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**Influence of Nitrogen Source and Availability on Amino Acids,**

**Pigments and Tissue Nitrogen of *Gracilaria edulis***

**(Rhodophyta)**

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**Influence of Nitrogen Source and Availability on Amino  
Acids, Pigments and Tissue Nitrogen of  
*Gracilaria edulis* (Rhodophyta)**

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This thesis is being submitted in the form of a scientific report for subsequent publishing in the Journal of Phycology. A table of contents and an appendix of photographs are attached for the purpose of the thesis.

I declare that this thesis does not contain any material which has been submitted by me previously for any degree or diploma to any university, and to the best of my knowledge, it does not contain any material published or written by another person, except where due reference is made in the text.

Adrian B. Jones.

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## ABSTRACT

Increasing eutrophication of coastal marine environments has led to the development of nutrient sampling programs to monitor water quality. Various shortcomings of chemical analyses have identified the need to develop biological indicators (bioindicators) that can be used to detect available nutrient concentrations. Macroalgal tissue nutrient content, pigments, and amino acids appear to be responsive to water column nutrient availability. The responses of the red alga, *Gracilaria edulis* (Gmelin) Silva, were related to nitrogen (N) source and availability in laboratory and field incubations to identify characteristics that would serve as bioindicators of N. The amino acid, pigment, and tissue N composition of *G. edulis* was analysed after incubation in different N sources ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and urea) and a range of  $[\text{NH}_4^+]$  in laboratory aquaria. These results were compared to field responses after incubation of *G. edulis* along a N gradient in the Brisbane River (3 sites) and Moreton Bay (5 sites), Queensland, Australia. Photosynthetic pigments (phycoerythrin and chlorophyll *a*) increased in laboratory experiments, in response to increasing  $[\text{NH}_4^+]$ , but not  $[\text{NO}_3^-]$  or [urea]. Phycoerythrin was observed to be the more responsive of the two. Total tissue N increased linearly with increasing  $[\text{NH}_4^+]$  in the laboratory but did not respond to  $[\text{NO}_3^-]$  or [urea]. In the field both phycoerythrin and tissue N appeared to respond equally to  $\text{NH}_4^+$  and  $\text{NO}_3^-$  availability. The amino acid composition provided the best representation of the concentration and source of available N. Citrulline was the most responsive of all amino acids to changes in concentrations of available N, with citrulline concentrations increasing linearly ( $r^2 = 0.84$ ) with  $[\text{NH}_4^+]$  in laboratory experiments.  $\text{NH}_4^+$  treatments produced increases in citrulline, phenylalanine, serine and free  $\text{NH}_4^+$ , and decreases in alanine;

$\text{NO}_3^-$  treatments produced increases in glutamic acid, citrulline and alanine; urea treatments produced increases in free  $\text{NH}_4^+$  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. The N source index was used to predict the dominant source of N being assimilated by the macroalgae (i.e., metabolic profiling). Results demonstrated strong correlations between the N source index value and water column nitrogen concentrations ( $r^2 = 0.99$  for  $\text{NO}_3^-$  and  $r^2 = 0.79$  for  $\text{NH}_4^+$ ) in Brisbane River field trials. In Moreton Bay where dissolved inorganic nitrogen values were low ( $< 2 \mu\text{M}$ ), N source indices proved valuable in identifying predominant N sources. Physiological responses to N by this macroalga could be related to N source and availability in both laboratory and field situations, thus providing a sensitive bioindicator of N. In particular, the analysis of macroalgal amino acid content to infer the source and availability of N in a variety of field situations, appears to be a very sensitive biological indicator.

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

## INTRODUCTION

The world's rivers and bays are becoming more eutrophic, particularly with respect to the levels of N and phosphorus (P) which are naturally the two most commonly limiting nutrients to the growth of aquatic plants (Valiela, 1984). Standard methods of analysing dissolved nutrient concentrations provide only an instantaneous concentration at the time of water collection, whereas in reality there can be large fluctuations in the concentrations of these dissolved nutrients on short time scales (Valiela, 1984; Wheeler and Björnsäter, 1992). 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 which are available for uptake and assimilation by plants. For assessment of water quality, the effects of nutrients on marine life may be more relevant than the instantaneous physical concentrations. Particularly relevant are 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

a) integrating short-term nutrient pulses which may go undetected; and b) by representing nutrient concentrations available for uptake and assimilation (Lapointe, 1985; Lyngby, 1990; Wheeler and Björnsäter, 1992).

Macroalgae are frequently used as biological indicators of water column nutrients 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, 1993).

Macroalgae rapidly assimilate surrounding nutrients, and as such can represent the local nutrient regime within a relatively short time period. *Gracilaria tikvahiae*, for example, can assimilate and store enough N in 6 h to allow it to grow for 2 weeks at non-nitrogen-limited levels (Ryther *et al.*, 1981). Pigments, total tissue N and amino acid concentrations of macroalgae are influenced by the availability of dissolved nutrients (Bird *et al.*, 1982). Macroalgal tissue N is probably the most widely analysed of these parameters for determining the macroalgae's response to dissolved inorganic nitrogen (DIN) concentrations in the water column (e.g., Gerloff and Krombholz, 1966; Lapointe and Ryther, 1979; Bird *et al.*, 1982; Lyngby, 1990). Total tissue N content of the red algae (Rhodophyta), has been observed to correlate more closely with water column N concentrations than green (Chlorophyta) or brown (Phaeophyta) macroalgae (e.g., Chapman *et al.*, 1978; D'Elia and DeBoer, 1978; Lapointe and Ryther, 1979; 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 (e.g., Ryther *et al.*, 1981; Bird *et al.*, 1982; Horrocks, 1993), and are characteristically tolerant to changes in salinity, temperature and light, all of which are known to affect the physiological parameters being analysed (Bird *et al.*, 1979; Yarish and Edwards, 1982).

The responsiveness of red algae to nutrient availability may be attributable in part to their phycobilisomes. Phycobilisomes are pigment complexes located on the surface of the thylakoid lamellae, and are exclusive to cyanobacteria and red algae (Gantt, 1990). The photosynthetic accessory pigments, phycoerythrin, phycocyanin, and allophycocyanin are arranged concentrically, with phycoerythrin as the outermost of the three pigments and the most sensitive to external changes in both light and nutrient availability (Kursar and Alberte, 1983).

When internal N reserves are depleted, algae start to lose their dark reddish-brown pigment, become a pale straw-yellow colour, and cease growing (Ryther *et al.*, 1981). This loss of colour may be a result of phycoerythrin being metabolised as a source of protein (Lapointe, 1981; Bird *et al.*, 1982). The responsiveness of macroalgal pigments to changes in nutrient availability makes them effective bioindicators.

Another macroalgal bioindicator of dissolved inorganic nitrogen is the amino acid content of the tissue (Horrocks, 1993). The source (i.e.,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  or urea) as well as the concentration of N being assimilated affects the free amino acid pool in algae (Bird *et al.*, 1982; Vona *et al.*, 1992). For N to be assimilated into amino acids, it must be in the form of  $\text{NH}_4^+$  (Arnstein, 1975). Consequently,  $\text{NO}_3^-$  is reduced and urea is hydrolysed to  $\text{NH}_4^+$  which is then assimilated through the glutamine synthetase (GS) / glutamate synthase (GOGAT) pathway (Figure VI) (Arnstein, 1975). Citrulline (a non - protein amino acid) is responsive to changes in DIN and appears to function as the major "luxury" N store in *Gracilaria* spp., and some other genera of macroalgae (e.g., Horrocks, 1993). The storage of N in the inorganic form, or biomechanical metabolites, is known as luxury consumption, and is an ecological adaptation to N limitation (Gerloff and Krombholz, 1966). Luxury reserves constitute that portion of tissue N above the species critical nitrogen concentration (i.e., the concentration which just limits maximal growth) (Hanisak, 1983). These reserves may be metabolised in times of low N availability (Vona *et al.*, 1992; Horrocks, 1993).

In the present study a number of characteristics (pigments, tissue N and amino acids) were analysed 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* spp. in Moreton Bay was greatly stimulated by N (Horrocks, 1993). The aims

of this research were a) to investigate the influence of different N sources (e.g.,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and urea) and  $[\text{NH}_4^+]$  on *G. edulis* in the laboratory, and b) to develop the use of *G. edulis* as an bioindicator of available nitrogen concentrations along an established dissolved inorganic nitrogen (DIN) gradient in the field.

## MATERIALS AND METHODS

### Algal Collection, Culture and Incubation

*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) (Figure I) and transported back to the university algal culture facilities in 25 l plastic containers. Several kilograms of macroalgae were placed in well aerated aquaria with low nutrient seawater (collected from Moreton Bay and sand filtered) for 6 - 8 weeks to deplete the algae of N stores before incubation in laboratory and field. Aquaria were maintained at 20 - 23°C, and exposed to light on a 12:12 day/night cycle using a mixture of triton, actinic, and daylight fluorescent lights which provided approximately 230  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ .

Macroalgal incubations were undertaken in the laboratory and field for a period of 3 d. This period appears to be essential in maximising amino acid responses to different N sources. Initial laboratory experiments indicated that after 7 days 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 in field incubations it would sacrifice the benefits of integrating the nutrient regime over time.

## Laboratory Experiments

*Gracilaria edulis* was incubated in different N sources and concentrations in laboratory aquaria. Nitrogen source experiments involved incubation for 3 d in aquaria with 200  $\mu\text{M}$  concentrations of three different N sources;  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , urea and a control with no added N.

The [ $\text{NH}_4^+$ ] experiments involved two different concentration ranges. The first range (0 - 800  $\mu\text{M}$ ) encompassed concentrations in excess of realistic biological values and was designed to amplify responses and determine a saturation level. Seven aquaria were used with the following concentrations: 0, 25, 50, 100, 200, 400, 800  $\mu\text{M}$   $\text{NH}_4^+$ . The second range (0 - 80  $\mu\text{M}$ ) was more biologically realistic and used the following concentrations: 0, 5, 10, 20, 40, 60, 80  $\mu\text{M}$   $\text{NH}_4^+$ . Three replicates of 4 g fresh weight were incubated for each treatment. Algae were placed in clear polycarbonate containers with 20 holes placed in the container to facilitate water flow.

## Field Incubations

In the field, two types of apparatus were used to contain the algae during the 3 d incubation period (Figure II). Water column macroalgal chambers consisted of an electrical conduit frame with attached polycarbonate containers. The chambers were anchored using a car tyre 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 which 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 (Figure I). 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 from constant dredging 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 fertiliser plant are located near the river mouth.

The results from laboratory analysis of pigments, 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 ( $< 2 \mu\text{M}$ ) in the bay to highly eutrophic concentrations (up to  $120 \mu\text{M}$ ) in the river, with large variations in source of N in the river.

There were 3 sites along the Brisbane River for macroalgal incubations, 1) - 4 km, Fort Lytton National Park ( $27^{\circ}24.69' \text{ S}$ ;  $153^{\circ}08.89' \text{ E}$ ) opposite the Luggage Point Sewage Treatment Plant, 2) - 32 km, the university rowing shed at the University of Queensland ( $27^{\circ}29.52' \text{ S}$ ;  $153^{\circ}00.75' \text{ E}$ ), 3) - 82 km, Kookaburra Park at Karana Downs ( $27^{\circ}32.48' \text{ S}$ ;  $152^{\circ}50.38' \text{ E}$ ). Three additional sites were sampled solely for water column nutrients, - 14 km ( $27^{\circ}29.55' \text{ S}$ ;  $153^{\circ}03.58' \text{ E}$ ), - 47 km ( $27^{\circ}32.55' \text{ S}$ ;  $152^{\circ}58.41' \text{ E}$ ), - 64 km ( $27^{\circ}36.21' \text{ S}$ ;  $152^{\circ}54.03' \text{ E}$ ).

There were 5 sites in Moreton Bay. Water column macroalgal chambers were used at Dunwich, + 27 km (27°29.50' S; 153°23.91' E) and at Amity Point, + 27 km (27°23.36' S; 153°25.80' E). The Dunwich site was subject to terrestrial runoff from the island's main settlement. The Amity site was located near the South Passage between North Stradbroke Island and Moreton Island where oceanic waters exchange with Moreton Bay. Sediment chambers were located close to the outfall pipe from a septic tank system at Dunwich and at two sites at Amity over fertilised and control seagrass plots. As part of a seagrass fertilisation study being conducted by the University of Queensland Marine Botany Group, *Zostera capricorni* seagrass beds on the Wanga Wallen banks at Amity Point (+ 27 km) have a number of 1 m<sup>2</sup> plots at which slow release Osmocote fertiliser has been placed into the sediment. Fertiliser containing 88 g N m<sup>-2</sup> (50:50 NH<sub>4</sub><sup>+</sup> : NO<sub>3</sub><sup>-</sup>) was applied to each plot. Chambers were placed in the sediment over both control and N fertilised plots.

### Macroalgal Tissue

After 3 days incubation in either 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 and chlorophyll *a*), tissue N analysis (organic and inorganic) and amino acid analysis.

### *Pigment Analysis*

Approximately 0.5 g fresh weight of *G. edulis* tissue was separated, blotted dry and weighed (fresh wt). 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 mins at 2500 rpm) to produce a supernatant containing phycoerythrin and a pellet with the remaining tissue. The supernatant was transferred to a cuvette and absorption at 565 nm and 710 nm determined on a spectrophotometer for phycoerythrin and turbidity blank, respectively. The pellet was resuspended in 5 ml of 80% acetone (analytical reagent grade) and disrupted with a tissue homogeniser to extract chlorophyll. Samples were re-centrifuged (20 mins at 2500 rpm) and absorbance at 664 nm and 710 nm determined for chlorophyll *a* and turbidity blank, respectively. Pigment concentrations as mg g<sup>-1</sup> dry wt were calculated with specific formulas for phycoerythrin (Rowan, 1989), and chlorophyll *a* (Parsons *et al.*, 1987)

### *Tissue Nitrogen Analysis*

Approximately 2.5 g fresh 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 ( $\pm$  0.1 mg) and placed into Kjeldahl digestion tubes. Samples were subject to hot acid digestion using a solution of concentrated H<sub>2</sub> SO<sub>4</sub> (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, 3 blanks and 2 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  $\text{NH}_4^+$  by the digestion was analysed using a Chemlab Mark 7 System autoanalyser with an isocyanurate, sodium salicylate/nitroprusside technique. Absorbance was determined by a colourimeter, and plotted on a chart recorder. Concentrations were then calculated and expressed as %N dry weight (Oweczkin and Kerven, 1987).

#### *Amino Acid Analysis*

Approximately 1.0 g fresh weight of algal tissue was placed in 5 ml of 100% methanol (analytical reagent grade) for 24 hours to extract amino acids. The methanol extract was filtered through Millipore Millex - HV<sub>13</sub> (0.45  $\mu\text{m}$ ) filters, and injected into a Beckman System 6300 post column derivatisation HPLC amino acid analyser, for detection of ninhydrin positive free amino acid groups at 570 nm. Results were calculated and expressed as  $\text{nmol g}^{-1}$  wet weight. As well as detecting free amino acids, this technique also measures the concentration of free  $\text{NH}_4^+$  in plant tissue. For ease of discussion throughout the paper, this  $\text{NH}_4^+$  will be referred to as an amino 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). The NSI related amino acid concentrations for each N source ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , or urea) present at the incubation site to a control value which was determined from a laboratory incubation in very low nutrient seawater. Equations for amino acid metabolic profiling were as follows:

$$\text{NH}_4^+ \text{ Source Index} = (\% \text{ Citrulline} \times \% \text{ Free NH}_4^+) / (\% \text{ Alanine})$$

$$\text{NO}_3^- \text{ Source Index} = (\% \text{ Glutamic Acid} \times \% \text{ Alanine})$$

$$\text{Urea Source Index} = (\% \text{ Free NH}_4^+) / (\% \text{ Phenylalanine} \times \% \text{ Serine})$$

## Water Column Nutrients

Concentrations of dissolved inorganic N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) 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 fibre filters to remove any solids (sediment and plankton) and frozen using dry ice. Analysis of  $\text{NH}_4^+$  in the laboratory was determined using a phenol / nitroprusside / hypochlorite method; and analysis of  $\text{NO}_3^- / \text{NO}_2^-$  using a copper - cadmium nitrate reduction column / N- (1-naphthyl)-ethylenediamine / sulphanilamide method (Parsons *et al.*, 1984). Measurement errors for these methods have been estimated as  $\pm 0.5 \mu\text{M}$  for  $\text{NO}_3^-$  and  $\pm 0.1 \mu\text{M}$  for  $\text{NH}_4^+$ . Because  $\text{NO}_2^-$  is rapidly oxidised to  $\text{NO}_3^-$ , the concentrations of  $\text{NO}_2^-$  in the water column are considerably less than  $\text{NO}_3^-$  and rarely significant (D'Elia and DeBoer, 1978; Baldwin, 1990). Therefore, the combined measurements of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  were considered together as the oxidised form of N and referred to as  $\text{NO}_3^-$ .

## Statistical Analysis

For all experiments in the field and laboratory, three replicates were analysed and from this means and standard errors were calculated. Differences between treatments were tested for significance using one way analysis of variance (ANOVA) and Tukey's Test for multiple comparison of means.

## RESULTS

### Nitrogen Source Experiments

Pigment, tissue N and amino acid content varied as a function of N source (Table I). Phycoerythrin increased significantly ( $p < 0.05$ ) in response to  $\text{NH}_4^+$  as a N source but no increases were observed with  $\text{NO}_3^-$  or urea. Chlorophyll *a*, in contrast, did not vary significantly between treatments ( $p > 0.05$ ). The only marked increase in total tissue N content (%N dry wt) was in response to the  $\text{NH}_4^+$  source which resulted in an increase from 1.9% N to 2.5% N. Although this increase was not statistically significant ( $p > 0.05$ ) it may be physiologically significant. The amino acid composition was observed to reflect the different N sources. With  $\text{NO}_3^-$  as N source, glutamic, alanine and citrulline increased.  $\text{NH}_4^+$ , however resulted in a marked decrease in alanine and significant ( $p < 0.05$ ) increases in citrulline, phenylalanine and free  $\text{NH}_4^+$ . Urea produced increases in free  $\text{NH}_4^+$  and glutamic acid, and decreases in phenylalanine and serine.

### $[\text{NH}_4^+]$ Experiments

In the laboratory source experiments phycoerythrin and tissue N concentrations in *G. edulis* responded to the supply of  $\text{NH}_4^+$ , but not to  $\text{NO}_3^-$  or urea (Table I). Consequently,  $\text{NH}_4^+$  was chosen for further studies to test the responses of *G. edulis* to changes in N concentrations. Phycoerythrin increased at a greater rate than chlorophyll *a* over the 0 to 80  $\mu\text{M}$   $[\text{NH}_4^+]$  treatments, indicating that phycoerythrin is more sensitive than chlorophyll to changes in  $[\text{NH}_4^+]$  (Figure III). With the 0 - 800  $\mu\text{M}$   $[\text{NH}_4^+]$  range increases in phycoerythrin and

chlorophyll were more closely related, probably due to saturation in the level of phycoerythrin (Figure IV). In the 0 to 80  $\mu\text{M NH}_4^+$  concentration range, phycoerythrin increased from 0.9 mg PE  $\text{g}^{-1}$  to 1.4 mg PE  $\text{g}^{-1}$ , while chlorophyll increased from 0.3 mg Chl  $a \text{ g}^{-1}$  to 0.5 mg Chl  $a \text{ g}^{-1}$ . However, in the 0 to 800  $\mu\text{M NH}_4^+$  range, phycoerythrin increased from 0.4 mg Chl  $a \text{ g}^{-1}$  to 0.7 mg Chl  $a \text{ g}^{-1}$ , while chlorophyll increased from 1.2 mg PE  $\text{g}^{-1}$  to 1.6 mg PE  $\text{g}^{-1}$ .

Total tissue N levels determined after incubation in the 0 to 80  $\mu\text{M NH}_4^+$  treatments, increased linearly with  $[\text{NH}_4^+]$  (Figure III). The %N levels from the 0 to 800  $\mu\text{M NH}_4^+$  treatments appeared to reach a saturation level at approximately 3.2% N (Figure IV).

The three amino acids which produced the greatest increases in response to  $\text{NH}_4^+$  in the N source experiments (i.e., citrulline, phenylalanine and free  $\text{NH}_4^+$ ) increased linearly with increasing  $[\text{NH}_4^+]$  with both 0 to 80  $\mu\text{M NH}_4^+$  (Figure III) and 0 to 800  $\mu\text{M NH}_4^+$  (Figure IV) treatments. There was no observed saturation in concentrations of these amino acids even with the 0 to 800  $\mu\text{M}$  treatments, indicating the ability of free amino acids to act as large stores of luxury N for use by the algae.

## Field Analysis

### *Dissolved Nitrogen Analysis*

Dissolved inorganic nitrogen concentrations in the Brisbane River ranged from 11  $\mu\text{M}$  at the - 82 km site to 38  $\mu\text{M}$  at the - 4 km site for  $\text{NH}_4^+$ , and 41  $\mu\text{M}$  at - 82 km to 113  $\mu\text{M}$  at the - 32 km site for  $\text{NO}_3^- / \text{NO}_2^-$  (Table II). These three were chosen from the six sites for macroalgal incubation because they represented the highest and lowest DIN concentrations

(Figure V). The peak in  $\text{NH}_4^+$  near the river mouth was most likely from the fertiliser plant at the - 7 km mark of the river (Moss, 1990), and the luggage point sewage treatment plant which processes 3 to 4 times more waste than all of Brisbane's other plants combined (Moss *et al.*, 1992). Much higher concentrations of  $\text{NO}_3^-$  compared to  $\text{NH}_4^+$  throughout most of the mid 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 on N cycling processes (i.e., phytoplankton are severely light limited and therefore are not incorporating large amounts of  $\text{NO}_3^-$  into the organic pool) (Moss, 1990).

Water column nutrient concentrations at the Moreton Bay sites were much lower than the Brisbane River; less than  $2 \mu\text{M}$  for  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , providing a test of the sensitivity of macroalgae to pulses of nutrients not normally detected by periodic chemical analysis.

### *Macroalgal Analysis*

Pigment contents of algae from different sites along the Brisbane River were not significantly different ( $p > 0.05$ ). However, chlorophyll concentrations were observed to increase downstream with increasing concentrations of water column  $\text{NH}_4^+$ . At the - 82 km site which had the lowest DIN concentration, phycoerythrin content was lower ( $1.03 \text{ mg PE g}^{-1}$ ) than the other two sites, - 32 km ( $1.39 \text{ mg PE g}^{-1}$ ) and - 4 km ( $1.41 \text{ mg PE g}^{-1}$ ), which have much higher DIN concentrations (Table II).

Tissue N concentrations between the river sites were also not significantly different ( $p > 0.05$ ), although the concentrations at the - 32 km (2.94% N) and - 4 km sites (2.92% N) were higher than the - 82 km site (2.82% N). These responses are consistent with DIN concentrations (Table II).

Amino acid composition of the algae varied markedly between sites, presumably associated with variations in N source dominance in the river (Table II). Glutamic acid and alanine were significantly ( $p < 0.05$ ) higher at the - 32 km site where  $\text{NO}_3^-$  was the dominant N source. Phenylalanine increased downstream with increasing  $\text{NH}_4^+$  concentrations. Citrulline concentrations were more greatly elevated at the - 32 km site ( $\text{NO}_3^-$  peak) than the - 4 km site ( $\text{NH}_4^+$  peak).

The relatively low dissolved N concentration at the Moreton Bay sites (0.5 - 1.5  $\mu\text{M}$  DIN, compared to 10 - 110  $\mu\text{M}$  for the Brisbane River), resulted in virtually no detectable water column N differences between sites. However, analysis of the macroalgal tissue revealed some relatively strong trends (Table II).

Phycocyanin was higher at the sediment N fertilised (1.16 mg PE  $\text{g}^{-1}$ ) and sewage pipe sites (1.16 mg PE  $\text{g}^{-1}$ ), than the other three sites ( $< 1.0$  mg PE  $\text{g}^{-1}$ ). However, no significant ( $p > 0.05$ ) differences could be observed in the concentrations of chlorophyll between the Moreton Bay sites.

At the Moreton Bay sites the lowest tissue N concentration in the algae (2.1% N) was observed at the Dunwich site, and the highest (3.1% N) at the sewage pipe. The algae incubated at the Amity site which is flushed by oceanic waters had the second highest value (2.45% N).

The macroalgal free amino acid composition at the sewage pipe site was significantly higher in citrulline ( $440 \text{ nmol g}^{-1}$ ) and free  $\text{NH}_4^+$  ( $1224 \text{ nmol g}^{-1}$ ) than all other sites. At the sediment N fertilised site, there were much higher concentrations of alanine ( $85 \text{ nmol g}^{-1}$ ) and to some extent, glutamic acid ( $181 \text{ nmol g}^{-1}$ ).

### Metabolic Profiling

Citrulline and free  $\text{NH}_4^+$  constituted a major proportion of the total amino acid pool when external N concentrations were high, and increased from 0 to  $2500 \text{ nmol g}^{-1}$  for citrulline and  $19000 \text{ nmol g}^{-1}$  for free  $\text{NH}_4^+$ . The size of the total amino acid pool increased in response to increases in external N concentrations. Therefore, rather than incorporating amino acid concentrations, the equations incorporated the percent amino acid values (i.e., the percentage that the particular amino acid contributed to the total amino acid pool). The use of this variable ensured that the equation was making sample comparisons independent of absolute concentrations.

The N source index (Table III) indicated the dominance of the particular N source assimilated by the algae, relative to the control treatment in the laboratory source experiment. The  $\text{NH}_4^+$  NSI for the algae incubated in the laboratory  $\text{NH}_4^+$  source treatment was 288. This compared to  $\text{NH}_4^+$  NSI values of 14.7 and 31 for the  $\text{NO}_3^-$  and urea treatments, respectively. The  $\text{NO}_3^-$  NSI for the algae incubated in the laboratory  $\text{NO}_3^-$  source was 27 versus 0.6 and 14 for the other two treatments, and the urea NSI was 4.9 versus 0.4 and 3.8 for the various treatments. When applied to the field incubations the NSI

values indicated similar trends based on the correlation with water column analysis of DIN.

At the - 4 km site (high  $\text{NH}_4^+$  concentrations) the  $\text{NH}_4^+$  NSI was 33 versus 18.6 and 1.4 for the other 2 river sites. The - 32 km site (high  $\text{NO}_3^-$  concentrations) values were 150 versus 1.3 and

14. Although water column concentrations of dissolved urea were not determined, the urea NSI produced the highest rating at the

- 4 km site.

The  $\text{NH}_4^+$  NSI values from the Moreton Bay incubations had the highest rating of 490 at the sewage pipe, which also had the highest urea NSI of 15. The highest  $\text{NO}_3^-$  rating of 33 was recorded at the seagrass N fertilised sediment site, and the second highest  $\text{NO}_3^-$  rating was recorded at the well flushed Amity site.

## DISCUSSION

In laboratory experiments, pigments, total tissue nitrogen and amino acids of *Gracilaria edulis* accumulated in response to the supply of  $\text{NH}_4^+$  but not to  $\text{NO}_3^-$  supply. The uptake of nitrate in algae is known to be more strongly dependent on light than ammonium (Hanisak, 1979; Falkowski, 1983). Under the relatively high light regimes of the Brisbane River and Moreton Bay, the algae appeared capable of using  $\text{NO}_3^-$  and  $\text{NH}_4^+$  equally to increase the levels of their internal N stores. This is consistent with the results of DeBoer *et al.* (1978) and Lapointe and Ryther (1978) who found that *Gracilaria tikvahiae* grows more successfully on  $\text{NH}_4^+$  than  $\text{NO}_3^-$  in the laboratory, but equally as well on  $\text{NH}_4^+$  or  $\text{NO}_3^-$  in high light outdoor tanks. 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 high light conditions necessary for assimilation of  $\text{NO}_3^-$ . This increased ability for growth, probably coincided with an increase in the production of pigments and amino acids, which contribute the majority of the increases in the level of total tissue N.

The highest tissue N level of river algae was 2.9% N compared with 1.9% N for the laboratory control treatment. Most of thallus N increase, as a result of  $\text{NH}_4^+$  supply to N deficient *G. tikvahiae*, is in the form of amino acids (Bird *et al.*, 1982). However, at the onset of N deficiency, amino acid concentrations decline rapidly, indicating these are also the first source of N to be utilised when ambient concentrations fall (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).

It has been demonstrated that the free amino acid composition and concentration of marine macroalgae is influenced by the source of externally available N (Nasr *et al.*, 1968; Bird *et al.*, 1982). The development of the N source index (NSI) based on the relative concentrations of a number of particularly responsive amino acids has demonstrated strong correlations with different sources of available N, in both laboratory and field experiments. The nitrogen source index (NSI) ratings for the field were usually higher than those for the laboratory experiments, even though the 200  $\mu\text{M}$  concentrations of N used in the laboratory experiments were much higher than any DIN concentrations recorded in the field (except for the sewage pipe). Also, note that some of the  $\text{NH}_4^+$  NSI values for the Moreton Bay sites were higher than those for the river. These are probably functions of the higher light levels, as the availability of light also affects the algal free amino acid pool (Bird *et al.*, 1982). Consequently, it would be useful to incorporate a light correction factor into the N source equations to compensate for differences between sites.

Amino acid levels proved to be very sensitive to changes in N availability, even at low nutrient sites in Moreton Bay. Citrulline was the major amino acid store of N in *G. edulis*, particularly when  $\text{NH}_4^+$  was the predominant N source. Its ability to function as a large store of N is due to its structure (i.e., it contains 3 N atoms per molecule), and the alternative pathway by which  $\text{NH}_4^+$  can be assimilated by some species of algae. Free  $\text{NH}_4^+$  is combined with carbon dioxide to form carbamyl phosphate, which donates its carbamyl group to ornithine to directly produce citrulline (Figure VI) (Lehninger, 1973). This process provides an important means of  $\text{NH}_4^+$  assimilation for some algae (Chen *et al.*, 1987). The

lack of arginine in the amino acid pool of *G. edulis* appears to be unusual as several studies on other species of red algae have observed accumulation of arginine in response to an increase in N availability (e.g Vona *et al.*, 1992). Arginine's structure (4 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 N source index from the laboratory source experiments can be used as a guide to speculate on the bioavailable N sources at the field incubation sites. In the Brisbane River, there was little doubt as to the predominant source of DIN at each site, because of the high concentrations, all greater than 50  $\mu\text{M}$  N. This made it an ideal location for testing the observed responses from the laboratory in a field situation. The observed relationships between NSI values and dissolved N concentrations were consistent with those in the laboratory. The - 4 km site which is predominantly  $\text{NH}_4^+$  had the highest  $\text{NH}_4^+$  NSI value. The - 32 km site which is predominantly  $\text{NO}_3^-$  had the highest  $\text{NO}_3^-$  NSI value. The urea NSI value is only speculative, as no dissolved organic N concentrations were measured. However, the algae from the - 4 km site which is located in close proximity to the outfall from Brisbane's largest sewage plant, had the highest urea NSI value. Due to the high sewage outfall in this region, the water column is likely to be high in urea, because sewage is typically high in this form of organic N (Albertson, 1983). 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  $\text{NH}_4^+$  or  $\text{NO}_3^-$ , but by analysing the relative concentrations of various amino acids, the NSI determined the N source to be predominantly  $\text{NO}_3^-$ .

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 which usually go undetected by traditional chemical analysis of the water column. For example, the algae incubated at N fertilised seagrass plots had the highest  $\text{NO}_3^-$  NSI of all Moreton Bay sites. The fertiliser used on the seagrass beds was 50%  $\text{NH}_4^+$  and 50%  $\text{NO}_3^-$ , but the NSI indicated that the predominant source being released from the sediment is  $\text{NO}_3^-$ . This may be explained by considering three factors. Firstly, seagrasses are known to preferentially assimilate  $\text{NH}_4^+$  (e.g., McRoy and McMillan, 1977; Iizumi and Hattori, 1982; Short and McRoy, 1984); secondly, the seagrass sediments contain nitrifying bacteria which oxidise  $\text{NH}_4^+$  to  $\text{NO}_3^-$  (Moriarty and Boon, 1989; Valiela, 1984); and thirdly,  $\text{NH}_4^+$  can often become bound up in the sediments by adsorbing onto soil particles (Albertson, 1983). Relatively high phycoerythrin concentrations were found at the N fertilised seagrass site. This is consistent with the response of phycoerythrin to high  $[\text{NO}_3^-]$  in the river incubations.

The NSI indices derived from the algae incubated at the sewage pipe site were indicative of high concentrations of  $\text{NH}_4^+$  and urea ( $\text{NH}_4^+$  NSI = 490 and urea NSI = 15). This is consistent with the major sources of N present in sewage waste;  $\text{NH}_4^+$  and urea (Albertson, 1983). The concentrations of both phycoerythrin and tissue N are high at this site, indicating the presence of increased dissolved nitrogen availability.

Although little could be inferred from the DIN, pigments, or tissue N concentrations, the NSI for the algae from the water column chamber at the Dunwich site indicated that there may

be a reasonably high concentration of urea present in the water column. This is consistent with the location of this chamber in the bay where the sewage outfall pipe is located. The algae from the Amity water column site had the second highest  $\text{NO}_3^-$  NSI rating. This site is well flushed by oceanic waters, and therefore this response may be due to pulses from the relatively  $\text{NO}_3^-$  enriched waters of the East Australian Current offshore (Anderson, 1987). This may explain the relatively high tissue N concentration obtained at this site, noting that algae's tissue N from Brisbane River incubations was observed to increase in high a  $\text{NO}_3^-$  environment, and not just in response to  $\text{NH}_4^+$  as observed in the laboratory experiments.

The use of metabolic profiling could feasibly be used to measure phosphate availability, as addition of P is known to yield responses in the concentrations of the various amino acids which are different to those observed after the addition of N (Vona *et al.*, 1992). For example, it was found that 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 dissolved inorganic N concentrations can be very close to detection limits, (e.g.,  $0.03 \mu\text{M NO}_3^-$  and  $0.02 \mu\text{M NH}_4^+$ ; Furnas, 1990). 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 the actual nutrient availability for marine plants (Raine and Patching, 1980). The measurement of  $\delta^{15}\text{N}$  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 (e.g., Sweeney and Kaplan, 1980; Van Dover *et al.*, 1992).

The ability of macroalgae such as *G. edulis* to store large amounts of N in their tissue may provide an efficient means of removing nutrients from eutrophic waterways. A particularly useful application may be in the removal of nutrients from point sources such as the wastewater from aquaculture farms, sewage treatment plants, and fertiliser plants, before the effluent is released into the waterways (Trono and Ganzon-Fortes, 1988). This algae could be harvested for commercial use in the production of agar and carrageenin, or perhaps used as fish food in aquaculture farms. Their high internal nutrient reserves also makes them ideal for use in the production of organic fertilisers (Trono and Ganzon-Fortes, 1988).

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 which would normally 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 nitrogen source index, 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, even when water column concentrations were very low.

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TABLE I. Pigments (PE = phycoerythrin; Chl = Chlorophyll *a*), tissue N and amino acids of *Gracilaria edulis* after 3 d in aquaria with control, 200  $\mu\text{M}$   $[\text{NO}_3^-]$ ,  $[\text{NH}_4^+]$  or urea.

Treatment	Phycoerythrin		Tissue Nitrogen	Amino Acids					Free $\text{NH}_4^+$
	PE	Chl		Serine	Glutamic Acid	Alanine	Citrulline	Phenyl-alanine	
	(mg/g dry wt)		(%N dry wt)		(nmol/g wet wt)				
Control	0.9 <sup>a</sup>	1.0	1.9	141	227	85 <sup>ab</sup>	232 <sup>c</sup>	45 <sup>b</sup>	263 <sup>b</sup>
$\text{NO}_3^-$	0.9 <sup>a</sup>	0.6	1.8	151	335	108 <sup>a</sup>	778 <sup>b</sup>	47 <sup>b</sup>	450 <sup>b</sup>
$\text{NH}_4^+$	1.3 <sup>b</sup>	0.7	2.5	199	227	21 <sup>b</sup>	2274 <sup>a</sup>	114 <sup>a</sup>	3901 <sup>a</sup>
Urea	0.9 <sup>ab</sup>	0.9	2.0	108	392	96 <sup>ab</sup>	734 <sup>b</sup>	21 <sup>b</sup>	1263 <sup>b</sup>
F-value	5.5 <sup>*</sup>	1.2	2.8	2.3	4.3 <sup>*</sup>	4.8 <sup>*</sup>	52.9 <sup>***</sup>	10.4 <sup>**</sup>	30.6 <sup>***</sup>

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$

<sup>abc</sup> means with different letters are significantly different at  $p < 0.05$

TABLE II. Dissolved inorganic nutrients (DIN) in water column and pigments (PE = phycoerythrin; Chl = Chlorophyll *a*), 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 Brisbane River Mouth (0 km). Negative = upstream.

Incubation Site	DIN		Pigments		Tissue	Serine	Amino Acids			Free NH <sub>4</sub> <sup>+</sup>	
	[NH <sub>4</sub> <sup>+</sup> ]	[NO <sub>3</sub> <sup>-</sup> /NO <sub>2</sub> <sup>-</sup> ]	PE	Chl	Nitrogen		Glutamic Acid	Alanine	Citrulline		Phenyl-alanine
	(μM)		(mg/g dry wt)		(%N dry wt)		(nmol/g wet wt)				
<b>Brisbane River</b>											
(- 82 km)	11 <sup>cd</sup>	41 <sup>b</sup>	1.03 <sup>ab</sup>	0.31 <sup>d</sup>	2.82 <sup>abc</sup>	22.4	55.2 <sup>e</sup>	6.3 <sup>a</sup>	115.5 <sup>b</sup>	12.5 <sup>b</sup>	101.5 <sup>cd</sup>
(- 32 km)	16 <sup>c</sup>	113 <sup>a</sup>	1.39 <sup>a</sup>	0.36 <sup>cd</sup>	2.94 <sup>a</sup>	13.2	330.7 <sup>a</sup>	222.2 <sup>b</sup>	397.9 <sup>a</sup>	26.1 <sup>a</sup>	102.2 <sup>cd</sup>
(- 4 km)	38 <sup>b</sup>	47 <sup>b</sup>	1.41 <sup>a</sup>	0.41 <sup>bcd</sup>	2.92 <sup>ab</sup>	1.31	196.9 <sup>b</sup>	8.4 <sup>a</sup>	121.5 <sup>b</sup>	28.8 <sup>a</sup>	150.7 <sup>c</sup>
<b>Moreton Bay</b>											
Amity	1.18 <sup>d</sup>	0.88 <sup>c</sup>	0.97 <sup>ab</sup>	1.02 <sup>a</sup>	2.45 <sup>abcd</sup>	10.3	70.9 <sup>de</sup>	5.6 <sup>a</sup>	5.6 <sup>b</sup>	0.0 <sup>c</sup>	5.8 <sup>d</sup>
Dunwich	1.19 <sup>d</sup>	1.17 <sup>c</sup>	0.83 <sup>b</sup>	0.99 <sup>ab</sup>	2.10 <sup>d</sup>	11.3	107.8 <sup>cd</sup>	9.5 <sup>a</sup>	120.4 <sup>b</sup>	0.0 <sup>c</sup>	588.4 <sup>b</sup>
Sed Control	1.21 <sup>d</sup>	0.84 <sup>c</sup>	0.98 <sup>ab</sup>	1.09 <sup>a</sup>	2.24 <sup>bcd</sup>	9.8	153.3 <sup>bc</sup>	8.9 <sup>a</sup>	116.8 <sup>b</sup>	0.0 <sup>c</sup>	496.3 <sup>b</sup>
Sed N+	1.35 <sup>d</sup>	0.86 <sup>c</sup>	1.16 <sup>ab</sup>	1.09 <sup>a</sup>	2.23 <sup>cd</sup>	11.3	181 <sup>b</sup>	85.2 <sup>a</sup>	178.4 <sup>b</sup>	0.0 <sup>c</sup>	594.1 <sup>b</sup>
Sew Pipe	940 <sup>a</sup>	10.7 <sup>c</sup>	1.16 <sup>ab</sup>	0.93 <sup>abc</sup>	3.11 <sup>a</sup>	11.26	84.5 <sup>de</sup>	5.3 <sup>a</sup>	440.4 <sup>a</sup>	0.0 <sup>c</sup>	1224.6 <sup>a</sup>
<b>F value</b>	<b>13434<sup>***</sup></b>	<b>54.8<sup>***</sup></b>	<b>3.81<sup>**</sup></b>	<b>8.54<sup>***</sup></b>	<b>7.62<sup>***</sup></b>	<b>0.93</b>	<b>90.9<sup>***</sup></b>	<b>17.3<sup>***</sup></b>	<b>14.5<sup>***</sup></b>	<b>31.5<sup>***</sup></b>	<b>373<sup>***</sup></b>

\* p ≤ 0.05; \*\* p ≤ 0.01; \*\*\* p ≤ 0.001

abcde means with different letters are significantly different at p < 0.05

TABLE III. Nitrogen Source Index results for laboratory source incubations and field incubations. Bold type figures indicate the highest value for each N source at the incubation sites; laboratory, Brisbane River, and Moreton Bay.

Place of Incubation	Source/ Site	NH <sub>4</sub> <sup>+</sup> Source Index	NO <sub>3</sub> <sup>-</sup> Source Index	Urea Source Index
Laboratory	Control	1.0	1.0	1.0
	NO <sub>3</sub> <sup>-</sup>	14.7	<b>27</b>	0.4
	NH <sub>4</sub> <sup>+</sup>	<b>288</b>	0.6	3.8
	Urea	31	14	<b>4.9</b>
Brisbane	- 82 km	18.6	1.3	1.13
River	- 32 km	1.4	<b>150</b>	1.1
	- 4 km	<b>33</b>	14	<b>7.6</b>
Moreton	Amity	0.2	11	0.01
Bay (+ 27 km)	Dunwich	44	1.3	5.7
	Sed Control	48	1.9	6.3
	Sed N+	8	<b>33</b>	3.
	Sew Pipe	<b>490</b>	0.4	<b>15</b>

## FIGURE LEGEND

Figure I. Map showing the location of the field sites along the Brisbane River and in southern Moreton Bay (off North Stradbroke Island).

Figure II. Water column macroalgal chamber and the sediment macroalgal chamber as used in the field incubations of *Gracilaria edulis*.

Figure III. Responses in pigments, tissue nitrogen and amino acids of *G. edulis* to increasing  $\text{NH}_4^+$  concentration (0 to 80  $\mu\text{M}$  scale).

Figure IV. Responses in pigments, tissue nitrogen and amino acids of *G. edulis* to increasing  $\text{NH}_4^+$  concentration (0 to 800  $\mu\text{M}$  scale).

Figure V. Dissolved inorganic nitrogen concentrations in the Brisbane River, measured from January to April, 1994.

Figure VI. Simplified diagram of the nitrogen assimilation pathways in *G. edulis*. The amino acids marked in bold text were the most responsive to changes in nitrogen source and availability.

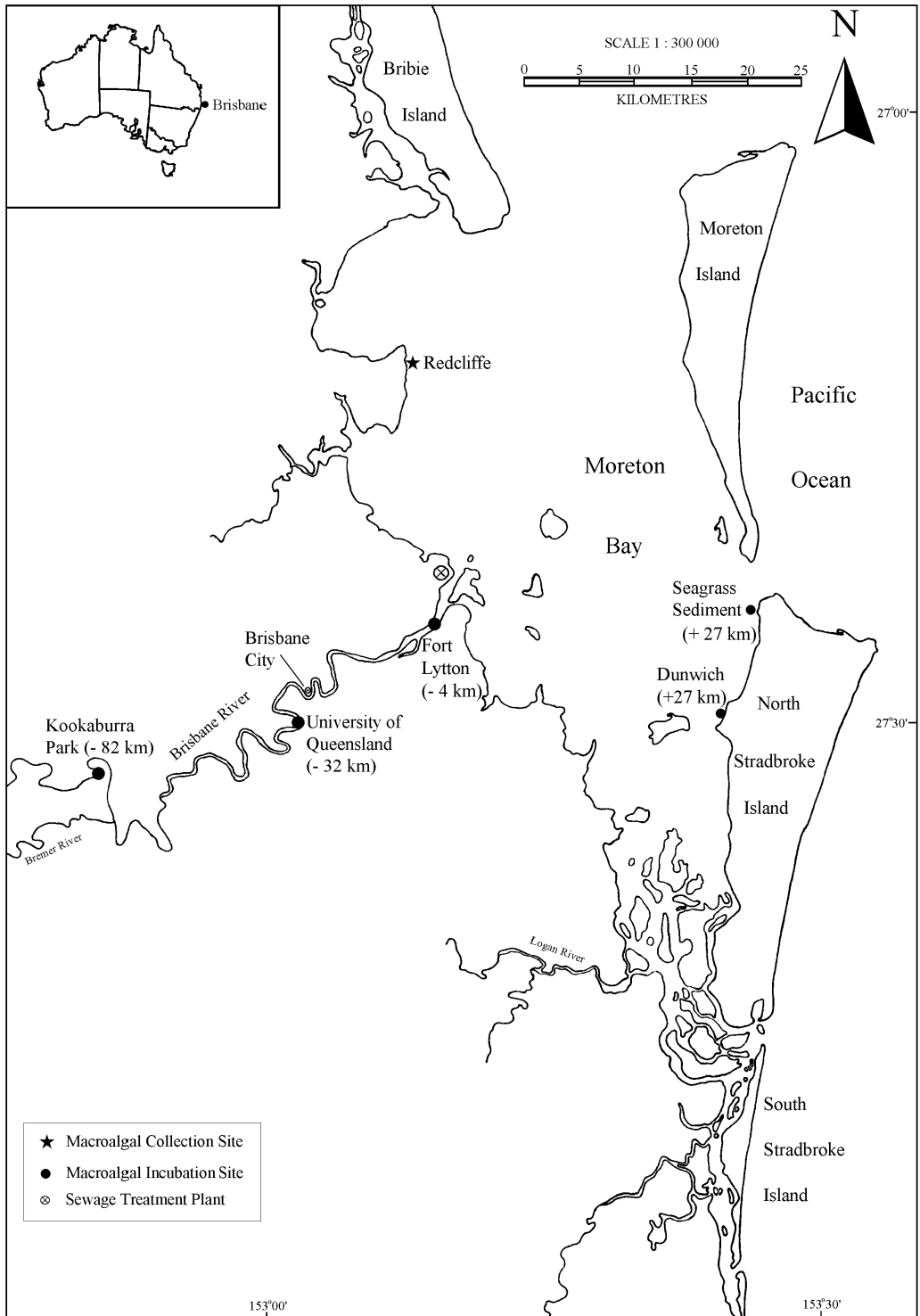


Figure I. Map showing the location of the field sites along the Brisbane River and in Moreton Bay.

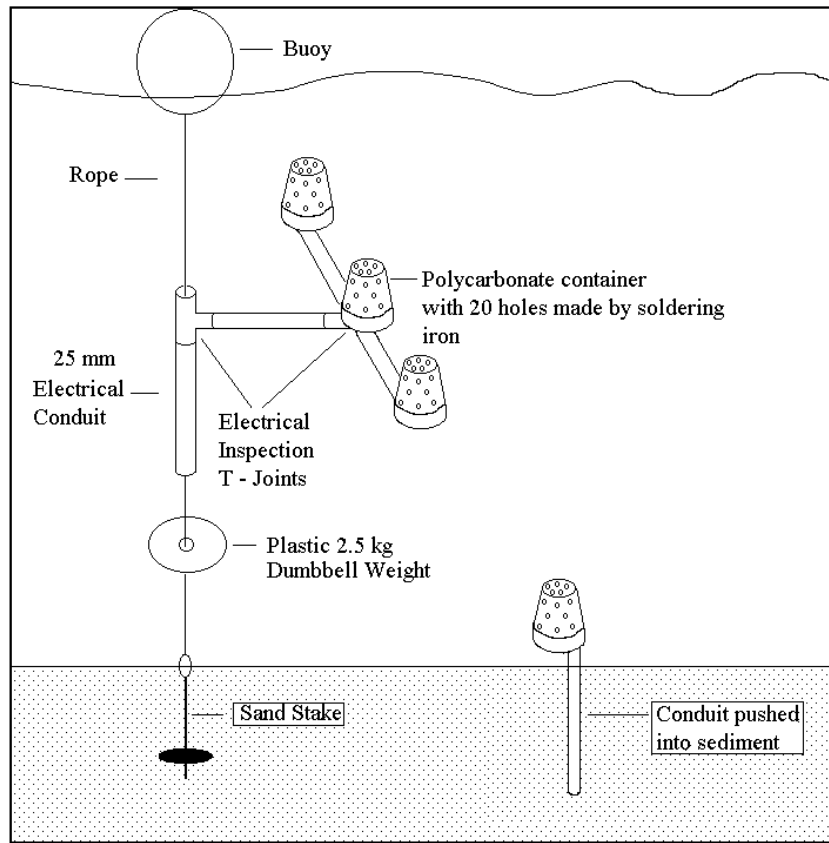


Figure II. Water column macroalgal chamber and the sediment macroalgal chamber as used in the field incubations of *Gracilaria edulis*.

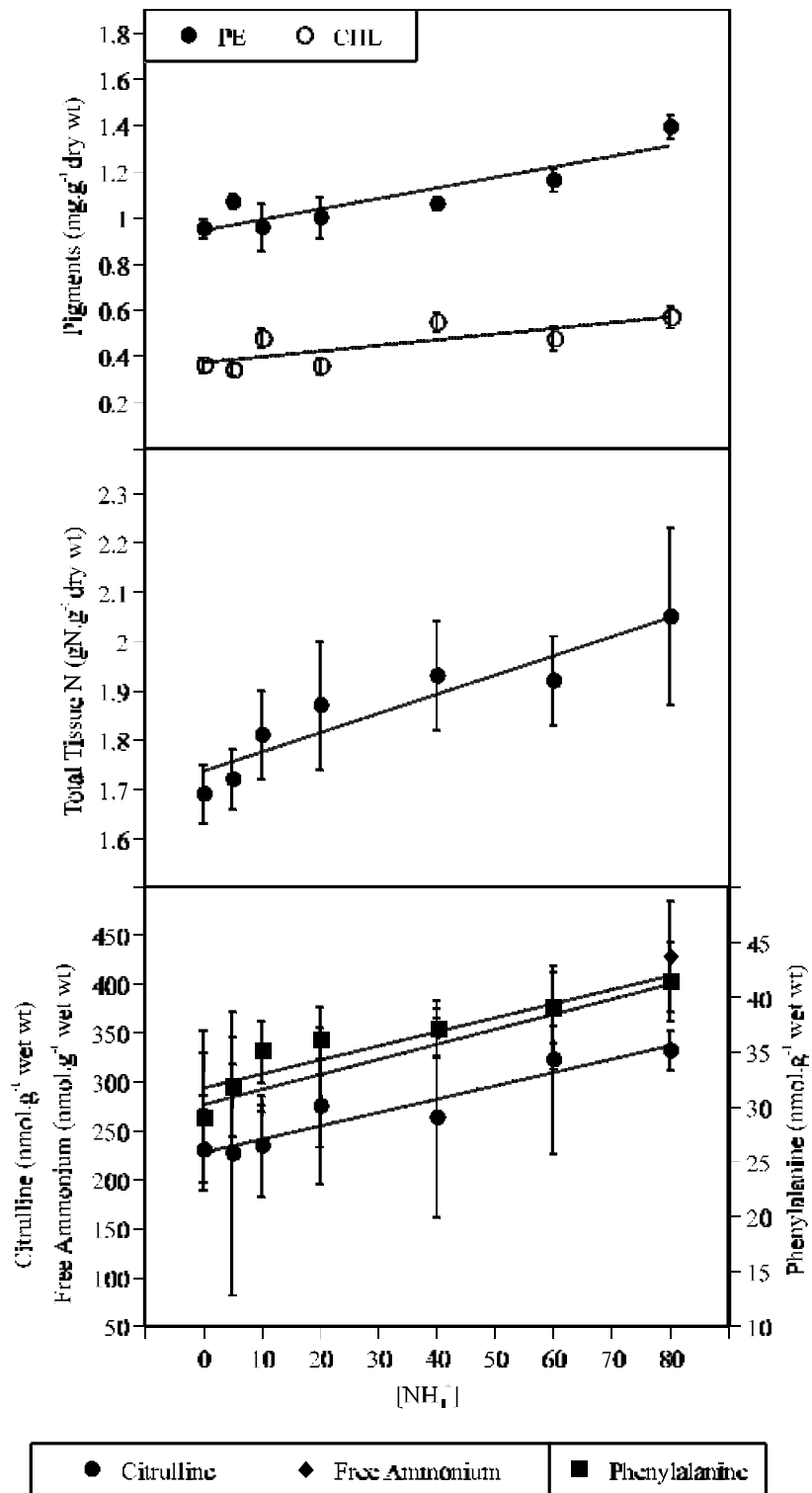


Figure III. Responses in pigments, total tissue nitrogen and amino acids of *Gracilaria edulis* to increasing  $NH_4^+$  concentrations (0 to 80  $\mu M$  scale).

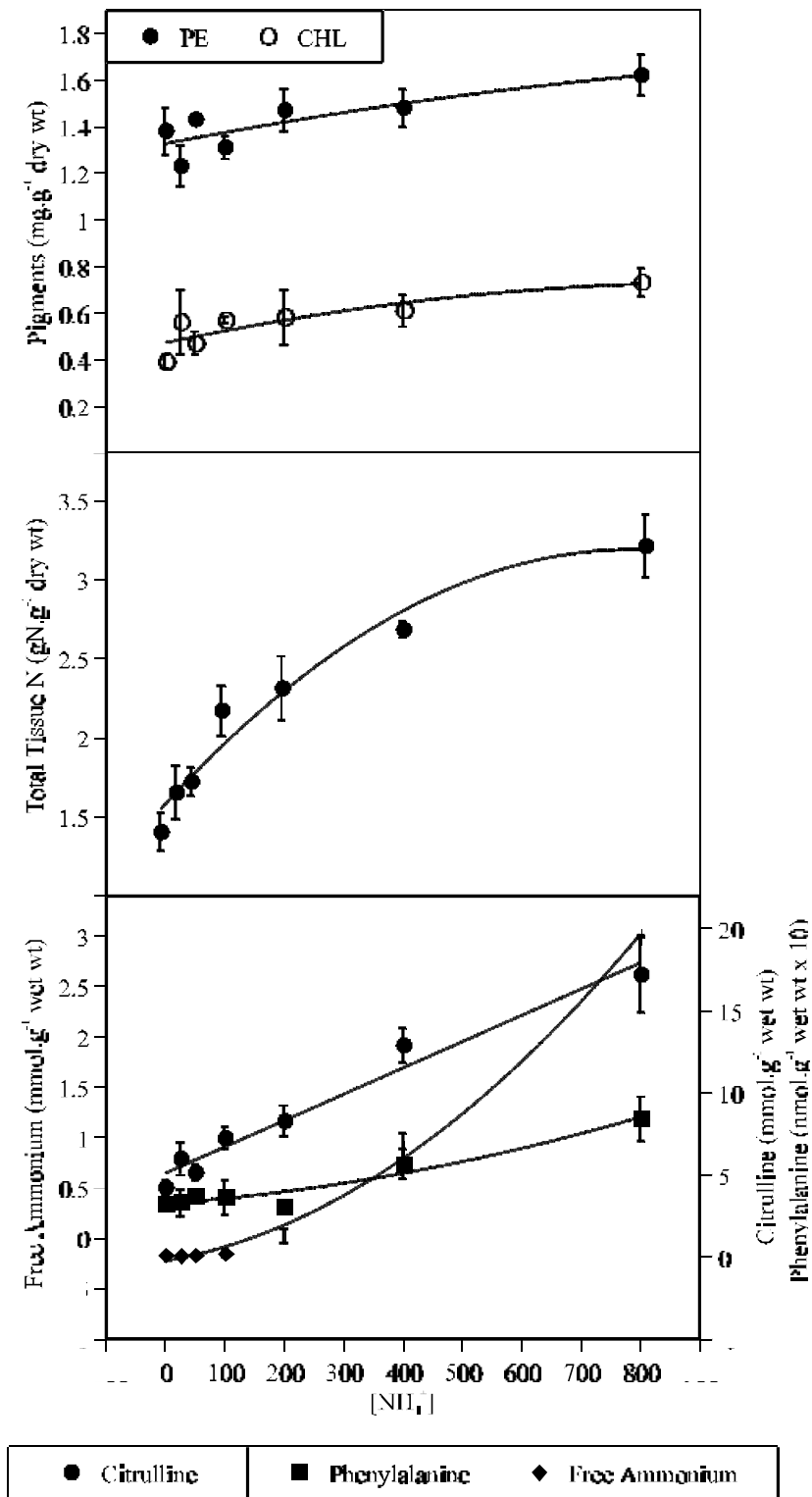


Figure IV. Responses in pigments, total tissue nitrogen and amino acids of *Gracilaria edulis* to increasing  $NH_4^+$  concentrations (0 to 800  $\mu M$  scale).

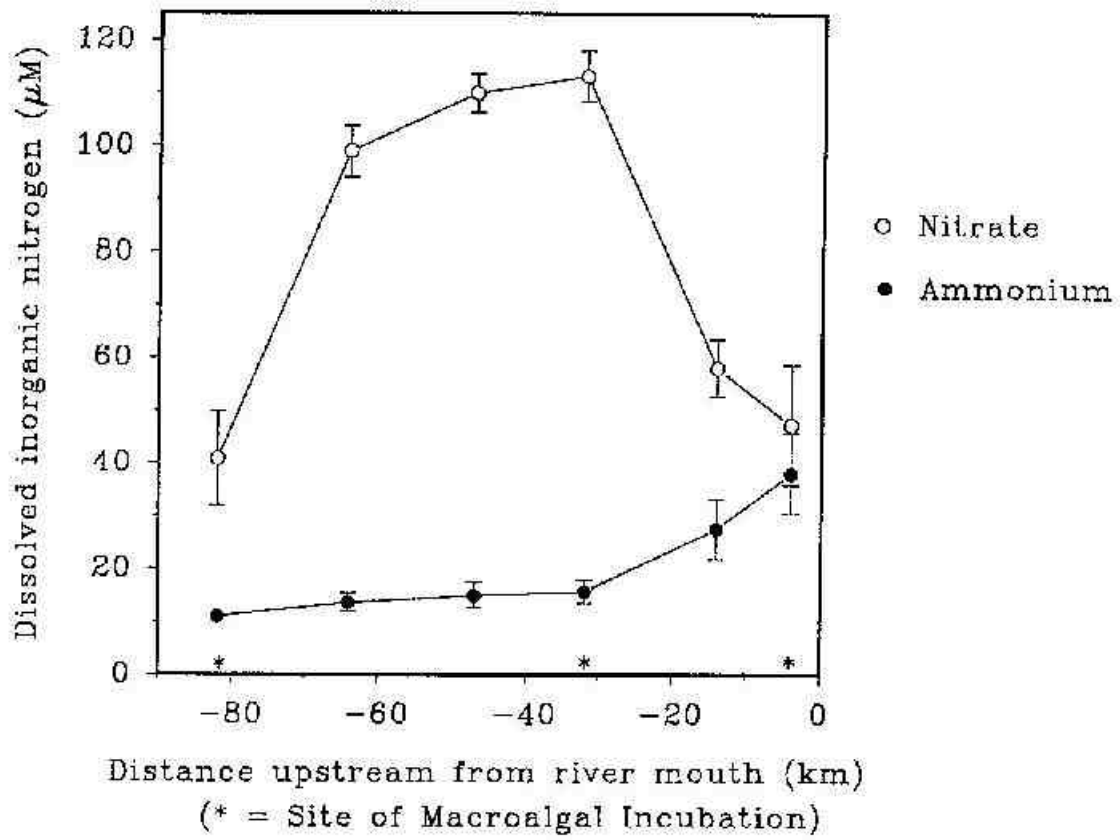


Figure V. Dissolved inorganic nitrogen concentrations in the Brisbane River, measured from January to April, 1994.

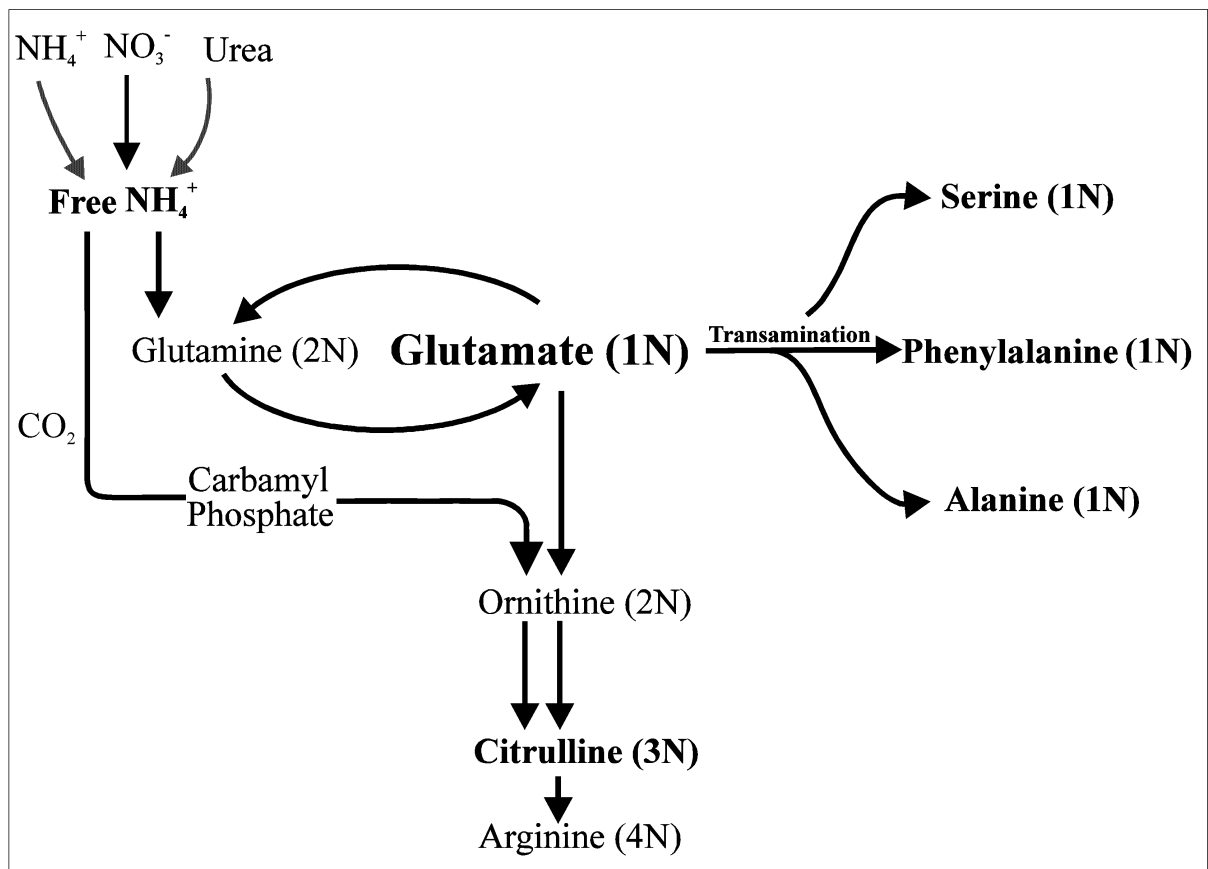


Figure VI. Simplified diagram of the nitrogen assimilation pathways in *G. edulis*. The amino acids marked in bold text were the most responsive to changes in nitrogen source and availability.

## PLATE LEGEND

Plate 1a. Laboratory culture facility showing the depletion aquarium and incubation aquaria.

Plate 1b. Laboratory culture facility showing a polycarbonate container containing *Gracilaria edulis* being incubated.

Plate 2a. Water column macroalgal chamber as used on North Stradbroke Island and in the Brisbane River.

Plate 2b. Sediment macroalgal chamber as used on the fertilised seagrass beds at Amity Point and next to the sewage pipe at Dunwich, both on North Stradbroke Island.

Plate 3a. Pontoon at the University of Queensland incubation site to which the macroalgal chamber was attached.

Plate 3b. Variations in the thallus colour of *Gracilaria edulis* as a result of differences in nitrogen availability.

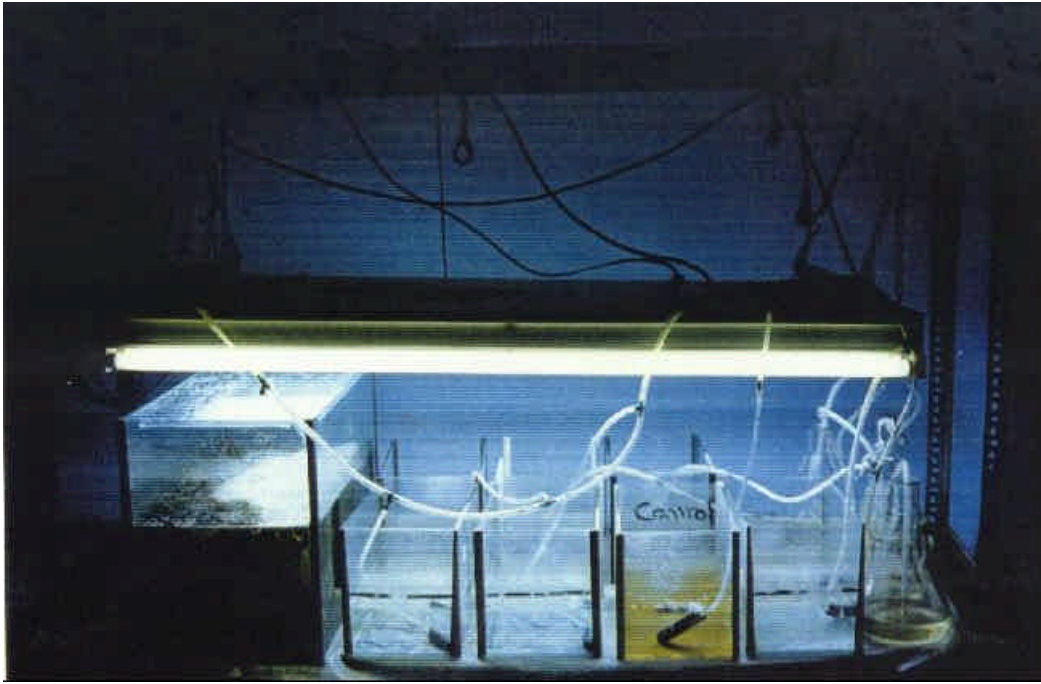


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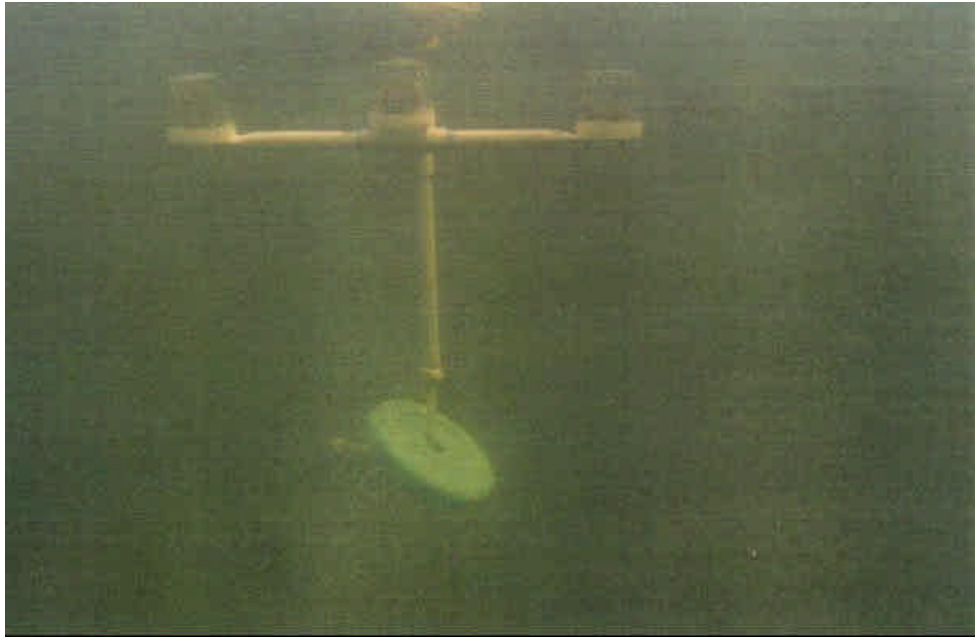


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