

Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake

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ABSTRACT: The ability to sustain growth at low availability of nitrogen (N) was examined in 6 species of macroalgae with different growth strategies by comparing substrate dependent growth kinetics. The N required to support optimal growth and the N uptake kinetics of 2 slow-growing algae, *Fucus vesiculosus* and *Codium fragile*, and 4 fast-growing species, *Chaetomorpha linum*, *Cladophora sericea*, *Ceramium rubrum* and *Ulva lactuca*, were experimentally determined in summer when the algae were N limited. The N required to support maximum growth varied 16-fold among species, with fast-growing algae having the highest N demands. The high N requirements of ephemeral algae were caused by up to 13-fold higher growth rates and 2- to 3-fold higher N content at maximum growth. Also, the fast-growing species took up ammonium and nitrate 4 to 6 times faster per unit of biomass than slow-growing species at both low and high substrate concentrations, but the ratios of maximum N uptake to requirements were larger among the slow-growing algae. Thus, the fast-growing species tended to require relatively higher external concentrations of inorganic N to saturate their growth. Under N limited conditions, all 6 macroalgae were able to exploit pulses of high concentrations of ammonium by taking up ammonium at transiently enhanced rates (i.e. surge uptake). Uptake was, however, only marginally enhanced at low, and naturally occurring, concentrations of ammonium, suggesting that surge uptake is of minor ecological importance. Our results show that large, slow-growing macroalgae may be better able to meet their N requirements at low N availability than fast-growing species. This is consistent with the common observation that nutrient-poor coastal areas are dominated by slow-growing macroalgae rather than ephemeral species, although ephemeral species have higher N uptake capacities.

KEY WORDS: Marine macroalgae · Nitrogen requirement · Nitrogen uptake · Competition

INTRODUCTION

Nutrient availability is an important factor controlling plant performance and species composition of plant communities in shallow coastal waters (Borum 1983, Orth & Moore 1983, Twilley et al. 1985, Neundorfer & Kemp 1993, Taylor et al. 1995, Borum 1996). These shallow water plant communities are composed of a large number of species representing various growth strategies and life forms, and the contribution

of the different plant types to total autotrophic biomass and production is regulated by several factors that influence growth and loss processes (Sand-Jensen & Borum 1991, Duarte 1995). The distribution of macroalgae along nutrient gradients is typically characterized by slow-growing species in nutrient-poor regions or seasons, while fast-growing, ephemeral species dominate under nutrient-rich conditions (Kautsky et al. 1986, Sfriso et al. 1987, Ménesguen & Piriou 1995, Taylor et al. 1995, Borum & Sand-Jensen 1996)

The association between high nutrient availability and increased dominance of ephemeral macroalgae suggests that fast-growing species require high nutrient inputs to sustain growth, while large, slow-grow-

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ing species are better adapted to nutrient-poor conditions. In support of this idea, Pedersen & Borum (1996) demonstrated that ephemeral macroalgae suffered from N limitation during longer periods of low N availability in summer than did slow-growing species. The observed variation in N limitation among algae of different growth strategy could partly be explained by species-specific differences in N requirements and storage capacity. However, the work also indicated that, compared to ephemeral species, slow-growing macroalgae were better able to meet their N requirements by exploiting low external N concentrations (Pedersen & Borum 1996).

Ephemeral macroalgae with thin thalli and simple morphology take up N faster per unit of biomass and, further, have higher affinities for uptake at low N concentrations than large, slow-growing species (Wallentinus 1984, Fujita 1985, Hein et al. 1995). Fast N uptake and high affinity among ephemeral algae has formerly been used to imply that these algae were well suited to compete for nutrients at low availability of N. It has also been hypothesized that ephemeral species utilize short-term pulses of ammonium by taking it up at transiently enhanced rates (i.e. surge uptake), thereby compensating for low uptake during subsequent periods of low N availability (Fujita 1985). Surge uptake has been documented for a large number of macroalgae (e.g. Fujita 1985, Thomas & Harrison 1987, Harrison et al. 1989, Pedersen 1994, McGlathery et al. 1996), and McGlathery et al. (1996) showed that the length and magnitude of the surge uptake were inversely related to the size of intracellular pools of ammonium in the ephemeral macroalga *Chaetomorpha linum*. If this mechanism is a general feature among algae, we expect all algal species to be able to perform surge uptake independently of growth strategy.

The advantage of fast uptake at low availability of N gained by ephemeral algae may, however, be offset if the N requirements of these species are comparably higher than the requirements for large, slow-growing species. The only way to evaluate species-specific differences in sensitivity to nutrient stress is to combine the kinetics for N uptake and N dependent growth into substrate dependent growth kinetics (Dugdale 1967) and then compare the ability of different species to sustain growth at low substrate concentrations (Turpin 1988).

The objective of this study was to test the hypothesis that large, slow-growing macroalgae are better adapted to grow under low N availability than fast-growing, ephemeral species, because their N requirements and uptake of N are better balanced at potentially limiting N concentrations. We addressed this question by comparing substrate dependent growth kinetics, modeled from experimentally determined kinet-

ics of N uptake and N requirements for growth among 6 species of macroalgae with inherently different maximum growth rates. We also tested whether the presence and magnitude of surge uptake differed significantly between fast- and slow-growing macroalgae.

METHODS

Whole thalli of 6 macroalgal species with different growth strategies were collected in Roskilde Fjord, Denmark, during late July and August 1991, when the algae were N limited (Pedersen 1995, Pedersen & Borum 1996). *Fucus vesiculosus* L. and *Codium fragile* (Suringar) Hariot. Lucas are slow-growing, perennial macroalgae with thick and structurally complex thalli, while *Ulva lactuca* L., *Cladophora serica* (Huds.) Lhtz., *Chaetomorpha linum* (O.F. Müll.) Lhtz. and *Ceramium rubrum* (Huds.) C.Ag. are annual species having thin thalli and high maximum growth rates. The algae were rinsed of sediment, debris and epiphytes and kept in laboratory cultures for 5 to 7 d before being used for assessment of N requirements for growth or for uptake experiments. The culture tanks contained freshly collected water from the sampling site [salinity = 17‰; dissolved inorganic N (DIN) < 1 µM; dissolved inorganic P (DIP) = 4 to 6 µM] and were kept at constant irradiance (300 µmol m⁻² s⁻¹ PAR in a 16 h light:8 h dark cycle) and temperature (15°C).

Nitrogen dependent growth. Data for growth of *Fucus vesiculosus*, *Ulva lactuca*, *Ceramium rubrum*, *Chaetomorpha linum* and *Cladophora serica* as functions of internal N contents in the algal tissues were obtained from Pedersen & Borum (1996), while N dependent growth of *Codium fragile* was experimentally determined in the present study, using the same method as in Pedersen & Borum (1996). All 6 species of macroalgae were grown in laboratory cultures under constant light (300 µmol m⁻² s⁻¹ PAR in a 16 h light:8 h dark cycle) and temperature (15°C). Nitrogen loadings were varied to obtain specimens with a range of different tissue N concentrations. Growth rates (µ) were measured as the increase in biomass assuming exponential growth:

$$\mu = (\ln B_t - \ln B_0) / (T_t - T_0) \quad (1)$$

where B_0 and B_t are the dry weight biomass of algae at the beginning (T_0) and at the end (T_t) of the experimental period. Observed growth rates were plotted against tissue N concentrations (N) and the Droop equation (Droop 1983) was fitted to the data using non-linear, least squares regression:

$$\mu = \mu_{\max} [1 - (N_Q/N)] \quad (2)$$

where μ_{\max} is the maximum growth rate at infinite

tissue N concentrations, N_Q is the minimum tissue N content needed to sustain growth (the subsistence N quota), and N is the actual tissue N concentration. The critical N concentration (N_C) needed to sustain growth at maximum rates was estimated as the intercept between the initial slope of the curve and the maximum growth rate (μ_{\max}), while N requirements for maximum growth (N_{req}) were estimated as the product of the maximum growth rate and critical N concentration (i.e. $\mu_{\max} \times N_C$).

Uptake experiments. Uptake of ammonium may initially be enhanced and nitrate uptake suppressed, when N depleted algae are exposed to sudden pulses of inorganic N (McGlathery et al. 1996). To discern the different phases of N uptake, we measured uptake rates of ammonium and nitrate by several time-course depletion experiments, lasting 4 to 10 h each. The depletion of substrate due to uptake was measured over different time intervals and substrate concentrations in each experiment, so that any transient phases appearing during the initial stage of the experiments could be separated from the more constant uptake rates occurring subsequently (referred to as 'assimilation rates', for details see Pedersen 1994, McGlathery et al. 1996).

The uptake experiments were carried out in translucent plexiglas chambers containing 2 l of medium and kept under constant irradiance ($400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) and temperature (15°C). Water circulation was generated by submersible pumps (6 l min^{-1}). Filtered (Whatman GF/C) seawater was enriched with N by adding either NH_4Cl or NaNO_3 from stock solutions. The uptake experiments were carried out with at least 6 different starting concentrations of N, ranging from 3.5 to 85 μM ammonium or from 3.5 to 45 μM nitrate. Algal material (0.1 to 0.7 g DW) was fixed on a Nitex mesh and submersed in the medium at time $t = 0$. Triplicate water samples (5 ml) were collected every 15 min during the first 90 min and subsequently every 30 min until all inorganic N had been depleted from the medium (within 240 to 600 min). Ammonium was immediately analyzed using the phenol-hypochlorite method (Solórzano 1969), while nitrate samples were frozen for later analysis on an Alpha Chem[®] Auto Analyzer. Algal dry weight was determined after drying to constant weight at 90°C for 48 h.

Uptake rates [V ($\mu\text{mol g}^{-1} \text{DW h}^{-1}$)] were calculated for each time interval during the depletion according to:

$$V = [(S_0 \times \text{vol}_0) - (S_t \times \text{vol}_t)] / (t \times B) \quad (3)$$

where S_0 and S_t are the substrate concentrations and vol_0 and vol_t the volumes before and after a sampling period (t), and B is algal dry weight biomass. Uptake rates (V) were plotted against the mean substrate con-

centration (S) for each time interval, and the Michaelis-Menten function was fitted to the data using non-linear, least squares regression:

$$V = (V_{\max} \times S) / (K_m + S) \quad (4)$$

where V_{\max} is the maximum uptake rate and K_m the half-saturation constant for uptake. The initial slope of the V versus S curve, equal to V_{\max}/K_m , was calculated and used as an index of N uptake affinity at low substrate concentrations (Healy 1980).

Estimation of substrate dependent growth. The association between growth rate and the concentration of ammonium or nitrate in the water was estimated for each species using the uptake kinetics (Eq. 4) for either ammonium (the 'assimilation' kinetics) or nitrate and the relationship between growth rate and tissue N content (Eq. 2), assuming steady state conditions. The relation between growth rate and substrate concentration under steady state conditions can be described by a Monod type equation:

$$\mu = (\mu^*_{\max} \times S^*) / (K_\mu + S^*) \quad (5)$$

where μ^*_{\max} is the maximum growth rate under steady-state conditions, K_μ is the half-saturation constant for growth, and S^* is the steady-state substrate concentration. Eqs. (2) & (4) can be combined and solved for N_Q , which is then substituted into Eq. (2) to estimate the constants μ^*_{\max} and K_μ of Eq. (5). Turpin (1988) provide a detailed description for the derivation of the equations defining μ^*_{\max} and K_μ :

$$\mu^*_{\max} = (\mu_{\max} \times V_{\text{inax}}) / [(\mu_{\max} \times N_Q) + V_{\text{inax}}] \quad (6)$$

and

$$K_\mu = (K_m \times \mu_{\max} \times N_Q) / [(\mu_{\max} \times N_Q) + V_{\text{inax}}] \quad (7)$$

where V_{inax} , K_m , μ_{\max} and N_Q are defined as described for Eqs. (2) & (4). Both μ^*_{\max} and K_μ of Eq. (5) were estimated from measured values of V_{inax} , K_m , μ_{\max} and N_Q , and used to model growth rates at typical *in situ* concentrations of ammonium and nitrate, respectively. The computation was performed assuming no effects of stored N in the algae.

Error estimation. The substrate dependent growth rate (μ^*) and the half-saturation constant for growth (K_μ) were both calculated as combinations of several individual variables, each measured with their own error. The bootstrap procedure (Efron & Tibshirani 1986) was, therefore, applied to estimate means and confidence limits for the combined results. All the individual variables were assumed to be normally distributed with observed means and standard deviations. Bias-adjusted bootstrapped mean values ($\bar{x}_{\text{B,adj}}$) of μ^*_{\max} and K_μ were computed from the individual variables (Eqs. 6 & 7) using Monte Carlo resampling (100 mean values were estimated), according to Meyer

et al. (1986). Bias-adjusted 95% confidence limits were finally computed from the 2.5 and 97.5% percentiles of the bootstrapped data sets following the procedure presented by Meyer et al. (1986).

RESULTS

Nitrogen requirements

Maximum growth rates (μ_{\max}), subsistence quotas (N_Q) and critical tissue N concentrations (N_C) obtained in the laboratory experiments (this study and Pedersen & Borum 1996) are summarized in Table 1. In general, growth rates increased as functions of tissue N concentration as exemplified by the relationship between growth and tissue N in *Codium fragile* (Fig. 1). The maximum growth rate at infinite tissue N concentration varied 13-fold among species and was lowest for *Fucus vesiculosus* and *C. fragile* (0.039 and 0.083 d^{-1}) and highest among the 4 ephemeral species (0.218 to 0.513 d^{-1} ; Table 1).

The subsistence quota (N_Q) ranged from 0.38% N of DW in *Chaetomorpha linum* to 1.02% of DW in *Ceramium rubrum*, with the remaining values being clustered around 0.6% N of DW. The critical tissue N

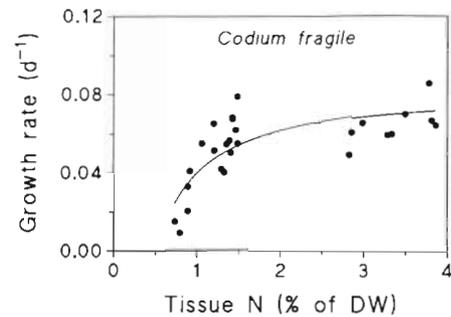


Fig. 1 *Codium fragile*. Relationship between growth rate and tissue N concentration as determined in laboratory experiments

concentration (N_C) ranged from 1.15% N of DW in *C. linum* to 3.1% N of DW in *C. rubrum* (Table 1). Both N_Q and N_C tended to increase with increasing maximum growth rate of the algal species, but the patterns were not consistent since N_Q and N_C were low for the relatively fast-growing *C. linum*. The amount of N required to sustain maximum algal growth varied 16-fold, from 48 $\mu\text{mol N g}^{-1} \text{DW d}^{-1}$ in *Fucus vesiculosus* to 795 $\mu\text{mol N g}^{-1} \text{DW d}^{-1}$ in *Ulva lactuca* (Table 1).

Table 1 Maximum growth rates (μ_{\max}), subsistence quotas (N_Q), critical tissue N concentrations (N_C) and estimated N requirements for growth under laboratory conditions in 6 species of macroalgae. Estimates of μ_{\max} and N_Q were obtained by non-linear least squares regression. Numbers are mean values with 95% confidence intervals in parentheses. Data for all algae except for *Codium fragile* are from Pedersen & Borum (1996)

	μ_{\max} (d^{-1})	N_Q (% N of DW)	N_C (% N of DW)	N_{req} ($\mu\text{mol N g}^{-1} \text{DW d}^{-1}$)
<i>Fucus vesiculosus</i>	0.039 (0.003–0.127)	0.55 (0.35–0.64)	1.71 (–1.48–4.38)	48 (–95–219)
<i>Codium fragile</i>	0.083 (0.072–0.094)	0.52 (0.40–0.64)	1.58 (0.45–2.61)	96 (25–169)
<i>Chaetomorpha linum</i>	0.218 (0.176–0.260)	0.38 (0.37–0.39)	1.15 (0.96–1.38)	179 (143–229)
<i>Cladophora serica</i>	0.251 (0.189–0.313)	0.68 (0.57–0.79)	2.05 (0.93–3.31)	364 (157–607)
<i>Ceramium rubrum</i>	0.223 (0.176–0.270)	1.02 (0.97–1.08)	3.10 (2.59–3.64)	493 (379–614)
<i>Ulva lactuca</i>	0.513 (0.479–0.547)	0.71 (0.66–0.76)	2.17 (1.33–2.93)	795 (595–987)

Table 2 Nitrogen uptake kinetics. The kinetic parameters V_{\max} ($\mu\text{mol N g}^{-1} \text{DW h}^{-1}$), K_m (μM) and the affinity for uptake at low concentrations (V_{\max}/K_m ; Healy 1980) for surge uptake of ammonium (surge; V^S), uptake and assimilation of ammonium (V^{ass}) and uptake of nitrate (V) for 6 species of macroalgae. Parameter estimates were obtained by non-linear least squares regression. Numbers are mean values $\pm 95\%$ confidence intervals. The coefficient of determination (r^2) is also given; all regressions were highly significant ($p < 0.001$)

	Surge uptake of ammonium (V^S)				Assimilation of ammonium (V^{ass})				Nitrate uptake (V)			
	V_{\max}	K_m	V_{\max}/K_m	r^2	V_{\max}	K_m	V_{\max}/K_m	r^2	V_{\max}	K_m	V_{\max}/K_m	r^2
<i>Fucus vesiculosus</i>	41 \pm 7	21 \pm 10	2.0	0.99	22 \pm 5	14 \pm 7	1.6	0.77	8 \pm 1	11 \pm 5	0.7	0.91
<i>Codium fragile</i>	81 \pm 15	25 \pm 12	3.3	0.94	44 \pm 5	12 \pm 3	3.8	0.93	9 \pm 1	5 \pm 1	2.0	0.92
<i>Chaetomorpha linum</i>	132 \pm 29	13 \pm 12	10.2	0.89	47 \pm 5	3 \pm 1	13.6	0.89	30 \pm 5	3 \pm 1	9.9	0.95
<i>Cladophora serica</i>	122 \pm 33	13 \pm 10	9.8	0.94	43 \pm 6	5 \pm 3	9.4	0.87	17 \pm 2	5 \pm 2	3.5	0.84
<i>Ceramium rubrum</i>	271 \pm 81	29 \pm 21	9.4	0.95	59 \pm 6	5 \pm 2	12.3	0.89	–	–	–	–
<i>Ulva lactuca</i>	240 \pm 61	21 \pm 16	11.3	0.88	72 \pm 6	6 \pm 2	12.4	0.95	20 \pm 3	5 \pm 2	4.5	0.86

Uptake rates

The uptake of ammonium was initially enhanced when N depleted algae were suddenly exposed to ammonium. Uptake rates are, therefore, represented by 2 sets of uptake kinetics: (1) one representing transiently enhanced uptake (i.e. surge uptake), measured during the first 15 min after exposure to ammonium, and (2) one representing the uptake rates obtained later than 120 min after exposure (here denoted as the 'assimilation rate'; see Pedersen 1994, McGlathery et al. 1996). The surge uptake probably represents a combination of adsorption of ions onto the plant surface and the absorption of ions into the plant tissue, while assimilation uptake most likely represents the rate of amino acid synthesis within the algae (McGlathery et al. 1996). The rate of surge uptake increased and tended to saturate with increasing substrate concentration among all the algal species examined (Fig. 2), but the kinetic constants exhibited large species-specific variations (Table 2). The maximum rate of surge uptake (V_{\max}^S) ranged 6-fold, from 41 $\mu\text{mol N g}^{-1} \text{DW h}^{-1}$ in *Fucus vesiculosus* to 271 $\mu\text{mol N g}^{-1} \text{DW h}^{-1}$ in *Ceramium rubrum*. The half-saturation constant (K_m) ranged from 13 μM in *Cladophora serica* to 29 μM in *C. rubrum*, but all K_m values were characterized by large errors, making inter-specific differences non-significant at the 5% level. The $V_{\max}:K_m$ ratios for ammonium (i.e. the affinity for ammonium uptake at low substrate concentrations) were higher among the ephemeral algae than for slow-growing species (9 to 11 vs 2 to 3; Table 2).

The assimilation rate of ammonium (V^{ass}) also increased and saturated with increasing substrate concentration, but rates were generally 2- to 5-fold lower than surge uptake rates at high substrate concentrations (Fig. 2). Maximum assimilation rates (V_{\max}^{ass}) ranged from 22 $\mu\text{mol N g}^{-1} \text{DW h}^{-1}$ in *Fucus vesiculosus* to 72 $\mu\text{mol N g}^{-1} \text{DW h}^{-1}$ in *Ulva lactuca* (Table 2). The half-saturation constants for assimilation were also lower than those for surge uptake, so the $V_{\max}:K_m$ ratios for surge and assimilation uptake remained roughly the same. Identical $V_{\max}:K_m$ ratios of surge and assimilation uptake indicate that the rates of ammonium uptake were not initially enhanced at low substrate concentrations.

We evaluated the potential gain of N achieved by the algae during surge uptake, by comparing the amounts of N that could be accumulated in excess of N assimilation

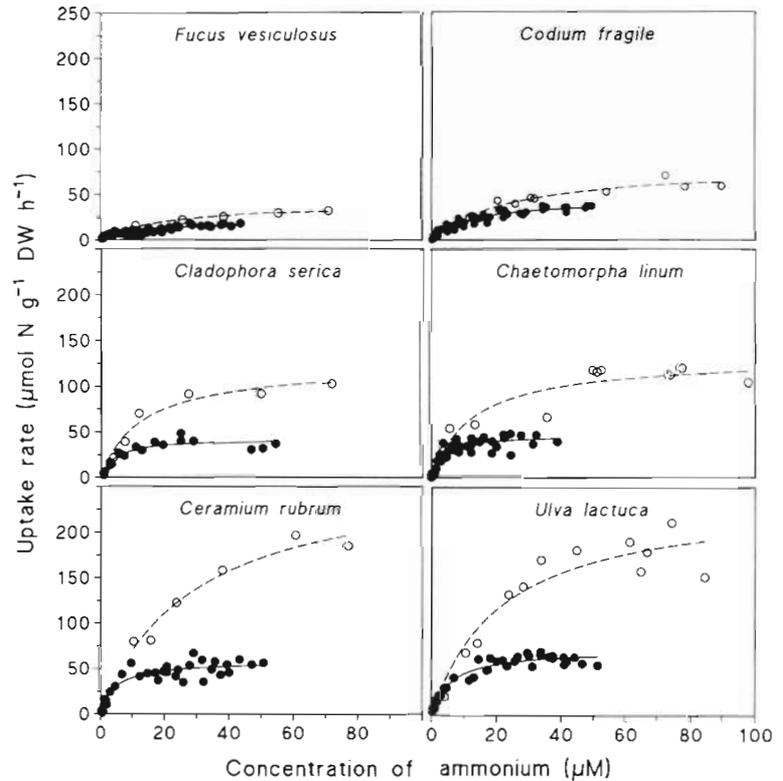


Fig. 2. Uptake rates of ammonium as a function of substrate concentration. Surge uptake (○) was measured over the initial 15 min after exposure to ammonium, while assimilation rates (●) were measured later than 120 min after the experiments were initiated

during the surge phase at high ammonium concentrations. The N gained during the surge phase ranged from 9.6 $\mu\text{mol N g}^{-1} \text{DW}$ in *Fucus vesiculosus* to 106.1 $\mu\text{mol N g}^{-1} \text{DW}$ in *Ceramium rubrum* (Table 3), and the ephemeral species accumulated in general more N per unit of biomass than the slow-growing algae. The higher gain by ephemeral algae was, however, balanced by higher N requirements for growth, and the N pool accumulated during the surge phase could sup-

Table 3. Role of surge uptake. Amount of ammonium taken up in excess of assimilation uptake during the surge phase (N gain), and the period of growth at maximum rates that can be supported by the ammonium taken up during the surge phase for 6 different species of macroalgae

	N gain ($\mu\text{mol N g}^{-1} \text{DW}$)	Growth period (h)
<i>Fucus vesiculosus</i>	9.6	4.6
<i>Codium fragile</i>	18.7	4.8
<i>Chaetomorpha linum</i>	42.6	6.0
<i>Cladophora serica</i>	39.3	2.9
<i>Ceramium rubrum</i>	106.1	3.3
<i>Ulva lactuca</i>	84.0	2.6

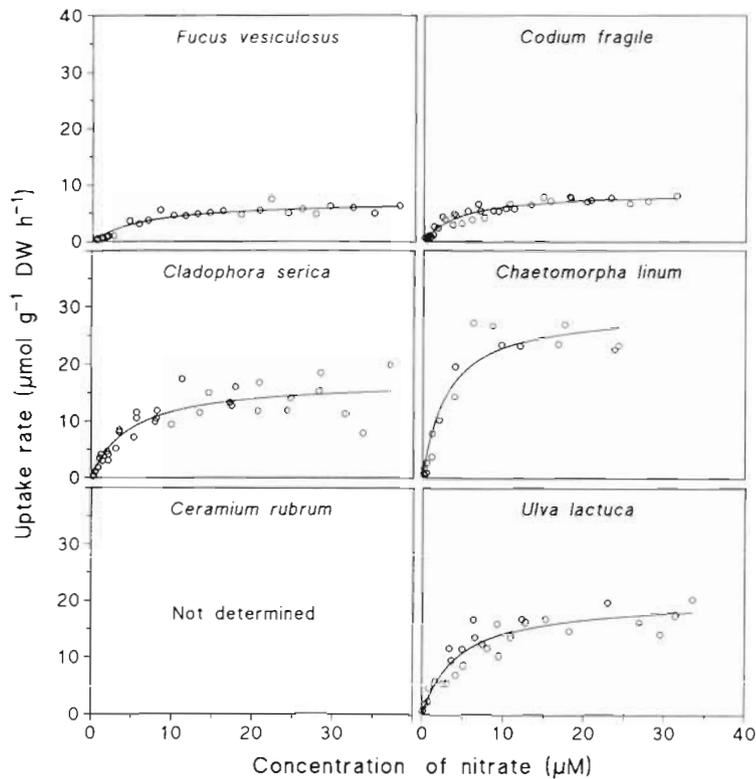


Fig. 3. Uptake rates of nitrate as a function of substrate concentration. Uptake rates (o) were measured later than 60 min after the experiments were initiated to avoid the initial phase of suppressed nitrate uptake

port growth for approximately the same period of time for all species (range: from 2.6 to 5.7 h; Table 3).

Uptake of nitrate was initially suppressed and was, therefore, taken as the rates measured after the initial lag phase had ceased, usually within 15 to 60 min after

exposure to nitrate. The uptake rates of nitrate were, like those of ammonium, hyperbolically related to substrate concentration (Fig. 3), but lower than the uptake rates of ammonium (Table 2). As for ammonium, maximum uptake rates of nitrate were related to species-specific differences in maximum growth rate, and maximum uptake rates for *Chaetomorpha linum*, *Ulva lactuca*, and *Cladophora serica* were 3- to 4-fold higher than those for *Codium fragile* and *Fucus vesiculosus*. The half-saturation constants varied 4-fold among species, and the ratio of $V_{max}:K_m$ tended to be highest among the fast-growing algae (Table 2).

The ratio between V_{max} and the amount of N required to sustain maximum growth varied substantially among species. All macroalgae could potentially take up ammonium and nitrate faster than needed for maximum growth, given that substrate concentrations were high enough, but the slow-growing species had ratios of $V_{max}:N$ requirement that were approximately 2-fold higher than for ephemeral species, suggesting that slow-growing species have a higher capacity for taking up N in excess of immediate demands for growth.

Balance between N availability and algal growth

The estimated maximum growth rates (μ_{max}^* ; Table 4) were slightly lower than the maximum growth rates estimated from the laboratory experiments (μ_{max} ;

Table 4. Growth kinetics. Predicted values of maximum growth rates (μ_{max}^*), half-saturation constants for growth (K_m) and of the substrate concentrations needed to saturate growth (S_{sat}^*) under steady state conditions in 6 different species of macroalgae with either ammonium or nitrate as inorganic N source. Numbers are bias-adjusted bootstrap means with 95% percentile-based confidence intervals in parentheses

Species	Ammonium			Nitrate		
	μ_{max}^* (d ⁻¹)	K_m (µM)	S_{sat}^* (µM)	μ_{max}^* (d ⁻¹)	K_m (µM)	S_{sat}^* (µM)
<i>Fucus vesiculosus</i>	0.040 (0.032 – 0.052)	0.29 (-0.74 – 1.51)	2.66 (-6.58 – 13.68)	0.038 (0.031 – 0.048)	0.81 (0.02 – 1.77)	7.32 (0.18 – 15.94)
<i>Codium fragile</i>	0.083 (0.071 – 0.095)	0.25 (0.00 – 0.58)	2.22 (0.03 – 5.19)	0.074 (0.065 – 0.086)	0.57 (0.36 – 0.92)	5.11 (3.24 – 8.31)
<i>Chaetomorpha linum</i>	0.142 (0.136 – 0.150)	0.11 (0.00 – 0.21)	1.00 (0.01 – 1.91)	0.139 (0.134 – 0.148)	0.13 (0.00 – 0.28)	1.18 (0.00 – 2.49)
<i>Cladophora serica</i>	0.208 (0.184 – 0.231)	0.36 (0.11 – 0.71)	3.28 (0.99 – 6.40)	0.188 (0.157 – 0.179)	0.83 (-0.02 – 1.77)	7.45 (-0.19 – 15.97)
<i>Ceramium rubrum</i>	0.299 (0.284 – 0.317)	0.63 (0.07 – 1.26)	5.65 (0.67 – 11.31)	-	-	-
<i>Ulva lactuca</i>	0.452 (0.422 – 0.482)	0.75 (0.52 – 1.10)	6.71 (4.61 – 9.90)	0.349 (0.322 – 0.373)	1.45 (0.87 – 2.10)	13.02 (7.84 – 18.89)

Table 1). Mean values of the half-saturation constant for growth (K_{μ}) with ammonium as the N source ranged from 0.11, 0.25, and 0.29 μM in *Chaetomorpha linum*, *Codium fragile*, and *Fucus vesiculosus*, respectively, to 0.36, 0.63, and 0.75 μM in *Cladophora serica*, *Ceramium rubrum*, and *Ulva lactuca*, respectively. K_{μ} ranged from 0.13 μM in *Chaetomorpha linum* to 1.45 μM in *Ulva lactuca* when nitrate was the N source. Hence, K_{μ} tended to increase with increasing maximum algal growth rate, but the mean values have quite wide confidence limits (Table 4) because K_{μ} was estimated from several independent factors (Eq. 7) each measured with its own error. The ability to convert available DIN in the water into growth through uptake (represented by the $\mu_{\text{max}}:K_{\mu}$ ratio) was highest among the ephemeral algae, but the predicted concentrations of ammonium and nitrate needed to saturate algal growth were higher for *U. lactuca*, *C. rubrum* and *C. serica* than for *C. linum*, *C. fragile* and *F. vesiculosus* (S_{sat}^* ; Table 4). These results suggest that ephemeral algae are less tolerant of low concentrations of inorganic N.

DISCUSSION

The ecological success of large, slow-growing macroalgae in nutrient-poor coastal areas could seem paradoxical because the relatively low ratios of surface area to volume (SA:V) of these species may confer less 'efficient' nutrient uptake. Indeed, absolute rates of N uptake are higher per unit of biomass and time for thin than for thick algae (Rosenberg & Ramus 1984, Hein et al. 1995, this study). When rates are expressed per unit of surface area, however, nutrient uptake 'efficiency' of thick algae tends to be higher than for thin species (Hein et al. 1995). The results presented here strongly support the suggestion that potential competitive success among algal species must be evaluated from the ability of their N uptake rates to meet the requirements for growth (Dugdale 1967, Turpin 1988). Such discussions of size-related algal competition within communities of micro- or macroalgae are often confused by inappropriate comparison of the efficiency of nutrient uptake alone.

The present study classifies the thick, slow-growing macroalgae as being at least as efficient as the thin, fast-growing species in exploiting limited N resources to meet their requirements. The variable N requirements among species were caused primarily by differences in growth rates and secondarily by differences in critical N concentrations. Species-specific maximum growth rates obtained in the laboratory experiments varied 13-fold and resembled rates measured for the same species *in situ* during summer (Pedersen &

Borum 1996). The observed variations in maximum growth rates with changing algal morphology (i.e. thin vs thick thallus) agreed with the general pattern found among algae of different size and thallus complexity (e.g. Littler & Littler 1980, Nielsen & Sand-Jensen 1990).

The experimentally obtained subsistence N quotas (N_Q) and critical tissue N concentrations (N_C) also tended to change with algal morphology and maximum growth rate. Subsistence quotas and critical tissue N concentrations resembled data from the literature (e.g. Hanisak 1979, Gordon et al. 1981, Rosenberg et al. 1984, Fujita et al. 1989, Lavery & McComb 1991). With the exception of *Chaetomorpha linum*, ephemeral species had slightly higher subsistence cell quotas and critical N concentrations than slow-growing species and, hence, species-specific N requirements to support maximum growth became several-fold larger among fast- than slow-growing macroalgae. This suggests that fast-growing species face a higher risk of N limitation when exposed to low N concentrations, unless their requirements are met by proportionally faster N uptake.

The uptake experiments confirmed that the ephemeral algae were superior to the slow-growing species in terms of nutrient uptake per unit of biomass and time. The maximum rate of surge and assimilation uptake of ammonium, the maximum uptake rate of nitrate and the affinity for both ammonium and nitrate were several-fold higher in the 4 ephemeral macroalgae than in the 2 slow-growing species. Such systematic variations in uptake kinetics are related to differences in algal morphology as thin macroalgae take up nutrients faster per unit of biomass than larger species with thick thalli (Rosenberg et al. 1984, Wallentinus 1984, Fujita 1985, Hein et al. 1995). This is because the small species have higher relative surface areas (i.e. SA:V) than large, thick algae, and uptake rates are primarily scaled to the exposed surface area relative to volume or biomass (Rosenberg & Ramus 1984, Hein et al. 1995).

The 6 algal species all took up ammonium at transiently enhanced rates when suddenly exposed to high ammonium concentrations after a period of N depletion. Surge uptake of ammonium seems to be controlled by small pools of intracellular ammonium, which may exert a negative feedback control on uptake rates as the pools are filled (Pedersen 1994, McGlathery et al. 1996). Initial uptake rates of ammonium are, therefore, enhanced for a short period of time only, thereafter rates are reduced to more constant levels likely controlled by the rates of amino acid synthesis (assimilation). The presence of surge uptake has been suggested to constitute a competitive advantage to ephemeral micro- and macroalgae living under

low, but fluctuating, N availability (e.g. Conway & Harrison 1977, Fujita 1985). Our data show, however, that surge uptake is not a special feature exclusive for ephemeral species.

The phase of surge uptake was short-lived, typically lasting for 60 to 120 min, and provided an extra uptake and accumulation of N in the range of 9 to 106 $\mu\text{mol N g}^{-1}$ DW. This short-term N storage could, however, only support maximum growth for 3 to 6 h independently of differences in intrinsic maximum growth rates among species. The advantage of faster surge uptake among ephemeral algae was offset by their higher N requirements. Furthermore, short-term storage of N gained through surge uptake could only be realized at high DIN concentrations ($>10 \mu\text{M}$) rarely experienced under field conditions. At low, and more natural, concentrations (0 to 10 μM DIN in Roskilde Fjord; Pedersen & Borum 1996), no significant differences appeared between rates of surge and assimilation uptake as reflected by the similar $V_{\text{max}}:K_m$ ratios. Hence, surge uptake may not be as ecologically important for algal N acquisition as formerly suggested in the literature.

Modeling steady-state growth, by combining the kinetics for growth and N uptake of each algal species, showed that the differences in the ability among species to sustain growth as a function of external N concentrations were small. The half-saturation constants for growth were generally low ($K_\mu = 0.11$ to $0.75 \mu\text{M}$ for ammonium, 0.13 to $1.45 \mu\text{M}$ for nitrate) but comparable to experimentally determined values for other species of macroalgae (DeBoer et al. 1978, Gordon et al. 1981, Rosenberg et al. 1984). The low K_μ values suggest that all the species examined are well suited to sustain high growth rates at low DIN concentrations. The ratio between the half-saturation constants for growth and uptake (K_μ/K_m) can be used as an index of sensitivity to nutrient stress; the lower K_μ is relative to K_m , the higher is the capacity for a given species to grow under low external nutrient concentrations because of variable cell quota (see Turpin 1988 for details). The ratio of $K_\mu:K_m$ for ammonium was substantially lower for *Fucus vesiculosus*, *Codium fragile* and *Chaetomorpha linum* (0.022 to 0.033) than for *Cladophora serica*, *Ceramium rubrum* and *Ulva lactuca* (0.076 to 0.127), and the 3 former species should, therefore, be better suited to sustain growth at low external N concentrations than the latter. According to our calculations of substrate dependent growth, the algal species would become N limited in the following sequence as the availability of DIN decreases: *U. lactuca*, *C. rubrum*, *C. serica*, *F. vesiculosus*, *C. fragile*, and finally *C. linum*. Hence, our data suggest that ephemeral algae, with the exception of *C. linum*, will experience N limitation at higher DIN concentrations than slow-growing species, making them more sensitive to N stress.

Overall, the present study has demonstrated that slow-growing macroalgae can exploit immediate N resources through uptake as efficiently as, or even more efficiently than, fast-growing algae. The slow-growing species have, in addition, a higher capacity for utilizing internal N stores to cover their requirements for growth at low external N availability than the ephemeral macroalgae or phytoplankton (Pedersen & Borum 1996). In combination, these abilities suggest that plants with inherently low maximum growth rates are better able to sustain maximum (i.e. non-nutrient limited) growth than ephemeral species in areas or seasons of low N availability. This is consistent with the observed differences in the temporal extent of N limitation observed among algae of different growth strategy in Roskilde Fjord (Pedersen & Borum 1996), where ephemeral macroalgae exhibited N limited growth for longer periods in summer than slow-growing species.

Since species-specific rates of biomass losses are almost directly scaled to intrinsic plant growth rates (Cebrian & Duarte 1994, Duarte 1995), we assume that the algae best able to sustain maximum growth rates under conditions of N limitation will have the competitive advantage to become dominant in mixed algal communities. At high and non-limiting N availability, ephemeral algae will be favored simply because of their high growth rates and will flourish at the expense of more slow-growing species (Sand-Jensen & Borum 1991, Duarte 1995). At low and potentially limiting N availability, the slow-growing species should be favored, while fast-growing species struggle to balance the continuously high biomass losses. These scenarios agree with the characteristic changes in plant communities described from a broad variety of natural and artificial coastal systems suffering from eutrophication (Borum 1983, Orth & Moore 1983, Kautsky et al. 1986, Sfriso et al. 1987, Neundorfer & Kemp 1993, Ménesguen & Piriou 1995, Taylor et al. 1995, Borum & Sand-Jensen 1996). Accordingly, we have provided a plausible, functional explanation for the effect of nutrients on the balance among algae of different growth strategy in coastal waters. Although we have primarily compared growth and nutrient kinetics of marine macroalgae, we expect that the functional explanation will be valid for size-related competition among algal species within micro- and macroalgal communities in general.

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