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Uptake kinetics and assimilation of inorganic nitrogen by *Catenella nipae* and *Ulva lactuca*

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Abstract

The kinetics of NH_4^+, the assimilation of NH_4^+ and nitrate uptake by *Catenella nipae* (Rhodophyta) were compared with *Ulva lactuca* (Chlorophyta). Both algal species demonstrated saturable NH_4^+ and nitrate uptake kinetics. Uptake of NH_3 by simple diffusion across the plasmalemma could not account for the observed saturation uptake kinetics of ammonia-N ($NH_3 + NH_4^+$), so NH_4^+ was the chemical form being taken up by the transport systems of the cells. Although the V_{max} of NH_4^+ uptake by *C. nipae* and *U. lactuca* was high (≈550 and 450 μmol g^{-1} DW h^{-1}, respectively), the K_m for *U. lactuca* (≈85 μM) was much lower than that for *C. nipae* (≈692 μM). The K_m and V_{max} values for nitrate uptake were much lower than for NH_4^+ for both *C. nipae* ($K_m \approx 5$ μM; $V_{max} \approx 8.3$ μmol g^{-1} DW h^{-1}) and *U. lactuca* ($K_m \approx 34$ μM; $V_{max} \approx 116$ μmol g^{-1} DW h^{-1}). Over the incubation times used (up to 28 min) there was no apparent induction of nitrate transport in either species. There was no evidence for induction of NH_4^+ transport in *C. nipae* but incubation time did affect the kinetics of NH_4^+ uptake in *U. lactuca*. At high concentrations of NH_4^+, *U. lactuca* rapidly assimilated it into organic N with limited build-up of intracellular NH_4^+ whereas *C. nipae* accumulated large amounts of NH_4^+ because uptake of NH_4^+ overtook the rate of assimilation. The effects of species-specific differences and experimental design on uptake-kinetic estimates are discussed in the light of the results of this other comparable studies. *C. nipae* is promising as a bioindicator species of the N-status of estuaries but *U. lactuca* changes its N-status too quickly for it to be a useful bioindicator of environmental conditions.

Keywords: Ammonia-N; NH_4^+; Nitrate; Assimilation; *Ulva lactuca*; *Catenella nipae*; Uptake kinetics; Bioindicator species

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1. Introduction

Catenella nipae Zanardini (Rhodophyta: Gigartinales) and its relative *Catenella repens* are commonly associated with mangroves in estuaries of most of the Indo-pacific Rim (Datta and Datta, 1999). *Catenella* is readily obtainable over a wide geographical area and is easily recognised and often present in nearly unialgal stands. It appears to be promising as a bioindicator for environmental studies of the impact of inputs of fixed nitrogen and phosphate in estuaries (Runcie, 2001). Unfortunately, very little is known about its response to eutrophication. To put a study of the nutrient responses of *C. nipae* into an ecophysiological context, findings on *C. nipae* were compared with results of similar experiments with *Ulva lactuca* Linnaeus (Chlorophyta) that has been the subject of many nutrient uptake studies (Fujita, 1985; Ho, 1987; Fujita et al., 1988; Lavery and McComb, 1991; Pedersen, 1994; Sfriso, 1995; Sfriso and Marcomini, 1996; Pedersen and Borum, 1996, 1997; Naldi and Wheeler, 1999). *U. lactuca* can be considered an almost universally obtainable bench-line species.

Charged ions such as NH_4^+ and NO_3^- are taken up by means of carrier-mediated mechanisms (Healey, 1980; Flynn, 1998). Both active and passive carrier-mediated transport mechanisms depend on a finite number of saturable carrier (enzyme) sites. In contrast, NH_3 may pass through the plasmalemma and tonoplast by simple diffusion: characterised by a linear relationship between increasing external concentration and uptake rate (Fick's Law).

The Michaelis–Menten relation describes carrier-mediated ion transport in terms of the two kinetic parameters V_{max} (the maximum uptake rate) and K_m (the concentration of substrate where uptake proceeds at half the maximum rate). The ratio V_{max}/K_m (often defined as the Affinity (A) incorporates both parameters and is the initial slope of the uptake rate versus substrate concentration curve. V_{max}/K_m or A provides an index describing uptake rates versus substrate concentration at very low substrate concentrations, with higher values suggesting competitive advantage and a higher affinity (Healey, 1980).

Recent molecular studies of a wide range of plant species including algae have identified and characterised nitrate (Forde, 2000) and NH_4^+ (Howitt and Udvardi, 2000) transporters. These mechanisms operate at low and high external concentrations and are known as High Affinity Transport Systems (HATS) and Low Affinity Transport Systems (LATS), respectively. The activity of some transporters may be induced by elevated external inorganic N concentrations (thus iHATS would be an induced HATS mechanism), while other transporters maintain a consistent activity regardless of external conditions (constitutive systems, thus cLATS is a constitutive LATS mechanism).

The goals of this study were:

(a) to demonstrate the appropriate methods to determine K_m and V_{max} and V_{max}/K_m (A) of NH_4^+ and nitrate uptake by *C. nipae* and *U. lactuca* in short-term incubations. These parameters are needed to develop ecological models (Fong et al., 1994, 1998; Smith et al., 1999);
(b) to compare the rates of NH_4^+ influx with rates of assimilation;
(c) to determine the extent to which increasing internal pools of NH_4^+ influence rates of NH_4^+ uptake;
(d) to interpret the observed uptake kinetics in terms of the current molecular understanding of inorganic N transport;

(e) draw conclusions about the utility of *C. nipae* compared to *U. lactuca* as a bioindicator of the N-status of marine estuaries in the Indo-Pacific region (Ho, 1987; Horrocks et al., 1995; Smith et al., 1999; Costanzo et al., 2000).

2. Materials and methods

U. lactuca was collected from an intertidal rock-shelf at a beach in metropolitan Sydney (Maroubra 33°57′S, 151°14′30″E) and *C. nipae* from mangrove pneumatophores on mangroves growing in the Hawkesbury River estuary north of Sydney (Mooney Mooney, 33°31′30″S, 151°12′E), Australia from April to October 1998. Except after episodes of heavy rain, the salinity at the Hawkesbury River site is close to normal seawater (Kerr, 1994). *U. lactuca* was transported in seawater from the collection site, while *C. nipae* was transported moistened by seawater but not immersed. Like several other intertidal algae, *C. repens* (a close relative of *C. nipae* found in India) is known to change its nutrient uptake behaviour after immersion (Thomas et al., 1987; Datta and Datta, 1999) and we found that *C. nipae* will not tolerate immersion in stagnant seawater for more than a few days.

2.1. Experimental design

Ambient concentrations of nitrate, NH_4^+ and phosphate were measured from filtered (0.45 μm cellulose acetate) samples taken concurrently with the algal collections. Nutrient concentrations in field collections of seawater and in experimental media were analysed in duplicate with a Technicon Multilyzer using a Cadmium column (Technicon, Tarrytown, USA) using standard automated methods (American Public Health Association, 1998). The cadmium column step of the assay converted NO_3^- to NO_2^- and so both forms of oxidised nitrogen are reported here as nitrate.

All uptake and assimilation experiments were performed on freshly collected algal material to avoid acclimation effects as much as possible. Care was taken to do similar experiments on both *C. nipae* and *U. lactuca*. Prior to experimentation, algal material was rinsed thoroughly in nutrient-free Artificial SeaWater (ASW) with trace elements and vitamins added according to the *f*/2 formula (Ritchie, 1988). Nutrient stocks were made up with NH_4Cl or $NaNO_3$. Epiphytes and mud were removed, and thalli were cut into small portions of approximately 0.5 g fresh weight (g FW) with holdfast and unpigmented tissue removed. As pointed out by Fujita et al. (1988), it was necessary to thoroughly clean each portion to obtain consistent results. A 1000 W metal halide lamp suspended above a water bath (acting as a heat filter) provided irradiance of approximately 450 μmol quanta m^{-2} s^{-1} (400–700 nm, Li-Cor 192-SA sensor) for both acclimation of the material and for running experiments. Material for each experiment was acclimated in ~11 ASW with gentle agitation for approximately 1 h. After acclimation, the material was drained and gently blotted dry, any dead material removed, and the portions were added to the treatment beakers (with ASW). Treatments were agitated vigorously for a further 10 min under the same irradiance before the addition of nutrient stock. The ambient temperature in the laboratory was 20 °C.

Uptake was measured over brief exposure intervals in order to avoid the inhibitory effect of elevated internal ion pools on further ion uptake (substrate inhibition: Fujita et al., 1988;

McGlathery et al., 1996). Confounding effects of the incubation treatments themselves (substrate concentration, incubation time, the balance between uptake and assimilation) were carefully minimised as outlined by Flynn (1998). As the assimilation of NH_4^+ into amino acids drains the internal NH_4^+ pool and reverses the inhibitory effect (McGlathery et al., 1996), experiments were also performed to determine the extent to which assimilation influences uptake-kinetic parameters over time.

Each uptake-kinetic experiment used twelve 50 ml treatment beakers each with 24 ml ASW. Aliquots of nutrient ions were added to each beaker, to provide a range of nutrient concentrations (usually 1–1200 μM). Uptake was followed by taking aliquots (usually 1 ml) of the incubation medium at set time intervals. Firstly, pilot experiments for each nutrient–alga combination were performed with a wide range of concentrations (μM to mM) to provide rough estimates of V_{max} and K_m. The range of nutrient concentrations used in subsequent experiments was then modified to more accurately estimate K_m (Ritchie and Prvan, 1996), where (ideally) 6 of the 12 treatments of each experiment would have substrate concentrations less than the K_m estimated from the pilot experiment (Ritchie and Prvan, 1996).

After all the beakers of algae had been pre-incubated, nutrient uptake was measured in a staggered series of incubations. Each experiment was performed by sequentially administering aliquots of concentrated nutrient stock at 30 s intervals to the treatment beakers, and removing a 1 ml sample of medium immediately after brief stirring (approximately 8 s after addition of the stock). These initial nutrient concentrations were taken as representative of substrate concentration at $t = 0$. Samples were removed at defined intervals after stock addition (e.g. 0, 6, 12 and 18 min), and all samples were frozen for later analysis. Afterwards, algal material was blotted dry to remove saltwater and oven-dried to constant weight at 60 °C. Substrate concentrations, with which the uptake rates were compared, were calculated from the mean concentration in the medium during the interval of uptake measured.

Additional uptake experiments were designed to measure changes in internal ammonia-N content over intervals of 0, 6, 12, 18 and 30 min (*U. lactuca*); and 0, 6, 12 and 18 min (*C. nipae*). Two experiments using portions of *U. lactuca* collected from the same site on the same day were performed with initial medium concentrations of 780 and 1240 μM NH_4^+. Two similar experiments were performed on portions of *C. nipae* with an initial concentration of 170 μM NH_4^+. At the end of each interval three replicate portions were removed from the medium, briefly rinsed in high-purity water, added to 90% ethanol, and ground to facilitate overnight release of NH_4^+ (McGlathery et al., 1996; Hwang et al., 1987). The ammonia-N concentration of the incubation medium was also monitored, as was the concentration of ammonia-N in the control beakers (without plant material). It was shown that NH_3 volatilisation and adsorption of NH_4^+ onto the surface of the beakers was negligible. Plant samples from the same stock, not exposed to added ammonia-N, were analysed to determine internal ammonia-N content prior to exposure to NH_4^+.

2.2. Data analysis

Substrate concentration was calculated as the arithmetic mean of the initial ($t = 0$) and final concentration for a time interval. Uptake rate was calculated as the quantity of nutrient removed by the plant per unit time per gram dry weight (μmol g^{-1} DW h^{-1}) and corrections were made for quantities of nutrient removed by sampling of the medium. Where

needed, fresh weight to dry weight conversions were calculated using conversion factors of 5.507 g FW g^{-1} DW for *C. nipae* ($n = 64$, this study) and 3.752 g FW g^{-1} DW for *U. lactuca* ($n = 100$, Ritchie, 1988).

Data for each kinetic experiment were fitted to a rectangular hyperbola (the Michaelis–Menten model) by non-linear least squares iterative techniques (ORIGIN 5.0 software, Microcal Software, Northampton, MA, USA). Curves were tested for significance using product-moment correlation coefficients (Rohlf and Sokal, 1969). Estimates of the parameters V_{max} and K_m (and their associated variance) from different experiments were compared and tested for heteroscedasticity using Cochran's test (Rohlf and Sokal, 1969). Where homogeneous, these estimates were pooled together and recalculated to provide an overall mean value with ±95% confidence limits. Using Tukey's test (Rohlf and Sokal, 1969), parameter estimates of experiments with different incubation intervals were examined by comparing the differences between each overall mean relative to the smallest overall mean. Errors for V_{max}/K_m (A) were determined by propagating errors from V_{max} and K_m.

Assimilation of ammonia-N was inferred from the difference between ammonia-N removed from the medium (uptake) and chemically assayable ammonia-N that accumulated in the algal material. Transport of NH_4^+ across the plasmalemma (uptake), or the assimilation of ammonia-N was calculated for intervals up to 18 min. Time courses were fitted to a single exponential function using a single compartment model for NH_4^+ uptake. Curve fitting was conducted by non-linear least squares on $1/y^2$ weighted mean data (Di Cera, 1992), and estimates of variance for each parameter were calculated. From these parameters, biological half-lives, pool sizes and flux rates were calculated for uptake of NH_4^+ into the algae. The rate of assimilation of the internal ammonia-N into organic-N was calculated by difference.

3. Results

3.1. Ambient nutrient concentrations

Ambient inorganic N concentrations in the estuary were consistently greater than concentrations in marine waters over the sampling interval (April to October 1998), and nitrate concentrations were greater than NH_4^+ concentrations at both estuarine (*C. nipae*) and marine (*U. lactuca*) collection sites during this period. The nitrate levels at Hawkesbury River were 15 μM (range 5–48 μM), compared to only 1.7 μM (range 0.5–7.3 μM) at the coastal site. Ammonia-N levels were low at both sites: Hawkesbury River 2.3 μM (range 0.4–5.2 μM); coastal site 0.9 μM (range 0.4–1.9 μM). High nutrient levels were found during or after episodes of heavy rain. Episodes of heavy rain occur in the Sydney region irregularly with little obvious seasonality. The *C. nipae* and *U. lactuca* used in this study were not collected during flooding.

3.2. Uptake kinetics of NH_4^+

NH_3 and NH_4^+ were measured together in this study and are referred to as ammonia-N ($NH_3 + NH_4^+$). At the pH of seawater (pH 8.1), NH_4^+ is the dominant form (pK_a $NH_3 \approx 9.25$). Diffusion of NH_3 could not be responsible for uptake of ammonia-N by either species

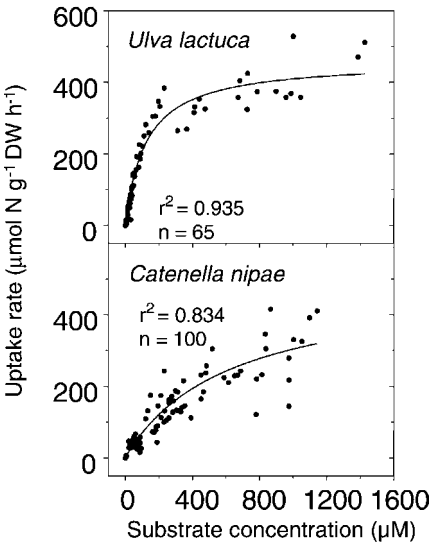

Fig. 1. Uptake rate of NH_4^+ by *Ulva lactuca* and *Catenella nipae* over 6, 12, and 18 min incubation intervals as a function of substrate (NH_4^+) concentration.

because uptake was clearly a saturable transport process (Fig. 1) and hence NH_4^+ was the substrate recognised by the transport mechanism.

Uptake rate of NH_4^+ versus concentration curves, for both *U. lactuca* and *C. nipae*, could be described by the rectangular hyperbola model (Fig. 1) (Healey, 1980; Pedersen, 1994). This indicates the action of saturable uptake mechanisms. Kinetic parameters of individual replicated uptake experiments were similar enough to permit pooling of data to obtain overall means with ±95% confidence intervals (Table 1). The incubation time (0–6 to 0–28 min) generally had no significant effect upon K_m and V_{max} of nitrate or NH_4^+ in either species and so combined values are shown in Table 1. However, the K_m and V_{max} parameters for uptake of NH_4^+ by *U. lactuca* were found to be considerably different depending on the incubation time used indicating either an acclimation or pool-filling effect during the course of incubation.

Millimolar concentrations of NH_4^+ were required to achieve the saturated rate of uptake. During 18 min of incubation at high concentrations, V_{max} of NH_4^+ uptake in *U. lactuca* remained constant at ≈450 μmol g^{-1} DW h^{-1}, indicating that ammonia-N pools did not fill sufficiently to cause declines in V_{max} (i.e. substrate inhibition was not apparent) (Table 1). In contrast, K_m increased from 85 μM (measured over 6 min incubation) to 150 μM (over 18 min incubation). The combination of invariant V_{max} and increasing K_m with increasing incubation interval is often an artefact resulting from a significant decline in substrate concentration of the less concentrated treatments causing a bias in parameter estimates (Flynn, 1998). Thus, the kinetic parameters of NH_4^+ uptake by *U. lactuca* over increasing incubation time intervals were influenced to an increasing extent as the NH_4^+ content of the incubation medium declined. Hence, the K_m and V_{max} parameters determined using different incubation times have been presented separately in Table 1.

Table 1
Inorganic nitrogen uptake kinetics

	Incubation duration (min)	NH_4^+ uptake			Nitrate uptake		
		V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m
Ulva lactuca	6	427 ± 48 (2)	85 ± 29 (2)	5.1 ± 1.8	–	–	–
	12	469 ± 34 (2)	127 ± 31 (2)	3.7 ± 0.9	–	–	–
	18	466 ± 32 (2)	147 ± 30 (2)	3.2 ± 0.7	–	–	–
	7, 14, 21 and 28	–	–	–	116 ± 18 (11)	34 ± 9 (11)	3.47 ± 1.03 (11)
Catenella nipae	12 and 18	–	–	–	8.3 ± 0.82 (6)	5 ± 1.7 (6)	1.7 ± 0.061 (6)
	6, 12 and 18	547 ± 68 (15)	692 ± 131 (15)	0.78 ± 0.07 (15)	–	–	–

The kinetic parameters V_{max} (μmol N g^{-1} DW h^{-1}), K_m (μM) and the affinity for uptake at low concentrations (V_{max}/K_m) (l g^{-1} DW h^{-1}) for uptake of ammonia-N (NH_4^+ and NH_3) and nitrate for the macroalgae *Ulva lactuca* and *Catenella nipae*. Values are mean parameter estimates derived from (*n*) replicate uptake experiments ±95% confidence limits. Nitrate uptake rates based upon 0–6 min incubation times were not accurate enough to give an estimate of K_m that was significantly different from zero. All other K_m and V_{max} determinations were significant to at least $P < 0.05$.

In contrast, *C. nipae* showed no significant variation in either K_m or V_{max} during 18 min of uptake. Therefore, the uptake-kinetic parameters were uninfluenced by a decline in the NH_4^+ concentration of the medium and so it was valid to pool the data (Table 1).

NH_4^+ uptake by *U. lactuca* and *C. nipae*, at low ammonia-N concentrations more like those found in the field, was linearly related to the NH_4^+ concentration in the medium (Fig. 1 and Table 1; see Healey, 1980). For *U. lactuca*, V_{max}/K_m (or A) derived from the first 6 min of NH_4^+ uptake was least affected by errors attributable to declining medium concentrations and therefore best estimated the affinity of *U. lactuca* for NH_4^+. Estimates of V_{max}/K_m by *C. nipae* were constant for incubation times up to 18 min and were four times less than for *U. lactuca* (Table 1).

3.3. Nitrate uptake kinetics

Nitrate uptake demonstrated saturation kinetics in both species (Table 1, Fig. 2). Both V_{max} and K_m of nitrate uptake by *U. lactuca* ($V_{max} \approx 116\ \mu mol\ g^{-1}\ DW\ h^{-1}$ and $K_m \approx 34\ \mu M$) and *C. nipae* ($V_{max} \approx 8.3\ \mu mol\ g^{-1}\ DW\ h^{-1}$ and $K_m \approx 5\ \mu M$) were independent of the incubation time used to measure nitrate uptake. Neither substrate inhibition nor a decline in the medium substrate concentration influenced the parameter values using incubation times up to 28 min and so the data could be pooled (Table 1).

Although the estimates of the affinity of *U. lactuca* for nitrate were of a similar magnitude to the affinity for NH_4^+ (V_{max}/K_m: Table 1), the affinity for nitrate by *C. nipae* was two times higher than its affinity for NH_4^+ (Table 1). This implies that *C. nipae* is better suited to taking

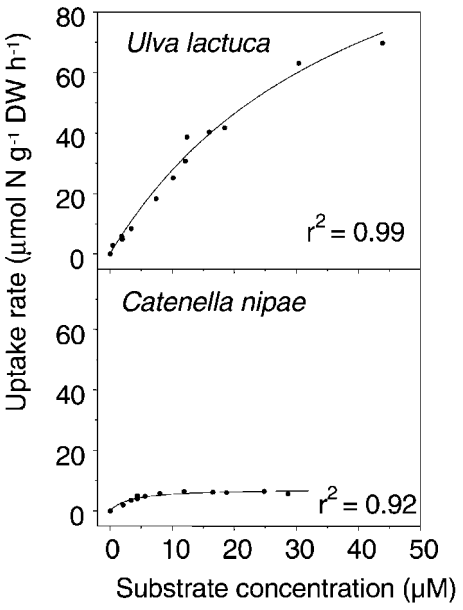

Fig. 2. Uptake rate of nitrate by *Ulva lactuca* and *Catenella nipae* as a function of substrate concentration. Curves are fitted to data derived from experiments with a 12 min incubation interval.

up nitrate relative to NH_4^+, whereas *U. lactuca* is equally able to take advantage of both nitrate and NH_4^+ in the environment when ammonia and nitrate-N are in the micromolar range.

Nitrate uptake by macroalgae is usually found to proceed more slowly than the uptake of NH_4^+ and will often saturate at relatively low concentrations (D'Elia and DeBoer, 1978; Pedersen and Borum, 1997). The highest nitrate concentration used in the present study was less than 50 μM. *U. lactuca* took up nitrate at high rates when exposed to high concentrations (Fig. 2) but the rates attainable at concentrations likely to be encountered in the field (≈2 μM) would be very low ($\phi \approx A \times [NO_3^-]_0$; Table 1). However, *C. nipae* demonstrated a maximum saturated uptake rate at approximately 10 μM ($K_m = 5$ μM) (Fig. 2), which is typical of the nitrate concentrations found in the Hawkesbury River and other eutrophic estuaries (Kerr, 1994).

3.4. Assimilation of NH_4^+

It was unclear from the kinetic experiments whether the assimilation of NH_4^+ influenced the apparent rates of uptake into the cells. A series of further incubations in ammonia-N were therefore performed at higher concentrations for longer time intervals. Estimates of NH_4^+ assimilation were inferred from the decline in NH_4^+ content of the medium taking into account the ammonia-N measured in the algal tissue. When exposed to 780 or

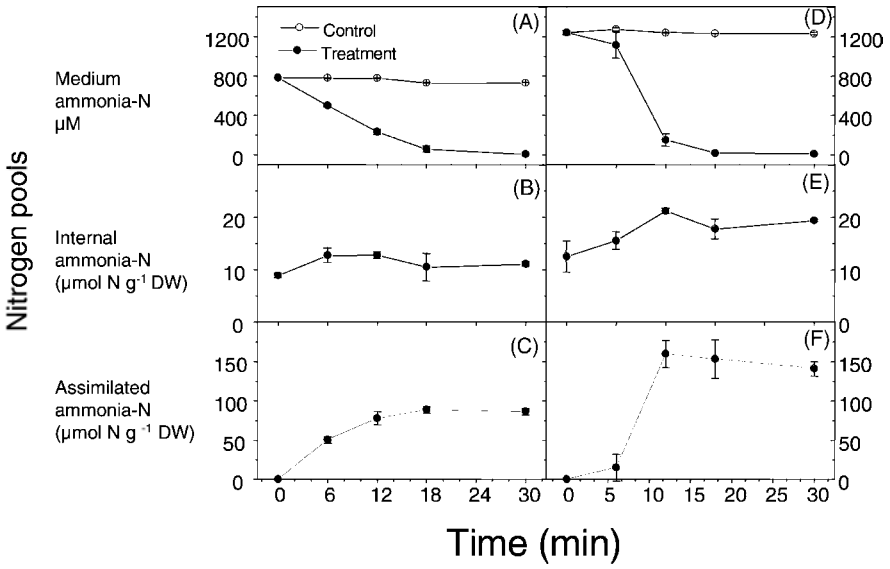

Fig. 3. Ammonium uptake and assimilation in *Ulva lactuca*. The left-hand panels (A–C) present results from incubations in 780 μM NH_4^+ and the right-hand panels (D–F) present results from incubations in 1240 μM. Error bars indicate standard error for three replicates; some bars are so small they are covered by the size of the symbols. The two upper panels (A and D) presents the medium NH_4^+ concentration of treatments with, and controls without *U. lactuca*; the two middle panels (B and E) presents the internal NH_4^+ content; and the two lower panels (C and F) presents the quantity of NH_4^+ removed from system by assimilation.

1240 μM NH_4^+ the internal ammonia-N content of *U. lactuca* increased to maximum levels of 13 or 21 μmol g^{-1} DW within 6 and 12 min, respectively (Fig. 3). Although in both experiments the internal ammonia-N content declined slightly after reaching the maximum concentration, it was not found to decline to the pre-exposure content. The corresponding internal ammonia-N concentrations were ≈8.4 and 13.8 mM (intracellular water content of *U. lactuca*: 2.72 ml g^{-1} DW from Ritchie, 1988).

U. lactuca incubated at 780 μM took up NH_4^+ steadily over 18 min, but demonstrated no increase in internal content after 6 min. Hence, the NH_4^+ content of the experimental system (medium + alga) declined significantly (Fig. 3A-C). NH_4^+ assimilated by *U. lactuca* over each time interval did not differ significantly from quantities taken up by the alga; thus, assimilation of NH_4^+ and uptake of NH_4^+ proceeded at similar rates. When incubated at a higher concentration (1240 μM NH_4^+), the quantity of NH_4^+ assimilated during the first 6 min was less than that assimilated by *U. lactuca* incubated at 780 μM (Fig. 3D-F). During the second 6 min interval the quantity assimilated was five times greater than that of the 780 μM NH_4^+ treatment, and subsequent intervals demonstrated minimal assimilation accompanied with minimal medium concentrations. Assimilation appeared to be related to the NH_4^+ concentration in the medium, but with a lag time of several min preceding any significant assimilation.

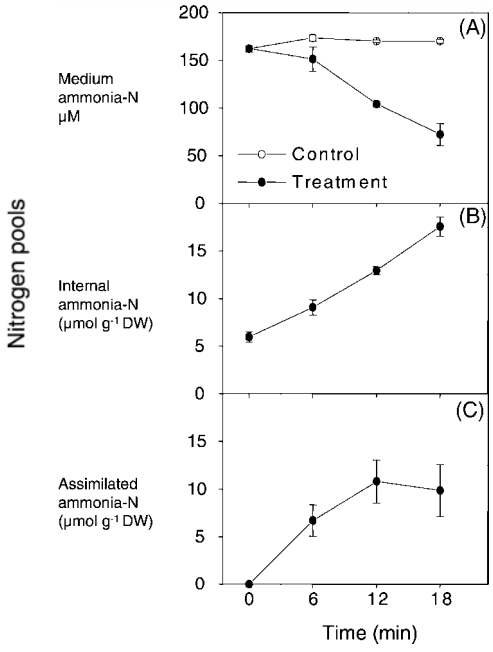

Fig. 4. Ammonium uptake and assimilation in *Catenella nipae* with initial medium NH_4^+ concentration of 170 μM. Panel A presents the medium NH_4^+ concentration of treatments with, and controls without *U. lactuca*; panel B presents the internal NH_4^+ content; and panel C presents the quantity of NH_4^+ removed from the seawater/alga system by assimilation. Data are pooled from two similar experiments and error bars indicate standard error for a total of six replicates, some error bars are covered by the symbols.

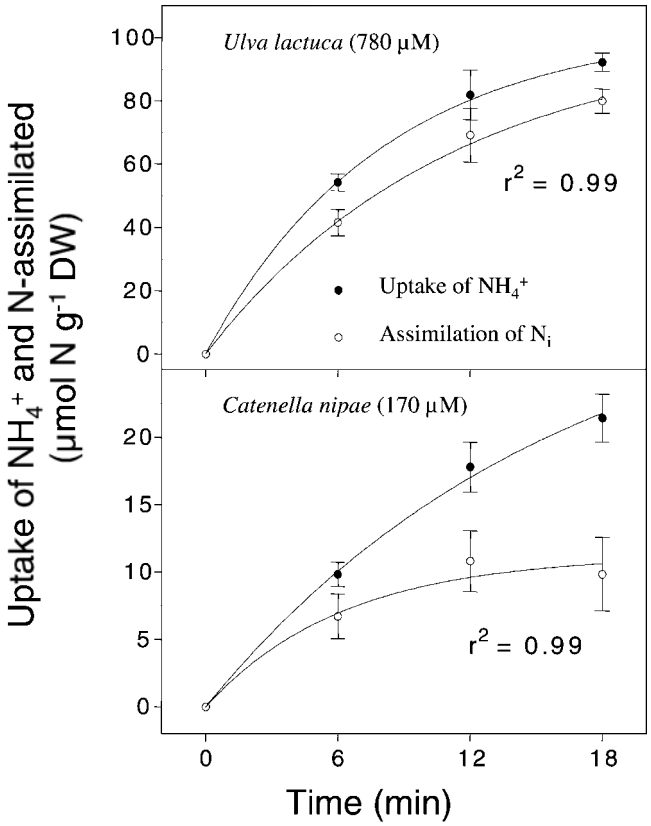

Fig. 5. Ammonium uptake by *Ulva lactuca* and *Catenella nipae* incubated in 780 and 170 µM NH_4^+, respectively. Data represent the quantity of NH_4^+ removed from the medium by the algae (uptake) and the quantity of NH_4^+ assimilated (assimilation) inferred from the molar difference in NH_4^+ taken up and the tissue NH_4^+ concentration at each time point. Data are presented with standard errors ($n = 3$).

C. nipae took up NH_4^+ at a constant rate when incubated in 170 µM NH_4^+, and the internal ammonia-N concentration continued to rise steadily over 18 min, although at 12 min assimilation reached a maximum rate (Fig. 4). In contrast, to *U. lactuca* (which assimilated most of the ammonia-N that was taken up) only 68% of the NH_4^+ taken up by *C. nipae* in the first 6 min of incubation was assimilated, and no decline in uptake rate was observed after 18 min incubation.

Fig. 5 shows that the fate of NH_4^+ taken up by *U. lactuca* and *C. nipae* are rather different. In *U. lactuca*, the intracellular [NH_4^+] builds up to about 10 mM in 6 min or less after initial exposure to high external concentrations of NH_4^+. After this time the intracellular ammonia-N remains constant because any extra NH_4^+ taken up is converted to assimilated N.

C. nipae rapidly ceased assimilating ammonia-N into organic nitrogen when fed high concentrations of NH_4^+. However, uptake of NH_4^+ continued to take place (Table 2, Fig. 4), indicating that NH_4^+ was being stored in the cells, probably in the large vacuoles of the

Table 2
Ammonia taken up from the medium compared with NH_4^+ removed by assimilation

Species	Conc. (μM)	Uptake				Assimilation			
		C_1 (μmol g^{-1} DW)	k_1 (min^{-1})	Half-life (min)	$V_{0(1)}$ (μmol g^{-1} DW h^{-1})	C_2 (μmol g^{-1} DW)	k_2 (min^{-1})	Half-life (min)	$V_{0(2)}$ (μmol g^{-1} DW h^{-1})
Ulva lactuca	780	103.38 ± 1.8	0.1247 ± 0.095	5.56 ± 4.23	773 ± 589	99.78 ± 2.7	(0.091 ± 0.12)	(7.60 ± 10.0)	545 ± 719
Catenella nipae	170	32.38 ± 2.2	0.061 ± 0.012	11.32 ± 2.24	119 ± 24.7	11.13 ± 1.3	(0.167 ± 0.26)	(4.16 ± 6.46)	112 ± 174

C_1 represents the theoretical maximum quantity of ammonia (or NH_4^+) transported via uptake (or assimilation); k is the uptake constant; the biological half-life is derived from k; and V_0 is the initial rate of ammonia (or NH_4^+) transport. Values are derived from curves fitted to mean values of three replicate experiments (total $n = 12$) ±95% confidence intervals. All curves were significant to at least $P < 0.05$. Values of k_n in brackets are not significantly different to zero, half-lives in brackets are derived from these values.

internal cells. *C. nipae* seems to have a limited capacity to convert NH_4^+ to organic N at an accelerated rate. Thus, *C. nipae* stores ammonia-N, if offered unusually high concentrations of NH_4^+, whereas *U. lactuca* increases its ammonia-N assimilation rate and stores proportionally less as ammonia-N.

4. Discussion

Nutrient uptake-kinetic data can be used to define the potential of an alga to utilise nutrients in the environment, and clearly distinguishes an opportunist (*U. lactuca*) from a late successional species (*C. nipae*). Ecological simulation models such as that by Fong et al. (1994, 1998) require nutrient uptake-kinetics data.

4.1. Uptake of ammonia-N

Uptake of ammonia-N in both *U. lactuca* and *C. nipae* exhibited saturable kinetics (Fig. 1) (Fujita, 1985; Pedersen, 1994; Pedersen and Borum, 1997; Flynn, 1998), which is inconsistent with significant uptake in the form of NH_3 by simple diffusion (cf. Taylor et al., 1998). No evidence was found for ammonia toxicity over the range of concentrations and incubation times used in the present study for *U. lactuca* or *C. nipae*.

Uptake experiments must be performed and data analysed using appropriate experimental protocols, such as the use of adequate replication, a wide range of substrate concentrations and short incubation intervals to avoid negative feedback effects upon the uptake process (Fujita, 1985; Fujita et al., 1988; Ritchie and Prvan, 1996; Flynn, 1998; Howitt and Udvardi, 2000). However, the calculation of uptake rates from declining medium ion concentration using chemical methods of determination measures net not unidirectional fluxes. Unidirectional fluxes (^{13}N is the only practicable method) are needed for a full thermodynamic interpretation of ion transport rates.

Ammonia-N uptake in the freshwater *Chlamydomonas reinhardtii* and in vascular plants has been characterised by Howitt and Udvardi (2000) to be an iHATS system regulated by plant N-status. In contrast to the present study, uptake by *C. reinhardtii* at very high ammonia-N concentrations shows linear kinetics (cf. Fig. 1).

The K_m values for NH_4^+ uptake shown in Table 1 for *U. lactuca* (≈85 μM) and *C. nipae* (≈692 μM) are very much higher than concentrations of ammonia-N found in the waters of the Sydney region. Hence, in the field, the uptake rate for ammonia-N in both species would be directly proportional to the ambient concentration of NH_4^+ (Healey, 1980). *U. lactuca* shows a tendency for both K_m and V_{max} to increase as the incubation time was increased from 0–6 to 0–18 min (Table 1). Activation of existing constitutive HATS and LATS systems could occur quite quickly (Hwang et al., 1987; McGlathery et al., 1996; Berges, 1997; Forde, 2000; Howitt and Udvardi, 2000). It is therefore important in measuring K_m and V_{max} in *U. lactuca* to use as short incubation times as practicable. Induction phenomena are a real problem in working with an opportunist like *Ulva*: it is known to be able to change its N-status very quickly. *C. nipae* showed a consistent K_m (≈692 μM) and V_{max} (≈547 μmol N g^{-1} DW h^{-1}) independent of the incubation time used and there was no induction or activation.

Table 1 shows that incubation time had some effect on estimates of K_m and V_{max} of NH_4^+ uptake in *U. lactuca* but not in *C. nipae* indicating rapid feedback control effects on NH_4^+ uptake in *U. lactuca*. Algae assimilate internal ammonia-N converting it into amino acids. This conversion can relieve substrate inhibition and enable further uptake. Fig. 3 shows that in *U. lactuca* there is evidence for activation of NH_4^+ assimilation over a time course of about 20 min but the intracellular ammonia-N pool remains at ≈10–20 μmol N g^{-1} DW (or ≈8–15 mM). Additionally, Fig. 3D shows some evidence for an activation time of about 5–10 min for uptake of NH_4^+ when offered very high external concentrations of this nutrient. This enabled *U. lactuca* to take up NH_4^+ rapidly for extended intervals when excess NH_4^+ was offered. Although *C. nipae* also took up NH_4^+ at high rates, initially high assimilation rates rapidly declined while internal pools continued to increase (Figs. 4 and 5). After 12–18 min incubation there was negligible assimilation. *C. nipae* stores NH_4^+ for later assimilation whereas *U. lactuca*, with much less capacity to store ammonia-N, accelerates ammonia-N assimilation when offered high ammonia-N concentrations.

The K_m (≈85 μM) of ammonia-N uptake by *U. lactuca* was much higher than found in previous studies (K_m ≈ 10–30 μM, Table 3). Both Pedersen (1994) and O'Brien and Wheeler (1987) used very long incubation times, which would have allowed activation and induction of HATS systems. Taylor and Rees (1999) were not able to estimate K_m and V_{max} of NH_4^+ uptake in *Enteromorpha* spp. because they did not include concentrations high enough to approach saturation of the uptake mechanism but found that V_{max}/K_m of NH_4^+ assimilation was about 1.51 g^{-1} DW h^{-1} (consistent with other values shown in Table 1).

Table 4 presents results from published NH_4^+ uptake studies for a variety of red algae. In terms of biomass, *C. nipae* is able to take up NH_4^+ at a rate about 10 times higher than found in many other red algae. The K_m for *C. nipae* (≈692 μM) was much higher than found previously in other red algae (K_m ≈ 10–30 μM), resulting in a low affinity value (V_{max}/K_m). The high V_{max} and K_m suggest that over any range of concentrations of NH_4^+ likely to be encountered in the environment, *C. nipae* will take up NH_4^+ at a rate directly proportional to the ambient concentration.

4.2. Nitrate uptake

Nitrate uptake by macroalgae generally proceeds at considerably lower rates (and demonstrates smaller V_{max} and K_m values: Fig. 2, Table 1) than NH_4^+ uptake (D'Elia and DeBoer, 1978; Pedersen and Borum, 1997). The negative charge on nitrate means that nitrate uptake would always be energy-dependent (Ritchie, 1988). There was no significant effect of the incubation time on nitrate uptake kinetics in either *U. lactuca* or *C. nipae* and so overall mean K_m and V_{max} values were calculated (Table 1). Table 3 shows that the K_m and V_{max} estimates made for *U. lactuca* in the present study compare well with previous estimates on *Ulva rigida* (Lavery and McComb, 1991) and *Enteromorpha prolifera* (O'Brien and Wheeler, 1987). *U. lactuca* is able to take up nitrate at rates about one-fourth of the uptake rates found for NH_4^+. Affinity of both species of algae for NH_4^+ and nitrate are rather similar and vary by a factor of no more than about 5 (Table 1). In environmental concentrations of NH_4^+ and nitrate, nitrate and NH_4^+ uptake rates would be similar.

Recent studies of nitrate uptake by a variety of plants demonstrate both LATS and HATS (iHATS and cHATS), and the activity of the iHATS is generally induced in the presence of an

Table 3
Ammonia-N, and nitrate transport kinetics of various uni- and bistromatic algae

Species/nutrient	Incubation duration (min)	Temp (°C)	V_{max} (μmol g^{-1} DW h^{-1})	K_m (μM)	V_{max}/K_m (l g^{-1} DW h^{-1})	Technique	Reference
Ammonia-N							
Ulva lactuca	6	20	427 ± 48	85 ± 29	5.1 ± 1.8	Constant interval, variable conc.	From Table 2 of this study
Ulva lactuca	15	15	211 ± 23	20 ± 6	10.6	Constant interval, variable conc.	Pedersen, 1994
Ulva sp.	15	15	146	14.4	10.1	Constant interval, variable conc.	Campbell, 1999
Enteromorpha prolifera	45–120	15–20	188 ± 30.3 S.D.	13.4 ± 7.35 S.D.	14.0 ± 7.98 S.D.	Constant interval, variable conc.	O'Brien and Wheeler, 1987
Ulva lactuca	300	15	111 ± 19	27 ± 10	4.1	Constant interval, variable conc.	Pedersen, 1994
Nitrate							
Ulva lactuca	7–28	20	116 ± 18	34 ± 9	3.47 ± 1.03	Constant interval, variable conc.	From Table 2 of this study
Ulva rigida	15–75	25	90	33	2.7	Constant interval, variable conc.	Lavery and McComb, 1991
Enteromorpha prolifera	120	12–14	169 ± 19.3 S.D.	13.3 ± 3.6 S.D.	12.7 ± 3.73 S.D.	Constant interval, variable conc.	O'Brien and Wheeler, 1987

The kinetic parameters V_{max}, K_m, and V_{max}/K_m for nutrient uptake (net influx) are shown. The nutrient status of material is field-collected unless stated otherwise; incubation time is *n* min after nutrient addition; kinetic parameter error is ±95% confidence intervals except where authors quoted the standard deviation (S.D.). Where necessary, rates on a fresh weight basis were converted to g DW assuming 1:2.752 DW:FW ratio.

Table 4
Ammonia-N and nitrate uptake kinetics of various red algae. The kinetic parameters V_{max}, K_m, and V_{max}/K_m for nutrient uptake (net influx) are shown

Species/nutrient	Incubation duration (min)	Temp (°C)	V_{max} (μmol g^{-1} DW h^{-1})	K_m (μM)	V_{max}/K_m (l g^{-1} DW h^{-1})	Technique	Reference
Ammonia-N							
Catenella nipae	18	20	547 ± 68	692 ± 131	0.78 ± 0.07	Constant interval, variable conc.	From Table 2 of this study
Polysiphonia decipiens	60	15	57.4	2.6	22.3	Constant interval, variable conc.	Campbell, 1999
Gracilaria foliifera	~180	20	23.8	1.6	14.9	Depletion	D'Elia and DeBoer, 1978
Neoagardhiella baileyi	~180	20	29.0	4.5	6.67	Depletion	D'Elia and DeBoer, 1978
Nitrate							
Catenella nipae	6–18	20	8.3 ± 0.82	5 ± 1.7	1.7 ± 0.61	Constant interval, variable conc.	From Table 2 of this study
Hypnea musciformis	60	26	6.2	1.1	5.8	Depletion	Haines and Wheeler, 1978
Gracilaria tikvahiae	180	20	9.7	2.5 ± 0.5 S.E.	3.9	Depletion	D'Elia and DeBoer, 1978

The nutrient status of material is field-collected unless stated otherwise; incubation time is *n* min after nutrient addition; kinetic parameter error is 95% confidence intervals except where the authors quoted the standard error (S.E.).

external supply of nitrate (Forde, 2000). In *U. lactuca* and *C. nipae* there was no activation and induction effects on nitrate uptake, at least over the concentration range used in the present study. O'Brien and Wheeler (1987) estimated a K_m for nitrate in *Enteromorpha prolifera* which is considerably lower than found in the present study (Table 3). Lavery and McComb (1991) found K_m values for *U. rigida* more similar to those found in the present study even though they used long incubation times (Table 3). If nitrate induction occurs in *Ulva* species it must take several hours. This is much slower than found in *Chaetomorpha linum* (Chlorophyta) (McGlathery et al., 1996). Under high ambient nitrate-N conditions, McGlathery et al. (1996) found that *Chaetomorpha* ceased N assimilation and nitrate uptake rates were then suppressed.

Table 3 shows a comparison of estimates of K_m, V_{max} and V_{max}/K_m from a number of studies of *Ulva* spp. and its close relative *Enteromorpha*. In general, the V_{max} found in the present study was about twice as high as found in other studies. However, the cited examples were generally done at considerably lower temperature (15 °C) on cool temperate populations, rather than material from the much warmer climate of the Sydney region, which is influenced by the warm East Australian current.

Table 4 presents data for nitrate uptake in *Hypnea musciformis* (Haines and Wheeler, 1978) and *Gracilaria tikvahiae* (D'Elia and DeBoer, 1978), which were run at similar temperatures to the present study. K_m, V_{max} and V_{max}/K_m are similar to those found here in *C. nipae*. Despite using long incubation times, Haines and Wheeler (1978) and D'Elia and DeBoer (1978) reported that they had found little, if any, induction or activation effects. In the longer term, elevated internal nitrate concentrations are known to inhibit nitrate influx rates (Fujita et al., 1988; McGlathery et al., 1996; Naldi and Wheeler, 1999).

4.3. Morphological differences

All cells of a bistromatic alga (like *U. lactuca*) are in contact with the external medium. Apparent net influx rates are the result of several processes going on:

(a) transport across the plasmalemma (influx);
(b) assimilation into organic N;
(c) transport across the tonoplast into the vacuole;
(d) transport across the plasmalemma (efflux).

Not only are all cells equally exposed to nutrients, they are also (generally) equally exposed to irradiance and inorganic carbon. *U. lactuca* demonstrated continued NH_4^+ assimilation rates equivalent to uptake rates for prolonged intervals (Fig. 5).

Polystromatic algae (like *C. nipae*) are integrated organisms with several kinds of tissues and organs that can potentially act as storage organs for nutrients. Internal cells of polystromatic algae are separated from the external medium, and nutrient ions migrating into this inner region must pass through a number of cells including those in the epidermis-like cortical cells. Initial uptake rates will reflect transport of nutrient ions into the surface cells and across the cortex (and be mostly uninfluenced by substrate inhibition). Afterwards, internal nutrient pools would begin to increase as the maximum assimilation rate is exceeded. Once nutrient pools in internal tissue increase, substrate inhibition would become apparent and the feedback reaction would eventually result in a decline in the uptake rate. *C. nipae*

demonstrated high initial assimilation rates of NH_4^+ that rapidly declined while uptake of NH_4^+ continued unabated (Fig. 4): internal pools continued to increase during the course of the incubation. The initial NH_4^+ assimilation rate of *Catenella* plants may also have been sufficiently rapid to exceed the photosynthetic carbon supply resulting in the rapid consumption of stored carbon by amino acid synthesis and a decline in the rate of NH_4^+ assimilation as found in phytoplankton (Berges, 1997).

4.4. Potential as bioindicator species

Care must be taken to design experiments such that they measure the constitutive potential for uptake rather than an experimentally induced state reflecting prolonged incubation under the experimental conditions being used (Pedersen, 1994; Pedersen and Borum, 1996, 1997). The time required for activation, induction or suppression of NH_4^+ and NO_3^- transporters varies widely amongst species and probably within species as well.

Opportunists like *Ulva* and *Enteromorpha* demonstrate rapid nutrient uptake rates when subjected to high nutrient concentrations (Ho, 1987; Fong et al., 1994, 1998; Smith et al., 1999). *U. lactuca* stores fixed nitrogen in relatively small quantities and rapidly remobilises the stored product when required (Hwang et al., 1987). *U. lactuca* and *Enteromorpha* sp. are commonly found in locations receiving frequent episodic pulses of nutrient-rich water, or locations with permanently elevated nutrient concentrations (such as the rock-shelf population used in the present study) (Sfriso, 1995; Sfriso and Marcomini, 1996; Smith et al., 1999). Figs. 3 and 5 show the classic responses that would be expected of an opportunistic species offered high levels of nutrients (Fujita, 1985; Fujita et al., 1988; Pedersen and Borum, 1997).

From the present study, the N-status of *Ulva* seems unpromising as a bioindicator: *Ulva* adjusts too rapidly to the ambient conditions. Some red algae such as *Gracilaria* spp. and *Gracilaria edulis* characteristic of waters receiving episodic pulses of high nutrients respond to nutrients in a similar way (Horrocks et al., 1995; Costanzo et al., 2000).

C. nipae is a late successionist species. *C. nipae* had generally lower uptake rates relative to *U. lactuca* and stored significant NH_4^+ during assimilation experiments and is not able to fully exploit high nutrients levels when offered (Figs. 4 and 5). *C. nipae* probably does not quickly change its N-status in response to ambient ammonia or nitrate-N. This is a desirable property for a bioindicator species because its N-status should reflect ambient conditions integrated over the previous several days.

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