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Nitrogen assimilation characteristics of polar seaweeds from differing nutrient environments

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ABSTRACT: Nitrogen uptake and assimilation strategies were compared in polar macroalgae from differing dissolved inorganic nitrogen (DIN) regimes. The antarctic endemic, *Himantothallus grandifolius*, experiences high nitrate concentrations year-round and occasionally high, but variable, ammonium levels. The arctic endemic, *Laminaria solidungula*, is exposed to seasonal fluctuations in DIN, with N-limitation occurring during the summer. Both species demonstrated saturation kinetics for nitrate and ammonium uptake. *L. solidungula* showed 'storage-specialist' characteristics of nitrate uptake, with high V_{max} allowing this species to take advantage of seasonally elevated nitrate concentrations. *H. grandifolius* had a high V_{max} for ammonium, allowing the alga to utilise pulses of this nutrient. In the presence of both DIN forms, nitrate uptake was significantly reduced in both species. Furthermore, *H. grandifolius* and *L. solidungula* demonstrated significantly reduced uptake and assimilation of nitrate during short-term and prolonged periods of darkness, while ammonium uptake and assimilation were relatively unaffected by light. Although preferential uptake of ammonium, particularly in the dark, allows both species to conserve energy in their cold, low-light environments, the antarctic species, which does not have the additional problem of N-limitation, showed stronger energy-conserving traits. Nitrogen assimilation characteristics of the arctic species appeared to balance energy conservation with the need to minimise N-limitation in an environment that alternates between low light and low N-availability.

KEY WORDS: *Himantothallus grandifolius* · *Laminaria solidungula* · Nitrogen assimilation · Algae · Antarctic · Arctic

INTRODUCTION

High-latitude marine environments are characterised by low and almost constant water temperatures (Drew & Hastings 1992, Sellman et al. 1992), and polar endemic algae exhibit adaptations which allow them to maintain relatively high rates of primary production at near-freezing temperature (Wiencke & tom Dieck 1989, Bischoff & Wiencke 1993, Dunton & Dayton 1995, Newkirk 1997, V.A.G. unpubl. data). Light and nutrient availability, in contrast, are highly variable in polar marine environments, and are the most important factors influencing seasonal variation in algal production.

Growth of *Himantothallus grandifolius* (A. & E. S. Gepp) Moe & Silva, a brown macroalga endemic to the Antarctic, is largely restricted to the summer months when sea-ice breaks up and underwater light levels are high (Drew & Hastings 1992). Growth patterns of the arctic kelp *Laminaria solidungula* J. Ag. are very different from those of its antarctic counterpart; stored carbon is used to produce new tissue during the dark winter months, while growth virtually stops during the ice-free summer (Dunton & Schell 1986, Dunton 1990). Differences in seasonal growth patterns of these 2 species are primarily a response to the availability of nutrients. In the Southern Ocean, dissolved inorganic nitrogen (DIN) levels remain high throughout the year; growth of *H. grandifolius* is rarely, if ever, N-limited and follows the seasonal light pattern (Drew & Hastings

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1992). In arctic seas, DIN levels are depleted during the summer by production of microalgal biomass, but increase over the winter months. Growth of *L. solidungula* is strongly N-limited during the summer and, therefore, follows the seasonal pattern of DIN availability (Chapman & Lindley 1980, Henley & Dunton 1995).

DIN uptake and assimilation characteristics of temperate seaweeds have been shown to vary among populations and species in ways that optimise survival and growth under local nutrient supply conditions (Davison & Stewart 1983, Espinoza & Chapman 1983, Wheeler & Weidner 1983, Davison et al. 1984, Kopczak 1994). Arctic and antarctic macroalgae, which occur in environments with similar low temperature/low irradiance conditions but very different N-supply regimes, may also exhibit adaptive differences in nutritional strategies. Thus, *Laminaria solidungula* has been shown to accumulate large internal reserves of nitrogenous compounds which can support growth for months during periods of low external N-supply, while internal N-reserves in *Himantothallus grandifolius* are minimal (Korb & Gerard 2000, in this issue). The present study compared DIN uptake and assimilation in *L. solidungula* and *H. grandifolius*. Both of these species are major biomass components and primary producers in their respective polar, shallow marine ecosystems (Dunton 1984, Amsler et al. 1995). Although *H. grandifolius* was recently reclassified from the order Laminariales to the closely related Desmarestiales (Tan & Druehl 1996), its morphology, physiology, and life history are similar to *L. solidungula* (Moe & Silva 1981).

MATERIALS AND METHODS

Sporophytes of *Himantothallus grandifolius*, 10 to 30 cm in blade length, were collected using SCUBA at 15 to 20 m depths from a site located near Palmer Station (64° 46' S, 64° 04' W) on Anvers Island, Antarctic Peninsula, during March and April 1997. Sporophytes of *Laminaria solidungula*, approximately 15 cm in length, were collected from the Canadian High Arctic at Resolute Bay, Cornwallis Island (74° 30' N, 95° W) during May 1997. Plants were maintained in 8 l batch cultures with 5 plants per tub, and 0°C aerated seawater enriched with 240 µM NO_3^- and 16 µM PO_4^{-3}. Seawater was changed twice weekly and nutrients added at this time. Irradiance was provided by cool white fluorescent lamps at a photon flux density (PFD) of 50 µmol m^{-2} s^{-1}, on a 16 h light:8 h dark cycle. Plants were held under these constant conditions for at least 4 wk prior to experimental use.

Nitrate and ammonium uptake. Plants were placed in 2 l of nitrate-free seawater for approximately 30 min to remove excess NO_3^- from intercellular tissue space (Gerard 1982). All measurements were performed at 0°C. Standard solutions of either 10 mM sodium nitrate or ammonium chloride were added to 4.8 l of seawater to give initial concentrations between 0 and 120 µM NO_3^- or 0 to 25 µM NH_4^+. The water was mixed well, and triplicate 1.0 ml samples were immediately removed for NO_3^- analysis. For NH_4^+, 12.5 ml of seawater was removed and added to 0.5 ml of phenol to fix samples. Aliquots (800 ml) of the seawater/N mix were added to 5 shallow, white plastic trays (1.5 l total volume). Water motion was generated by aeration, and irradiance was maintained at 50 µmol m^{-2} s^{-1}. For measurements of uptake in the light, individual plants were blotted to remove excess water, then placed in one of the trays for 5 to 7 h, after which plants were removed, the water was mixed well, and a second set of triplicate samples was removed from each tray. NO_3^- uptake in the presence of NH_4^+ was examined using the previously described experimental procedure in seawater containing both 5 µM NO_3^- and 5 µM NH_4^+. Short-term, dark uptake rates were measured over 7 h in the presence of either NO_3^- or NH_4^+ at saturating or close to saturating N-concentrations, as determined in light experiments, to give maximum rates of uptake in the dark. Lights in the incubator were turned off, and the trays covered with black plastic bags. At the end of each experiment, blotted wet weight was measured for each plant, and blade area was determined by paper tracings. The change in NO_3^- concentration over time was determined using the method of A. Gao (pers. comm.) modified from Jones (1984) as described in Korb & Gerard (2000). Changes in NH_4^+ concentrations over time were determined using the alternative ammonium assay described by Parsons et al. (1984).

Enzyme activities. Crude enzyme extracts were prepared using a modified version of the method described by Hurd et al. (1995). Discs (2.27 cm^2) were cut from mature blade tissue with a cork borer, frozen immediately in liquid nitrogen, and stored at −80°C until required for use. Fresh algal thalli could be frozen for several weeks without loss of enzyme activity (data not shown). Frozen tissue was ground to a fine powder under liquid nitrogen with a mortar and pestle. For each 0.1 g wet wt of tissue (approximately 1 disc), 2 ml of ice-cold extraction buffer containing 200 mM phosphate buffer (pH 7.9), 5 mM EDTA, 20 mM dithiothreitol (DTT), 7.5 µM PVP and 1% (v/v) Triton X-100 were added; the sample was reground to homogeneity and centrifuged for 5 min at 4°C (10 000 × *g*). Extracts were kept on ice and assayed within 30 min.

Nitrate reductase (NR) activity in the supernatant of the crude extracts was determined in an assay mixture containing 0.2 mM NADH, 10 mM KNO_3, and 200 mM phosphate buffer (pH 7.9). Reactions were started by

adding 0.4 ml crude enzyme extract to 1.6 ml assay mix; 1 ml was removed immediately and added to 1 ml of 550 mM zinc acetate. The remaining 1 ml was incubated for 30 min at 10°C and the reaction stopped by the addition of 1 ml zinc acetate. Samples were centrifuged for 5 min (10 000 × *g*). Of the resulting supernatant, 0.5 ml was removed, added to 20 µl of 825 µM phenazine methylsulphate, and allowed to stand for 20 min. NO_2^- was measured spectrophotometrically at 540 nm (Parsons et al. 1984) after the addition of 0.5 ml each of 58 mM sulfanilamide (in 1 M HCl) and 3.86 mM *N*-1-napthylethylenediamine.

Curves of NR activity versus substrate concentration were generated by measuring NR activity at concentrations of KNO_3^- ranging from 0 to 20 mM. *Laminaria solidungula* stores up to 3 µmol cm^{-2} NO_3^- in its tissues, which masks actual NR activity in the presence of low external NO_3^- concentrations. Therefore, this alga was maintained in nitrate-free seawater for 12 wk prior to experimental use. Diel NR activity of *Himantothallus grandifolius* was measured every 2 h over a 24 h period.

For glutamine synthetase (GS) activity, enzyme extracts were prepared as for NR with the following exceptions: for each 0.1 g fresh wt of tissue, 1 ml of ice-cold extraction buffer containing 200 mM HEPES buffer (pH 7.9), 50 mM $MgCl_2$, 5 mM EDTA, 20 mM DTT, 7.5 µM PVP and 1% (v/v) Triton X-100 was added. GS activity was measured following the method of Rees et al. (1995) in an assay mix containing 50 µl crude enzyme extract, 8 mM ATP, 100 mM glutamate, and 50 mM ammonium chloride added to basic HEPES buffer (minus PVP and Triton-X) to give a final volume of 750 µl. Reactions were started by addition of ATP. Blanks consisted of the same additions minus glutamate. Samples were incubated for 15 min at 30°C, the reaction stopped by adding 0.25 ml 1 M H_2SO_4, and the samples centrifuged for 1 min (16 000 × *g*). Of the resulting supernatant, 25 µl was added to 975 µl of distilled water, and inorganic phosphate was measured as in Rees et al. (1995).

Effects of prolonged darkness on N-assimilation. To determine which, if any, nitrogen source is taken up during periods of prolonged darkness and the effect on enzyme activity, sporophytes of *Himantothallus grandifolius* and *Laminaria solidungula* were grown in the dark as 8 l batch cultures for 1 mo. All other culture conditions were as described previously. After 1 mo in darkness, plants were placed under a PFD of 50 µmol photons m^{-2} s^{-1}, on a 16 h light:8 h dark cycle. At 0, 2, and 7 d in the light, NR, GS, and NO_3^-/NH_4^+ uptake rates were measured. Discs of mature blade tissue were cut for NR and GS, frozen immediately in liquid nitrogen, and stored at −80°C until analysis. For uptake experiments, whole plants were incubated in either 5 µM NO_3^- or 5 µM NH_4^+ with a PFD of 50 µmol photons m^{-2} s^{-1}.

Effect of nitrogen limitation on N-assimilation. To examine the effect of nitrogen limitation on NR and NO_3^- uptake, *Himantothallus grandifolius* was grown as 8 l batch cultures in nitrate-free seawater for 1 mo and *Laminaria solidungula* for 3 mo (at which point internal NO_3^- pools had been depleted). All other culture conditions were as described previously. After 1 or 3 mo without NO_3^-, samples were taken for NR activity, frozen immediately in liquid nitrogen, and stored at −80°C. NO_3^- uptake experiments were performed on whole plants of *H. grandifolius* with 800 ml of seawater containing 30 µM NO_3^-. Due to the time constraints involved in producing *L. solidungula* plants with depleted internal NO_3^- pools, it was not possible to perform NO_3^- uptake experiments.

Statistical analyses. Maximum uptake rates (V_{max}) and half saturation constants (K_m) for N-uptake and NR were calculated from non-linear regressions (least squares analysis) using the curve fitting package Semi-Newton (SYSTAT NLIN procedure; see Berges et al. 1994). The NO_3^- and NH_4^+ uptake data of *Laminaria solidungula* did not fit a hyperbolic curve when data points from high N concentrations were included. To aid comparisons with *Himantothallus grandifolius*, these points were removed and V_{max} and K_m calculated using SYSTAT. Statistical analyses were made using either a 1- or 2-way ANOVA (Fully Factorial MGLH, SYSTAT) followed by multiple comparisons testing using the Tukey-Kramer HSD-test (testing for significance at $p < 0.05$) or a Student's *t*-test when only 1 factor was involved.

RESULTS

N-uptake and -assimilation in the light

Uptake measurements showed differences in characteristics between antarctic *Himantothallus grandifolius* and arctic *Laminaria solidungula* in relation to differences in ambient DIN availability. The arctic plants had a higher V_{max} but a lower affinity (higher K_m) for NO_3^- than the antarctic plants (Fig. 1, Table 1). In contrast, *H. grandifolius* had a higher V_{max} but a lower affinity for NH_4^+ than *L. solidungula*. Similar V_{max}/K_m ratios for both forms of DIN, however, indicated similar uptake efficiencies for the 2 species. The arctic species was more sensitive than the antarctic species to high concentrations of NO_3^- or NH_4^+. NO_3^- uptake by *L. solidungula* was 70% lower at 120 µM than at 60 µM, and NH_4^+ uptake was inhibited at 17 µM. *H. grandifolius*, in contrast, showed no deleterious effects of high substrate concentrations.

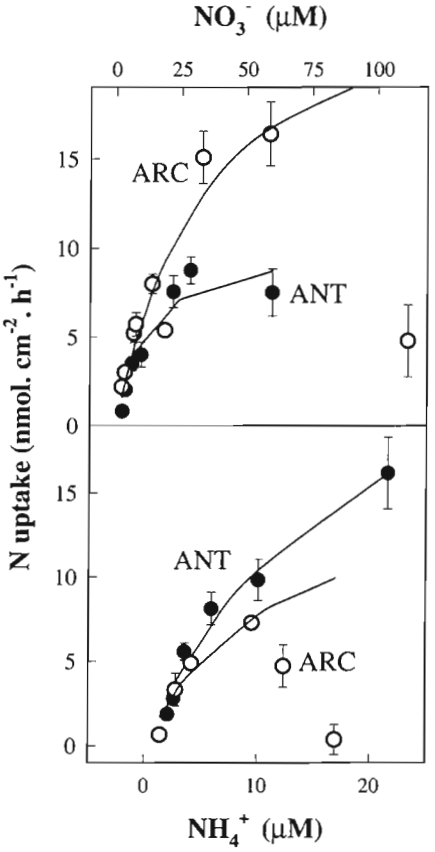

Fig. 1. NO_3^- and NH_4^+ uptake by antarctic *Himantothallus grandifolius* (ANT, filled symbols) and arctic *Laminaria solidungula* (ARC, open symbols). Results represent the mean (±1 SE) of 5 or 6 individual plants. Lines represent non-linear transformations using SYSTAT; data points for *L. solidungula* from the highest concentrations were not included. Correlation coefficients for NO_3^- and NH_4^+ were $r^2 = 0.90$ and 0.93, respectively, for *H. grandifolius*, and $r^2 = 0.93$ and 0.93 for *L. solidungula*

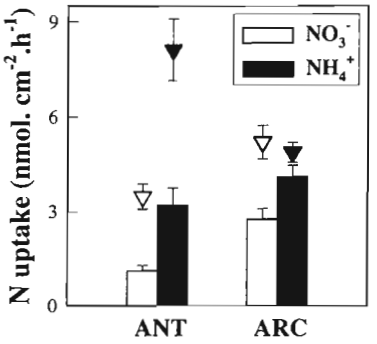

Fig. 2. Uptake rates of NO_3^- and NH_4^+ by antarctic *Himantothallus grandifolius* (ANT) and arctic *Laminaria solidungula* (ARC) when only 1 form was present (symbols) or when both forms were present together (bars). Open bars represent NO_3^- uptake and filled bars NH_4^+ uptake (mean ± 1 SE, n = 5 or 6). Initial concentrations were 5 μM in all cases

Table 1. Mean V_{max} and K_m values (±1 SE) of NO_3^- and NH_4^+ utake by whole plants of *Himantothallus grandifolius* and *Laminaria solidungula* (n = 5 or 6 individual plants at each concentration)

	H. grandifolius	*L. solidungula*
NO_3^-		
V_{max} (nmol cm^{-2} h^{-1})	10.6 ± 1.1	26.7 ± 4.3
K_m (μM)	12.8 ± 3.5	35.0 ± 10.6
V_{max}/K_m	0.83	0.76
NH_4^+		
V_{max} (nmol cm^{-2} h^{-1})	31.4 ± 6.7	17.4 ± 6.4
K_m (μM)	20.4 ± 7.4	12.7 ± 7.0
V_{max}/K_m	1.54	1.37

NH_4^+ and NO_3^- were taken up simultaneously by antarctic and arctic plants when both forms were present (Fig. 2). NO_3^- uptake was 65 to 70% lower than NH_4^+ uptake in *Himantothallus grandifolius*, regardless of whether N-forms were supplied singly or together. The presence of the N-forms together lowered uptake of both NO_3^- and NH_4^+. In *Laminaria solidungula*, the presence of both N-forms reduced NO_3^- uptake by 45%, but had no effect on NH_4^+ uptake. Differences between uptake rates measured for each N-form individually and rates measured in the presence of both N-forms were statistically significant (ANOVA, $F = 24.7$ for *H. grandifolius*, and $F = 7.7$ for *L. solidungula*, $p < 0.01$).

NR activity measured at varying NO_3^- concentrations followed Michaelis-Menton saturation kinetics for both *Himantothallus grandifolius* and *Laminaria solidungula* (Fig. 3). Similar to V_{max} of NO_3^- uptake, V_{max} of NR activity was higher in the arctic than in the antarctic species (Table 2); however, NR activity of arctic plants showed a higher affinity for NO_3^- and a higher V_{max}/K_m ratio, indicating more efficient enzyme activity than in antarctic plants.

Effects of darkness and NO_3^- availability on N-uptake and -assimilation

Comparison of N-uptake under short-term exposure to light and dark showed that effects depended on the form of nitrogen, i.e. there was a significant interactive effect of light and N-form on uptake rate for both *Himantothallus grandifolius* and *Laminaria solidungula* (2-way ANOVA, $F = 23.3$ and 6.7, respectively, $p < 0.05$). Both species were unable to take up significant amounts of

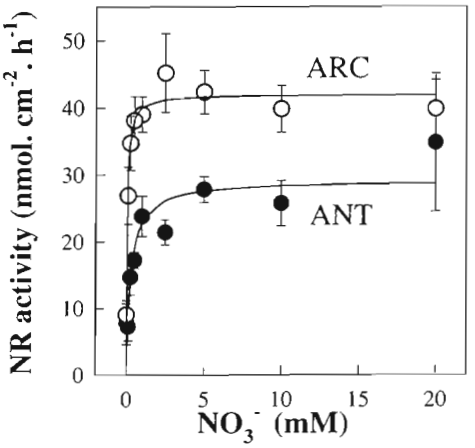

Fig. 3. Nitrate reductase (NR) activity at varying substrate concentrations in antarctic *Himantothallus grandifolius* (ANT, filled symbols) and arctic *Laminaria solidungula* (ARC, open symbols). Each point represents the mean (±1 SE) of 3 or 4 individual plants. Lines represent non-linear transformations using SYSTAT; $r^2 = 0.88$ for *H. grandifolius* and $r^2 = 0.95$ for *L. solidungula*

Table 2. Mean (±1 SE) V_{max} and K_m values for nitrate reductase activity versus substrate concentration for *Himantothallus grandifolius* and *Laminaria solidungula* (n = 3 or 4 individual plants)

	H. grandifolius	*L. solidungula*
V_{max} (nmol cm^{-2} h^{-1})	29.13 ± 2.41	42.03 ± 1.81
K_m (mM)	0.28 ± 0.12	0.05 ± 0.02
V_{max}/K_m	0.10	0.84

NO_3^- in the dark, and the antarctic plants actually appeared to release a small amount (Table 3). Dark uptake rates were significantly higher for NH_4^+ than for NO_3^- for both species (Tukey's HSD-test, $p < 0.05$), and although NH_4^+ uptake by both species was 20 to 26% lower in the dark than in the light, the effects of light on NH_4^+ uptake were not statistically significant.

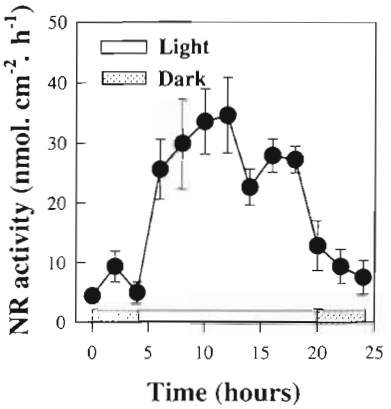

Fig. 4. Diel activity of nitrate reductase (NR) in antarctic *Himantothallus grandifolius*. Plants were grown on a 16 h light:8 h dark cycle in a photon flux density of 50 µmol m^{-2} s^{-1} at 0°C with 240 µM NO_3^- Each point represents the mean (±1 SE) of 3 individual plants

Long-term exposure to darkness had effects on N-uptake similar to effects of short-term exposure. After 1 mo darkness, both *Himantothallus grandifolius* and *Laminaria solidungula* exhibited significantly lower rates of NO_3^- uptake compared to control plants (Table 4, ANOVA, $F = 44.5$ and 32.3, respectively, $p < 0.01$). The antarctic species was still unable to take up NO_3^- 2 d after its return to a diel light cycle, but both species showed full recovery of V_{max} after a week. In contrast to NO_3^- uptake, both species took up significant amounts of NH_4^+ immediately following 1 mo in darkness. Initial rates of NH_4^+ uptake in *H. grandifolius* were only 20% of rates by control plants, while uptake rates of dark-treated *L. solidungula* were 75% of control plants.

NR activity in *Himantothallus grandifolius* declined during short-term exposure to darkness almost as markedly as NO_3^- uptake, resulting in a diel pattern (Fig. 4) similar to those found in other algal species (Davison & Stewart 1984, Gao et al. 1992, Lopes et al. 1997, Vegara et al. 1998). NR activity rose rapidly within 2 h of the start of the light cycle, peak activity (approximately 4 times higher than the dark rate)

Table 3. Effect of light on NO_3^- and NH_4^+ uptake (nmol cm^{-2} h^{-1}) by *Himantothallus grandifolius* and *Laminaria solidungula*. Results represent the mean (±1 SE) of 5 or 6 individual plants. Initial substrate concentrations are shown in parentheses

	H. grandifolius		*L. solidungula*	
	Dark	Light	Dark	Light
NO_3^-	−1.15 ± 0.39 (30 µM)	8.75 ± 0.76 (28 µM)	1.27 ± 0.58 (6 µM)	5.21 ± 0.53 (7 µM)
NH_4^+	7.88 ± 1.30 (10 µM)	9.83 ± 1.22 (10 µM)	3.6 ± 0.61 (5 µM)	4.88 ± 0.32 (4 µM)

Table 4. NO_3^- and NH_4^+ uptake rates (nmol cm^{-2} h^{-1}) of *Himantothallus grandifolius* and *Laminaria solidungula* after 1 mo in darkness. Results represent the mean (±1 SE) of 5 individual plants. Numbers in parentheses indicate activity as percentage of control plants (n = 5) grown on a 16 h light:8 h dark cycle at a photon flux density of 50 µmol m^{-2} s^{-1}. All uptake rates were measured in the light at 30 µM NO_3^- or NH_4^+. •Uptake rates were not measured on these days

	NO_3^-		NH_4^+	
	H. grandifolius	*L. solidungula*	*H. grandifolius*	*L. solidungula*
Controls	7.79 ± 0.73	5.21 ± 0.53	3.29 ± 0.85	4.88 ± 0.37
Dark plants (days in light)				
0	−0.34 ± 0.17 (0)	0.43 ± 0.31 (8)	0.66 ± 0.53 (20)	3.60 ± 0.47 (74)
2	−0.71 ± 0.49 (0)	1.26 ± 0.22 (24)	1.72 ± 0.47 (52)	•
7	6.43 ± 0.82 (83)	5.95 ± 0.69 (114)	3.77 ± 0.52 (115)	•

Table 5. Nitrate reductase (NR) activity (nmol cm^{-2} h^{-1}) and glutamine synthetase (GS) activity (µmol cm^{-2} h^{-1}), respectively, in *Himantothallus grandifolius* and *Laminaria solidungula* after 1 mo in darkness. Results represent the mean (±1 SE) of 5 individual plants. Numbers in parentheses indicate activity as a percentage of control plants (n = 5) grown on a 16 h light:8 h dark cycle at a photon flux density of 50 µmol m^{-2} s^{-1}. •Enzyme activities were not measured on these days

	NR		GS	
	H. grandifolius	*L. solidungula*	*H. grandifolius*	*L. solidungula*
Controls	41.2 ± 3.5	31.9 ± 6.0	193.8 ± 22.2	149.3 ± 61.8
Dark plants (days in light)				
0	1.3 ± 1.0 (3)	2.9 ± 2.6 (9)	126.2 ± 15.5 (65)	43.0 ± 20.2 (29)
2	6.9 ± 3.5 (17)	23.8 ± 6.5 (75)	174.6 ± 23.4 (90)	168.4 ± 33.1 (113)
7	9.2 ± 3.2 (22)	59.5 ± 3.0 (187)	•	•

occurred after 8 h in the light, and activity rapidly declined when darkness resumed. Exposure of both algal species to prolonged darkness caused changes in NR and GS activities (Table 5) similar to changes in NO_3^- and NH_4^+ uptake, respectively. NR activity was initially very low and significantly different from activity in control plants (ANOVA, $F = 23.4$, $p < 0.01$), while GS activity immediately after exposure to light was 29 to 65% of the activity in controls. Unlike NO_3^- uptake, which showed full recovery after a week of light exposure, NR activity in dark-treated *H. grandifolius* recovered to only 25% of control activity after 7 d under a diel light cycle. Dark-treated *Laminaria solidungula* showed complete recovery of NR activity.

Long-term absence of an external nitrogen source had a stimulatory effect on N-uptake and -assimilation by antarctic plants. *Himantothallus grandifolius* grown for 1 mo without NO_3^- demonstrated significantly higher NO_3^- uptake rates (Student's *t*-test, $p < 0.01$) and increased NR activity compared to control plants (Table 6). N-limited *Laminaria solidungula*, on the other hand, showed similar NR activity to control plants.

Table 6. NO_3^- uptake rates and nitrate reductase (NR) activity, (nmol cm^{-2} h^{-1}) of *Himantothallus grandifolius* and *Laminaria solidungula* (NR only) after containment in nitrate-free seawater for 1 and 3 mo, respectively. Results represent the mean (±1 SE) of 5 individual plants. Numbers in parentheses indicate activity as percentage of control plants (n = 5) grown in the presence of 240 µM NO_3^-

	H. grandifolius		*L. solidungula*
	NO_3^- uptake	NR activity	NR activity
Control plants	7.67 ± 1.01	28.66 ± 3.29	64.28 ± 11.38
N-limited plants	14.35 ± 1.48 (187)	41.6 ± 6.49 (145)	58.56 ± 6.85 (91)

DISCUSSION

Nutritional characteristics of seaweeds often show adaptations to local nutrient conditions. Temperate kelps from areas with different seasonal patterns of DIN availability, for example, exhibit variation in nitro-

gen uptake, activity of enzymes involved in N-assimilation, and nitrogen storage (e.g. Gagné et al. 1982, Espinoza & Chapman 1983, Wheeler & Weidner 1983, Davison et al. 1984, Druehl et al. 1989). In the present study, it was expected that polar macroalgae, from similar environments in terms of temperature and light availability, but which have evolved under different nutrient regimes, may have nitrogen uptake and assimilation characteristics suited to their specific environments. Around the Antarctic Peninsula, concentrations of NO_3^- as high as 30 µM are recorded yearround (M. Vernet pers. comm.). NH_4^+ levels are lower than NO_3^-, generally representing 2 to 10% of the DIN pool. The variability of NH_4^+ is the result of patches of excretion from krill, birds, and mammals (Olsen 1980). In contrast, arctic seas have much lower levels of both N-forms. Winter NO_3^- concentrations are approximately 4 to 5 µM, falling to undetectable levels in the summer (Dunton & Schell 1986). NH_4^+ concentrations are also low and variable, ranging from 0.03 to 0.4 µM (Wheeler & Kokkinnakis 1990).

Arctic *Laminaria solidungula*, which is periodically subjected to low DIN concentrations, was expected to have higher affinity and higher maximum uptake rates of NO_3^- and NH_4^+ than antarctic *Himantothallus grandifolius*, an alga exposed to constantly high NO_3^- concentrations. In fact, in a previous study, *L. solidungula* did not demonstrate saturation kinetics — both NO_3^- and NH_4^+ uptake rates continued to increase with increasing substrate concentration up to 80 µM (Dibble 1994). In the present study, NO_3^- uptake rates in both the arctic and antarctic species exhibited saturation kinetics, similar to a number of temperate kelp species (Haines & Wheeler 1978, Harlin & Craigie 1978, Gerard 1982, Kopczak 1994, Braga & Yoneshigue-Valentin 1996). The apparent contradiction is probably due to different experimental conditions. In the earlier study, uptake rates measured either in polythene bags or in the laboratory may have been limited by insufficient water motion and, therefore, reflected rates of boundary layer diffusion rather than active uptake (Gerard 1982). The present study found that *L. solidungula* did indeed have higher maximum NO_3^- uptake rates than *H. grandifolius*. However, affinity for NO_3^- was lower in the arctic plants, and the opposite pattern was seen for NH_4^+. Obviously, the differences between these species are not simply adaptive responses to low versus high nutrient regimes.

With respect to nutrient uptake kinetics, planktonic microalgae have been categorised as (1) 'affinity-adapted' species with low V_{max}, but also low K_m for efficient use of low nutrient concentrations; (2) 'velocity-adapted' species with high V_{max}, high K_m, and high maximum growth rates, which utilise nutrient pulses to support periods of rapid growth; and (3) 'storage specialists' with high V_{max} and high K_m, but low maximum growth rates, which utilise pulses for luxury consumption and storage (Sommer 1984). Based on results of the present study, *Laminaria solidungula* would fall into the 'storage specialist' category. The high V_{max} for NO_3^-, coupled with a low maximum growth rate, allows arctic plants to take advantage of seasonally elevated concentrations to accumulate large internal pools of NO_3^- and organic N-reserves (Henley & Dunton 1995, Korb & Gerard 2000). On the basis of NO_3^- uptake, *Himantothallus grandifolius* did not fit well into any of the 3 categories. Although K_m was low in antarctic plants relative to arctic plants, much lower values of K_m have been determined for temperate seaweeds (e.g. Haines & Wheeler 1978, Harlin & Craigie 1978, Probyn & Chapman 1982, Espinoza & Chapman 1983), and a high-affinity strategy would not be particularly valuable to a species living under constant, high DIN supply. Nor does *H. grandifolius* fit the definitions of 'velocity-adapted' or 'storage specialist' species, since it has a low maximum growth rate and does not accumulate significant internal N-reserves (Korb & Gerard 2000). Perhaps, the nutritional strategy of this species cannot be defined on the basis of a response to N-supply, because in its natural environment it is limited by low annual irradiance rather than N-availability. In other words, the N-uptake characteristics of *H. grandifolius* may be adapted to optimise energy consumption rather than N-assimilation.

Preferential use of NH_4^+ over NO_3^- can be considered as an energy-saving process, because NO_3^- must be reduced to NH_4^+ prior to incorporation into organic compounds, requiring the equivalent of 8 electrons per NO_3^- (Syrett 1981). The preferential use of NH_4^+ may, therefore, be viewed as an energetic advantage, especially in polar waters that receive low annual inputs of solar radiation (Dunton & Dayton 1995). *Himantothallus grandifolius* had a much higher uptake rate of NH_4^+ than of NO_3^-, whether presented with 1 form of DIN or both together (Fig. 2). High V_{max} values for NH_4^+ would allow the antarctic species to rapidly use pulses of this nutrient when it is available. DIN uptake by *H. grandifolius* may be similar to that of antarctic phytoplankton, which meet their nitrogenous needs largely through NH_4^+ uptake, despite high ambient concentrations of NO_3^- (Olsen 1980, Glibert et al. 1982, Koike et al. 1986). Similar preference for NH_4^+ was found in the deep-dwelling kelp *Laminaria abyssalis* (Braga & Yoneshigue-Valentin 1996) and in the freshwater macroalga *Lemanea mamillosa*, which grows rapidly in the winter (MacFarlane & Raven 1990). Arctic *Laminaria solidungula*, on the other hand, showed similar rates of NO_3^- and NH_4^+ uptake, whether the 2 DIN forms were presented individually or together. Compared to the antarctic alga, the arctic kelp is subjected

to lower ambient NO_3^- concentrations, which peak at 3 to 6 µM (Dunton 1985), and probably rarely experiences NH_4^+ concentrations >1 µM (Wheeler & Kokkinnakis 1990). Minimal interference of uptake between the 2 forms of DIN may enable *L. solidungula* to take advantage of available DIN, regardless of form.

Differences between *Himantothallus grandifolius* and *Laminaria solidungula* with respect to effects of darkness on nutrient uptake rates may also reflect a stronger influence of energy limitation in the antarctic species. Assimilation of both NO_3^- and NH_4^+ is dependent on photosynthesis or on stored photosynthetic products, so that uptake by most algae occurs at a reduced rate in the dark (e.g. DeBoer et al. 1978, Haines & Wheeler 1978, Syrett 1981, Gerard 1982, Harrison et al. 1986). Although both the antarctic and arctic species demonstrated reduced DIN uptake during short-term and prolonged periods of darkness, the effect was much greater in *H. grandifolius*, which took up no NO_3^- and little NH_4^+ in the dark (Tables 3 & 4). The N-assimilation enzymes similarly exhibited a greater dark-induced reduction and/or a slower recovery of activity in the antarctic alga (Table 5). Since *H. grandifolius* does not grow during the winter months of darkness (Drew & Hastings 1992) and does not store internal N-reserves, this species has no need to take up DIN in the dark. The arctic kelp, in contrast, completes over 90% of its annual growth during winter utilising stored carbohydrates (Dunton & Schell 1986, Dunton 1990). In *L. solidungula*, however, NO_3^- uptake in the dark was insufficient to meet growth requirements. The average growth rate of this species at 0°C is 0.45% d^{-1} (Korb & Gerard 2000), requiring 0.06 µmol N g^{-1} fresh wt h^{-1}. NO_3^- uptake occurred at one-third of this rate in the dark. It is possible that the arctic kelp supports winter growth at least partly on internal organic N-reserves accumulated during periods when both light and DIN are available. Alternatively, under-ice irradiance, although very low (Dunton 1990), may be sufficient to significantly enhance NO_3^- uptake compared to dark rates and thus support winter growth.

Although reduced rates of NO_3^- uptake and assimilation may reflect energy conservation, they may also be influenced by NO_3^- availability. Nitrate reductase is an inducible enzyme, regulated by factors such as nitrate or light (Crawford 1995), whereby activity occurs at constitutive levels in plants grown with no NO_3^- or light supply and increases with exposure to these factors. The temperate kelps *Laminaria saccharina* and *L. digitata* showed reduced NR activity under N-limitation, apparently to conserve enzyme protein (Wheeler & Weidner 1983, Davison et al. 1984). We expected a similar reduction of NR activity in arctic *L. solidungula* under N-limited conditions, and did find a small difference between N-replete and N-limited plants (Table 6). However, the effect was less dramatic than in the temperate species, possibly reflecting the greater ability of the arctic kelp to maintain growth on internal N-reserves for long periods of N-limitation (Henley & Dunton 1997, Korb & Gerard 2000). In *Himantothallus grandifolius*, on the other hand, both NR activity and nitrate uptake rate increased under N-limitation. As this species is never exposed to low nitrate concentrations in nature, it may not have evolved a mechanism to conserve nitrogen by reducing its complement of assimilatory enzymes.

Overall, results of the present study indicated that the large brown algae from the southern and northern polar regions exhibit marked differences in nutritional strategies. The antarctic endemic, although constantly exposed to high nitrate concentrations, preferentially takes up and assimilates NH_4^+ and, hence, is able to take advantage of periodically high concentrations of this nutrient to conserve energy. The arctic endemic utilises the 2 DIN forms more equitably, allowing the plant to minimise the impact of seasonally low concentrations via luxury consumption and storage. Both species conserve energy by reducing rates of NO_3^- uptake and assimilation during periods of darkness, but the dark effect is greater in the antarctic alga. Thus, *Himantothallus grandifolius* exhibits a nutritional strategy that is strongly oriented toward energy conservation, while *Laminaria solidungula* appears to balance the need to conserve energy with the need to avoid N-limitation in an environment that alternates between low light and low nitrogen availability.

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