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Nitrogen status and metabolism in the green seaweed *Enteromorpha intestinalis*: an examination of three natural populations

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ABSTRACT: Nitrogen metabolism in *Enteromorpha intestinalis* from 3 contrasting populations in the Auckland region, New Zealand, was investigated. The sites (intertidal flats at Laingholm in the Manukau Harbour and Mangemangeroa and rockpools at Waterfall Reef) were chosen as they provided a range of ammonium enrichment. Seawater at Laingholm had higher levels of ammonium and total inorganic nitrogen compared with the other 2 sites. However, mean nitrate levels in Mangemangeroa seawater were similar to those measured at Laingholm. Seawater at Waterfall Reef had the lowest concentrations of ammonium and nitrate. At all 3 sites, *E. intestinalis* was found high in the intertidal. At Laingholm, the ammonium-enriched site, plants were immersed for 90 min at high tide; during this period the plants took up sufficient ammonium to increase their nitrogen content by 10%. The higher levels of inorganic nitrogen in the seawater at Laingholm resulted in higher levels of tissue nitrogen, chl *a* and *b*, glutamine and asparagine, and lower nitrogen-specific rates of ammonium assimilation (measured in the laboratory in the presence of saturating concentrations of ammonium) in *E. intestinalis* at this site. There were strong positive correlations between seawater concentrations of ammonium and the level of glutamine and between levels of chl *a* and *b* and tissue nitrogen in *E. intestinalis*. The nitrogen-specific rate of ammonium assimilation decreased as the nitrogen content of the plant increased and reached a minimum value above a nitrogen content of 1 to 2% or a glutamine level of 5 µmol per g dry weight (DW). This glutamine level corresponded to a seawater ammonium concentration of 6 µM. The nitrogen-specific rate of ammonium assimilation provides a novel and important bioindicator of the link between the concentration of inorganic nitrogen in the environment and the nitrogen status of the plant.

KEY WORDS: Amino acids · Ammonium assimilation · Bioindicators · Chlorophyll · *Enteromorpha intestinalis* · Nitrogen status

INTRODUCTION

Increased nitrogen loading in the coastal environment can result in excessive growth of opportunistic seaweeds such as *Enteromorpha* spp., together with marked decreases in biodiversity (Nixon 1995, Morand & Briand 1996, Valiela et al. 1997, Schramm 1999). Opportunistic seaweeds have a high demand for nitrogen that, coupled with the higher levels of nitrogen required to saturate growth, explains the dominance of these fast-growing seaweeds in nitrogen-enriched coastal waters (Pedersen & Borum 1997). Though our knowledge of the responses of seaweeds to increased levels of inorganic nitrogen is reasonably extensive for both uptake of the nutrient and, through nitrogen status, growth rate (DeBoer 1981, Hanisak 1983, Lobban & Harrison 1997), we know little of the intervening metabolic responses of seaweeds to nitrogen enrichment.

There is often either a linear (Duke et al. 1989a, Hanisak 1990, Fong et al. 1994) or hyperbolic (Bird et al. 1981, Cohen & Neori 1991) relationship between the concentration of inorganic nitrogen in the environ-

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ment and the nitrogen status of seaweeds. These relationships were derived from either laboratory cultures or microcosm experiments, but a hyperbolic relationship has been demonstrated for a field population of *Gelidium latifolium* (Rico & Fernandez 1996). In nature, the supply of nutrients is likely to be episodic (Fong et al. 1994) and the effect of pulsed nutrient supply has been investigated in laboratory experiments (Rosenberg et al. 1984, Fujita 1985, Lapointe 1985, Ramus & Venable 1987). Consequently, there may be a poor correlation between the concentration of seawater inorganic nitrogen and the nitrogen status of a given seaweed in nature. An example of this is the absence of any effect of short-lived upwellings (despite attendant increases in seawater nitrate concentrations) on the tissue nitrogen content of *G. latifolium* (Rico & Fernandez 1996). Moreover, changes in both temperature and photon flux density will modify the relationship between nutrient supply and the nitrogen status of seaweeds (Lapointe & Duke 1984, Duke et al. 1989a). Though tissue nitrogen content integrates the recent nutrient history of the plant, with the exception of nitrate and ammonium uptake, the processes involved in determining the relationship between nutrient supply and nitrogen status in seaweeds are poorly understood. For example, our knowledge of the mechanisms and control of assimilation of nitrate and ammonium in seaweeds is limited (Hurd et al. 1995, Rees et al. 1998).

Glutamine is a key metabolite in plant cells. Ammonium is assimilated by glutamine synthetase to give glutamine, which, in combination with 2-oxoglutarate, is converted into 2 molecules of glutamate by glutamate synthase. Consequently, glutamine occupies a pivotal point in the interaction between carbon and nitrogen metabolism in plants. The level of glutamine and/or the ratio of glutamine:glutamate is an indicator of nitrogen status in bacteria (Ikeda et al. 1996), microalgae (Flynn 1990) and higher plants (Watanabe et al. 1997), because levels of glutamine increase in response to an increase in the supply of inorganic nitrogen. Clearly, if the glutamate level is constant, then the ratio and the glutamine level will both change by the same proportion. However, in cultured radish cells the cytosolic glutamine synthetase transcript level correlates with the glutamine:glutamate ratio and not the glutamine level (Watanabe et al. 1997). The glutamine:glutamate ratio has been measured in a number of subtidal and intertidal seaweeds (Al-Amoudi 1994), but this study did not attempt to relate levels of these amino acids to the concentration of inorganic nitrogen in the seawater or to differences in the nitrogen content of any given seaweed. A study by Jones et al. (1996) investigated the response of levels of certain amino acids to nitrogen loading in *Gracilaria edulis*, but these did not include measurements of either glutamine or the glutamine:glutamate ratio. Consequently, there is no study that specifically examines the relationship between glutamine and glutamate levels in seaweeds and nitrogen loading in nature.

The rate of ammonium assimilation is a measure of the rate at which ammonium is incorporated into glutamine, which is catalysed by glutamine synthetase (Taylor & Rees 1999). This rate is equal to the rate of ammonium uptake at low external concentrations of ammonium, but at higher external concentrations the rate of uptake exceeds assimilation (Taylor & Rees 1999). In some green algae (e.g. *Chlorella* spp.), it is thought that the rate of ammonium assimilation limits the overall rate of incorporation of nitrogen into macromolecules (Wheeler 1983). However, there is no available information on the relationship between the rate of ammonium assimilation, the level of available combined nitrogen present in the environment, and the nitrogen status of the plant for other green algae such as *Enteromorpha* spp.

In this paper we examine the effect of different levels of combined nitrogen (mainly ammonium) on nitrogen metabolism in 3 different populations of *Enteromorpha intestinalis* in the Auckland region. Irrespective of any other differences, the 3 sites covered a range of seawater inorganic nitrogen concentrations that enabled us to investigate the effect of increased nitrogen loading on nitrogen metabolism in natural populations of *E. intestinalis*. Rates of ammonium uptake and assimilation, levels of glutamine, glutamate, asparagine and aspartate and chlorophyll are related to the tissue nitrogen content of the plants and concentrations of inorganic nitrogen in the seawater.

MATERIALS AND METHODS

Three populations of *Enteromorpha intestinalis* (Blomster et al. 1998) from Laingholm, Mangemangeroa, and Waterfall Reef (Fig. 1) were investigated between June and November 1999. At Laingholm and Mangemangeroa, *E. intestinalis* was collected from the high intertidal, the plants being attached to the shell hash substrate common at both sites. Laingholm is situated on the Manukau Harbour, and the photosynthesis and growth of phytoplankton in this nutrient-enriched harbour have been studied extensively (Vant & Budd 1993, Vant & Safi 1996). At Waterfall Reef, *E. intestinalis* was collected from 2 rock pools in the upper intertidal. All plants were collected 1 h after immersion at high tide. Collections were made on days when high tide occurred close to midday, and analyses were made as quickly as possible after collection. Extraction of amino acids was conducted on site in all cases. Measurement of rates of uptake and assimilation were de-

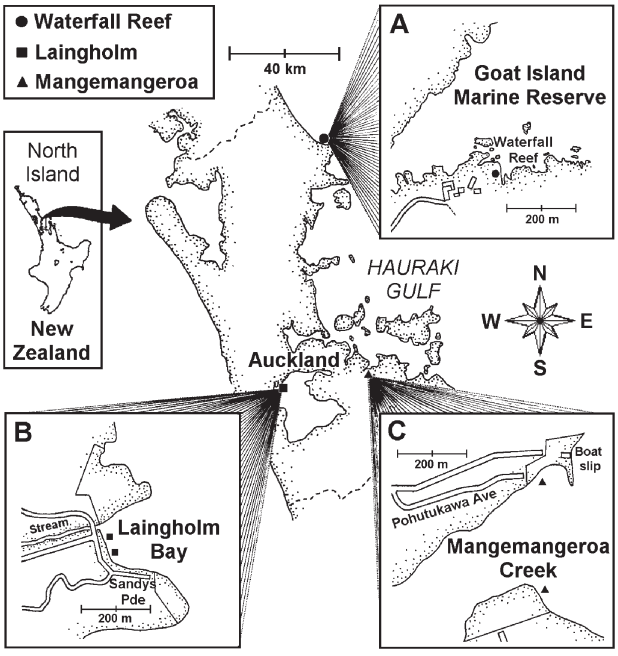

Fig. 1. Map showing location of study sites at Laingholm, Mangemangeroa, and Waterfall Reef, New Zealand

termined after 12 h incubation in seawater at 150 µE m^{-2} s^{-1} and 17.5°C. This incubation time allowed the plants to deplete their tissue ammonium pools.

Seawater samples at each site were collected at the same time as seaweed samples, placed on ice during transportation, and frozen (–18°C) unfiltered in acid-washed polycarbonate bottles within 8 h of collection. Independent experiments verified that this procedure had no effect on the results obtained (data not shown) and that there was no significant difference in nutrient concentrations between filtered and unfiltered seawater samples (Table 1). Nutrient (ammonium, nitrate, nitrite and phosphate) concentrations were determined, in triplicate, within 1 wk of collection. Ammonium was determined as described by Koroleff (1983b), nitrite and nitrate by Parsons et al. (1984), and phosphate as by Koroleff (1983a).

Table 1. Effect of filtration through GF/F filters on ammonium, nitrate and phosphate concentrations in seawater from Waterfall Reef. Three separate water samples that were collected at the same time were stored frozen after filtration or no filtration until analysis of nutrient concentrations. Values are means ± SD; p-values are results of a Student's *t*-test

Nutrient	Unfiltered (µM)	Filtered (µM)	p
Ammonium	0.93 ± 0.24	0.87 ± 0.02	0.70
Nitrate	1.87 ± 0.03	1.97 ± 0.20	0.48
Phosphate	0.37 ± 0.04	0.33 ± 0.07	0.36

Amino acids were extracted from *Enteromorpha intestinalis* (1 g fresh weight) after the plants had been immersed for 1 h at high tide. Amino acids were extracted in 5 ml ice-cold 1 M perchloric acid for 10 min, before neutralising with 1 M KOH/0.2 M MOPS (3-(N-morpholino)propanesulfonic acid). After 60 min the plant tissue was removed. Although it was not possible to centrifuge samples immediately after the extraction procedure, neutralised samples were kept on ice and centrifuged for 2 min at 1750 × *g* at 4°C within 10 h of collection, the supernatant decanted and stored at –80°C for later HPLC analysis. Independent experiments verified that this procedure had no effect on the results obtained (Table 2). Standards were treated in the same manner as the samples. The amount of neutralising solution required to give pH 7 was determined by titration and pH values of neutralised extracts were checked.

Amino acids were analysed using a method modified from Bergeron & Jolivet (1991). Extracts (20 µl) were added to 10 µl 3 M lithium acetate, briefly vortexed, centrifuged, frozen in liquid nitrogen and dried in a vacuum desiccator for 2 h. The dried samples were incubated with 30 µl methanol:10% PITC in acetonitrile:triethylamine:water (7:1:1:1, v:v) at room temperature for 20 min, after which 150 µl ultrapure water and 150 µl heptane was added to each of the samples. The samples were stored at 4°C prior to HPLC analysis, which was done in all cases within 20 h; the samples were stable for at least 48 h.

Amino acids were analysed with reversed phase chromatography on a Shimadzu high pressure binary HPLC system using an Altima 4.6 × 250 mm, C18 column. Solvent A consisted of acetonitrile and ultrapure water (4:1 v/v). Solvent B consisted of 0.14 M sodium

Table 2. *Enteromorpha intestinalis.* Effect of time after extraction and before centrifugation and freezing on amino acid levels in *E. intestinalis* from Waterfall Reef. Amino acids were extracted from *E. intestinalis* (1 g fresh weight) after the plants had been immersed for 1 h at high tide. Amino acids were extracted in 5 ml ice-cold 1 M perchloric acid for 10 min, before neutralising with 1 M KOH/0.2 M MOPS (3-(N-morpholino)propanesulfonic acid). After 60 min the plant tissue was removed. Neutralised samples were kept on ice and centrifuged for 2 min at 1750 × *g* at 4°C either immediately or 10 h later, the supernatant decanted and stored at –80°C for later HPLC analysis. Values are means ± SD for 3 replicates; p-values are results of Student's *t*-test

Amino acid	Centrifuged and frozen immediately (µmol g DW^{-1})	Centrifuged and frozen after 10 h (µmol g DW^{-1})	p
Aspartate	0.67 ± 0.04	0.67 ± 0.04	0.91
Asparagine	0.91 ± 0.10	0.91 ± 0.11	0.97
Glutamate	3.05 ± 0.30	3.08 ± 0.33	0.90
Glutamine	2.38 ± 0.21	2.41 ± 0.22	0.91

acetate, 60 ml acetonitrile, 940 ml ultrapure water, 0.5 ml triethylamine and 1 ml 10 mM Na_2EDTA. The pH was adjusted to 6.4 with 10% acetic acid and the solution filtered through 0.2 µm nylon filter. The solvents were sparged with helium for 10 min before a set of samples were run. Samples and standards were run using 20 µl of the lower phase of the derivatised samples that were injected 0.5 min after gradient control was started. In the gradient control programme, Solvent B went from 6 to 100% over a 15.5 min period using a Type +5 curve (Class-VP, Version 5.0). After a 3 min purging period with 100% Solvent B, the column was allowed to equilibrate to 6% Solvent B for 11 min before the next run was started. Concentrations of amino acids were calculated using standard curves that were linear over the range of concentrations obtained from plant extracts.

Levels of tissue ammonium in *Enteromorpha intestinalis* were determined *in situ*. Portions (1 g fresh weight) of plant were added to 20 ml seawater containing 100 µM carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) for 140 min (Rees et al. 1998). The plant was then removed and kept for determination of dry weight and the ammonium concentration in the medium determined as described by Koroleff (1983b). Dry weight was determined by drying plant tissue at 80°C to a constant weight.

For rates of ammonium uptake, 0.6 g fresh weight portions of *Enteromorpha intestinalis* were incubated in 400 µM ammonium in 150 ml seawater for 15 min and rates of uptake determined as described in Taylor et al. (1998). Rates of ammonium assimilation were determined over a 60 min period with 0.6 g fresh weight plant incubated in 150 ml seawater as described in Rees et al. (1998), with the following modifications. Unassimilated ammonium was released from the plant by transferring the plant to 50 ml seawater containing 100 µM CCCP in polypropylene pots. This modification improved release of ammonium from the plant, because release did not occur into a high external concentration of ammonium. Ammonium concentration in the samples was determined as described (Koroleff 1983b), with reference to standard curves with known amounts of ammonium in seawater plus 100 µM CCCP. Nitrogen-specific rates of uptake and assimilation were calculated as:

$$\frac{\text{biomass-specific rate (µmol ammonium g DW}^{-1}\,\text{h}^{-1})}{\text{tissue nitrogen content (µmol nitrogen g DW}^{-1})}$$

Glutamine synthetase activity was determined as described previously (Rees et al. 1995, Taylor & Rees 1999) at 17.5°C. Rates were normalised to dry weight using a dry weight:fresh weight ratio of 0.25.

Samples for tissue carbon and nitrogen analyses were dried at 80°C immediately after being collected from the field. Analysis of carbon and nitrogen content was conducted on a Carlo Erba EA 1108 CHSN element analyser. Chlorophyll was extracted in a methanol:dimethylsulphoxide ratio of 4:1 (v:v) (Duncan & Harrison 1982) for 24 h at 4°C. Absorbances were converted to concentrations of chl *a* and *b* using standard formulae (Holden 1965).

For comparisons of seawater nutrient concentrations, post hoc comparisons were made using the Tukey's HSD test following a significant ANOVA. Linear regression was used to examine relationships between variables. Where there was no obvious independent and dependent variables in the linear relationships, reduced major axis (RMA) regression was used to describe the relationship between the *x* and *y* variables. If there was an obvious response variable then standard least squares regression was used. Where relationships were obviously nonlinear, data were fitted with logistic functions (Sigmastat). The r^2 value is reported for all regressions that were significant ($p < 0.05$) and conformed to the assumptions of the analysis.

RESULTS

Seawater concentrations of ammonium and total inorganic nitrogen were significantly greater at Laingholm compared with the other 2 sites (Fig. 2). However, mean nitrate and phosphate concentrations in seawater from Mangemangeroa were similar to those measured at Laingholm. Seawater at both of these sites had higher levels of ammonium, nitrate and phosphate compared with Waterfall Reef. The effect of these differences in both the composition and concentration of total inorganic nitrogen on the nitrogen metabolism of *Enteromorpha intestinalis* from the 3 sites was investigated.

Levels of tissue ammonium in *Enteromorpha intestinalis* at Laingholm increased rapidly as soon as the plants were immersed and declined when the plants became emersed (Fig. 3). Tissue ammonium levels reached a peak of 67 (±2) µmol per g dry weight (DW) immediately prior to emersion. In contrast, the highest level we measured in *E. intestinalis* from Waterfall Reef is 2 µmol g DW^{-1}. Levels of ammonium extracted from the plants were compared with tissue levels of 4 amino acids (glutamine, glutamate, asparagine and aspartate) and their ratios (Fig. 3). Glutamine levels increased immediately after the plants were immersed and started to decline about 60 min after plants were emersed. Levels of asparagine also increased when the plants were immersed, but levels continued to increase, albeit at a lower rate, after levels of tissue ammonium and glutamine had declined. Both gluta-

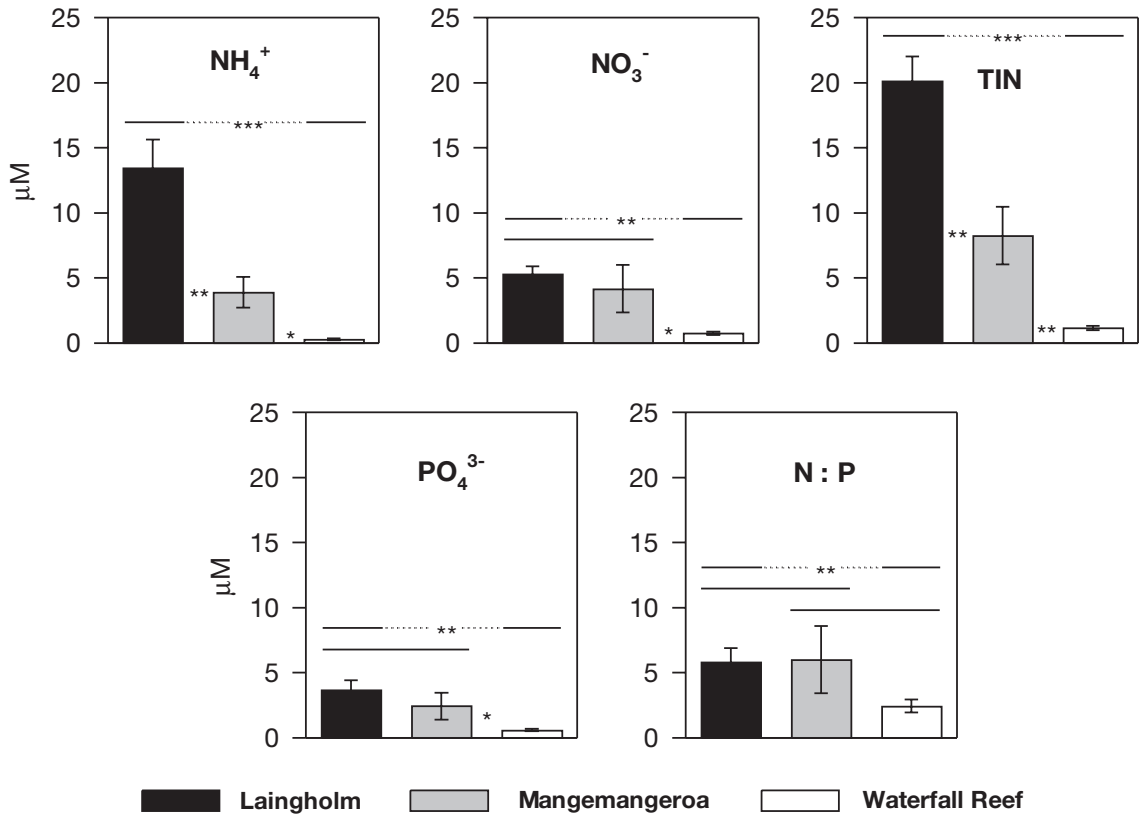

Fig. 2. Concentrations of ammonium, nitrate, total inorganic nitrogen (TIN) and phosphate, and total inorganic nitrogen:phosphate ratio in seawater at Laingholm, Mangemangeroa, and Waterfall Reef. Post hoc comparisons were made using the Tukey's HSD test following a significant ANOVA (*p < 0.05, **p < 0.01, ***p < 0.001). Values are means ± SE for all values obtained during the study period

mate and aspartate remained at relatively constant levels but there were minor increases about 45 min after plants were immersed. Initial decreases in these tissue constituents were probably due to the experiment commencing at dawn (i.e. utilisation of stored ammonium and amino acids in light prior to immersion). The glutamine:glutamate and asparagine:aspartate ratios both increased. There was a strong positive relationship between seawater ammonium concentrations and levels of glutamine extracted from plants at the 3 sites (r^2 = 0.85, $F_{1,10}$ = 87, p < 0.001) (Fig. 4A). There was no apparent correlation between levels of glutamine in the plant and concentrations of seawater nitrate across the 3 sites (Fig. 4B). However, there was a strong, non-linear relationship between seawater total inorganic nitrogen and tissue levels of glutamine (r^2 = 0.95, $F_{3,7}$ = 55.4, p < 0.001; Fig. 4C). Glutamine: glutamate ratios were poorly correlated with seawater ammonium (Fig. 4D), nitrate (Fig. 4E) or total inorganic nitrogen (Fig. 4F).

The differences in total inorganic nitrogen concentrations at the 3 sites resulted in a range of tissue nitrogen contents (0.49 to 3.36%) in their populations of *Enteromorpha intestinalis*. This range of values enabled us to determine the effect of natural variation in nitrogen status on nitrogen metabolism in this green alga.

The dry weight:fresh weight ratio decreased with increasing levels of tissue-nitrogen (Fig. 5). However, this relationship could not be accounted for by changes in stored carbon (data not shown).

Biomass-specific rates of ammonium uptake and assimilation were compared with tissue nitrogen content of plants from the 3 sites. *Enteromorpha intestinalis* from Laingholm generally had higher levels of tissue nitrogen and higher rates of ammonium uptake (Fig. 6A) and assimilation (Fig. 6B). Though there was a general trend for biomass-specific rates of ammonium assimilation to increase with increased levels of tissue nitrogen, they were poorly correlated. Biomass-specific rates of ammonium assimilation in *E. intestinalis* and *E. compressa* were compared with activities of glutamine synthetase (GS), which is the primary ammonium-assimilating enzyme. Despite a range of

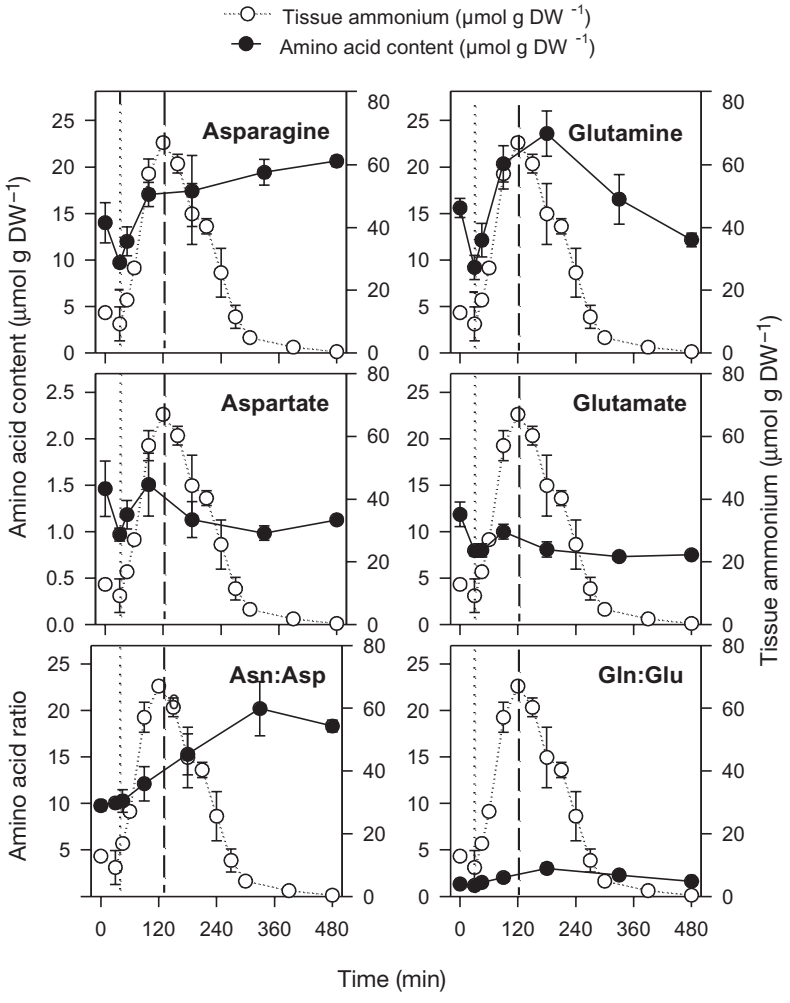

Fig. 3. *Enteromorpha intestinalis.* Tissue levels of ammonium, asparagine, aspartate, glutamine and glutamate, and ratios of asparagine:aspartate and glutamine:glutamate during tidal immersion (dotted vertical lines) and emersion (dashed vertical lines) at Laingholm in August 1999. The concentration of seawater ammonium was 17.3 µM and the experiment was started at dawn. Values are means ±SE for 3 separate determinations

values for both rates of ammonium assimilation (54 to 96 µmol g DW^{-1} h^{-1}) and GS activity (79 to 125 µmol g DW^{-1} h^{-1}), the ratio of assimilation:GS activity (0.74 ± 0.02) was remarkably constant.

Nitrogen-specific rates of both uptake (Fig. 6C) and assimilation (Fig. 6D) decreased to a constant value at tissue nitrogen levels above 1 to 2%. There was a strong positive correlation between levels of chl *a* and *b* and tissue nitrogen (Fig. 7). Consequently, the relationship between rates of ammonium uptake (Fig. 6E) and assimilation (Fig. 6F) normalised to chl *a* and *b* content and tissue nitrogen were very similar to that between nitrogen-specific rates of ammonium uptake (Fig. 6C) and assimilation (Fig. 6D) and tissue nitrogen.

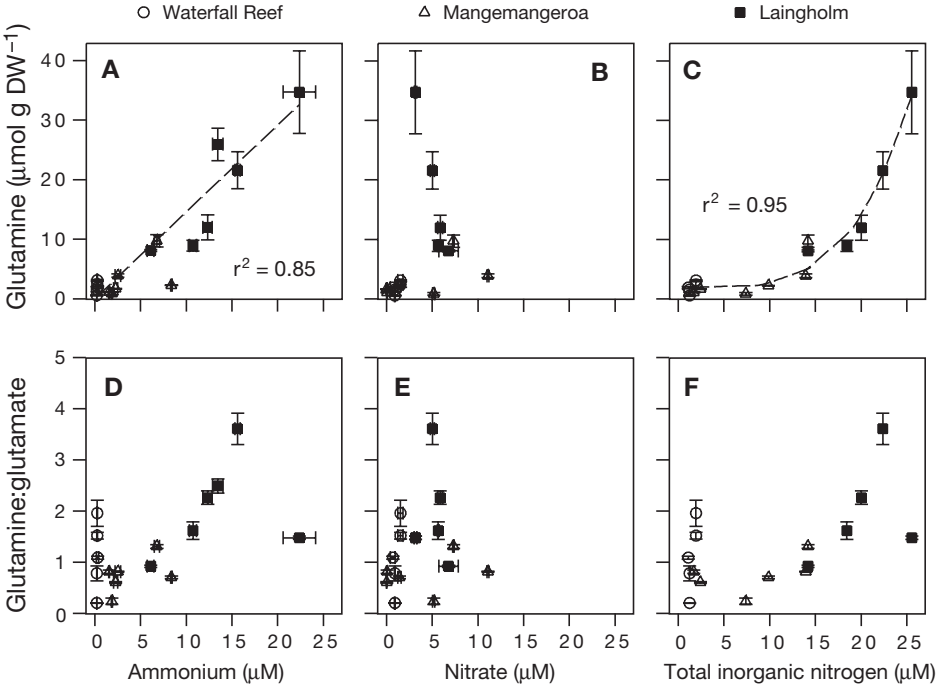

Fig. 4. *Enteromorpha intestinalis.* Tissue levels of glutamine, and glutamine:glutamate ratio in *E. intestinalis* versus concentrations of seawater ammonium, nitrate or total inorganic nitrogen at Laingholm, Mangemangeroa, and Waterfall Reef. For non-linear regression of levels of glutamine versus total inorganic nitrogen, an adjusted r^2 value was used. The equation for tissue glutamine versus concentration of seawater ammonium is Gln (µmol g DW^{-1}) = (1.46 × µM NH_4^+). The equation for tissue glutamine versus total seawater inorganic nitrogen concentration is Gln (µmol g DW^{-1}) = 1280/[1 + (µM NH_4^+/63.7)$^{-4}$] + 1.97 ($p < 0.0001$). Values are means ±SE for 3 separate determinations

In contrast to the strong positive correlation between tissue glutamine levels and seawater ammonium concentrations (Fig. 4A), glutamine levels showed a weak positive correlation with nitrogen content in plants from the 3 sites (Fig. 8A) and the glutamine:glutamate ratio showed an even poorer relationship with tissue nitrogen (Fig. 8B).

The relationship between nitrogen-specific rates of ammonium assimilation and levels of glutamine (Fig. 9) was similar to that between nitrogen-specific rates of ammonium assimilation and tissue nitrogen content (Fig. 6D). With levels of glutamine greater than 5 µmol g DW^{-1}, only low nitrogen-specific rates of ammonium assimilation were measured (Fig. 9). From the linear regression for the relationship between seawater ammonium concentration and tissue glutamine levels in *Enteromorpha intestinalis* (Fig. 4), these concentrations of glutamine would occur with concentrations of seawater ammonium greater than 6 µM.

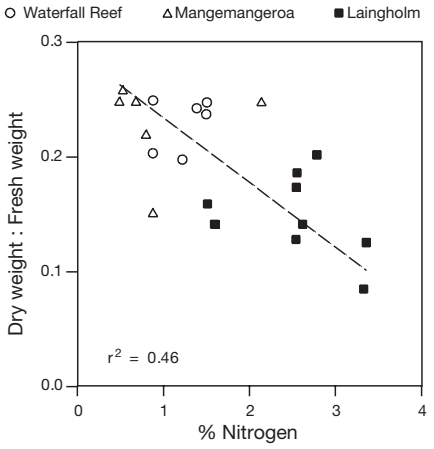

Fig. 5. *Enteromorpha intestinalis.* Relationship between dry weight:fresh weight ratio and tissue nitrogen in *E. intestinalis* from Laingholm, Mangemangeroa, and Waterfall Reef. Dry weight:fresh weight ratio = −0.056 × %N + 0.29. Values are means ± SE for at least 3 separate determinations

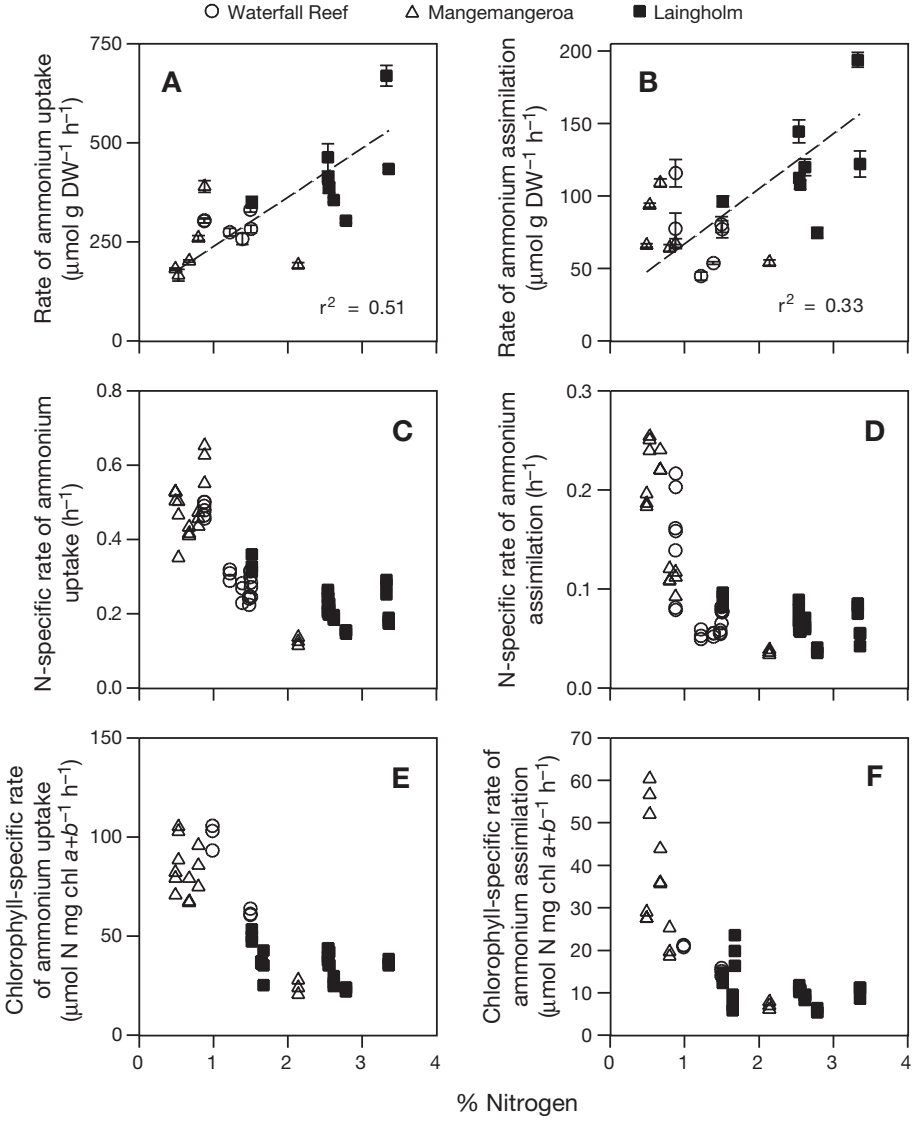

Fig. 6. *Enteromorpha intestinalis.* Relationship between biomass-specific rates of ammonium uptake (A) and assimilation (B), nitrogen-specific rates of ammonium uptake (C) and assimilation (D), and chlorophyll-specific rates of ammonium uptake (E) and assimilation (F) and tissue nitrogen in *E. intestinalis* from Laingholm, Mangemangeroa, and Waterfall Reef. The equations are ammonium uptake (µmol g DW^{-1} h^{-1}) = (124 × %N) + 114 and ammonium assimilation (µmol g DW^{-1} h^{-1}) = (38 × %N) + 29. Rates of ammonium uptake and assimilation were determined in the presence of 400 µM ammonium and are means ± SE for 3 separate determinations

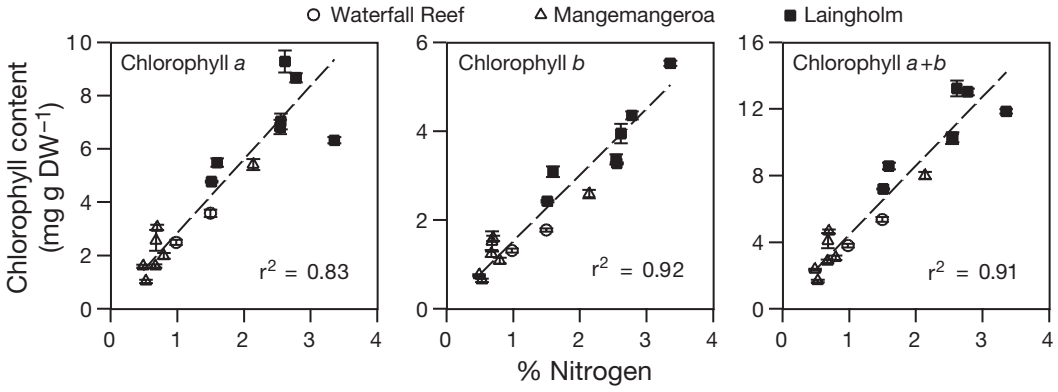

Fig. 7. *Enteromorpha intestinalis.* Relationship between chl *a* and *b* and tissue nitrogen in *E. intestinalis* from Laingholm, Mangemangeroa, and Waterfall Reef. The equations are chl *a* (mg g DW^{-1}) = (2.8 × %N) + 0.09, chl *b* (mg g DW^{-1}) = (1.5 × %N) – 0.03, and chl *a* + *b* (mg g DW^{-1}) = (4.1 × %N) – 0.3. Values are means ± SE for 3 separate determinations

DISCUSSION

The 3 populations of *Enteromorpha intestinalis* that were investigated in this study covered a range of nitrogen content. Plants from Mangemangeroa (0.9 ± 0.2% DW) had the lowest average nitrogen content, followed by plants from Waterfall Reef (1.2 ± 0.1% DW) and Laingholm (2.3 ± 0.3% DW). These values partially reflect the concentrations of inorganic nitrogen in the seawater at these sites. However, it is important to emphasise that the correspondence between the concentrations of seawater inorganic nitrogen and the nitrogen status of the plants at these sites was far from precise. For example, the concentrations of nitrate and ammonium in the seawater at Mangemangeroa were significantly greater than those at Waterfall Reef, but this was not reflected in the apparent nitrogen status of the plants at these sites. Moreover, the nitrogen status of the plants from the 3 sites bore no relationship to seawater nitrate concentrations. Possible interpretations of this apparent discrepancy are that there were other sources of nitrogen (e.g. organic nitrogen) available to these plants that we did not measure, that nutrient supply was episodic and/or that there were other unmeasured variables that affected the nitrogen status of the plants. For example, one possible source of ammonium was excretion by amphipods and gastropods that were commonly observed associated with *E. intestinalis* at Waterfall Reef. Excretory products from epifauna (Taylor & Rees 1998), barnacles (Williamson & Rees 1994) and bryozoans (Hurd et al. 1994) can represent important sources of nitrogen for seaweeds. Irrespective of this, our data suggest that any attempt to infer the nitrogen status of a plant from the concentration of inorganic nitrogen apparently available to that plant should be treated with caution.

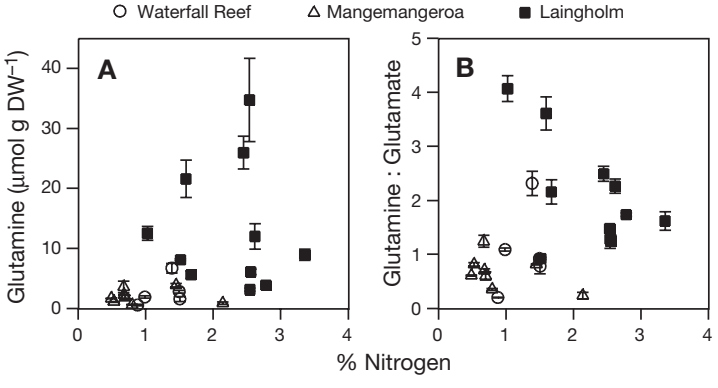

Fig. 8. *Enteromorpha intestinalis.* Levels of glutamine (A) and glutamine:glutamate ratio (B) versus tissue nitrogen in *E. intestinalis* from Laingholm, Mangemangeroa, and Waterfall Reef. Values for glutamine are means ± SE for 3 separate determinations

Seaweeds with high surface area:volume ratios, such as *Enteromorpha intestinalis*, have high rates of am-monium uptake (Rosenberg & Ramus 1984, Hein et al. 1995, Taylor et al. 1998, 1999), which, in part, explains their ability to dominate nutrient-enriched coastal waters (Pedersen & Borum 1997). An indication of the extent of this uptake ability in nature was provided by the experiment in which tissue ammonium levels were monitored over a tidal cycle at the ammonium-enriched site (Laingholm) (Table 3). About 10% of the total nitrogen content of the plant was taken up during 90 min immersion.

Tissue levels of glutamine in *Enteromorpha intestinalis* were positively correlated with seawater concentrations of ammonium and total inorganic nitrogen, but not with nitrate. Similar,

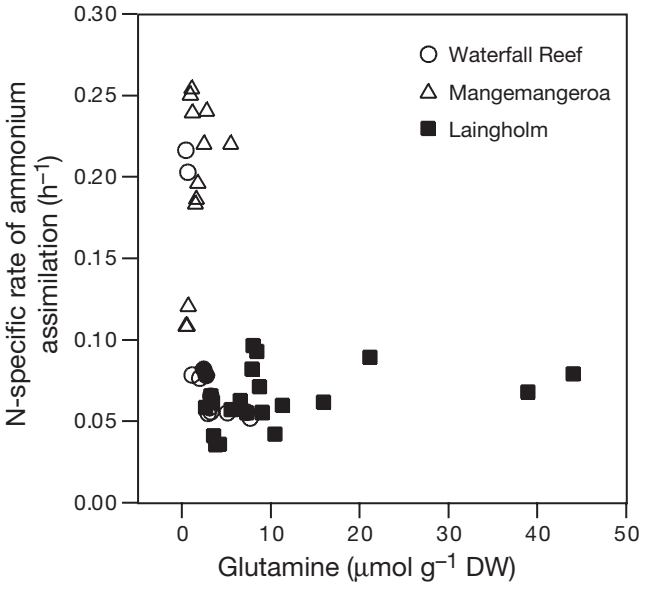

Fig. 9. *Enteromorpha intestinalis*. Relationship between maximum nitrogen-specific rates of ammonium assimilation and levels of glutamine in *E. intestinalis* from Laingholm, Mangemangeroa, and Waterfall Reef

but less convincing, relationships were obtained with the glutamine:glutamate ratio and seawater inorganic nitrogen concentrations. The rate of ammonium uptake in *E. intestinalis* is greater than that for nitrate, and the uptake of nitrate (at 30 µM) is inhibited by 50% in the presence of 15 µM ammonium (Thomas & Harrison 1987). Seawater concentrations of nitrate (0.01 to 11.1 µM) in this study coincided with comparable concentrations of ammonium (0.1 to 22.4 µM), suggesting that there was often sufficient ammonium present to inhibit the uptake of nitrate. In addition to inhibiting uptake of nitrate, ammonium can inactivate nitrate reductase, and growth on ammonium down-regulates synthesis of both the nitrate transport system and nitrate reductase in microalgae (Syrett 1981). A combination of these factors probably caused the observed relationship between tissue glutamine levels in *E. intestinalis* and seawater concentrations of ammonium or nitrate.

There was a poor correlation between either the tissue glutamine level or the glutamine:glutamate ratio and nitrogen content of *Enteromorpha intestinalis*. Though few studies have examined changes in levels of specific amino acids in seaweeds in response to nitrogen loading (Jones et al. 1996), levels of total amino acids do reflect the nitrogen status of seaweeds (Naldi & Wheeler 1999). The ratio of glutamine:glutamate provides a useful indicator of recent nitrogen status in microalgae (Flynn 1990). In earlier work on seaweeds, Al-Amoudi (1994) suggested that the ratio could be used as a sensitive indicator of nitrogen status. Our data suggests that the glutamine level is an excellent indicator of recent nutrient history, but is a poor indicator of the longer-term nutrient history as represented by the nitrogen content of the plant. The probable reason for this difference is that the nitrogen content of the plant represents the integration of a variety of external nutrient concentrations experienced by the plant over a period of time (Fong et al. 1994). In addition, the results of this study suggest that levels of glutamine (normalised to tissue dry weight) provide better information about nitrogen loading than the glutamine:glutamate ratio.

Asparagine levels were broadly correlated with tissue nitrogen in *Enteromorpha intestinalis* from the 3 sites (data not shown). Although levels of asparagine showed a slightly better relationship to tissue nitrogen than glutamine, they did not correlate well with seawater ammonium concentrations.

Biomass-specific rates of ammonium uptake are negatively correlated with tissue nitrogen in a number of green algae, including *Enteromorpha* spp. (Fujita 1985), *Ulva lactuca* (Fujita 1985, Pedersen 1994), *U. curvata* (Duke et al. 1989b), and *Chaetomorpha linum* (McGlathery et al. 1996), and red algae *Gracilaria tikvahiae* (Fujita 1985), *G. foliifera* and *Neoagardhiella baileyi* (D'Elia & DeBoer 1978). In contrast, biomass-specific rates of ammonium uptake in *Cladophora vagabunda* (Peckol et al. 1994) and *E. intestinalis* (this study) are positively correlated with tissue nitrogen.

Table 3. *Enteromorpha intestinalis*. Values used to calculate the contribution made by stored and assimilated ammonium during 90 min immersion at high tide to the nitrogen content of *E. intestinalis* at Laingholm. All values were derived from the experiment in August 1999 shown in Fig. 2. The half-saturation constant (K_m) value for ammonium assimilation was obtained from Taylor & Rees (1999)

Parameter	Value
Seawater ammonium concentration	17 µM
Plant nitrogen content (z)	18.5 mg g DW^{-1}
Increase in ammonium content of plant tissue during immersion (a)	58 µmol g DW^{-1}
K_m for ammonium assimilation	18 µM
Maximum rate of ammonium assimilation	96 µmol g DW^{-1} h^{-1}
Rate of ammonium assimilation at ambient ammonium concentration	46 µmol g DW^{-1} h^{-1}
Ammonium assimilated during immersion (b)	69 µmol g DW^{-1} h^{-1}
Total ammonium acquired during immersion ($a + b$)	127 µmol g DW^{-1} h^{-1}
Increase in nitrogen content during immersion (y)	1.8 mg g DW^{-1}
Increase as % of total nitrogen content [(y/z) × 100]	9.7%

There was a slight increase in the rate of ammonium assimilation expressed per g dry weight with increasing tissue nitrogen content in *Enteromorpha intestinalis*, but the correlation was poor. Two studies (Pedersen 1994, McGlathery et al. 1996) have examined changes in response to nitrogen status of the internally controlled rate of ammonium uptake (V_i), which is equal to the maximum rate of ammonium assimilation (Rees et al. 1998, Taylor et al. 1999). There is little change in the rate (expressed per unit dry weight) of ammonium assimilation in nitrogen-starved cultures of *Ulva lactuca* (Pedersen 1994). In contrast, V_i in *Chaetomorpha linum* decreases in response to nitrate- or ammonium-enrichment, and increases when tissue nitrogen pools are depleted (McGlathery et al. 1996). There was a distinct, non-linear relationship between the nitrogen-specific rate of ammonium assimilation and tissue nitrogen content in *E. intestinalis*, with nitrogen-deficient plants having the highest rates. Nitrogen-specific rates of ammonium assimilation increase in *U. lactuca* (Pedersen 1994), *C. linum* (McGlathery et al. 1996) and the diatom *Phaeodactylum tricornutum* (Rees et al. 1998) following nitrogen deprivation. However, the nature of the relationship between nitrogen content and the nitrogen-specific rate of ammonium assimilation in these algae are not clear and preclude any comparison with *E. intestinalis*.

Chl *a* and *b* levels in *Enteromorpha intestinalis* were positively correlated with tissue nitrogen. As any nutrient deficiency will result in a decrease in chlorophyll (Healey 1973), the strength of this relationship suggests that chlorophyll levels were responding primarily, if not exclusively, to the nitrogen status of the plants. Positive correlations between chl *a* and tissue nitrogen have been observed in *Ulva fasciata* (Lapointe & Tenore 1981). Similarly, in *Gracilaria tikvahiae*, increased levels of chl *a* ($r^2 = 0.46$) and phycoerythrin ($r^2 = 0.70$) are positively correlated with increases in tissue nitrogen (Bird et al. 1982, Lapointe & Duke 1984), despite differences in photon flux densities during growth. *E. intestinalis* at all 3 sites was located in similar light environments (unshaded areas, high in the intertidal), suggesting that photon flux densities were not limiting growth. Accessory pigments may provide a better proxy for tissue nitrogen than chl *a*. The r^2 values for plots of chl *b* in *E. intestinalis* from this study and phycoerythrin in *G. tikvahiae* (see above) against tissue nitrogen were greater than those for chl *a*. Expressing indices of nitrogen status (e.g. rates of ammonium assimilation) per unit chl *a* and *b* may prove more useful than expressing these parameters per unit dry weight.

The minimum nitrogen-specific rate of ammonium assimilation coincided with a seawater ammonium concentration of 6 µM, a glutamine level of 5 µmol g DW^{-1} and a nitrogen content of 1 to 2%. This suggests that the nitrogen-specific rate of ammonium assimilation may provide an informative link between the concentration of available inorganic nitrogen in the plant's environment and its nitrogen status as measured by tissue nitrogen content. The critical tissue nitrogen content of green seaweeds (defined as the minimum percentage tissue nitrogen level that saturates growth rate) ranges from 1.2 to 3.2% (Björnsäter & Wheeler 1990, Lavery & McComb 1991). Given the defined relationship between tissue nitrogen content and growth rate (Hanisak 1983), the nitrogen-specific rate of ammonium assimilation may provide a rapid measure of relative growth rate in nature, and we are currently investigating this possibility.

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