occurs as free-living, largely monospecific beds in a limited number of sheltered coastal water bodies on the west coast of southern Africa. Studies on the ecology, ecophysiology and cultivation of *Gracilaria gracilis* in Namibia and South Africa have been reported by Anderson *et al.* (1989), Rotmann (1990), Molloy (1992), Anderson *et al.* (1993), Dawes (1995), Anderson *et al.* (1996a, 1996b), Smit *et al.* (1997), Smit (1998), Anderson *et al.* (1998, 1999) and Smit and Bolton (1999). According to one study (Anderson *et al.* 1996a), low environmental nutrient concentrations appear to be responsible for the low growth rates of *Gracilaria* at certain times of the year. In other systems, similar seasonal changes in growth rates and production of seaweeds have also been ascribed to nutrient limitation (e.g. Rosenberg and Ramus 1982, Lapointe and Duke 1984, Fujita *et al.* 1989, Borum and Sand-Jensen 1996). It is well known that the addition of N can greatly enhance the growth rate

and production of seaweeds under certain conditions (Lapointe and Ryther 1979, Smit *et al.* 1997). Anderson *et al.* (1996a) also suggest that site-related differences in growth rate of *G. gracilis* may be caused by differences in water movement at these sites.

The growth rate and productivity of algae is, in part, controlled by the concentration of dissolved inorganic nitrogen (DIN) in the aqueous medium surrounding the thallus (Dugdale 1967, Chapman and Craigie 1977, Rosenberg and Ramus 1982, Lavery and McComb 1991). The ability of an alga to utilise N for biomass production is determined by the rate at which DIN can traverse the boundary layer adjacent to the outer cell layer of the thallus, and the rate at which this N takes part in biochemical processes (Wheeler 1980, Koch 1994, Sanford and Crawford 2000). Consequently, external physical processes acting on the boundary layer govern the rate of diffusion, whereas enzymatic processes (i. e. within the algal cells) determine the second stage of uptake. Understanding N nutrition of seaweeds thus requires the integration of at least three controlling factors, i. e. N concentration in the growth

medium, the rate of diffusion through the boundary layer, and the rate at which the seaweed can utilise N in metabolic processes.

Nutrient uptake is often determined by measuring the disappearance of the nutrient from the incubation medium over a given time interval after the addition of the alga. The uptake rate (V) of nutrients is usually described as a hyperbolic function of substrate concentration (S), by analogy to the Michaelis-Menten expression (Equation 1; also called the Monod equation, e. g. Sanford and Crawford 2000) used to mathematically represent enzyme-catalysed reactions (Dowd and Riggs 1965):

$$V = V_{max} \cdot \frac{S}{K_s + S}$$

This equation requires that uptake is unidirectional so that no losses occur after uptake. Two parameters have to be estimated, namely V_{max} and K_s . The theoretical maximal rate of uptake of a nutrient (V_{max}) indicates rate saturation above a certain S , i. e. V ceases to increase with increasing substrate concentration. The half saturation constant, K_s , is numerically equivalent to the value of S where $V = \frac{1}{2} V_{max}$ (Dowd and Riggs 1965). The hyperbolic geometry of the V vs. S curve suggests that uptake is not simply a passive process relying on diffusion alone, but that it is also controlled to some extent by factors intrinsic to the alga itself (Lobban and Harrison 1994). Alternatively, it may be that some other upper limit is imposed on the rate at which nutrients can be incorporated into thallus tissue (Pedersen 1994). The parameters V_{max} and K_s contain ecologically relevant information relating to the nutrient uptake ability of a species under conditions of varying nutrient availability, allowing for comparisons of nutrient uptake characteristics among species and independent studies (Harrison *et al.* 1989). The nutritional history of the seaweed has a marked effect on the shape of the V vs. S relationship. For example, N limitation may change the typical hyperbolic response to a biphasic (D'Elia and DeBoer 1978) or linear relationship (Fujita 1985). In some instances the uptake of nutrients (e. g. ammonium) does not appear to be saturated even at high experimental concentrations irrespective of nutritional history, necessitating the application of other models that more adequately describe the process (D'Elia and DeBoer 1978).

Usually, kinetic studies focus on S since it is an integral part of the Michaelis-Menten relationship. However, water motion also affects V , which is positively related to the rate of water movement past a thallus or cell via its effect on the boundary layer (Wheeler 1980, Neushul *et al.* 1992). Water motion alters V by acting on K_s , the model parameter that describes the phase of uptake that is under physical (external) control. The maximum velocity of V , on the other hand, should be independent of any external process, since it represents the maximum rate at which

the alga is able to metabolise DIN made available to the inside of the boundary layer subsequent to physical transport. The velocity of nutrient uptake at a given S approaches V_{max} only when the rate of water motion is sufficient to suppress transport limitation; alternatively, an adequately high concentration gradient can overcome transport limitation.

The aim of this paper is to evaluate the ammonium ($\text{NH}_4^+\text{-N}$) and nitrate ($\text{NO}_3^-\text{-N}$) uptake kinetics of *Gracilaria gracilis*. In doing so, I explore the nutrient physiological response of the seaweed in context of the highly transient N environment under which it persists. The specific ecophysiological attributes in question are represented by the parameters V_{max} and K_s , and the geometry of the V vs. S relationship in response to external determinants such as temperature, N concentration and water motion. No attempts are made to relate N uptake rate and cellular N status to growth rate. I also determine whether transport limitation or generally low nutrient concentration limit nutrient acquisition. A knowledge of the factors affecting the ability of the seaweed to acquire N in a transient N system could be used to understand the apparent large fluctuations in *Gracilaria* biomass that occur (Anderson, unpublished; personal observations). This knowledge can also be applied to strain- and site-selection purposes important for *Gracilaria* cultivation.

Material and Methods

Collection site

Gracilaria gracilis was collected during winter (May – August) from Saldanha Bay on the west coast of South Africa (~ 33° 5'S, 18° 0'W). The Bay is sheltered from direct oceanic wave-action by a breakwater and iron-ore jetty. The seaweed forms unattached beds of varying size, and is often infested with the red algal epiphyte *Ceramium diaphanum* (Lightfoot) Roth (Anderson *et al.* 1998) as well as a variety of microalgae, mainly diatoms. The hydrodynamics of Saldanha Bay and the conditions giving rise to the nutrient impoverished surface waters are discussed in detail by Anderson *et al.* (1996a) and Monteiro and Largier (1999). Despite Saldanha Bay being situated adjacent to the highly productive Benguela upwelling system, the Bay is dominated by oligotrophic surface waters in summer, receiving transient inputs of DIN resulting from fish factory waste disposal and the intrusion of nutrient-rich upwelled water (Smit 1998, Anderson *et al.* 1999, Monteiro and Largier 1999). The fluctuation between summer oligotrophic conditions and the upwelling events leads to a range of temperatures of between 12°C and 23°C. Dissolved inorganic nitrogen from fish factory waste has been shown to sustain *Gracilaria* production in certain areas of the Bay on the occasions when the upwelled source is in short supply; this is highly dependent on hydrodynamic

conditions (Smit 1998, Anderson *et al.* 1999). Consequently, DIN availability is temporally transient and spatially variable with NO_3^- -N concentrations ranging from undetectable levels to values as high as 30–40 μM in some areas. Ammonium-nitrogen levels seldom exceed 4 μM , but are more-often present at much lower (undetectable) concentrations.

Seaweed preparation

Clean, healthy *Gracilaria* specimens were collected and acclimatised in aquaria of recirculating seawater. Separate batches were maintained in order to induce two levels of nutrient status at two temperatures; these temperature and internal N levels were chosen in order to represent physical conditions (temperature) and N status levels (Smit, unpublished data) experienced or attained in the field. A N-replete state was attained by transferring individual thalli to Erlenmeyer flasks containing 500 mL enriched (16 μM NO_3^- -N and 20 μM NH_4^+ -N), filtered seawater (0.45 μm) and kept at temperatures of 15 and 20 °C at a stocking density of approximately 4 g fresh seaweed per 500 mL. The culture medium was replaced daily over a period of two weeks to prevent N limitation, and water movement was achieved by supplying compressed air to the flasks via disposable pipette tips attached to plastic airlines. Illumination was provided by cool white fluorescent tubes at 90 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ which was sufficient to saturate growth (Engledow and Bolton 1992). The other two batches were incubated under the same physical conditions as described above, but the nutrient medium was replaced only once a week over the two weeks to bring about N limitation (termed N-limited *Gracilaria* hereafter). Carbon to nitrogen ratios (C:N) were determined for the two nutrient treatments at the end of the acclimation period, prior to the experiment, using a Carlo-Erba NA 1500NC elemental analyser. The N uptake parameters were determined for N-replete and N-limited *Gracilaria* acclimatised to 15 and 20 °C in a series of perturbation experiments.

Perturbation experiments

Ammonium-nitrogen and NO_3^- -N uptake kinetics were determined by following the depletion of either species of DIN from the incubation medium over short time intervals after an initial spiking to a high concentration (perturbation method: Harrison *et al.* 1989, Pedersen 1994). The experiment was initiated by adding 150 mL, 0.45 μm filtered seawater enriched to 50 μM NH_4^+ -N or NO_3^- -N to three replicate 250 mL Schott bottles, each containing approximately 1.5 g fresh seaweed. The bottles were capped and attached to a Stuart Scientific Model SF1 flask shaker set to 150 oscillations min^{-1} to provide turbulent water movement, illuminated with 90 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ and held at a constant 15 or 20 °C. Water samples were

taken at intervals over the course of the experiment until all N had been taken up. Controls showed that volatilisation of NH_4^+ -N and/or uptake of NH_4^+ -N and NO_3^- -N were undetectable over the experimental period. Ammonium-nitrogen concentration remaining in the incubation medium was determined manually using the phenol-hypochlorite method (Solórzano 1969); NO_3^- -N and NO_2^- -N were analysed independently on a Technicon AutoAnalyser II (Grasshoff 1983). The analysis of water samples for NH_4^+ -N concentration was done in duplicate but this was not necessary for NO_3^- -N analysis due to the high analytical precision of the instrument. At the end of the experiment the seaweed samples were dried in an oven at 60 °C for determination of dry mass and analysed for the content of C and N on a Carlo-Erba CN analyser.

Data reduction and statistical treatment

The effect of DIN concentration on the uptake rate of *Gracilaria* was analysed using the Michaelis-Menten equation when uptake rate was saturated at high substrate concentrations. To obtain the V ($\mu\text{g N g}^{-1}$ dry h^{-1}) vs. S ($\mu\text{M NH}_4^+$ -N or NO_3^- -N) plot, the slope between consecutive sample pairs on the S vs. time (t) depletion curve (obtained from the perturbation experiment) was determined and graphed against the average S of that interval. In other words, the slope for each segment of the line provides an estimate of V at the corresponding (average) S . Corrections were made for seaweed dry mass and change in incubation volume due to interval sampling. The Michaelis-Menten model was fitted to transformed data and V_{max} ($\mu\text{mol N g}^{-1}$ dry h^{-1}) and K_s ($\mu\text{M N}$) estimated using a nonlinear least squares procedure in the statistical package, *R* (Ihaka and Gentleman 1996) on the Linux operating system. A third parameter, alpha (α), was introduced here, and was calculated as the initial slope of the Michaelis-Menten curve at substrate concentrations of less than K_s . The interpretation of this parameter is analogous to α describing the initial slope of photosynthesis-irradiance curves (quantum efficiency) – it provides an indication of how readily uptake rate responds to changes in substrate concentration. This parameter is directly proportional to the magnitude of the change in uptake rate that is associated with a corresponding shift in substrate concentration. Alpha used here is similar in concept to V_{max}/K_s (Harrison *et al.* 1989), also denoted as α .

To test for the suitability of the Michaelis-Menten model, the data were Hanes-Woolf transformed (S/V against S) to obtain linearity (Dowd and Riggs 1965); Pearson's product-moments correlation and t -tests were then used to determine whether the slope and intercept differed significantly from zero (Selvin 1998). Various diagnostic plots were used to evaluate for departures from assumptions pertaining to linear models (Venables and Ripley 1999). The Hanes-Woolf

transformation was chosen above the Lineweaver-Burk (double-reciprocal; $1/V$ vs. $1/S$) and Eadie-Hofstee (V vs. V/S) transformations for reasons given by Dowd and Riggs (1965) and because it maintains an independent variable (S) on the ordinate. The kinetic parameters are obtainable from the Hanes-Woolf plot with the intercept on the horizontal axis as $-K_s$ and the slope as $1/V_{max}$ if need be, but nonlinear estimation was the preferred method in this study.

Estimates of V_{max} and K_s obtained from the nonlinear Michaelis-Menten models were compared using the procedures involving incremental parameters, full and partial models and extra sum of squares analysis as outlined by Bates and Watts (1988) using the package, *R*. Analysis of covariance (ANCOVA) was used to test whether linear regressions, in particular the slopes, displayed significant differences between treatments (Venables and Ripley 1999) using the same software.

The effect of water movement on nutrient uptake rate

Gracilaria collected from Saldanha Bay was cleaned of epiphytes and maintained for one week in an aquarium of recirculating seawater ($0 \mu\text{M}$ N-at DIN and $2 \mu\text{M}$ inorganic-P) under saturating light conditions ($90 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) at 15°C . To determine the uptake rate of $\text{NH}_4^+\text{-N}$ under different rates of water movement, thalli each weighing approximately 1.5 g (fresh), were placed in Schott bottles containing 150 mL enriched filtered seawater ($4 \mu\text{M}$ $\text{NH}_4^+\text{-N}$) with separate bottles for each rate of water motion. Three replicate incubation vessels were randomly attached to flask shakers set to 0 (control), 250 or 500 oscillations min^{-1} (later referred to as still, low or high respectively). The experimental temperature and light conditions were 15°C and $90 \mu\text{mol photons m}^{-2}\text{s}^{-1}$. The rate of nutrient uptake was determined by measuring the disappearance of the nutrient from the medium over one 30-min incubation period. Ammonium-nitrogen concentration remaining in the incubation medium was analysed as above. Rates of disappearance were corrected for incubation time, volume and seaweed dry mass; uptake was expressed as $\mu\text{mol NH}_4^+\text{-N g}^{-1}$ dry h^{-1} .

In a subsequent experiment, the effect of water motion on the Michaelis-Menten parameters was examined by using the perturbation method as described previously. Only $\text{NO}_3^-\text{-N}$ ($\sim 60 \mu\text{M}$) was included in the incubation medium. The seaweed was acclimated for a week in Erlenmeyer flasks (4 g per 500 mL) containing low nutrient ($< 4 \mu\text{M}$ $\text{NO}_3^-\text{-N}$; $0 \mu\text{M}$ $\text{NH}_4^+\text{-N}$) water under $90 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ at 15°C . This experiment allowed the assessment of the interaction between nutrient concentration and the rate of water movement (0, 250 and 500 oscillations min^{-1}). Data were treated as described previously.

Results

Nutrient uptake kinetics

Tissue C:N ratios at the start of the uptake experiments were 6.9 ± 1.6 and 21.1 ± 0.1 for the N-replete and N-limited treatments, respectively (mean \pm SD, using pooled data for the temperature treatments). This difference was significant as determined by a Student's *t*-test ($P < 0.05$, $t = -12.89$, $df = 2$). Ammonium-nitrogen depletion curves of N-limited and N-replete *Gracilaria* incubated at 15 and 20°C are shown in Figure 1, and indicate that $\text{NH}_4^+\text{-N}$ concentration decreases over the incubation period and is nonlinear with time. The shapes of the curves are virtually identical for the two temperature treatments.

Statistically, both linear and Michaelis-Menten models fit the V vs. S data for N-replete and N-limited *Gracilaria* extremely well. However, because linear Hanes-Woolf transformations did not yield significant correlations and resulted in deviations from the expected distribution of residuals (not shown), it was felt that linear regressions were most appropriate (Fig. 2). Also, the extremely high estimates of K_s and V_{max} (not shown) were seen as unrealistic and artificial. The linear response shows that uptake rate does not reach an asymptote within the range of $\text{NH}_4^+\text{-N}$ concentrations used in these experiments for either N-replete or N-limited material at either of the temperatures. Slopes of the regression lines were 1.8 ± 0.1 and 2.1 ± 0.1 for N-replete *Gracilaria* at 15 and 20°C respectively, and increased to 2.5 ± 0.2 and 3.2 ± 0.2 in the N-limited material (Table I). Statistical comparisons of

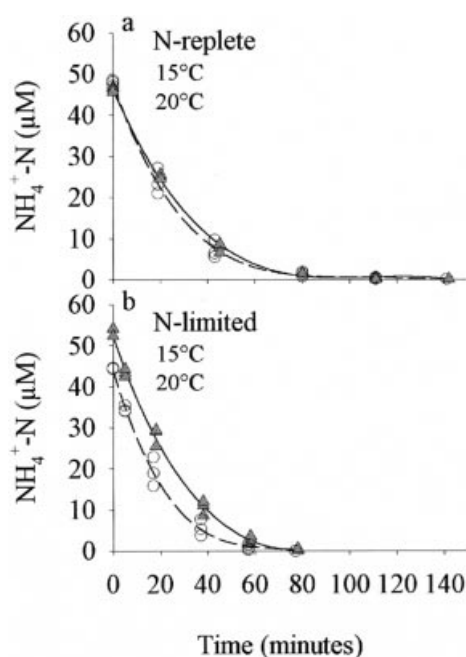


Fig. 1. Time-course of $\text{NH}_4^+\text{-N}$ depletion for (a) N-replete and (b) N-limited *Gracilaria* at 15 and 20°C . Curves fitted using least-squares estimation.

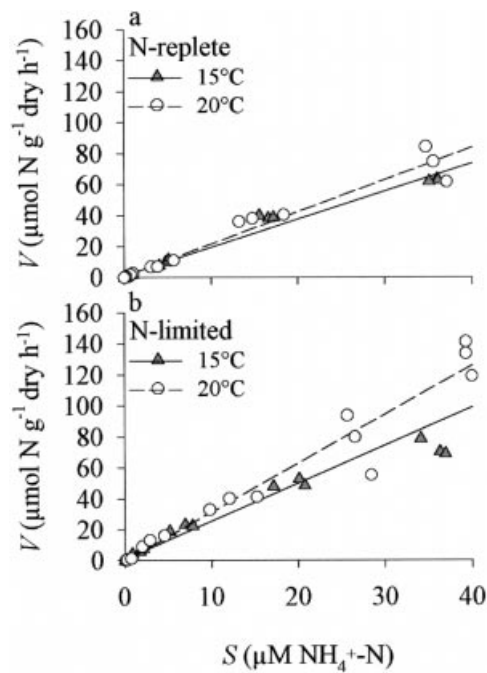


Fig. 2. Linear regressions of NH_4^+ -N uptake results for (a) N-replete and (b) N-limited *Gracilaria*. Regression statistics are given in Table I.

the slopes of the regression equations using ANCOVA showed that this increase was significant at $P < 0.01$ for both of the temperature treatments. For the N-replete nutrient treatment, an increase in temperature significantly affected the rate at which nutrients were removed from the incubation medium ($P < 0.05$); however, this was not the case for the N-limited treatments ($P > 0.05$).

The uptake kinetics of NO_3^- -N are distinctly different from those of NH_4^+ -N. Examples of NO_3^- -N depletion over time typical of Michaelis-Menten kinetics are shown in Figure 3. The 'linear' part of the curve between the start of the experiment and 80 min shows concentration-independent or rate-saturated uptake. The second part of the curve (after 80 min) indicates the concentration-dependent or rate-unsaturated uptake. As is the case with NH_4^+ -N, the depletion curves at 15 and 20 °C are very similar.

Michaelis-Menten curves for N-replete *Gracilaria* at 15 and 20 °C are virtually identical (Fig. 4a), with similar V_{max} values obtained at the two temperatures (34.6–35.0 $\mu\text{mol N g}^{-1} \text{ dry h}^{-1}$; ANOVA, $P = 0.8873$, Table II). The half saturation constant, K_s , at 15 °C is somewhat higher than at 20 °C (6.9 and 5.6 $\mu\text{M NO}_3^-$ -N at 15 and 20 °C respectively), but not significantly so (ANOVA, $P = 0.6141$). The α -parameter was also not significantly different between the two temperatures (ANCOVA, $P > 0.05$). Linear Hanes-Woolf transformations of the same data are shown graphically on

Table I. Estimated kinetic parameters for NH_4^+ -N uptake (mean \pm SE) and statistics associated with linear and Michaelis-Menten models ($K_s = \mu\text{M}$; $V_{max} = \mu\text{mol N g}^{-1} \text{ dry h}^{-1}$).

Linear model:	Parameter estimates		Correlation
	Intercept [t, P]	Slope [t, P]	r
N-repl., 15 °C	1.4 \pm 1.1 [1.2, 0.2337]	1.8 \pm 0.1 [25.7, < 0.0001]	0.988
N-repl., 20 °C	0.8 \pm 1.6 [0.5, 0.6438]	2.1 \pm 0.1 [20.4, < 0.0001]	0.981
N-lim., 15 °C	1.0 \pm 4.0 [0.3, 0.8043]	2.5 \pm 0.2 [16.0, < 0.0001]	0.970
N-lim., 20 °C	-0.3 \pm 4.6 [-0.1, 0.9538]	3.2 \pm 0.2 [15.1, < 0.0001]	0.973
Michaelis-Menten model:	Parameter estimates		
	V_{max} [t, P]	K_s [t, P]	α^{\S}
N-repl., 15 °C	160.5 \pm 17.2 [9.4, < 0.0001]	55.1 \pm 8.9 [6.2, < 0.0001]	1.8 \pm 0.1
N-repl., 20 °C	272.9 \pm 122.8 [2.2, 0.0410]	97.8 \pm 57.5 [1.7, 0.1080]	2.1 \pm 0.1

\S The α -parameter is the slope of the Michaelis-Menten curve estimated below the K_s concentration. V_{max} and K_s estimates for N-limited *Gracilaria* were seen as unrealistically high and are omitted from the Table.

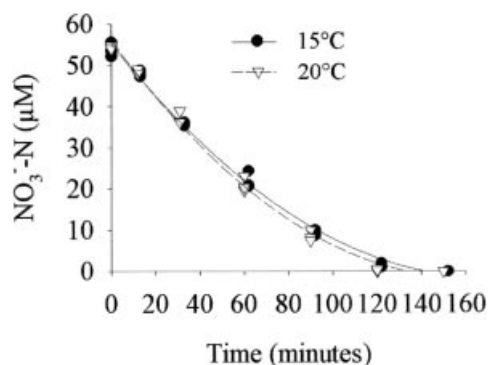


Fig. 3. Time-course of NO_3^- -N depletion measured for N-replete *Gracilaria*. Curves fitted are smoothed splines.

Figure 4b–c, showing highly significant linear correlations of S/V and S for each plot ($r > 0.93$). The Michaelis-Menten model (Fig. 4a) is clearly well suited to describe the data.

Table II provides kinetic parameters and curve-fit statistics of Michaelis-Menten and Hanes-Woolf plots for NO_3^- -N uptake by N-limited *Gracilaria* at 15 and 20°C. Highly significant ($P < 0.001$) linear models were obtained when S/V was regressed with respect to

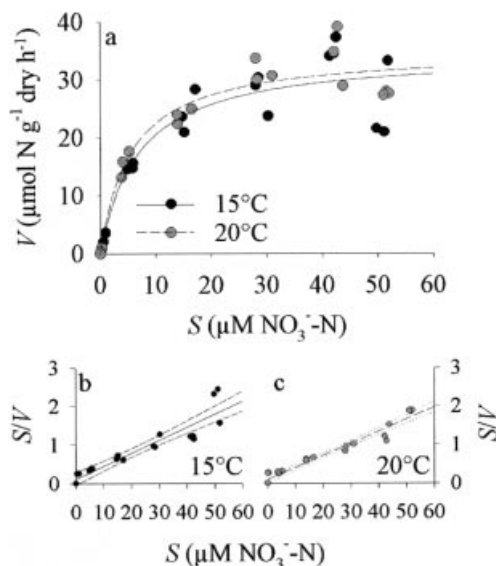


Fig. 4. (a) Michaelis-Menten curves of N-replete *Gracilaria* NO_3^- -N uptake data at 15 and 20 °C and (b) Hanes-Woolf transformed plots of the same data. 95 % confidence intervals are given for the linear regressions. Estimates of kinetic parameters and statistical comparisons are given in Tables II and III, respectively.

Table II. Kinetic parameters (mean \pm SE) estimated for four NO_3^- -N perturbation experiments from Michaelis-Menten curves, together with Hanes-Woolf transformations S/V vs. S .

Nonlinear model – Michaelis-Menten:

	Parameter estimates				
	V_{max} [t, P]	K_s [t, P]	Residual SE	d. f.	α
N-repl., 15 °C	34.6 \pm 2.8 [12.5, < 0.0001]	6.9 \pm 2.3 [3.0, 0.0073]	4.2	19	1.3 \pm 0.3
N-repl., 20 °C	35.0 \pm 1.8 [19.6, < 0.0001]	5.6 \pm 1.3 [4.2, 0.0005]	3.0	19	1.1 \pm 0.1
N-lim., 15 °C	15.5 \pm 1.8 [8.5, < 0.0001]	6.5 \pm 3.4 [1.9, 0.0745]	3.3	19	2.6 \pm 0.1
N-lim., 20 °C	20.0 \pm 2.7 [7.4, < 0.0001]	4.2 \pm 3.2 [1.4, 0.1840]	5.5	15	3.5 \pm 0.1

Linear model – Hanes-Woolf transformed:

	Parameter estimates		Correlation r
	Intercept [t, P]	Slope [t, P]	
N-repl., 15 °C	0.12 \pm 0.08 [1.4, 0.1725]	0.03 \pm 0.00 [11.4, < 0.0001]	0.951
N-repl., 20 °C	0.10 \pm 0.04 [2.2, 0.0382]	0.03 \pm 0.00 [19.8, < 0.0001]	0.967
N-lim., 15 °C	0.30 \pm 0.28 [1.0, 0.3200]	0.08 \pm 0.01 [10.9, < 0.0001]	0.915
N-lim., 20 °C	0.08 \pm 0.32 [0.3, 0.7990]	0.07 \pm 0.01 [9.3, < 0.0001]	0.892

Correlations are Pearson's product-moment correlations. $K_s = \mu\text{M NO}_3^-$ -N; $V_{max} = \mu\text{mol N g}^{-1} \text{ dry h}^{-1}$. Corresponding fitted curves are shown in Figures 4 and 5.

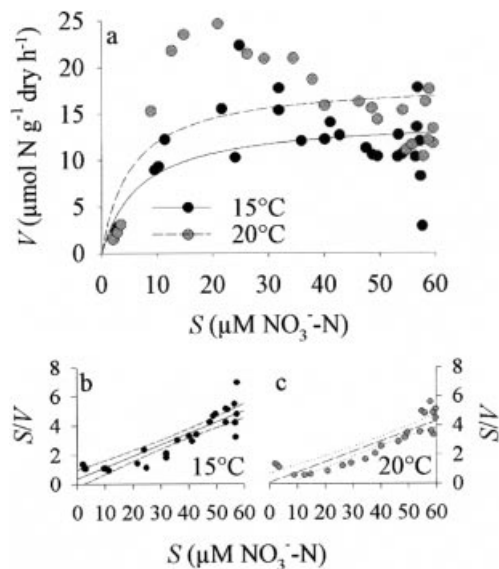


Fig. 5. Michaelis-Menten curves of N-limited *Gracilaria* NO_3^- -N uptake data (a), and Hanes-Woolf transformed plots of the same data at (b) 15 °C and (c) 20 °C. 95 % confidence intervals are given for the linear regressions. Estimates of kinetic parameters and statistical comparisons are given in Tables II and III, respectively.

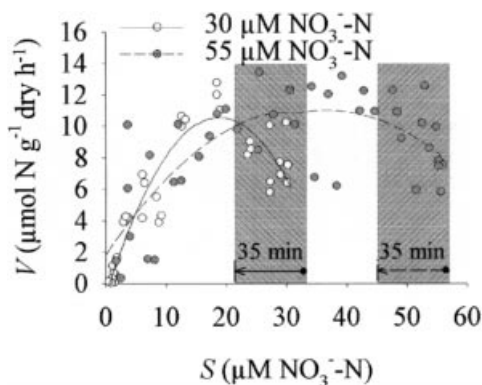


Fig. 6. NO_3^- -N uptake rates during incubation in two media containing different initial NO_3^- -N concentrations. Arrows indicate the first 35 min of each experiment (arrows are not meant to be used as a second independent variable); experiments terminated when substrate concentrations reached about 0 μM NO_3^- -N. Fitted lines are smoothed splines.

S . Analysis of variance comparing K_s and V_{max} between the two temperature treatments did not reveal any significant differences ($P = 0.5921$ and 0.1531 for K_s and V_{max} , respectively), but an ANCOVA showed that α was higher at 20 °C ($P < 0.0001$).

Both K_s and V_{max} are lower in N-limited seaweed than in N-replete material, with the lowest V_{max} values estimated for the thalli acclimatised to 15 °C. Never-

theless, these differences are not statistically significant ($P > 0.05$ in all cases). Alpha differed between N-replete and N-limited treatments at both temperatures ($P = 0.0004$ and < 0.0001 at 15 and 20 °C, respectively). A feature of the curve fitted to the data obtained in the experiment at 20 °C (Fig. 5a) is the low uptake rate at high NO_3^- -N concentrations (at the start of the experiment) followed by an increase approximately 30 min later. The phenomenon led to a curvature in the linearised plots (Fig. 5b–c). The possibility of inhibition of uptake at high NO_3^- -N concentrations and the inhibition of NO_3^- -N uptake due to the presence of NH_4^+ -N was therefore evaluated in subsequent perturbation experiments below.

Inhibition of NO_3^- -N uptake at high nitrate concentrations

The effect of NO_3^- -N concentration in inhibiting uptake at high concentrations was examined by conducting two perturbation experiments, one with an initial NO_3^- -N concentration of 55 μM and another at 30 μM (Fig. 6). Results show that uptake rate is low during the first 35 min of incubation where after it increases to about 10 – 11 $\mu\text{mol N g}^{-1}$ dry h^{-1} at both 30 and 55 μM NO_3^- -N concentrations. Model parameters were not obtained for these data.

NH_4^+ -N – NO_3^- -N interaction

Since the low rate of NO_3^- -N uptake at the start of the experiment does not seem to depend on NO_3^- -N concentration within the range of experimental concentrations, the role of NH_4^+ -N in the inhibition of nitrate uptake was determined. A perturbation experiment was conducted in which N-limited *Gracilaria* was placed in an incubation medium containing 50 μM NO_3^- -N and 25 μM NH_4^+ -N and the depletion of both N species monitored over time. The control treatment used NH_4^+ -free seawater spiked to an initial NO_3^- -N concentration of about 60 μM . Depletion curves of NH_4^+ -N and NO_3^- -N (Fig. 7a) show the low rate of NO_3^- -N uptake in the presence of NH_4^+ -N at concentration above 5 μM . After the concentration of NH_4^+ -N was reduced from about 30 μM to less than 5 μM within the first 50 min, the rate of NO_3^- -N uptake increased substantially (Fig. 7b). Uptake rates as high as 16 $\mu\text{mol N g}^{-1}$ dry h^{-1} in the absence of NH_4^+ -N were maintained until the NO_3^- -N concentration reached 20 μM . In comparison, the control had high rates of NO_3^- -N uptake from the onset of the experiment at 60 μM NO_3^- -N. Although kinetic parameters were not estimated, a visual comparison of curves (Figs 5a and 7b) indicates that fit properties are comparable in both experiments involving N-limited seaweed.

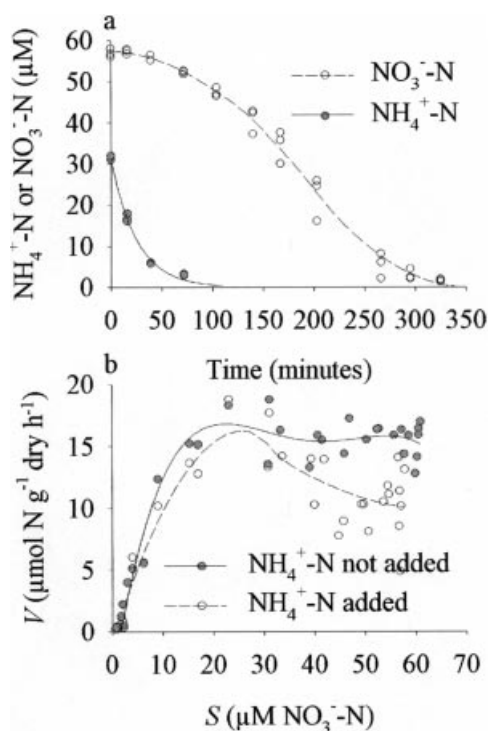


Fig. 7. (a) Time-course of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ depletion after spiking one medium with both $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$. (b) Spline smoothed curves fitted to $\text{NO}_3^-\text{-N}$ uptake rate vs. substrate concentration in the presence and absence of $\text{NH}_4^+\text{-N}$. Data for $\text{NO}_3^-\text{-N}$ uptake in the presence of $\text{NH}_4^+\text{-N}$ was obtained from curve shown in (a).

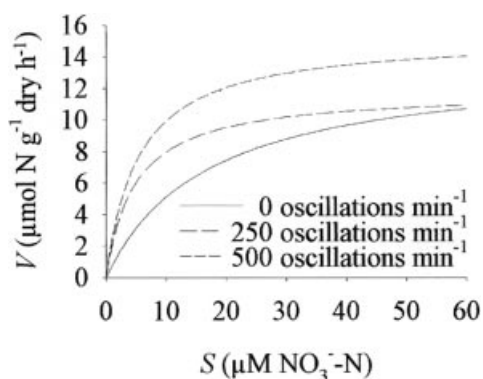


Fig. 8. Michaelis-Menten curves for $\text{NO}_3^-\text{-N}$ uptake by *Gracilaria* under three rates of water movement. Data-points are not shown to prevent clutter. Curve-fit statistics and kinetic parameters are given in Table III.

The effect of water movement on $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ uptake

A one-way ANOVA indicates a significant effect of water motion on the rate of $\text{NH}_4^+\text{-N}$ uptake by *Gracilaria* ($P < 0.001$, d. f. = 1, 4). Ammonium-nitrogen uptake rates at shaker settings of 0 and 250 oscillations min^{-1} are 1.1 ± 0.2 and 1.2 ± 0.2 $\mu\text{mol NH}_4^+\text{-N g}^{-1}$ dry h^{-1}

respectively and are not significantly different as shown by the Tukey Honest Significant Difference (HSD) test ($P > 0.05$). At 500 oscillations min^{-1} the uptake rate significantly increases to 2.0 ± 0.4 $\mu\text{mol NH}_4^+\text{-N g}^{-1}$ dry h^{-1} (Tukey HSD, $P < 0.005$).

Michaelis-Menten curves for $\text{NO}_3^-\text{-N}$ uptake at three rates of water motion are shown in Figure 8. An increase in water motion from still (0 oscillations min^{-1}) conditions to settings of 250 and 500 oscillations min^{-1} affected the Michaelis 'constant', K_s (Tables III and IV), so that the affinity of *Gracilaria* for $\text{NO}_3^-\text{-N}$ increases at higher rates of water motion ($P < 0.05$ in both instances; Table IV). The effect on V_{max} is less clear, but the highest maximal rate of uptake occurs at 500 oscillations min^{-1} where it is significantly higher than at the intermediate setting ($P < 0.05$; Table IV). The α -parameter was strongly dependent on the rate of water movement, with higher rates resulting in a higher α -value. Alpha was significantly greater at high and intermediate rates with respect to still conditions ($P < 0.0001$), but the value did not differ appreciably between intermediate and high rates ($P = 0.2035$). Estimates of V_{max} and K_s were significant in all cases ($P < 0.05$ for K_s and $P < 0.0001$ for V_{max}).

Discussion and Conclusion

The transport of solutes across solid-fluid boundaries plays an integral role in nutrient cycling in marine and aquatic systems. The movement of nutrients from the aqueous medium into seaweeds is described by a two-stage process (Sanford and Crawford 2000). First, uptake rate is influenced by the mass transfer across the boundary layer, the thin layer of water adjacent to the seaweed surface. Second, reaction kinetics inside the thallus, mediated by the seaweed itself, makes up the final stage of uptake. Under very fast mass transfer rates, nutrient acquisition is kinetically controlled; conversely, uptake is mass transfer controlled when kinetic reactions are faster than mass transfer rates (Wheeler 1980).

Although $\text{NH}_4^+\text{-N}$ uptake may be described by a Michaelis-Menten relationship in N-replete *Gracilaria*, extrapolated K_s and V_{max} values fall well outside the actual DIN concentration measured in the Saldanha Bay, where $\text{NH}_4^+\text{-N}$ concentrations seldom exceed $4 \mu\text{M}$. In reality such high Michaelis-Menten parameters would not be ecologically meaningful, and it is suggested that a linear, rate-unsaturated response would for all practical purposes adequately describe the uptake response. In the case of N-limited seaweed, selection of the best model is considerably simplified by the lack of sufficient statistical support for the nonlinear model (Table I). Non-saturable kinetics for $\text{NH}_4^+\text{-N}$ has also been shown for corals with symbiotic algae (Muscatine and D'Elia 1978), *Neoagardhiella baileyi* (Harvey ex Kützing) Wynne et Taylor and *Gracilaria foliifera* (Forsskål) Børgesen (D'Elia and DeBoer 1978), *Gracilaria tikvahiae* McLachlan

Table III. Kinetic parameters estimated for NO_3^- -N uptake under three rates of water movement, and statistics associated with the fit of the model to the data.

	Parameter estimates		
	V_{max} [t, P]	K_s [t, P]	α
0 osc. min^{-1}	13.7 ± 1.5 [9.1, < 0.0001]	16.8 ± 5.5 [3.0, 0.0040]	0.4 ± 0.0
250 osc. min^{-1}	11.8 ± 0.8 [15.1, < 0.0001]	4.8 ± 1.9 [2.6, 0.0128]	1.2 ± 0.1
500 osc. min^{-1}	15.3 ± 1.1 [13.8, < 0.0001]	5.4 ± 1.9 [2.8, 0.0075]	1.4 ± 0.1

$K_s = \mu\text{M NO}_3^-$ -N; $V_{max} = \mu\text{mol N g}^{-1} \text{ dry h}^{-1}$. Figure 8 shows corresponding Michaelis-Menten curves.

Table IV. ANOVA tables showing comparisons of Michaelis-Menten parameter estimates obtained from NO_3^- -N uptake perturbation experiments at three rates of water motion.

	Model	Residual d. f.	Residual sum of squares	d. f.	Sum of squares	F-value	P
$K_s - 0 \text{ osc. min}^{-1}$	Full	86	487.55	1	48.27	8.5083	0.0045
vs. 250 osc. min^{-1}	Partial	87	536.14				
$V_{max} - 0 \text{ osc. min}^{-1}$	Full	86	487.87	1	10.26	1.8084	0.1822
vs. 250 osc. min^{-1}	Partial	87	498.13				
$K_s - 0 \text{ osc. min}^{-1}$	Full	86	682.15	1	55.54	7.0019	0.0097
vs. 500 osc. min^{-1}	Partial	87	737.69				
$V_{max} - 0 \text{ osc. min}^{-1}$	Full	86	682.15	1	4.65	0.5857	0.4462
vs. 500 osc. min^{-1}	Partial	87	688.80				
$K_s - 250 \text{ osc. min}^{-1}$	Full	80	677.35	1	0.54	0.0640	0.8010
vs. 500 osc. min^{-1}	Partial	81	677.89				
$V_{max} - 250 \text{ osc. min}^{-1}$	Full	80	677.35	1	65.99	7.7942	0.0066
vs. 500 osc. min^{-1}	Partial	81	743.34				

Estimated parameters are presented in Table III.

(Friedlander and Dawes 1985) and *Chaetomorpha linum* Kützing and *Ulva rigida* C. Agardh (MacFarlane and Smith 1982, McGlathery *et al.* 1996). It appears that linear uptake kinetics is more common in the acquisition of reduced forms of DIN (Fujita 1985).

Fujita (1985) showed that the geometry of the uptake response is variable in *Ulva lactuca* L., with specimens grown at low N flux displaying a rate-unsaturated (linear) response, while those grown at high N flux having a characteristic Michaelis-Menten response. A similar switch has been demonstrated for *Neogardhiella baileyi* (D'Elia and DeBoer 1978) and *Ulva rigida* (Lavery and McComb 1991). There may be some evidence for this in *Gracilaria gracilis* in this study, since statistical evidence does not completely rule out support for Michaelis-Menten kinetics in N-replete material. However this would be limited to conditions of high N concentrations that rarely occur in nature.

Two parameters of ecological relevance are con-

tained in the linear model: the slope and the y-intercept. In the linear regression, the slope has exactly the same meaning as α in the nonlinear case, and they are also mathematically equivalent. Consider the case of NH_4^+ -N uptake by N-replete *Gracilaria* where the extrapolated K_m -value is $55.0 \pm 8.9 \mu\text{M}$ at 15°C . Since the experiment was conducted at NH_4^+ -N concentrations of $50 \mu\text{M}$ or less, it implies that the entire dataset be used for the calculation of α (which is calculated as the slope of the linear part of the Michaelis-Menten curve using V, S pairs at $S < K_m$). This is effectively the same as fitting a linear model to the dataset (compare the slope estimate and α in Table I).

A steep slope (high α) shows that uptake rate is readily enhanced as soon as elevated NH_4^+ -N concentrations are encountered; this is an indication of the sensitivity of uptake rate increase in response to elevated ambient DIN concentrations (i. e. affinity; Fujita 1985). A high affinity implies that the seaweed can effectively acquire nutrients at low concentrations.

The slopes of the linear models in this study clearly respond to changes in temperature and N stress, with the lowest slope for N-replete *Gracilaria gracilis* at 15 °C and the highest at 20 °C for N-limited material. These results are in agreement with those of Fujita (1985) who examined the NH_4^+ -N uptake and kinetic parameters of *Ulva lactuca*, *Enteromorpha* spp. and *Gracilaria tikvahiae* grown under high and low N-flux, both before and after starvation. Although the mechanism for uptake differed, he also found that N-limitation increased the affinity for NH_4^+ -N. Similarly, Peckol *et al.* (1994) found that NH_4^+ -N uptake of *G. tikvahiae* is faster under N-limiting conditions. The enhancement of nutrient uptake rate following stress in the form of N limitation has often been proposed as a mechanism that enables algae to maintain growth in an environment with fluctuating nutrient concentrations (Fujita 1985). The increase in the slope of the regression line with ensuing nutrient limitation, as demonstrated in the present study, is also supportive of such a mechanism.

A linear uptake response, particularly in N stressed specimens growing at low nutrient concentrations, also points to a relatively large N storage capacity. Although this was not directly confirmed by multiple exposures to high nutrient media, it is known from previous work that the same strain of *Gracilaria gracilis* indeed has the ability to grow for extended periods of time at high growth rates despite being cultivated at low N concentrations (Smit *et al.* 1997). The ability of *G. gracilis* to maintain relatively high growth rates during periods of N limitation, combined with its ability to increase NH_4^+ -N uptake rate upon exposure to increased nutrient levels is therefore an extremely effective mechanism for persistence in a temporally transient N environment. Baroclinic pressure gradients that exist across the coastal (southern Benguela upwelling system) and Bay (Saldanha Bay) environments allow nutrient-rich upwelled water to intrude into Saldanha Bay at a period of 6–8 days (Monteiro and Largier 1999). The periodicity of N availability in Saldanha Bay closely matches the previously reported storage capacity of *Gracilaria* (Smit *et al.* 1997). Temperature increase has a limited effect on nutrient uptake because it is influenced by physical processes such as diffusion (Lobban and Harrison 1994).

The second ecologically meaningful parameter in the linear model is the y-intercept. A positive y-intercept has been suggested to indicate the possibility of the existence of a strong diffusive component (Lavery and McComb 1991). This does not appear to be true in the present study as none of the y-intercept estimates differ significantly from 0 (Table 1).

Gracilaria gracilis appears to have a higher affinity for NH_4^+ -N than for NO_3^- -N at low temperatures (compare α in Tables I and II), but this difference is lost at 20 °C. A greater affinity for NH_4^+ -N has also been demonstrated for other Rhodophyta (D'Elia and DeBoer 1978) and phytoplankton (Caperon and Zie-

mann 1976), and is related to the fact that NO_3^- -N uptake is more energetically costly since it is somewhat dependent on photosynthesis (Wheeler 1982). Nevertheless, NO_3^- -N rather than NH_4^+ -N would probably contribute more towards the total N consumption by species because the oxidised form of N comprises the bulk of the total environmental DIN pool. A high affinity for NO_3^- -N, together with the ability of *Gracilaria gracilis* to store N internally in excess of immediate growth requirement (Rosenberg and Ramus 1982, Lapointe and Duke 1984, Peckol *et al.* 1994, Vergara *et al.* 1995, Smit *et al.* 1997), allow it to be productive in an environment dominated by transient pulses of nutrients, such as Saldanha Bay. These same characteristics make this species highly effective in mariculture conditions, where nutrients are supplied in pulses at a similar frequency that is experienced by plants growing *in situ* (Smit *et al.* 1997). Such adaptations may also make *G. gracilis* competitively superior to its main epiphyte, *Ceramium diaphanum*, but this has yet to be confirmed through comparative physiological studies. The competitive strengths of *Gracilaria tikvahiae* relative to that of its epiphyte have been demonstrated by Peckol *et al.* (1994).

The geometry of the relationship between V and S clearly depends on the form of DIN assimilated. It was shown, above, that a linear relationship exists between NH_4^+ -N uptake rate and concentration of this nutrient. In the case of NO_3^- -N the shape of the relationship is best approximated by the Michaelis-Menten equation. This model is widely used in algal nutrient uptake studies (e.g. Dugdale 1967, Chrisholm and Stross 1976, Topinka 1978, Probyn and Chapman 1982, Williams and Fisher 1985, Parker 1993, Peckol *et al.* 1994, Portielje and Lijklema 1994, Vergara *et al.* 1995). The hyperbolic geometry of the $V - S$ relationship is characteristic of facilitated diffusion or active uptake, indicating saturation of carriers responsible for transporting nutrients across membranes (Lobban and Harrison 1994). Alternatively, the saturation response has been linked to the rate at which amino acids are incorporated into biomass (Pedersen 1994).

Nitrate-nitrogen uptake by *Gracilaria gracilis* is associated with a rate-saturating mechanism that appears stable across temperature fluctuations or historic nutrient exposure within the limits of these variables in Saldanha Bay. Temperature influenced α only in N-limited thalli, with the higher value for specimens kept at 20 °C. Nutrient depletion, however, affected α irrespective of temperature, so that the seaweed's sensitivity to fluctuations in ambient N was more pronounced when internal N pools were depleted. These results are similar to those of D'Elia and DeBoer (1978) who also failed to show any relationship between C:N ratio and the kinetic parameters (V_{max} and K_s) for NO_3^- -N uptake; no mention was made to α (as V_{max}/K_s). In contrast, results of O'Brien and Wheeler (1987) show that NH_4^+ -N uptake of *Enteromorpha prolifera* (Müller) J. Agardh was not correlated with

tissue N content while both kinetic parameters were higher for NO_3^- -N uptake under reduced tissue N content. Other studies give evidence for and against enhanced NH_4^+ -N or NO_3^- -N uptake under different degrees of N-limitation (e.g. Probyn and Chapman 1982, Rosenberg *et al.* 1984, Friedlander and Dawes 1985, Probyn and McQuaid 1985, McGlathery *et al.* 1996), suggesting that the mechanism for N acquisition is greatly species or strain dependent.

High α -values indicate that uptake rate is rapidly enhanced as soon as NO_3^- -N availability transiently increases in the growing medium, and it follows that high α -values are indicative of being efficient in taking up nutrients at low concentrations. Harrison *et al.* (1989) and Pedersen (1994) employ V_{max}/K_s as an indication of the initial slope of the Michaelis-Menten curve, and suggest that it is more meaningful than using K_s , since K_s is somewhat dependent on V_{max} . However, V_{max}/K_s yields a value that is very different from α (data not shown). It is therefore suggested that α be used as a measure of affinity rather than V_{max}/K_s , as it is mathematically representative of the true slope of the initial part of the Michaelis-Menten curve. It is also equivalent to the slope in the linear model, and can thus be used to compare the affinity component between two types of kinetic models.

Uptake rate of NO_3^- -N is reduced by up to 38 % in the presence of NH_4^+ -N. The reduction in uptake rate lasts for as long as NH_4^+ -N is present in the culture medium at concentrations above 5 μM ; typically 30–35 min in the experiments discussed here. Since the NH_4^+ -N concentration in Saldanha Bay is usually lower than 5 μM , it is unlikely that inhibition of NO_3^- -N uptake will take place in nature. The inhibition of NO_3^- -N uptake in the presence of NH_4^+ -N has also been shown for *Enteromorpha intestinalis* (L.) Grev., *Gracilaria pacifica* Abbott (Thomas and Harrison 1987), *Enteromorpha prolifera* and *Ulva* sp. (O'Brien and Wheeler 1987) and phytoplankton (Parker 1993). Ecologically, inhibition of NO_3^- -N uptake in the presence of the reduced form of DIN has an advantage, as NO_3^- -N assimilation can only occur after its reduction by nitrate-reductase, making it energetically costly.

A detailed study on the effect of N-limitation on uptake by Pedersen (1994) indicated three stages of NH_4^+ -N uptake (surge, internally-controlled and externally-controlled uptake) that are a function of internal N concentration. Only the externally controlled phase [or physically controlled phase; Munk and Riley (1952)] is of relevance to this study since it was shown to be independent of N-limitation due to a limit being imposed on uptake by the rate of mass transport of N across the boundary layer. The limitation of uptake by externally controlled physical factors is called 'diffusion transport limitation' (Pasciak and Gavis 1974), and can be alleviated through an increased concentration of nutrients in the external medium, or the rate of water movement across the boundary layer (Gavis 1976, DeBoer *et al.* 1978, Parker 1982).

Several workers have demonstrated that increased rates of water movement enhance algal growth, nutrient uptake and photosynthetic rates above rates obtained under still conditions. For example, DIN or dissolved inorganic carbon (DIC) uptake rates are affected by the velocity of currents or waves past attached seaweeds (e.g. Munk and Riley 1952, Parker 1981, 1982, Neushul *et al.* 1992, Koch 1994) or the sinking rate or swimming speed of planktonic algae (e.g. Pasciak and Gavis 1974, Canelli and Fuhs 1976, Gavis 1976). Most studies on the kinetics of nutrient uptake by seaweeds neglect the fact that under certain hydrodynamic conditions, low rates of diffusion may limit the nutrient supply to the seaweed surface. One method for determining whether nutrient uptake is under kinetic or mass transfer control is by measuring the rate of nutrient uptake under a range of hydrodynamic conditions (e.g. Koch 1994). Examining the results obtained in this study, it does indeed appear that transport limitation controls the rate at which NO_3^- -N can be assimilated by *Gracilaria gracilis*, at least under conditions of low water movement. Such conditions often occur in Saldanha Bay during summer (personal observations). Increased turbulent water motion specifically affects the values of α and K_s , but no discernable trend is apparent in V_{max} . This result is not surprising, as the former two parameters relate to the initial part of the Michaelis-Menten curve describing the externally controlled phase – the phase during which NO_3^- -N diffuses across the boundary layer. The maximal velocity of nutrient uptake remains relatively unchanged irrespective of turbulence, and points to that part of the uptake process that is controlled by factors intrinsic to the seaweed itself.

In this study an increase in shaker speed from still conditions to 250 oscillations min^{-1} is accompanied by a 2.8 fold increase in α . Doubling the water motion resulted in a 4.5-fold increase of α above still conditions. The K_s value is about three times lower at higher rates of water movement so that the efficiency of NO_3^- -N uptake is greatly enhanced. Clearly, water motion enhances diffusion transport and reduces transport limitation. Water motion is especially important in facilitating nutrient uptake under low ambient levels of NO_3^- -N often experienced *in situ*. For example, at 4 μM NO_3^- -N, uptake rate is increased by a factor of 2.5 when water motion is increased from completely still conditions to 500 oscillations min^{-1} . In environments with low DIN concentrations, either the rate of diffusion across the boundary layer or the affinity for N may limit growth or determine the nutrient status of the alga (Fujita and Goldman 1985). If the affinity for N is low, an increased rate of diffusion to the algal surface might exceed uptake capacity. On the other hand, if the affinity for DIN is high, N uptake might be limited by the rate at which N can be supplied to the surface.

Variable K_s and α values indicate that caution needs to be exercised when Michaelis-Menten parameters

are compared between different studies. The 'true' value of K_s (i.e. measured when transport limitation is not an issue, and is controlled solely by the plant's physiological characteristics such as nitrate reductase activity) can only be measured under conditions of relatively high rates of water motion. The point at which the mass transport of nutrients is maximal will vary from seaweed to seaweed, and will also be affected by characteristics such as the presence of epiphytes, surface texture and roughness (Nowell and Jumars 1984). The maximal velocity of nutrient uptake, on the other hand, can be determined under any range of hydrodynamic conditions, but substrate concentrations need to be sufficiently high in order for it to be measured.

Although it is not possible to relate experimental findings to conditions experienced in the field unless the exact hydrodynamic nature of the water body surrounding the suspended seaweed raft is known (Neushul *et al.* 1992), support is given to the suggestion of Anderson *et al.* (1996a) that site-related differences in growth of *Gracilaria gracilis* at Saldanha Bay may be caused by seasonal differences in water movement. In terms of seaweed cultivation, results stress the importance of proper raft maintenance (in terms of tensioning of the seaweed lines) and positioning in areas to allow sufficient water motion to overcome nutrient transport limitation. This is especially important in environments where ambient nutrient concentrations are already low. It also shows that a comparison of nutrient uptake studies from various authors is seldom valid because the effect of water motion on the kinetic parameters is often overlooked.

From an ecological perspective, rates of nutrient uptake are most likely of limited value in allowing one to judge a species' growth performance and it is suggested that future studies attempt to apply the Droop (1974, 1977) model that allows one to examine the de-coupling between growth rate and external substrate concentration. Through application of this model it should be possible to show the extent to which growth rate is controlled by the availability of intern-

ally stored N, while simultaneously showing the dependence of thallus N on environmental nutrient availability through an understanding of uptake kinetics.

Results of this first study on the N uptake ecophysiology of a southern African species of *Gracilaria* in some cases mirror and in others contrast with uptake mechanisms determined for other strains or species of *Gracilaria* worldwide. The study discusses the mechanisms by which *Gracilaria* is able to remain productive in an environment dominated by temporally variable concentrations of DIN. Specifically, the use of rate-unsaturated kinetics for NH_4^+ -N uptake and a high affinity system for NO_3^- -N uptake allow it to utilise both forms of DIN over wide concentration ranges. This N uptake strategy also underlies the principle of 'luxury consumption', which provides a seaweed with the ability to take up nutrients well in excess of its immediate requirement for growth, thereby uncoupling the growth response from N uptake. A practical application of this phenomenon is illustrated in tank cultivation systems in which the culture medium is enriched only once or twice a week to concentrations of up to $1200 \mu\text{M}$ NH_4^+ -N (e.g. Lapointe 1985, Smit *et al.* 1997). The same authors have shown that *G. gracilis* is capable of growing rapidly using only internally stored N for extended periods of time while DIN is completely lacking from the external culture medium. This property of the *Gracilaria* also makes it well suited for growing in a system in which nutrient supply is primarily driven by upwelling.

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