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Tissue Nutrient Content of *Gracilaria* spp. (Rhodophyta) and Water Quality along an Estuarine Gradient

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Abstract. Tissue nutrient content of *Gracilaria* spp. (Rhodophyta) was tested as a bioindicator of water column nutrient availability in the Logan River and southern Moreton Bay, south-eastern Queensland. Macroalgae were incubated for one to two weeks within flow-through incubation chambers suspended in the water column. Tissue nutrient content of *Gracilaria* spp. and water column nutrients were measured at five sites over a five-month period. Tissue nitrogen content (%N) was correlated with dissolved inorganic nitrogen (DIN) at a site 15 km upstream from the Logan River mouth ($r^2 = 0{\cdot}81$), at the Logan River mouth ($r^2 = 0{\cdot}50$), and at a Moreton Bay site 8 km from the Logan River mouth ($r^2 = 0{\cdot}71$). Time-course analyses of water column nutrients and plant tissue content showed more significant correlations with nitrogen (N) than with phosphorus (P). Plant tissue nitrogen-to-phosphorus (N:P) molar ratios ranged between 19 and 23 whereas water column N:P ratios were between 2 and 6, suggesting low nitrogen availability relative to plant requirements and possible N limitation.

In the laboratory, *Gracilaria verrucosa* was subjected to treatments of N, P or N + P nutrient additions. Deepening of the thallus colouration was observed after additions of N. Chlorophyll and phycoerythrin concentrations increased in treatments with N addition; however, owing to wide variability between phycoerythrin replicates, only chlorophyll increases were significant. The amino acid citrulline also increased with the addition of N and accounted for up to 16% of the total tissue N. Macroalgae may be more useful than traditional water quality sampling for integrating biologically available pulses of nutrients, especially for a limiting nutrient such as N in coastal marine ecosystems.

Introduction

Eutrophication of coastal waters caused by nitrogen (N) and phosphorus (P) has made it relevant to study the extent to which these two nutrients control macroalgal growth (Valiela 1984, chapters 1 and 2). The question of N or P limitation in coastal, estuarine waters is important in resource management and in developing nutrient reduction strategies for the control of cultural eutrophication. Monitoring of nutrient availability through the use of aquatic macrophytes is a potential method of evaluating the significance of N and P as potential growth-limiting factors (Gerloff and Krombholz 1966; Gerloff and Fishbeck 1973; Chapman and Craigie 1977; Birch *et al.* 1981; Lapointe 1987; Lyngby 1990).

The use of bioindicators such as macroalgae to infer changes in habitat characteristics is based on the ability of the plants to reflect water column concentrations of a preferred nutrient form (Fujita 1985; Hwang *et al.* 1987). This may give more relevant results than do chemical water measurements, which probably do not assess actual availability of a particular nutrient for plant growth (Lyngby 1990). Tissue nutrient composition also integrates the nutrient regime over a time period prior to sampling and thus is likely to provide a more reliable indicator of nutrient status (Wheeler and Bjornsater 1992). Macroalgal tissue composition can be used to detect and integrate pulses of nutrients, which may go unreported by fortnightly to monthly sampling in typical water quality monitoring programmes (Lapointe 1985).

The algae are unique in the plant kingdom in having a variety of photosynthetic pigments in contrast to higher plants, which use chlorophyll *a* and *b* only (Rowan 1989, pp. 1–65). Red macroalgae contain several phycobiliproteins, including the red pigment R-phycoerythrin, which is an accessory photosynthetic pigment to chlorophyll. Phycobilisomes have been shown to increase in number in the presence of nitrogen (Lapointe 1981; Kursar and Alberte 1983), acting as a N store that is utilised when the thalli become N limited (Lapointe and Rythe 1979; Gantt 1981; Lapointe 1981; Ryther *et al.* 1981; Bird *et al.* 1982; Kursar and Alberte 1983). In addition, N allocation and storage also occur in amino acid and protein pools of red algae (Gantt 1981; Rigano *et al.* 1992; Vona *et al.* 1992), and the concentrations of certain amino acids are dependent upon nutrient availability (Bird *et al.* 1982).

The rhodophytes *Gracilaria* spp. have been used in a variety of studies investigating nutrient limitation and storage (DeBoer *et al.* 1978; D'Elia and DeBoer 1978; Lapointe and Ryther 1979; Ryther *et al.* 1981; Bird *et al.* 1982; Rosenberg and Ramus 1984; Fujita 1985; Lapointe 1985; Huang *et al.* 1987). Several marine species of *Gracilaria* are abundant and have widespread distributions in Moreton Bay. In addition, *Gracilaria verrucosa*, an

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estuarine species, is common in the rivers of south-eastern Queensland. In the present study, *Gracilaria* spp. were used to investigate water quality of the Logan River and southern Moreton Bay.

Materials and Methods

Study Area

Moreton Bay is located on the eastern coast of Australia, adjacent to Brisbane, Queensland, in a subtropical climate. The bay is bordered by the mainland to the west, sand islands to the east, and several vegetated mud islands to the south. The Logan River is a major river in south-eastern Queensland that drains from the Great Dividing Range and McPherson Range in the south-west and empties into southern Moreton Bay. The catchment area of the Logan River extends far beyond the sites in this study to encompass an area of 3650 km^2.

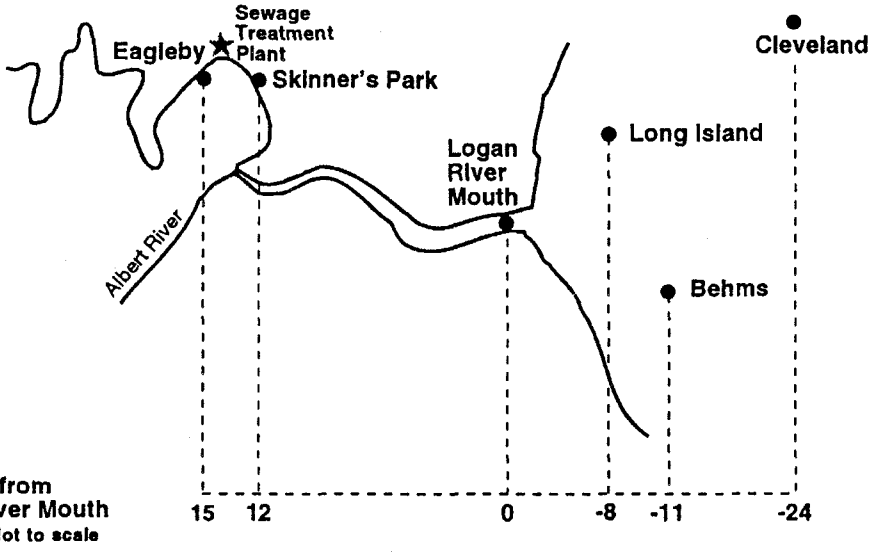
Five study sites were positioned along the Logan River and into southern Moreton Bay, providing a transect from areas of low salinity (0–14), high turbidity (total suspended solids, TSS, 25–104 mg L^{-1}) and shallow Secchi depths (0·15–0·77 m) to areas in the bay with more saline conditions (35–38), lower turbidity (TSS, 8–32 mg L^{-1}) and deeper Secchi depths (0·71–1·78 m). Sites were referred to as distances from the mouth of the Logan River (0 km), with positive values upstream and negative values out into Moreton Bay (Fig. 1).

15 km (Eagleby). The Logan River site was within the tidal influence and experienced wide fluctuations of salinity (0–14), depending on rainfall and tidal exchange. This site was approximately 1 km upstream of a large sewage treatment plant. The first two samplings in the field were from a site 12 km from the mouth of the Logan River and downstream of the sewage treatment plant (Skinner's Park), but subsequently the site was moved upstream 3 km for better accessibility (Fig. 1).

0 km (Logan River Mouth). The Logan River Mouth site was positioned 2 km upstream from the intersection of the river with the main channel in southern Moreton Bay. This area received strong tidal currents, producing very turbid conditions in the relatively shallow waters. Salinity at this site was variable (16–36) and depended primarily on upstream rainfall.

–8 km (Long Island). The position of the bay site closest to the river was over a large mud bank, north-east of the river mouth. This site was under the influence of the river plume, which travels north in Moreton Bay (Moss *et al.* 1992). Patchy areas of short, intertidal seagrass were present adjacent to the site.

–11 km (Behm's Creek). This site was positioned south of the river mouth in relatively protected waters surrounded by numerous mangrove islands. Small, patchy areas of intertidal seagrass were present adjacent to the site.



Latitude	27°41.60'S	27°42.20'S	27°38.81'S	27°45.90'S	27°32.03'S
Longitude	153°11.95'E	153°19.32'E	153°21.21'E	153°22.05'E	153°18.40'E
Salinity (PSS)	5 (0-14)	30 (16-36)	36 (35-38)	36 (35-37)	36 (35-38)
Secchi Depth (m)	0.15	0.77	0.71	0.90	1.78
Total Suspended Solids (mg/l)	104	25	32	20	8
Macrophytes	estuarine algae	none	seagrass	seagrass	seagrass

Fig. 1. Study transect and environmental parameters of Logan River and southern Moreton Bay sites.

–24 km (Cleveland). This site was the northern-most site and was situated in relatively open waters of the bay. Extensive intertidal and subtidal seagrass beds were present in the area and were used by dugongs as feeding grounds.

Collection of Gracilaria spp.

Approximately 40 g of *Gracilaria* spp. (*G. blodgetti* Harvey, *G. compressa* (Agardh) Greville, *G. cylindrica* Boergesen) were collected monthly from the Toondah Harbour/Oyster Bay area near the –24 km (Cleveland) site. These algae were used at the Moreton Bay sites, i.e. –11 km (Behm's Creek), –8 km (Long Island) and –24 km (Cleveland), and also at the Logan River Mouth site (0 km). Macroalgae were collected by snorkelling in 1 to 1·5 m water depth and by raking the bottom at low to mid tide and selecting the *Gracilaria* from the mixture of species. *Gracilaria* spp. were found to be extremely patchy and seasonal in distribution, with the greatest amount found in spring to early summer. Other species found growing with *Gracilaria* spp. included *Hypnea cervicornis* Agardh, *Hypnea spinella* (C. Agardh) Kuetzing, and to a lesser extent *Caulerpa* spp. Lamouroux, *Lobophora variegata* (Lamouroux) Womersley, and the seagrass *Halophila spinulosa* (R.Br.) Aschers. In mid summer very little *Gracilaria* was available, and when it was found it was entangled with *Hypnea* spp. Lamouroux in very shallow muddy waters.

The macroalga used for the 15 km (Eagleby) river site (*Gracilaria verrucosa* Hudson) was collected monthly from the base of a floating pontoon in the Brisbane River at the University of Queensland. This *Gracilaria* species also appeared to be seasonal in abundance, with very few algae present during summer. *G. verrucosa* has been noted as being extremely tolerant to low salinity ranges (May 1948) and thus appeared to be well suited to the 15 km (Eagleby) site.

Culture

All of the macroalgae collected were transported in 25-L carboys to a laboratory algal culture facility with glass aquaria containing freshly collected water from each of the respective collection sites. The water from the Logan River and Brisbane River was filtered through a GF/A glass-fibre filter in a Nalgene filtering apparatus to remove suspended solids.

Every six weeks, nutrients were added to the culture water to final concentrations of 140 μM NH_4Cl and 8 μM NaH_2PO_4. The temperature of the culture water was kept fairly constant at 19–23°C. The culture tanks were constantly aerated, and 12 Triton and Aktenic fluorescent lights provided 250 μmol m^{-2} s^{-1} (PAR, photosynthetically active radiation) in a 12:12 light:dark cycle.

Macroalgal Growth Chambers

Submersible macroalgal growth chambers were used for 1-week or 2-week incubations of macroalgae *in situ*. Growth chambers were made of clear, slitted, 150-mL polycarbonate containers inverted onto their lids, connected by cable ties to 25-mm electrical conduit. PVC junctions were used to join the frame. A buoy was joined to the frame and then to an anchor by 8-mm nylon rope. A car tyre rim was used as an anchor in soft, muddy sediment, and variable-length sand stakes were screwed into the sediment at sandy or vegetated sites. In the bay and at the river mouth, the submersible-chamber frames and the connected buoy and anchor were positioned with the aid of SCUBA. At the 15 km (Eagleby) site, macroalgal growth chambers were connected by a rope to a floating pontoon. All macroalgal chambers were positioned at half the average Secchi depth, resulting in macroalgal chamber depths of: 15 km (Eagleby), 0·08 m; 0 km (Logan River Mouth), 0·40 m; –8 km (Long Island), 0·35 m; –11 km (Behm's Creek), 0·45 m; –24 km (Cleveland), 0·90 m. The average light availability at half Secchi depth was 1270 μmol m^{-2} s^{-1} (PAR) at midday. However, owing to rapid colonization of microalgae and settling of sediment both on and in the macroalgal chambers, light availability within the chambers decreased to a midday value of 250 μmol m^{-2} s^{-1} (PAR), approximately the same light availability as provided at the culture facility.

Macroalgal specimens were changed at each sampling interval and replicate water samples were obtained from 1 m water depth. Water samples were filtered through a GF/F glass-fibre filter in a Nalgene filtering apparatus, transferred to 120-mL polycarbonate sample bottles, and immediately placed on dry ice for later analysis. Incubations were conducted during the summer months of October 1992 to February 1993.

A combination of *Gracilaria* spp. was used to make up the required biomass for each replicate at each bay site and for each incubation period. These species of *Gracilaria* were found mixed together in the field and this habit was maintained to avoid destroying the integrity of the species assemblage. *Gracilaria verrucosa* was cultured and incubated for the 15 km (Eagleby) river site.

Incubated algae were washed with deionized water, blotted dry for wet weight, and oven-dried for dry weight (60°C, 72–96 h). Originally, the algae were incubated for two weeks, but reductions in light availability due to fouling and sediment loads led to shorter incubation times (one week).

Analysis of Macroalgal Tissue

The dry algae were ground into a fine powder before Kjeldahl digestion; digested samples were stored at 4–6°C until analysis. N and P in replicate samples of standard leaf tissue were analysed with a Chemlab Mark 7 autoanalyser (N, ±0·21%; P, ±0·02%).

Water Column Nutrients

The analyses of dissolved inorganic nutrients (NO_3^-, NH_4^+, PO_4^{3-}) used a Skalar autoanalyser, with measurement errors estimated as ±0·143 μM for N and ±0·064 μM for P. Dissolved inorganic nitrogen (DIN) was taken as the sum of NO_3^- and NH_4^+ concentrations, and dissolved inorganic phosphorus (DIP) was taken as the PO_4^{3-} concentration.

Laboratory Experiment

Gracilaria verrucosa was collected from the Brisbane River and cultured for two weeks in the laboratory. Brisbane River water was used as the basic media (control), with treatments of 1400 μM NH_4Cl, 80 μM NaH_2PO_4 or 1400 μM NH_4Cl + 80 μM NaH_2PO_4. Specimens were placed in 1-L glass jars containing the various media and were grown under aerated conditions in a glasshouse. After the two-week incubation, the algal samples were weighed and divided up for pigment, nutrient and amino acid analyses.

Pigment Analysis

Phycoerythrin and chlorophyll content was determined from homogenates of 0·5–1·0 g of *Gracilaria verrucosa* tissue with the aid of 0·01 M phosphate buffer (pH 6·5) and an Ultra Turrax homogenizer. The homogenate was centrifuged at 426 *g* for 15 min, and optical density of the supernatant was recorded with a Hitachi U-1100 spectrophotometer at 565 nm (Rowan 1989, pp. 166–211).

Chlorophyll concentration was determined from the pellet from the phycoerythrin sample. Cold 80% acetone (2 mL) was added to the pellet and an Ultra Turrax homogenizer used to disrupt the cells. After centrifugation at 426 *g* for 15 min, the supernatant was transferred to a spectrophotometer cuvette. Absorbances were measured at wavelengths of 710 nm (turbidity) and 664 nm (maximum absorption of chlorophyll *a*), with acetone as a blank (Parsons *et al.* 1984, pp. 3–24).

Amino Acid Analysis

The wet weight of 0·5–1·0 g of *Gracilaria verrucosa* was recorded and the tissue then added to a graduated centrifuge tube. Cold methanol (2 mL) was added and an Ultra Turrax homogenizer used to disrupt the cells. The homogenate was centrifuged at 426 *g* for 15 min and the supernatant transferred to a vial and stored at 4°C for amino acid analysis with a Beckman amino acid analyser.

Statistical Analysis

Means and standard errors of a range of parameters were calculated for each field site and laboratory treatment. One-way analysis of variance (ANOVA) and Tukey's test for multiple comparison of means were used to test for differences among field sites and laboratory treatments.

Results

Growth

Tissue losses resulting in negative growth rates were recorded for all sites when tissue wet weight at Time 0 was compared with tissue wet weight at the end of incubation periods. As a result, specific growth rates at all sites were generally negative. The *Gracilaria* within the macroalgal chambers received between 8% and 40% of the total light availability during the incubation, with the average value tending towards 8% owing to rapid fouling rates; therefore, photosynthesis was probably light limited.

Temporal Relationships

Tissue N corresponded with trends of water column DIN at Logan River sites, 15 km (Eagleby) and 0 km (Logan River Mouth) (Fig. 2). The two river sites recorded both relatively high water column DIN and tissue N at the beginning of the study in October, with DIN and tissue N gradually decreasing until December to early January and then increasing in late January to early February. The two bay sites closest to the river mouth, –8 km (Long Island) and –11 km (Behm's Creek), had tissue N concentrations that generally paralleled DIN concentrations but fluctuated widely. At the other bay site, –24 km (Cleveland), there was little correspondence between tissue N and DIN. In contrast, no distinct correspondence between water column DIP and tissue P was observed at any of the river or bay sites (Fig. 3).

Macroalgal and Water Column Nutrients

Water column nutrients, macroalgal tissue contents, correlation coefficients, and molar N:P ratios were compared at the five study sites (Table 1). Mean DIN values followed a general trend from relatively high DIN in the Logan River (24–57 μM) to low DIN in Moreton Bay (<3 μM). Similarly, DIP values decreased from 10 μM in the Logan River to 0·6 μM in Moreton Bay. Bay sites –8 km (Long Island) and –11 km (Behm's Creek) recorded similar values for DIN and DIP (DIN: 2·9 μM and 2·1 μM, respectively; DIP: 1·0 μM and 1.0 μM) and for tissue N and P (N: 1·1% and 1·4%; P: 0·2% and 0·2%). Tissue N content values were correspondingly high at the two river sites, and regression coefficients (r^2) for DIN and tissue N were

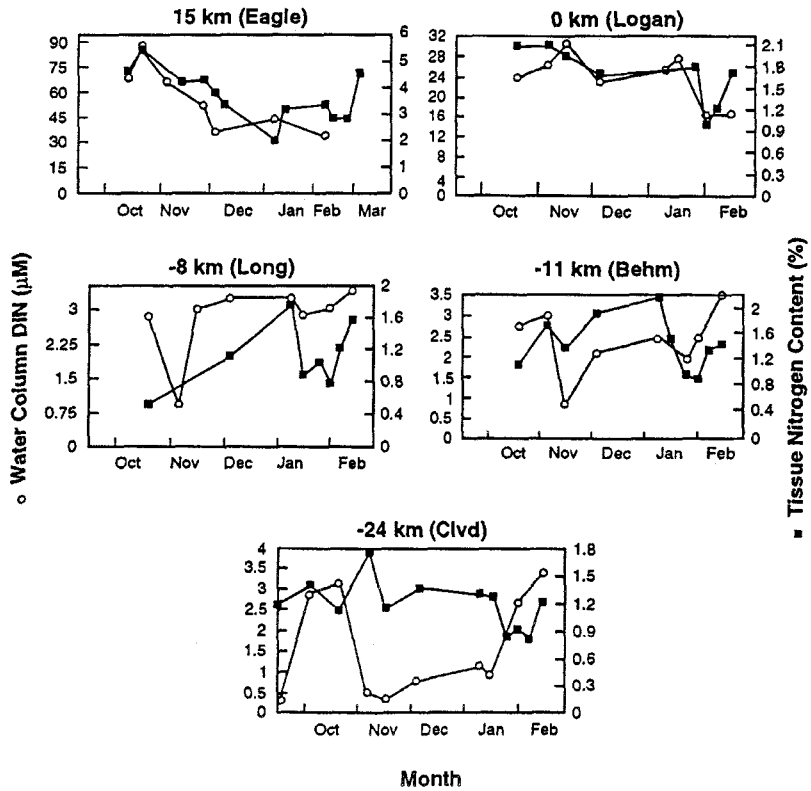


Fig. 2. Mean concentrations of dissolved inorganic nitrogen (μM) and *Gracilaria* spp. tissue nitrogen content (%) over five months (October–February).

calculated for 15 km (Eagleby), 0 km (Logan River Mouth) and –8 km (Long Island) as 0·81, 0·50 and 0·71, respectively ($P < 0{\cdot}001$). Regression coefficients (r^2) between water column DIP and tissue P were low ($r^2 \leq 0{\cdot}17$).

Correlations between algal nutrient content and water column concentrations were significant only for N, not for P; which could be due to N limitation of growth resulting from the relative availability of N *v.* P or to the ability of the plant to store greater concentrations of N than of P.

Water column N:P ratios (DIN:DIP) were also higher at the river sites (5·7–6·2) that at the bay sites (2·1–2·9). However, these were all much lower than tissue N:P ratios,

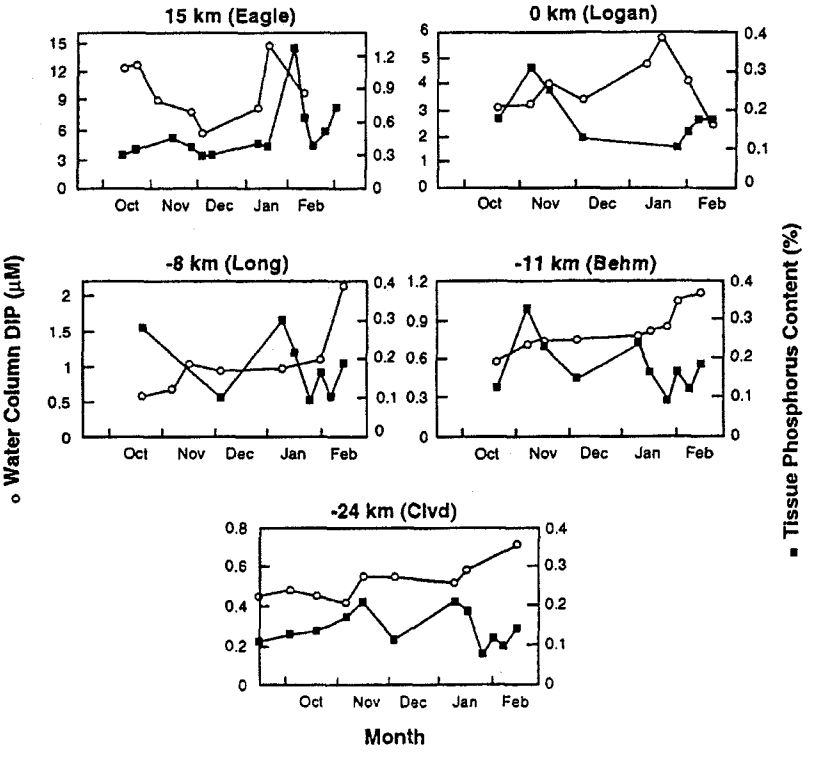


Fig. 3. Mean concentrations of dissolved inorganic phosphorus (µM) and *Gracilaria* spp. tissue phosphorus content (%) over five months (October–February).

Table 1. Nitrogen (N) and phosphorus (P) concentrations in water and *Gracilaria* spp. tissue in the Logan River estuary

Distances relative to the mouth of the Logan River; dissolved inorganic nitrogen (DIN) and phosphorus (DIP) concentrations in water; percent total N and P and molar ratio in tissue; exposure time one to two weeks; mean values (standard errors) over five months (October–February); total $n = 202$; ^{abc}means with different letters are significantly different at $P < 0{\cdot}001$; $^{*}P < 0{\cdot}001$

Study site	DIN (µM)	Tissue nitrogen (%N)	r^2 value, DIN *v.* %N	DIP (µM)	Tissue phosphorus (%P)	r^2 value, DIP *v.* %P	Water N:P ratio (DIN:DIP)	Tissue N:P ratio (mol N:mol P)
+15 km (Eagle)	57^{a} (6·1)	3·6^{a} (0·3)	0·81	10^{a} (1·0)	0·5^{a} (0·1)	0·17	5·7^{a} (7·1)	20·0 (0·3)
0 km (Logan)	24^{b} (1·6)	1·7^{b} (0·1)	0·50	3·9^{b} (0·4)	0·2^{b} (0·02)	0·00	6·2^{a} (1·4)	22·7 (0·2)
–8 km (Long)	2·9^{c} (0·3)	1·1^{b} (0·1)	0·71	1·0^{c} (0·2)	0·2^{b} (0·02)	0·08	2·9^{b} (0·3)	18·8 (0·2)
–11 km (Behm)	2·1^{c} (0·2)	1·4^{b} (0·1)	0·07	1·0^{c} (0·1)	0·2^{b} (0·02)	0·05	2·1^{b} (0·3)	20·0 (0·2)
–24 km (Clvd)	1·4^{c} (0·4)	1·2^{b} (0·1)	0·28	0·6^{c} (0·0)	0·1^{b} (0·01)	0·00	2·3^{b} (0·4)	21·0 (0·1)
F-value	75·87*	25·85*		139·84*	9·79*		10·90*	0·44

which ranged from 18·8 to 22·7, at the five sites. N:P ratios of tissue had less variability than did water column N:P ratios, indicating that tissue concentrations may be buffered better than water column nutrient concentrations.

Other species of macroalgae of the Rhodophyta and Phaeophyta from Moreton Bay showed tissue N and P content similar to that of the *Gracilaria* spp. at corresponding sites in the present study. Differences between the N:P ratios of the red and brown macroalgae were recorded and may be due to taxonomic or life-history differences (Niell 1976): *Chondria* sp. C. Agardh (Rhodophyta) collected from –11 km (Behm's Creek), N = 2·96%, P = 0·32%, N:P = 9·4; *Hypnea spinella* (Rhodophyta) collected from –11 km (Behm's Creek) and –24 km (Cleveland), N = 2·18%, P = 0·24%, N:P = 9·3; *Ectocarpus* sp. Lyngbye (Phaeophyta) collected from –8 km (Long Island) and –24 km (Cleveland), N = 2·27%, P = 0·40%, N:P = 5·7; *Hydroclathrus clathratus* (C. Agardh) Howe (Phaeophyta) collected as drift near –24 km (Cleveland), N = 2·49%, P = 0·14%, N:P = 17·4.

Laboratory Experiment

The relationships between *Gracilaria verrucosa* tissue N and P content and the respective N and P additions to the media were highly significant ($P < 0·001$) (Table 2). *G. verrucosa* also responded to the addition of N by increasing concentrations of photosynthetic pigments, but this was significant only for chlorophyll. Thallus colouration changed from light green to red-brown after addition of N. Phycoerythrin concentration increased from 0·18 mg g^{-1} dry wt in the control to 0·32 mg g^{-1} dry wt in the +N treatment. The concentration of chlorophyll with N addition also increased: from 0·04 mg g^{-1} dry wt (control) to 0·56 mg g^{-1} dry wt (+N) and 0·11 mg g^{-1} dry wt (+N+P). The percentage of N as free amino acids increased significantly ($P < 0·001$) in response to N availability from 10·5% (control) to 17·6% (+N) and 18·0% (+N+P). The percentage of tissue N existing as the amino acid citrulline also increased significantly ($P < 0·001$) with the N addition from 8·9% (control) to 13·9% (+N) and 16·4% (+N+P). Citrulline represented 70–90% of the total tissue %N in free amino acids within *Gracilaria verrucosa*.

Discussion

Tissue N:P ratios (18·8–22·7) were much higher than the DIN:DIP ratios of the water column (2·1–6·2). The water column DIN:DIP ratios deviate substantially from the oceanic Redfield molar ratio of N:P = 16:1 (Redfield 1958; Atkinson and Smith 1983) probably as a result of freshwater inputs and point-source nutrients (Wheeler and Bjornsater 1992). The discrepancy in molar N:P ratios of *Gracilaria* spp. plant tissue relative to nutrient availability indicates potential N limitation of growth. The macroalgal tissue content of N and P also indicates potential N limitation. The values of %P (0·1–0·5) are higher than typical tropical and subtropical macroalgae values (Lapointe *et al.* 1992), but the values of %N (1·1–1·7, except at the +15 km site) are similar to those of other tropical and subtropical macroalgae (Lapointe *et al.* 1992).

Deviations in phytoplankton composition from the Redfield ratio (N:P = 16:1) are often used to infer nutrient limitation (Atkinson and Smith 1983; Wheeler and Bjornsater 1992). The range of N:P ratios in the present study (18·8–22·7) was within the overall range for macroalgae. A survey of benthic marine macroalgae and seagrasses worldwide established a median N:P ratio of 30:1 (Atkinson and Smith 1983), with *Gracilaria* spp. ranging from 16 to 124 (Atkinson and Smith 1983; Lapointe *et al.* 1992).

Table 2. Laboratory experiments on the effects of adding nitrogen and phosphorus to river water on tissue nitrogen, phosphorus, pigments and amino acids of *Gracilaria verrucosa*

Control, Brisbane River water; Phy, phycoerythrin; Chl, chlorophyll; duration, two weeks; means with (standard errors); total $n = 202$; ^{abc}means with different letters are significantly different at $P < 0·001$; $^{*}P < 0·001$

Treatment	Tissue nutrients		Pigments		Amino acids	
	%N	%P	Phy (mg g^{-1} dry wt)	Chl (mg g^{-1} dry wt)	%N as amino acids	%N as citrulline
Control	1·85^{a}	0·24^{a}	0·18	0·04^{a}	10·5^{a}	8·9^{a}
	(0·19)	(0·03)	(0·03)	(0·01)	(7·40)	(0·40)
+N	4·78^{b}	0·32^{ab}	0·32	0·56^{b}	17·6^{b}	13·9^{b}
	(0·42)	(0·03)	(0·07)	(0·05)	(18·02)	(0·64)
+P	1·89^{a}	0·51^{bc}	0·22	0·08^{a}	11·5^{a}	8·1^{a}
	(0·14)	(0·07)	(0·01)	(0·03)	(2·84)	(0·32)
+N+P	4·03^{b}	0·43^{c}	0·20	0·11^{a}	18·0^{b}	16·4^{b}
	(0·53)	(0·05)	(2·24)	(0·03)	(11·06)	(1·05)
F-value	14·60*	11·42*	2·14	24·42*	39·21*	38·27*

N:P ratios of macroalgae also vary as a function of taxonomic affinity (Niell 1976) and seasonality of nutrient availability (Chapman and Craigie 1977; Lapointe 1987, 1989). Macroalgal tissue nutrient content has been used in other seasonal studies to infer nutrient limitation: *Laminaria longicruris* (Phaeophyta), N limitation during summer in Nova Scotia, Canada (Chapman and Craigie 1977); *Cladophora* sp. (Chlorophyta), P limitation in the Peel–Harvey estuarine system, Western Australia (Birch *et al.* 1981); *Gracilaria tikvahiae* (Rhodophyta), P limitation during summer in the Florida Keys (Lapointe *et al.* 1987); *Ceramium rubrum* (Rhodophyta), P limitation in spring and N limitation during summer in Limfjord, Denmark (Lyngby 1990).

Reservoirs of protein pigment complexes of plants that harvest light may be affected by both N and light availability (Lapointe 1981). Light availability in the laboratory culture was similar to that within the macroalgal chambers in the field, so concentrations of phycoerythrin and chlorophyll and thallus colouration are likely due to N availability/limitation rather than adaptation to different light availability.

An ecological adaptation by algae to N limitation appears to be uptake and storage of N beyond immediate growth requirements (Chapman and Craigie 1977; Lapointe 1981; Bird *et al.* 1982). Phycoerythrin is considered a N storage pool in *Gracilaria tikvahiae* (Ryther *et al.* 1981; Bird *et al.* 1982; Fujita 1985; Hwang *et al.* 1987) and *G. foliifera* (Lapointe and Ryther 1979; Rosenberg and Ramus 1982). Ratios of phycoerythrin to chlorophyll *a* have been used as an indication of complementary chromatic adaptation for algae growing at different water depths (Ramus *et al.* 1976). Increases in the chlorophyll *a* content with increase in cellular N are also well known in the algae (Fogg 1959; Bird *et al.* 1982), and the relatively large increase in chlorophyll concentration compared with that of phycoerythrin in the present study may indicate that the chlorophyll pigments are primary stores of N in these species of *Gracilaria*. Previous studies have reported lag periods between N addition and chlorophyll synthesis (Lapointe and Ryther 1979; Lapointe 1981); however, this was not the case in the present study.

Species of the Rhodophyta seem more appropriate than other algal divisions for use as bioindicators of water quality and habitat parameters. Comparisons between water nutrient concentrations and tissue nutrient content of both red and green algal species show the best correspondence when a red alga, *Ceramium rubrum*, is compared with green algae (Birch *et al.* 1981; Lyngby 1990; Lavery and McComb 1991; Lohman and Priscu 1992). The ability of red algae to store significant amounts of N in pigments when N is not limiting may enable red algae to persist in unfavourable nutrient environments (Fujita 1985). When N availability is reduced, algae without these N reserves need to fragment or produce propagules to survive the nutrient stress. N pulses in nature may prevent opportunistic species from excluding other, more persistent species (Fujita 1985).

Pigmentation and thallus colour can be related to water nutrient concentrations (Ryther *et al.* 1981). With extended periods of low DIN, *Gracilaria* spp. lose their dark red-brown colouration and become a pale straw yellow colour (Lapointe and Ryther 1979; Ryther *et al.* 1981). In the present study, higher tissue nutrient content was often associated with dark thallus colouration, and pale tissue was capable of rapid colour deepening (hours) in the presence of higher water column N. In the future, it may be possible to use thallus colouration of *Gracilaria* spp. or other Rhodophyta as a preliminary visual indicator or 'litmus test' for water column N availability.

The concentration of free amino acids, including glutamine, glutamate and especially citrulline, significantly increased with N availability ($P < 0{\cdot}001$). Amino acids form a large proportion of the biochemical constituents of algal tissue and are believed to form the initial pool of N that is metabolized when ambient N concentrations become reduced (Bird *et al.* 1982). Both amino acid and protein concentrations decrease with the onset of N limitation and thus are considered primary N reserves (Dawes *et al.* 1974). Citrulline is an intermediate involved in the transformation of glutamate to arginine (Miflin and Lea 1977) and has also been reported as a translocation product of N_2 fixation, especially in plants that have symbiotic N-fixing bacteria (Sellstedt and Atkins 1991; Rigano *et al.* 1992). In the case of *Gracilaria verrucosa*, citrulline may be a N storage product as 70–90% of the free amino acid N was contained in citrulline. This has been reported in the red microalga *Cyanidium caldarium*, where N-limited cells showed a continuous increase of glutamine, citrulline and arginine following the addition of ammonium, and where citrulline values increased 40× within 5 h of ammonium addition (Rigano *et al.* 1992). N limitation may reduce transfer of citrulline to arginine by reducing N metabolism and causing citrulline to function as a storage product in the chloroplast (Vona *et al.* 1992).

Summary

In regions where nutrients are limiting to growth, tissue nutrient content may be a more reliable method of water quality monitoring than is periodic chemical analysis. In the present study, macroalgal tissue N was shown to be positively correlated with DIN at most study sites. Nitrogen-to-phosphorus ratios (N:P) of the macroalgal tissue (19–23) were 3–10 times higher than the water column N:P (2–6), indicating potential N limitation. Extensive fouling occurring on and within growth chambers reduced light availability and caused negative growth rates. Nitrogen addition to *Gracilaria verrucosa* in the laboratory resulted in increases

in tissue N, %N as free amino acids, and photosynthetic pigments. Amino acids represented 14–16% of the total tissue N after N addition, with the amino acid citrulline constituting 70–90% of the total N in the free amino acids. This suggests that citrulline may be a primary reservoir for N. Thallus pigmentation may be useful for visual correlation of N availability *in situ* as pigment content in *Gracilaria* spp. appeared to be responsive to N availability both in the field and in the laboratory. By storing N as amino acids and pigments, *Gracilaria verrucosa* was able to integrate N availability continuously. The maintenance of field incubation chambers was logistically difficult and monitoring was limited to the seasonal availability of the chosen macroalgal species. However, the use of macroalgae as bioindicators of water quality is useful in terms of monitoring biologically available nutrient pulses that might otherwise go undetected by traditional sampling methods.

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