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MACROALGAL RESPONSES TO NITROGEN SOURCE AND AVAILABILITY: AMINO ACID METABOLIC PROFILING AS A BIOINDICATOR USING *GRACILARIA EDULIS* (RHODOPHYTA)[1]

Adrian B. Jones,[2] *William C. Dennison, and George R. Stewart*

Botany Department, The University of Queensland, Brisbane, Queensland, 4072, Australia

ABSTRACT

The use of macroalgae as biological indicators of dissolved nutrient source and availability in the water column was investigated. Total tissue nitrogen (N) content, pigments, and amino acids of the red alga Gracilaria edulis *(Gmelin) Silva were compared to N source and availability in laboratory and field incubations to identify responses that would serve as bioindicators of N. Field-collected algae were preincubated (6–8 wk) in low-nutrient seawater to deplete their luxury reserves of N. Incubations were then conducted for periods of 3 d in laboratory aquaria (N-spiked seawater) and in the field using macroalgal incubation chambers. After incubation in different N sources (NH_4^+, NO_3^-, and urea) in laboratory aquaria, photosynthetic pigments (phycoerythrin and chlorophyll* a*) and total tissue N increased, in response to increasing $[NH_4^+]$ but not to $[NO_3^-]$ or [urea]. Incubation in two ranges of $[NH_4^+]$, one from 0 to 80 μM and the other from 0 to 800 μM, in laboratory aquaria increased the total amino acid pool. Citrulline concentrations were the most responsive to $[NH_4^+]$ ($r^2 = 0.84$). NH_4^+ source treatments produced increases in citrulline, phenylala-*

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[2] Author for reprint requests.

nine, serine, and free NH_4^+ and decreases in alanine; NO_3^- treatments produced increases in glutamic acid, citrulline, and alanine; and urea treatments produced increases in free NH_4^+ and decreases in phenylalanine and serine. The observed variations in amino acid content facilitated the development of an index for each N source based on relative concentrations of various amino acids (i.e. metabolic profiling). Gracilaria edulis *was incubated along a field N gradient in the Brisbane River (three sites) and Moreton Bay (four sites), Queensland, Australia. Both phycoerythrin and tissue N appeared to respond equally to NH_4^+ and NO_3^- availability in the field. N source indices, based on amino acid concentration, were effective predictors of both $[NH_4^+]$ and $[NO_3^-]$ over a wide range of concentrations along the field gradient. Macroalgal physiological responses, particularly amino acid content, to changes in source and availability of N appear to be useful as sensitive bioindicators of N.*

Key index words: amino acids; bioindicator; Gracilaria edulis; *macroalgae; metabolic profiling; nutrients; pigments; Rhodophyta; tissue nitrogen; water quality*

Macroalgae can be used as a bioindicator of nutrients in the water column because of the relationships established between their tissue nutrient content and dissolved nutrient concentrations in the surrounding water (e.g. Steffensen 1976, Haines and Wheeler 1978, Lapointe and Ryther 1978, Ryther et al. 1981, Lyngby 1990, Horrocks et al. 1995). Macroalgae rapidly assimilate surrounding nutrients, and their tissue nutrient content can reflect the local nutrient regime within a relatively short time period. *Gracilaria tikvahiae,* for example, can assimilate and store enough nitrogen (N) in 6 h to allow it to grow for 2 wk at non-N-limited levels (Ryther et al. 1981). Pigments, total tissue N, and amino acid concentrations of macroalgae are influenced by the availability of dissolved nutrients (K. T. Bird et al. 1982). Macroalgal total tissue N is probably the most widely analyzed of these parameters for determining macroalgal response to dissolved inorganic nitrogen (DIN) concentrations in the water column (Gerloff and Krombholz 1966, Lapointe and Ryther 1979, K. T. Bird et al. 1982, Lyngby 1990). Total tissue N content of the red algae (Rhodophyta) correlates more closely with water column N concentrations than that of green (Chlorophyta) or brown (Phaeophyta) macroalgae (Chapman et al. 1978, D'Elia and DeBoer 1978, Lapointe and Ryther 1979, K. T. Bird et al. 1982, Björnsäter and Wheeler 1990, Friedlander et al. 1991, Lohman and Priscu 1992). In particular, species of the genus *Gracilaria* display a strong response to changes in nutrient concentrations (Ryther et al. 1981, K. T. Bird et al. 1982, Horrocks et al. 1995) yet are characteristically tolerant to other environmental factors (e.g. salinity, temperature, light), all of which are known to affect the physiological parameters being analyzed (N. L. Bird et al. 1979, Yarish and Edwards 1982).

The responsiveness of red algae to nutrient availability may be attributable in part to their phycobilisomes (Kursar and Alberte 1983, Gantt 1990). When internal N reserves are depleted, algae start to lose their dark reddish-brown pigments, become a pale straw-yellow color, and cease growing (Ryther et al. 1981). This loss of color may be a result of pigments being metabolized as a source of protein (Lapointe 1981, K. T. Bird et al. 1982).

Another macroalgal bioindicator of DIN is the amino acid content of the tissue (Horrocks et al. 1995). The source (NH_4^+, NO_3^-, NO_2^{2-}, or urea) as well as the concentration of N being assimilated affects the free amino acid pool in algae (K. T. Bird et al. 1982, Vona et al. 1992, Flynn et al. 1994). NO_3^- is reduced and urea is hydrolyzed to NH_4^+, which is then assimilated through the glutamine synthetase/glutamate synthase pathway (Arnstein 1975). Citrulline (a 3 N non-protein amino acid) is responsive to changes in DIN and appears to function as the major "luxury" N store in *Gracilaria* sp. and some other genera of macroalgae (Horrocks et al. 1995). The storage of N in the inorganic form, or biomechanical metabolites, is known as luxury consumption (i.e. luxury assimilation) and is an ecological adaptation to N limitation (Gerloff and Krombholz 1966). Luxury reserves constitute that portion of tissue N above the critical nitrogen concentration of the species (i.e. the concentration that just limits maximal growth) (Hanisak 1983). These reserves may be metabolized in times of low N availability (Vona et al. 1992, Horrocks et al. 1995).

In the present study, a number of characteristics (pigments, total tissue N, and amino acids) were analyzed in *Gracilaria edulis,* a macroalgal species found abundantly in Moreton Bay. The effects of dissolved N on this species were investigated, as previous studies determined that *Gracilaria* sp. in Moreton Bay was greatly stimulated by N (Horrocks et al. 1995). The aims of this research were to 1) investigate the influence of different N sources (NH_4^+, NO_3^-, and urea) and $[NH_4^+]$ on *G. edulis* in the laboratory and 2) develop the use of *G. edulis* as a bioindicator of available nitrogen concentrations along an established DIN gradient in the field.

MATERIALS AND METHODS

Gracilaria edulis (Gmelin) Silva was collected from approximately 1 m below the mean low-tide mark from the rocks near Redcliffe, Moreton Bay (27°13.1'S, 153°06.9'E) (Fig. 1) and transported back to the university algal culture facilities in 25-L plastic containers (containing seawater). Several kilograms of macroalgae were placed in well-aerated aquaria with low-nutrient seawater (collected from Moreton Bay and sand-filtered) for 6–8 wk to deplete the algae of N storage before incubation in the laboratory and field. The algae was stocked in the aquaria at low enough density to ensure adequate water flow and movement of the algae. Aquaria were maintained at 20°–23° C and exposed to light on a 12:12 h LD cycle using a mixture of triton, actinic, and daylight fluorescent tubes that provided approximately 230 μmol quanta$\cdot m^{-2}\cdot s^{-1}$.

Macroalgal incubations were undertaken in the laboratory and

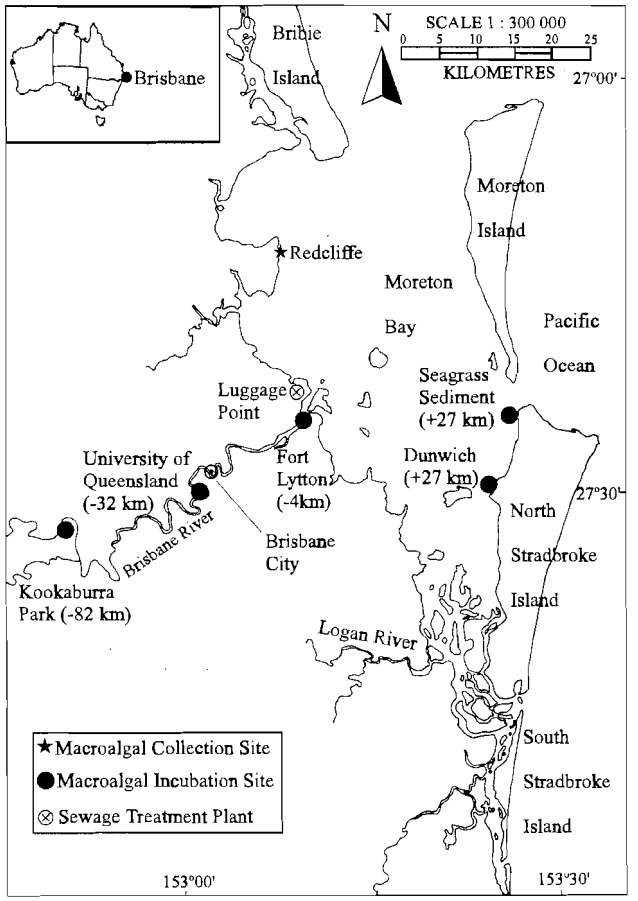

FIG. 1. Map showing the location of the field sites along the Brisbane River and in Moreton Bay.

field for a period of 3 d. This period appears to be optimal for maximizing the amino acid responses to different N sources. Initial laboratory experiments indicated that after 7 d the amino acid responses were considerably less pronounced, perhaps as a consequence of further metabolism. It may be that a time period of less than 3 d would produce even greater responses, but for field incubations it may reduce the ability of the algae to integrate nutrients available as pulses over longer periods of time.

Laboratory experiments. All the aquaria used for the following experiments were 30 × 15 × 20 cm and, like the aquaria used for algal nutrient depletion, were well aerated, maintained at 20°–23° C, and exposed to light on a 12:12 h LD cycle using a mixture of triton, actinic, and daylight fluorescent tubes that provided approximately 230 μmol quanta·m^{-2}·s^{-1}. The algae were incubated in N-spiked seawater. The concentrations of dissolved N stated are the initial values, and because the water was not exchanged during the experiment these concentrations would have decreased over time as the macroalgae assimilated the N. Nitrogen source experiments involved incubation for 3 d in aquaria with 200 μM concentrations of three different N sources (NH_4^+, NO_3^-, urea) and a control with no added N.

The [NH_4^+] experiments involved two different concentration ranges. The first range (0–800 μM) encompassed concentrations in excess of realistic biological values and was designed to enhance responses and determine a saturation level. Seven aquaria were used with the following concentrations: 0, 25, 50, 100, 200, 400, and 800 μM NH_4^+. The second range (0–80 μM) was more biologically realistic and used the following concentrations: 0, 5, 10, 20, 40, 60, and 80 μM NH_4^+. Three replicate containers of 4 g fresh weight were incubated in the one aquarium for each

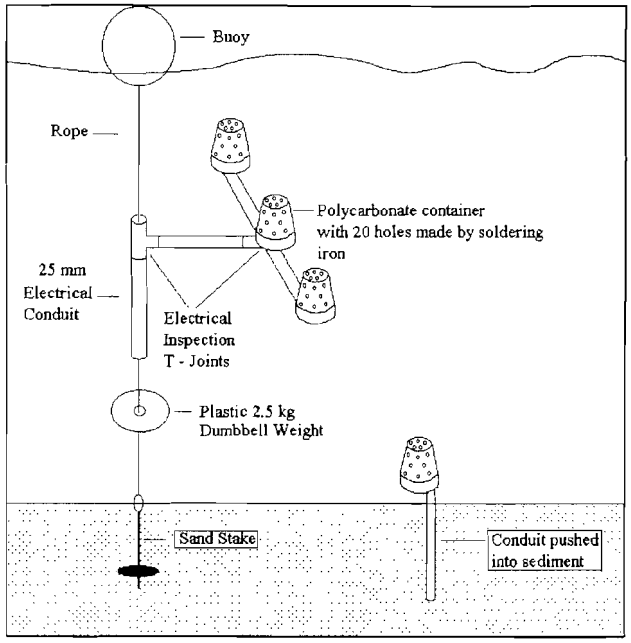

FIG. 2. Water column macroalgal chamber and the sediment macroalgal chamber used in the field incubations of *Gracilaria edulis*.

treatment. Algae were placed in clear polycarbonate containers (7 cm in diameter at lid) with 20 holes placed in the container to facilitate water flow (Fig. 2).

Field incubations. Two types of apparatus were used to contain the algae during the 3-d incubation period (Fig. 2). Water column macroalgal chambers consisted of an electrical conduit frame with attached polycarbonate containers. The chambers were anchored using a car tire rim or sandstake depending on the substrate and were floated just below the water surface using a combination of a buoy and weight, all connected with 8-mm rope. Sediment macroalgal chambers consisted of a polycarbonate container attached to electrical conduit that was pushed into the sediment. Holes in the polycarbonate container lid facilitated direct access to nutrients being released from the sediment.

There were two main areas in which field incubations were undertaken: Brisbane River and Moreton Bay (Fig. 1). Sites were expressed as kilometres from the river mouth, with negative values being upstream and positive values into the bay. The Brisbane River is subject to high turbidity and large inputs of nutrients from non-point source terrestrial runoff, sewage treatment plants, and release from sediments after resuspension by dredging (Moss 1990). Brisbane's largest sewage treatment facility, along with a fertilizer plant, is located near the river mouth.

The results from laboratory analysis of pigments, total tissue N, and amino acids were compared to the responses observed by incubation of the algae in the Brisbane River and Moreton Bay, to determine the predominant sources and concentrations of N available to marine plants at a number of sites at these two locations. The sites were chosen to provide a variable gradient from oceanic N concentrations (<2 μM) in Moreton Bay to highly eutrophic concentrations (up to 120 μM) in Brisbane River, with large variations in source of N in the river.

Three sites (measured as plus and minus distances from the river mouth) along the Brisbane River were used for macroalgal incubations: 1) −4 km, Fort Lytton National Park (27°24.69′S, 153°08.89′E) opposite the Luggage Point Sewage Treatment Plant, 2) −32 km, the university rowing shed at the University of Queensland (27°29.52′S, 153°00.75′E), and 3) 82 km, Kookaburra Park at Karana Downs (27°32.48′S, 152°50.38′E).

TABLE 1. *Pigments, total tissue N, and amino acids of* Gracilaria edulis *after 3 d in aquaria with control, 200 μM [NO_3^-], [NH_4^+], or urea. * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001. Within columns, means with different letters are significantly different at P < 0.05.*

Treatment	Pigments (mg·g^{-1} dry weight)		Total tissue nitrogen (%N dry weight)	Amino acids (nmol·g^{-1} wet weight)					
	PE	Chl *a*		Serine	Glutamic acid	Alanine	Citrulline	Phenyl-alanine	Free NH_4^+
Control	0.9^a	1.0	1.9	141	227^b	85^{ab}	232^c	45^b	263^b
NO_3^-	0.9^a	0.6	1.8	151	335^a	108^a	778^b	47^b	450^b
NH_4^+	1.3^b	0.7	2.5	199	227^b	21^b	2274^a	114^a	3901^a
Urea	0.9^{ab}	0.9	2.0	108	392^a	96^{ab}	734^b	21^b	1263^b
F-value	5.5*	1.2	2.8	2.3	4.3*	4.8*	52.9***	10.4**	30.6***

Four sites were in Moreton Bay. A water column macroalgal chamber was used at Dunwich (+27 km; 27°29.50'S, 153°23.91'E). This site was subject to terrestrial runoff from the island's main settlement. Sediment chambers were located close to the outfall pipe from a septic tank system at Dunwich and at two sites at Amity (+27 km; 27°23.36'S, 153°25.80'E) over fertilized and control seagrass plots. As part of a seagrass fertilization study conducted by the University of Queensland Marine Botany Group, *Zostera capricorni* seagrass beds on the Wanga Wallen banks at Amity Point have a number of 1-m^2 plots at which slow release Osmocote fertilizer had been placed into the sediment. Fertilizer containing 88 g N·m^{-2} (50:50 NH_4^+:NO_3^-) was applied to each plot. Chambers were placed in the sediment over both control and N-fertilized plots.

Macroalgal tissue. After 3 d incubation in either the field or laboratory, the algae were removed, rinsed in distilled water to remove any nutrients and sediment from the thallus surface, and then prepared for three types of analysis: pigment analysis (phycoerythrin [PE] and chlorophyll *a* [chl *a*]), total tissue N analysis (organic and inorganic), and amino acid analysis.

For pigment analysis, approximately 0.5 g fresh weight of *G. edulis* tissue was separated, blotted dry, and weighed (fresh weight). The tissue was ground with a mortar and pestle in a phosphate buffer solution (pH 6.5) to disrupt the cells. The extract was then poured into a glass-graduated centrifuge tube, made up to 10 mL, and centrifuged (20 min at 2500 rpm) to produce a supernatant containing PE and a pellet with the remaining tissue. The supernatant was transferred to a cuvette and absorption was determined on a spectrophotometer at 565 and 710 nm for PE and turbidity blank, respectively. The pellet was resuspended in 5 mL of 80% acetone (analytical reagent grade) and disrupted with a tissue homogenizer to extract chlorophyll. Samples were recentrifuged (20 min at 2500 rpm), and absorbance was determined at 664 and 710 nm for chlorophyll *a* and turbidity blank, respectively. Pigment concentrations as mg·g^{-1} dry weight (using a wet weight : dry weight ratio of 10:1) were calculated with specific formulas for phycoerythrin (Rowan 1989) and chl *a* (Parsons et al. 1984).

For total tissue N analysis, approximately 2.5 g wet weight was dried (60° C, 48 h) and ground to a fine powder using a ball mill. Approximately 200–300 mg of the ground material was then weighed (±0.1 mg) and placed into Kjeldahl digestion tubes. Samples were subject to hot acid digestion using a solution of concentrated H_2SO_4 (with 2.5% salicylic acid), sodium thiosulphate, and a Kjeldahl digestion tablet (selenium catalyst). Digest temperature (up to 395° C) was controlled by an A.I.M. 500 block digestion controller. Six standard solutions, three blanks and two references with known concentrations of N, were also included with every batch of 40 samples. Organic and inorganic N (from the algal tissue), converted to NH_4^+ by the digestion, was analyzed using a Chemlab Mark 7 System autoanalyzer with an isocyanurate, sodium salicylate/nitroprusside technique. Absorbance was determined by a colorimeter and plotted on a chart recorder. Concentrations were then calculated and expressed as %N dry weight.

For amino acid analysis, approximately 1.0 g wet weight of algal tissue was placed in 5 mL of 100% methanol (analytical reagent grade) for 24 h to extract amino acids. The methanol extract was filtered through Millipore Millex–HV_{13} (0.45-μm) filters and injected into a Beckman System 6300 postcolumn derivatization high-performance liquid chromatography amino acid analyzer, for detection of ninhydrin-positive free amino acid groups at 570 nm. Results were calculated and expressed as nmol·g^{-1} wet weight. As well as detecting free amino acids, this technique also measures the concentration of free NH_4^+ in plant tissue.

Water column nutrients. Concentrations of dissolved inorganic N (NH_4^+ and NO_3^-/NO_2^{2-}) were determined from both field areas using three replicate samples collected from just below the water surface. Water samples were immediately filtered using a Nalgene filtering apparatus with Whatman GF/F glass-fiber filters to remove any solids (sediment and plankton) and frozen immediately using dry ice. Analysis of NH_4^+ in the laboratory was conducted using a phenol/nitroprusside/hypochlorite method, and analysis of NO_3^-/NO_2^{2-} was conducted using a copper–cadmium nitrate reduction column/*N*-(1-naphthyl)-ethylenediamine/sulphanilamide method (Parsons et al. 1984). Because NO_2^{2-} is rapidly oxidized to NO_3^-, the concentrations of NO_2^{2-} in the water column are considerably less than NO_3^- and rarely significant (D'Elia and DeBoer 1978). Therefore, the combined measurements of NO_3^- and NO_2^{2-} were considered together as the oxidized form of N and referred to as NO_3^-.

Statistical analysis. For all experiments in the field and laboratory, three replicates were analyzed, and means and standard errors were calculated. Differences between treatments were tested for significance using one-way analysis of variance and Tukey's test for multiple comparison of means.

RESULTS

Nitrogen source experiments. Pigment, total tissue N, and amino acid content varied as a function of N source (Table 1). PE increased significantly ($P < 0.05$) in response to NH_4^+ as a N source, but no increases were observed with NO_3^- or urea. Chlorophyll *a*, in contrast, did not vary significantly between treatments ($P > 0.05$). There were no statistically significant increases in total tissue N content (%N dry weight), although the NH_4^+ source treatment resulted in an increase from 1.9–2.5% N. The amino acid composition was observed to reflect the different N sources. With NO_3^- as N source, glutamic acid, alanine, and citrulline increased. NH_4^+, however, resulted in a decrease in alanine (not significantly different from the control, but it was significantly different from the NO_3^- treatment) and significant ($P < 0.05$) increases in citrulline, phe-

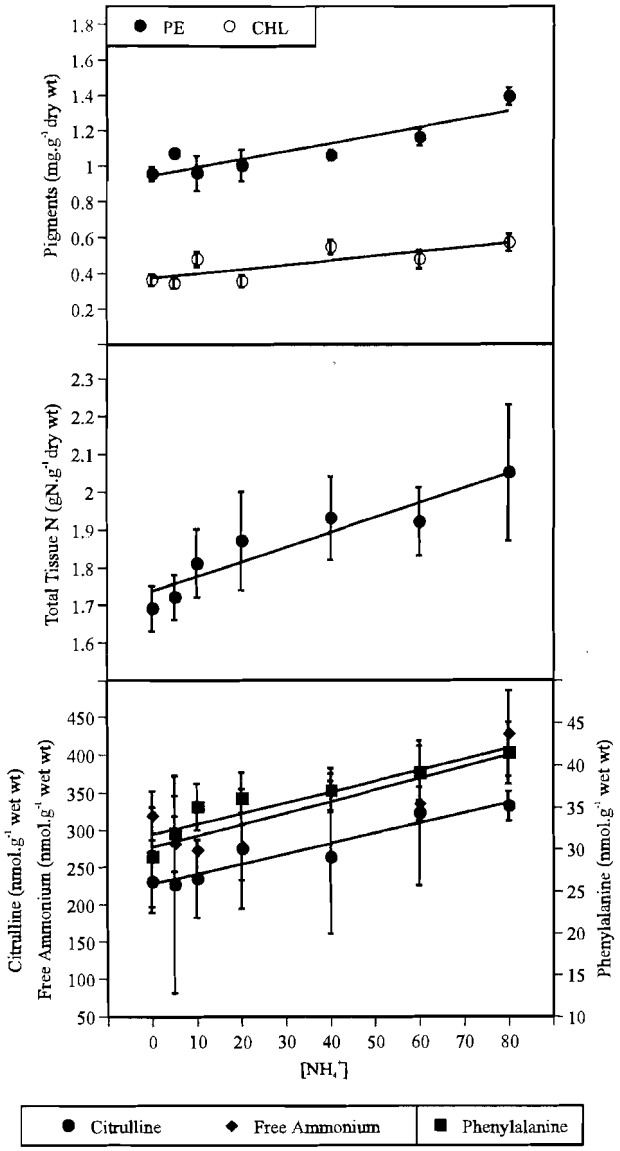

FIG. 3. Responses in pigments, total tissue nitrogen, and amino acids of *Gracilaria edulis* to increasing NH_4^+ concentrations (0–80-μM scale).

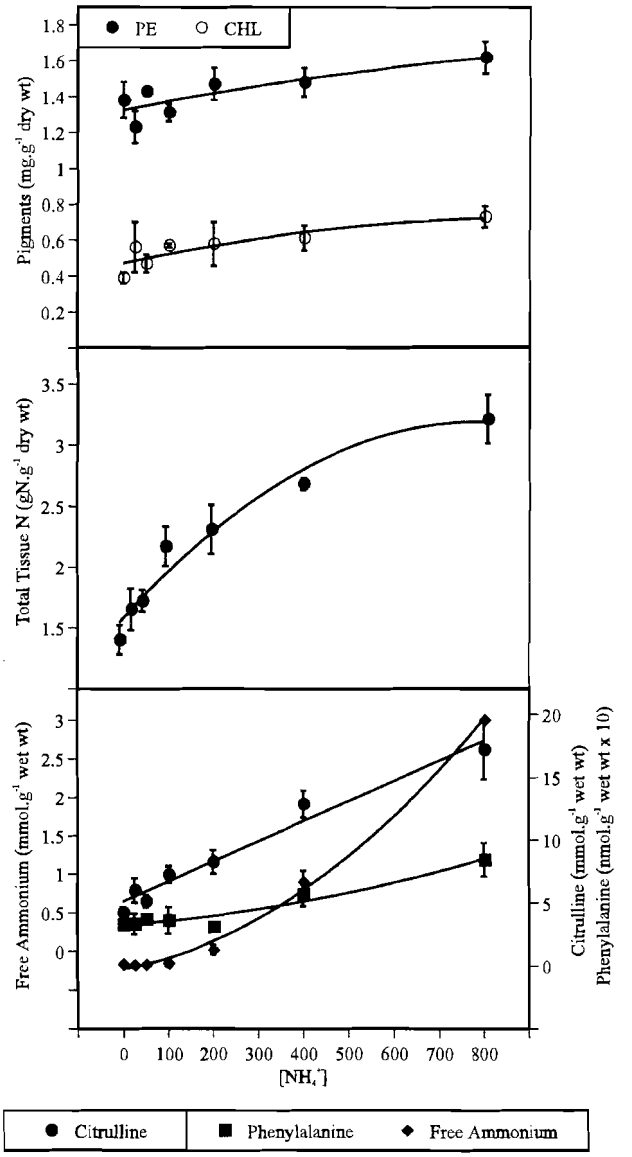

FIG. 4. Responses in pigments, total tissue nitrogen, and amino acids of *Gracilaria edulis* to increasing NH_4^+ concentrations (0–800 μM scale).

nylalanine, and free NH_4^+. Urea produced increases in free NH_4^+ (not statistically different, $P > 0.05$) and glutamic acid (statistically different, $P < 0.05$) and decreases in phenylalanine and serine (although both these were not statistically significant, $P > 0.05$).

[NH_4^+] experiments. In the laboratory source experiments, PE and total tissue N concentrations in *G. edulis* responded to the supply of NH_4^+ but not to NO_3^- or urea (Table 1). The stronger response of NH_4^+ across all three parameters measured (pigments, total tissue N, and amino acids) prompted us to use this form of N for further studies to test the responses of *G. edulis* to changes in N concentrations. In the 0–80-μM NH_4^+ concentration range (Fig. 3), PE increased from 0.9 to 1.4 mg PE·g^{-1}, whereas chl *a* increased from 0.3 to 0.5 mg chl *a*·g^{-1}. It appears that PE may be more sensitive than chl *a* to changes in [NH_4^+] (a 0.5-mg·g^{-1} increase in PE compared to a 0.2-mg·g^{-1} increase in chl *a*). However, in the 0–800-μM NH_4^+ range (Fig. 4), PE increased from 1.2 to 1.6 mg PE·g^{-1}, whereas chl *a* increased from 0.4 to 0.7 mg chl *a*·g^{-1} (Fig. 3). There was little difference between PE and chl *a* responses in the 0–800-μM NH_4^+ range, perhaps indicating saturation in the level of PE at the higher N concentrations.

Total tissue N levels determined after incubation in the 0–80-μM NH_4^+ treatments increased linearly with [NH_4^+] (Fig. 3). The %N levels from the 0–800-μM NH_4^+ treatments appeared to reach a saturation level at approximately 3.2% N (Fig. 4).

The three amino acids that showed the greatest

TABLE 2. *DINs in water column and pigments, total tissue N, and amino acids of* Gracilaria edulis *after 3 d field incubations in the Brisbane River and the eastern side of Moreton Bay (+27 km). Sites are marked as distance from the Brisbane River mouth (0 km). Negative = upstream. * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001. Within columns, means with different letters are significantly different at P < 0.05.*

Incubation site	Water column nutrients: DIN (μM)		Pigments		Total tissue nitrogen (%N dry weight)	Amino acids (nmol·g⁻¹ wet weight)					
	$[NH_4^+]$	$[NO_3^-/NO_2^{2-}]$	PE (mg·g⁻¹dry weight)	Chl *a*		Serine	Glutamic acid	Alanine	Citrulline	Phenylalanine	Free NH_4^+
Brisbane River											
−82 km	11^{cd}	41^{b}	1.0^{ab}	0.31^{d}	2.8^{abc}	22.4	55^{e}	6.3^{a}	116^{b}	12.5^{b}	102^{cd}
−32 km	16^{c}	113^{a}	1.4^{a}	0.36^{cd}	2.9^{a}	13.2	331^{a}	222.2^{b}	398^{a}	26.1^{a}	102^{cd}
−4 km	38^{b}	47^{b}	1.4^{a}	0.41^{bcd}	2.9^{ab}	1.3	197^{b}	8.4^{a}	122^{b}	28.8^{a}	151^{c}
Moreton Bay (+27 km)											
Dunwich	1.2^{d}	1.2^{c}	0.8^{b}	0.99^{ab}	2.1^{d}	11.3	108^{cd}	9.5^{a}	120^{b}	0.0^{c}	588^{b}
Septic pipe	940^{a}	10.7^{c}	1.2^{ab}	0.9^{abc}	3.1^{a}	11.3	85^{de}	5.3^{a}	440^{a}	0.0^{c}	1225^{a}
Sed control	1.2^{d}	0.8^{c}	1.0^{ab}	1.09^{a}	2.2^{bcd}	9.8	153^{bc}	8.9^{a}	117^{b}	0.0^{c}	496^{b}
Sed N+	1.4^{d}	0.9^{c}	1.2^{ab}	1.09^{a}	2.2^{cd}	11.3	181^{b}	85.2^{a}	178^{b}	0.0^{c}	594^{b}
F-value	13,434***	55***	3.8**	8.5***	7.6***	0.9	91***	17.3***	14.5***	31.5***	373***

increases in concentration in response to NH_4^+ in the N source experiments (citrulline, phenylalanine, and free NH_4^+) increased linearly with increasing $[NH_4^+]$ with both 0–80-μM NH_4^+ (Fig. 3) and 0–800-μM NH_4^+ (Fig. 4) treatments. There was no observed saturation in concentration of these amino acids even with the 0–800-μM treatments, indicating the ability of free amino acids to act as stores of luxury N for later use by the algae in times of N limitation.

Field analysis. DIN concentrations in the Brisbane River ranged from 11 to 38 μM for NH_4^+ and 41 to 113 μM for NO_3^-/NO_2^{2-} (Table 2). The peak in NH_4^+ near the river mouth was most likely due to the fertilizer plant at the −7-km mark of the river (Moss 1990) and the Luggage Point sewage treatment plant, which processes 3–4 times more waste than all of Brisbane's other plants combined (Moss et al. 1992). Much higher concentrations of NO_3^- compared to NH_4^+ throughout most of the middle and upper reaches of the estuary could be due to a range of non-point sources along the river, the lack of freshwater input to the river (due to Wivenhoe Dam and Mount Crosby Weir), and perhaps also the effect of high turbidity (low light) on N uptake by phytoplankton.

Pigment and total tissue N contents of algae along the Brisbane River did not vary significantly between sites ($P > 0.05$) (Table 2). In contrast, the amino acid composition of the algae varied markedly between sites, possibly associated with variations in N source dominance in the river (Table 2). Glutamic acid and alanine were significantly ($P < 0.05$) higher at the −32-km site, where NO_3^- was the dominant N source. Phenylalanine increased downstream with increasing NH_4^+ concentrations. Citrulline concentrations were greater at the −32-km site (NO_3^- peak) than the −4-km site (NH_4^+ peak).

Water column nutrient concentrations at Moreton Bay sites were much lower than at the Brisbane River: <2 μM for NO_3^- and NH_4^+, providing a test of the sensitivity of macroalgae to pulses of nutrients not normally detected by periodic chemical analysis. Relatively low dissolved N concentrations (except the septic overflow pipe site) were observed at Moreton Bay sites (0.5–1.5 μM DIN, compared to 10–110 μM for the Brisbane River). There was no significant difference ($P > 0.05$) in water column N concentration between Moreton Bay sites (except the septic overflow sites); however, analysis of the macroalgal tissue revealed some significant trends.

PE was significantly higher ($P < 0.05$) at the fertilized sediment (1.2 mg PE·g⁻¹) and septic overflow pipe (1.2 mg PE·g⁻¹) sites than at the other three sites (<1.0 mg PE·g⁻¹). However, no significant ($P > 0.05$) differences could be observed in the concentrations of chl *a* between the Moreton Bay sites.

The lowest total tissue N concentration in the algae (2.1% N) was observed at the Dunwich water column site, and the highest (3.1% N) at the septic overflow pipe.

The macroalgal free amino acid content at the septic pipe site was significantly higher in citrulline (440 nmol·g⁻¹) and free NH_4^+ (1225 nmol·g⁻¹) than at all other sites (next highest values were 178 and 594 nmol·g⁻¹ for citrulline and free NH_4^+, respectively, at the sediment N fertilized site). At the sediment N fertilized site, there were higher concentrations of alanine (85 nmol·g⁻¹) and glutamic acid (181 nmol·g⁻¹). The next highest were 9.5 nmol·g⁻¹ of alanine and 153 nmol·g⁻¹ of glutamic acid.

Metabolic profiling. Results obtained from the laboratory experiments revealed that the composition and concentration of various amino acids varied significantly with changes in concentration and N source in the water column. From the observed changes in amino acid composition, it was possible to construct an equation for each source of N involving a number of different amino acids and resulting in a N source index (NSI) (Table 3). Once these NSIs had been determined in the laboratory to known concentrations of the various N sources, they could be used

TABLE 3. *NSI equations and results for laboratory source incubations. Bold figures indicate the highest value for each N source. NH_4^+ source index = (% citrulline × % free NH_4^+)/(% alanine); NO_3^- source index = (% glutamic acid × % alanine); urea source index = (% free NH_4^+)/(% phenylalanine × % serine). Percent of each amino acid refers to their percentages of the total amino acid pool. The control treatment is low nitrogen (< 1 μM) seawater.*

N source treatment	NH_4^+ source index	NO_3^- source index	Urea source index
Control	1.0	1.0	1.0
NO_3^- (200 μM)	14.7	**27**	0.4
NH_4^+ (200 μM)	**288**	0.6	3.8
Urea (200 μM)	31	14	**4.9**

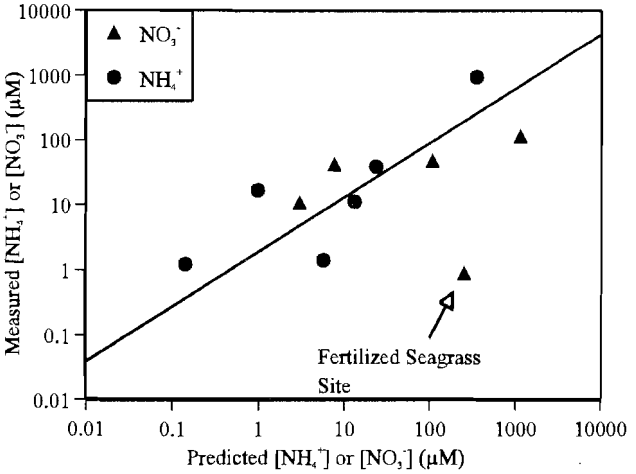

FIG. 5. Measured versus predicted concentrations of NH_4^+ and NO_3^- in Moreton Bay and the Brisbane River (log scale). These points were calculated from the NSIs. Note that the regression line was calculated by excluding the N fertilized seagrass site.

to predict the concentrations of each N source (NH_4^+, NO_3^-, or urea) present at the field incubation site. Correlation of the field measured NO_3^- and NH_4^+ concentrations versus the predicted [NO_3^-] and [NH_4^+] (calculated from the NSI) is shown in Figure 5. The regression line ($r^2 = 0.84$) has been plotted for all points except the N fertilized seagrass site, which demonstrates a significantly higher predicted [NO_3^-] than measured [NO_3^-].

DISCUSSION

Many of the world's waterways are becoming more eutrophic, particularly with respect to the levels of N and P, which are the two macronutrients often limiting the growth of aquatic plants (Valiela 1995). Standard chemical methods of analyzing dissolved nutrient concentrations provide only an instantaneous concentration at the time of water collection, whereas there can be large fluctuations in the concentrations of these dissolved nutrients on short time scales (Wheeler and Björnsäter 1992, Valiela 1995). In addition, chemical analysis of dissolved nutrient concentrations may not be indicative of the "bioavailable" concentrations (Lyngby 1990). Bioavailable refers to the concentration and form of nutrients that are available for uptake and assimilation by plants. For assessment of water quality, the effects of nutrients on marine life are probably more relevant than the instantaneous physical concentrations. Particularly relevant are the different sources of N in the water column, which are preferentially assimilated by the aquatic flora (Lyngby 1990). The use of marine plants as indicators of water column nutrient availability overcomes some of the limitations of standard chemical analysis by 1) integrating short-term nutrient pulses that may go undetected and 2) representing nutrient concentrations available for uptake and assimilation (Lapointe 1985, Lyngby 1990, Wheeler and Björnsäter 1992).

In laboratory experiments, pigments, total tissue nitrogen, and amino acids of *Gracilaria edulis* accumulated in response to the supply of NH_4^+ but not to NO_3^- supply. In contrast, the algae in the Brisbane River and Moreton Bay appeared capable of using both NO_3^- and NH_4^+ to increase the levels of their internal N stores. These results are consistent with the results of DeBoer et al. (1978) and Lapointe and Ryther (1978), who found that *Gracilaria tikvahiae* grows more successfully on NH_4^+ than NO_3^- in the laboratory, but equally as well on NH_4^+ or NO_3^- in high light outdoor tanks. The uptake of nitrate in algae is known to be more strongly dependent on light than ammonium (Hanisak 1979, Falkowski 1983). Despite the high turbidity in the Brisbane River, the light level available to the algae even at the most turbid river sites was between 3 and 9 times higher than those in the laboratory and as such probably provided the higher light conditions necessary for assimilation of NO_3^-. Due to the effect of the availability of light on the algal free amino acid pool (K. T. Bird et al. 1982), it would be useful to incorporate a light correction factor into the N source equations to compensate for differences between field incubation sites.

The highest tissue N level of river algae was 2.9% N compared to 1.9% N for the laboratory control treatment. Most of thallus N increase, as a result of NH_4^+ supply to N-deficient *G. tikvahiae,* is in the form of amino acids (K. T. Bird et al. 1982). However, at the onset of N deficiency, amino acid concentrations decline rapidly, indicating that these are also the first source of N to be utilized when ambient concentrations fall (K. T. Bird et al. 1982). In the red alga *Chondrus crispus,* half its organic N is in the form of the dipeptide citrullinylarginine, suggesting that amino acids and peptides are the major storage units (Laycock and Craigie 1977, Hanisak 1983).

Citrulline was the major amino acid store of N in *G. edulis,* particularly when NH_4^+ was the predominant N source. Its ability to function as a large store of N is due to its structure (i.e. it contains three N

atoms per molecule) and the alternative pathway by which NH_4^+ can be assimilated by some species of algae. Free NH_4^+ is combined with carbon dioxide to form carbamoyl phosphate, which donates its carbamoyl group to ornithine to directly produce citrulline (Lehninger 1973). This process provides an important means of NH_4^+ assimilation for some cyanobacteria (Chen et al. 1987) and perhaps also for some eukaryotic macroalgae. The lack of arginine in the amino acid pool of *G. edulis* appears to be unusual as other species of red algae have demonstrated accumulation of arginine in response to an increase in N availability (Vona et al. 1992). Arginine's structure (four N atoms per molecule) indicates its potential as a reserve of N (Steward and Pollard 1962). The lack of aspartate (required for the conversion of citrulline to argininosuccinate, which is then converted to arginine) in *G. edulis* may be responsible (Lehninger 1973), or perhaps citrulline is accumulated at a site removed from arginine biosynthesis.

The values of the NSI from the laboratory source experiments can be used as a guide to predict the concentration and source of bioavailable N at the field incubation sites. The concentration of DIN at the field incubation sites was analyzed and was used to confirm the macroalgal responses. However, we do not know the dissolved urea concentration, but we were able to use the laboratory NSI results to predict the field urea concentrations. The −4-km Brisbane River site had the highest NH_4^+ NSI value. The −32-km site (the source of N at this site is predominantly NO_3^-) had the highest NO_3^- NSI value. The analysis of various relationships between amino acids through the NSI proved to be a much more useful technique than simply examining increases in various amino acids. For example, the algae incubated in the river at the −32-km site had a much higher concentration of citrulline than either of the other sites. Based on the laboratory responses, this could be a result of high NH_4^+ or NO_3^-, but by analyzing the relative concentrations of various amino acids the NSI predicts the N source to be predominantly NO_3^-.

Due to the very low DIN values at the Moreton Bay sites, the NSI values are generally presumed to result from the ability of macroalgae to assimilate nutrients arising from nutrient pulses that usually go undetected by traditional chemical analysis of the water column. For example, the algae incubated at N fertilized seagrass plots had the highest predicted NO_3^- of all Moreton Bay sites. In addition, relatively high PE concentrations were found at the N fertilized seagrass site, consistent with the response of phycoerythrin to high [NO_3^-] observed in river incubations. The fertilizer used on the seagrass beds was 50% NH_4^+ and 50% NO_3^-, but the NSI indicated that the predominant source being released from the sediment was NO_3^-. The lack of NH_4^+ sediment release could be a result of the following. First, seagrasses preferentially assimilate NH_4^+ (McRoy and McMillan 1977, Iizumi and Hattori 1982, Short and McRoy 1984); second, the seagrass sediments contain nitrifying bacteria that oxidize NH_4^+ to NO_3^- (Moriarity and Boon 1989); and third, NH_4^+ can often become bound up in the sediments by adsorbing onto soil particles (Albertson 1983). It appears that a significant amount of NO_3^- was being released from the sediment; however, it was not being detected by traditional water column sampling, possibly due to the large dilution effect taking place into the surrounding water. Differences between measured and predicted may simply be reflecting the difference between available and useable (by the algae) nutrient concentrations.

Metabolic profiling could potentially be used to measure phosphorus availability, as concentrations of the various amino acids respond to P differently than to N (Vona et al. 1992). For example, P-deficient cells of *Cyanidium caldarium* respond with a significant decrease in glutamic acid upon addition of phosphate, while the other amino acids remained unchanged (Vona et al. 1992).

Other applications for the metabolic profiling technique may exist in environments such as the Great Barrier Reef, where DIN concentrations can be very close to lower detection limits, (e.g. 0.03 μM NO_3^- and 0.02 μM NH_4^+, Bell 1992). The use of macroalgae in detecting nutrient release from sediments may prove more useful than traditional methods using mesocosms or bell jars, which prevent water movement and other natural processes, thereby affecting diffusion processes (Raine and Patching 1980). The measurement of $\delta^{15}N$ values in the macroalgae after incubation in the field may also provide an indication of the source of nutrients, that is, from human inputs or from internal cycling processes (Sweeney and Kaplan 1980, Van Dover et al. 1992).

The utility of macroalgae as bioindicators of nutrient availability has been demonstrated, and further development of this approach could provide biologically valuable information on the source, fate, and transport of N in marine ecosystems. Incubation for 3 d in the field allowed *G. edulis* to integrate pulses of nutrients that may have gone undetected by chemical analysis of dissolved nutrient concentrations. The amino acid composition demonstrated a number of characteristic responses to changes in the availability of dissolved N, and through the use of the NSI variations in the amino acid composition were correlated with the availability and predominance of different N sources. In *G. edulis*, the amino acid composition appeared to be a sensitive parameter for detection of bioavailable N concentrations.

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