Vol. 209: 99–107, 2001 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published January 5

Ammonium requirements of fast-growing ephemeral macroalgae in a nutrient-enriched marine embayment (Port Phillip Bay, Australia)

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ABSTRACT: The observed high biomass of fast-growing macroalgae in Port Phillip Bay (PPB), Australia is of concern and may represent a shift from perennial macrophytes (e.g. kelps and seagrasses) to a dominance by fast-growing macroalgae. This study examined the limiting and optimum external ammonium-nitrogen concentrations for the growth of 3 fast-growing macroalgae that dominate reefs in northern Port Phillip Bay, Australia. The relationships between growth and tissue nutrients and the capacity of these algae to assimilate, store and survive on nutrients was examined. Winter and summer experiments aimed to determine the effects of tissue nutrient status on growth responses. An absence of any seasonal variation in critical N thresholds and N subsistence quotas in any of the species examined suggests that all species are able to tolerate low N availability. The utilisation of N reserves to support non-limited growth of *Hincksia sordida* (Clayton) and *Polysiphonia decipiens* (Montagne) was about 2- to 5-fold shorter in summer than winter, indicating potential N-limitation during summer. A high requirement for N by both *H. sordida* and *Ulva* sp. means that internal N reserves could support reduced growth for shorter periods compared to *P. decipiens*. Such high demands for N by *H. sordida* and *Ulva* sp. makes these taxa susceptible to N-limitation in summer, when inputs of N to coastal waters are low. Elevated nutrient inputs into PPB may allow these taxa to become nutrient-sufficient and colonise larger areas of nearshore reefs.

KEY WORDS: Fast-growing macroalgae · Ammonium · Tissue nutrients · Nutrient storage · Eutrophication

INTRODUCTION

Port Phillip Bay in southern Australia is a shallow marine embayment subject to nutrient inputs from sewage and catchment sources. Fast-growing macroalgae are dominant components of this nutrient-enriched nearshore habitat (Light & Woelkerling 1992). Elsewhere, eutrophication of shallow marine embayments has led to an increase in the biomass and production of fast-growing ephemeral macroalgae (Lapointe & O'Connell 1989, Björnsater & Wheeler 1990, Pedersen & Borum 1996, Valiela et al. 1997). These algae compete for available nutrients (Pedersen & Borum 1997) and are capable of replacing slower-growing primary producers such as perennial macroalgae and seagrasses (Cambridge et al. 1984, Fujita 1985, Pedersen & Borum 1996). The growth of these algae have generally shown higher growth rates during spring and summer than during autumn and winter (Geertz-Hansen & Sand-Jensen 1992, Pedersen & Borum 1996). Growth of macroalgae may also be influenced by nutrient availability irrespective of seasonal changes in temperature and light (Lavery & McComb 1991a). The rapid uptake of nutrients by macroalgae with high biomass and long residence times may also

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influence inorganic nutrient concentrations in the water column at the expense of other species (Fujita 1985, Pedersen & Borum 1996, 1997).

Rapid uptake and luxury accumulation of N have been reported for many species of macroalgae (Chapman & Cragie 1977, Lapointe & Tenore 1981, Pedersen & Borum 1997). Nutrient limitation is often dependent on seasonal *in situ* nutrient exposure and internal nutrient reserves (Lapointe 1985, Wheeler & Björnsäter 1992). Stored nutrients may support maximum growth for longer periods of time and may confer a competitive advantage in areas of episodic nutrient availability (Pedersen & Borum 1996). Interspecific differences in nutrient uptake rates may be explained by differences in morphology, especially the surface: area ratio (Wallentinus 1984, Hein et al. 1995). Nutrient requirements of macroalgae may also vary significantly with light, temperature and relative growth rates (Sfriso 1995).

Studies on nutrient requirements of macroalgae have largely focussed on differences in nutrient requirements between fast- and slow-growing taxa (Fujita 1985, Pedersen & Borum 1996), whilst few studies have examined seasonal differences in nutrient requirements of fast-growing macroalgae (Hurd & Dring 1990, Lavery & McComb 1991a,b, Lohman & Priscu 1992). It can be difficult to extrapolate findings on growth of macroalgae from culture experiments, where algae receive pulsed N-concentrations, to field situations where nutrients are in continual supply, because nutrients may be sufficiently available for growth demands at low concentrations (Larned & Stimson 1996, Schaffelke & Klumpp 1998). The use of experimental pulsed concentrations may, however, have relevance to field situations when pulses of nutrients are supplied to nearshore waters from sewage outfalls or other point-sources.

The aim of this study was to examine the relationships between growth and tissue nutrient concentrations in 3 species of fast-growing macroalgae in a shallow marine embayment in Port Phillip Bay, Victoria, Australia. The species examined were the filamentous phaeophyte *Hincksia sordida* (Clayton), the filamentous rhodophyte *Polysiphonia decipiens* Montagne and the sheet-like chlorophyte *Ulva* sp. All algae are dominant taxa in nearshore reef areas, and this paper aims to characterise their N requirements, N storage capacities, and growth patterns in relation to internal nutrient reserves and previous nutritional history.

METHODS

Sample and collection preparation. Whole thalli of *Hincksia sordida* (Clayton), *Polysiphonia decipiens* Montagne and *Ulva* sp. were collected during winter (June 1995 to August 1995) and summer (January and February 1996) from a site in Port Phillip Bay, at 3 m depth, approximately 500 m from shore. Plants were collected by SCUBA divers and kept at ambient temperature during transport to the laboratory.

Nutrient-enrichment experiments. Approximately 5 g (fresh weight) of plant material was washed in filtered (0.2 µm) seawater in the laboratory to remove sediment and epiphytic algae. Pieces of tissue were excised into portions on the day of collection and maintained overnight in aerated natural seawater in 20 l aquaria at 15°C, under a downwelling photo-flux density of 150 to 200 mmol m^{-2} s^{-1} (36 W 'cool white' fluorescent tubes) and a 12:12 h light:dark cycle. Photosynthesis-irradiance curves for each species showed that the photosynthesis of each species was saturated at <150 µmol m^{-2} s^{-1} (S.C. unpubl. data).

Experiments designed to investigate the relationship between macroalgal growth and tissue N over a range of ammonium-N concentrations were set up under the above conditions. Four replicate plants (each 2 g fresh weight) of *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. were placed in separate 4 l aquaria on the day after collection. This achieved initial dry weight to volume ratios of 0.04 to 0.06, enabling maintenance of optimal productivity rates (Littler 1979) and providing sufficient material for tissue-nutrient analysis. Each of the 4 replicates were subjected to 5 ammonium-N treatments (<0.4, 3.6, 7.1, 14.2, 28.5 µM) (20 aquaria per species), with all other physico-chemical parameters kept constant. The ammonium concentrations used were within the range of inorganic nitrogen concentrations (<1 to 30 µM) recorded in nearshore waters of Port Phillip Bay subject to sewage derived nutrient inputs (Campbell 1999). Experiments were performed on separate occasions for each species during July and August 1995 and January and February 1996, when ambient bottom water temperatures were 10 to 13°C and 17 to 20°C respectively. *In situ* water-column nutrient concentrations ranged from 20 to 28 µM NH_4-N and 10 to 15 µM PO_4-P during July and August 1995 and from 0 to 2 µM NH_4-N and 10 to 15 µM PO_4-P during January and February 1996.

Nitrogen was added to the cultures as NH_4Cl, and P was added as NaH_2PO_4 from stock solutions. Each ammonium treatment was made up and changed every 2 d for the duration of the experiment (14 d). The water-column concentrations of ammonium-N and phosphate-P were measured at the start and finish of each 2 day period. To prevent C limitation, carbon was added by additions of $NaHCO_3$ to concentrations of 3 mM DIC. The pH of all cultures was monitored daily and kept at 8.1 to 8.3. To account for the seasonal effects of nutritional history on growth and avoid other

seasonal cues, the cultures were maintained under constant saturating photosynthetic photon-flux density (200 µmol m^{-2} s^{-1} and a 12:12 h light:dark cycle) and temperature (15°C) to control for the effects of light and temperature. The aquaria were aerated to ensure water movement and gas equilibrium. After each experiment, algal material was removed from treatments for growth measurements (weighing) and sampled for tissue N and P analysis.

Growth rates. Growth was calculated from changes in fresh weight over a 14 d period. Plant material was trimmed and weighed at 7 d in order to maintain approximate biomass to water-volume ratios during the experiment.

The net growth rate (μ) was calculated from changes in fresh weight biomass after 7 and 14 d, and represent the means of these 2 values:

$$\mu = (\ln B_t - \ln B_0)\ t^{-1}\ 100$$

For this equation, B_0 represents the initial and B_t the final biomass after t days in the experimental treatment. Units of net growth rate are % d^{-1}.

Fresh and dry weights. Fresh weights of all plant material were determined after blotting with tissues. Dry weights were determined after drying at 70°C for 24 h.

Nutrient analysis. Spectrophotometric analysis of water samples for inorganic nitrogen (NH_4-N and NO_3-N) and inorganic phosphorus (PO_4-P) were undertaken using standard methods of Strickland & Parsons (1972).

Algal samples for tissue N and P were dried, homogenised and digested in concentrated sulphuric acid (H_2SO_4) and perchloric acid (H_2O_2) using a modification of the method outlined by Allen (1989). After digestion, N and P concentrations were determined with a Novaspec II nutrient autoanalyser (Pharmacia) using methods of the American Public Health Association (1995). Concentrations are expressed either as mg N or P g dry wt^{-1}.

Nitrogen growth parameters. Critical tissue-nitrogen concentrations (N_c = tissue N concentration where maximum growth is possible), N subsistence quotas (N_q = the tissue N concentration where growth is zero and the alga manages to subsist) of the 3 species were calculated according to the equations of Pedersen & Borum (1996). The critical N concentration, or N_c, was calculated from the intercept of the 2 lines represented by the maximum growth rate and the initial slope of the curve. The initial slope was approximated as the line going through the points where $\mu = 0.5\ \mu_{max.}$

Nitrogen storage capacity. The ability of algal species to accumulate N was examined by comparing the maximum tissue-N concentration measured during the laboratory experiments, as described by Pedersen & Borum (1996). These parameters include: the maximum tissue N contents (N_{max}); the N pool stored in excess of the requirements for maximum growth ($N_s = N_{max} - N_c$); the N storage capacity [$t_{max} = \ln (N_{max}/N_c)\mu_{max}^{-1}$], defined as the duration (in days) this storage can sustain maximum algal growth without any compensatory N uptake from the external media; and the period of reduced growth in days [$t_{red} = \ln(N_c/N_q)\ (0.5\ \mu_{max})^{-1}$] over which internal N pools are below the critical level ($N_{red} = N_c - N_q$) (i.e. when the tissue N content decreased below the critical limit [N_c] until the subsistence quota [N_q] was reached).

Statistical analyses. Data were examined for heterogeneity of variance (Cochran's test) and skewness of data (residuals and outliers). Non-normal data were subjected to log transformation, $\ln(x+1)$. A Student's t-test was used to examine seasonal differences in field tissue-N content for each species. A 3-way ANOVA was used to examine for effects of factors, season, ammonium-nitrogen enrichment and species on the growth rates calculated after 14 d. The significance level used was $p < 0.05$. Tukey's HSD test was used to make post-hoc multiple comparisons among treatment means from significant ANOVA tests.

Nitrogen growth parameters were calculated by plotting μ against the tissue N concentration for each sample, and fitting data to the Droop equation (Droop 1983) using non-linear least-square regression (SYSTAT Version 5.03, Systat Inc., Evanston, Illinois, USA).

$$\mu = \mu_{max}\ (1 - N_q/N)$$

where μ_{max} is the maximum growth rate, N_q is the minimum tissue N concentration needed to sustain growth (the subsistence quota), and N is the actual tissue concentration in the alga. The significance level of each regression is presented for each species on each graph in Fig. 2. Means and confidence intervals for μ_{max} and N_q were derived from the non-linear regression. Confidence intervals for the critical N concentrations (N_c) were derived from the error estimates of μ_{max}. The computer package software SYSTAT (Version 5.03, Systat Inc., Evanston, Illinois, USA) was used for all analyses.

RESULTS

Tissue-N

Tissue-N contents for field collected *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. are presented in Table 1; they were significantly higher ($p < 0.05$) for all species in winter than in summer.

Table 1. *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. Tissue-N content of macroalgae collected in winter (July and August) and summer (January and February) in Port Phillip Bay. Values are means (CI), n = 6. Values in any one row with different superscript letters are significantly different at $p < 0.05$

Species	Tissue-N (mg g^{-1} dry wt)	
	Winter	Summer
H. sordida	43.22^{a} (2.9)	25.12^{b} (4.9)
P. decipiens	44.30^{a} (4.6)	35.50^{b} (2.3)
Ulva sp.	43.78^{a} (5.2)	28.27^{b} (4.4)

Growth responses to nutrients

For all growth cultures after each 2 day enrichment period, ammonium-N was depleted in all <0.4 µM treatments and in 30% of 3.6 µM replicates, across all algae examined. In all other treatments (i.e. ≥7.1 µM) residual ammonium-N was found in the culture media after 2 d, indicating N sufficiency at these higher concentrations.

The mean net μ (% d^{-1}), obtained over the 14 d period increased asymptotically towards μ_{max} with increasing tissue-N content in all algae during winter (Fig. 1). Of all species, this relationship was strongest in *Hincksia sordida* (Clayton) during winter ($r^2 = 0.71$) and summer ($r^2 = 0.78$). This relationship was weak for *Polysiphonia decipiens* in winter ($r^2 = 0.63$), but stronger in summer ($r^2 = 0.79$). Weak relationships were found for *Ulva* sp. in winter ($r^2 = 0.25$) and in summer ($r^2 = 0.002$).

The estimated μ_{max} in the experiments varied seasonally for all 3 species (Table 2). There was a 2- to 3-fold difference in maximum growth rate between species with *Ulva* sp. having the highest rate. During winter, μ_{max} was lower in *Polysiphonia decipiens* (0.049 d^{-1}) than in both *Hincksia sordida* (0.093 d^{-1}) and *Ulva* sp. (0.118 d^{-1}). A similar pattern was evident in summer, with a higher μ_{max} recorded for *H. sordida* (0.116 d^{-1}) than for *P. decipiens* (0.088 d^{-1}). Growth of *Ulva* sp. in summer was negligible; data did not fit the Droop model, and therefore no μ_{max} was calculated.

The growth of all 3 species showed enhancement in response to ammonium-N additions (Fig. 2). Growth of *Hincksia sordida* in summer and winter and of *Polyishonia decipiens* in summer increased with ammonium-N additions from <0.4 to 14.2 µM. In winter there was no increase in growth rate in *P. decipiens* or *Ulva* sp. above 3.6 µM. In summer, *Ulva* sp. showed no growth enhancement with ammonium-N enrichment.

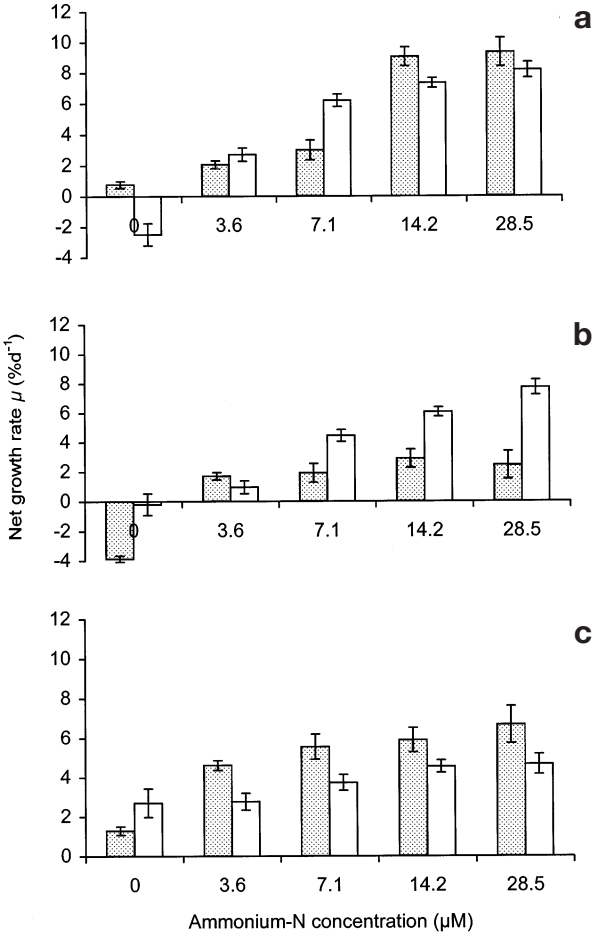

Fig. 1. (a) *Hincksia sordida*, (b) *Polysiphonia decipiens* and (c) *Ulva* sp. Net growth rates after exposure to ammonium-N over a 14 d period in winter (shaded bars) and summer (open bars). Values are means (±SE), n = 4

A 3-way ANOVA revealed significant effects of season, ammonium concentration and species on the specific growth rates of the 3 species examined (Table 3). A significant 3-way interaction was due to: higher

Table 2. *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. Maximum net growth rates (μ_{max}), obtained from laboratory experiments. Values are means (CI), n = 4. Values in any one row with different superscript letters are significantly different at $p < 0.05$

Species	μ_{max} (% d^{-1})	
	Winter	Summer
H. sordida	9.3^{a} (1.8)	11.6^{b} (2.1)
P. decipiens	4.9^{a} (1.4)	8.8^{b} (1.6)
Ulva sp.	11.8 (3.5)	

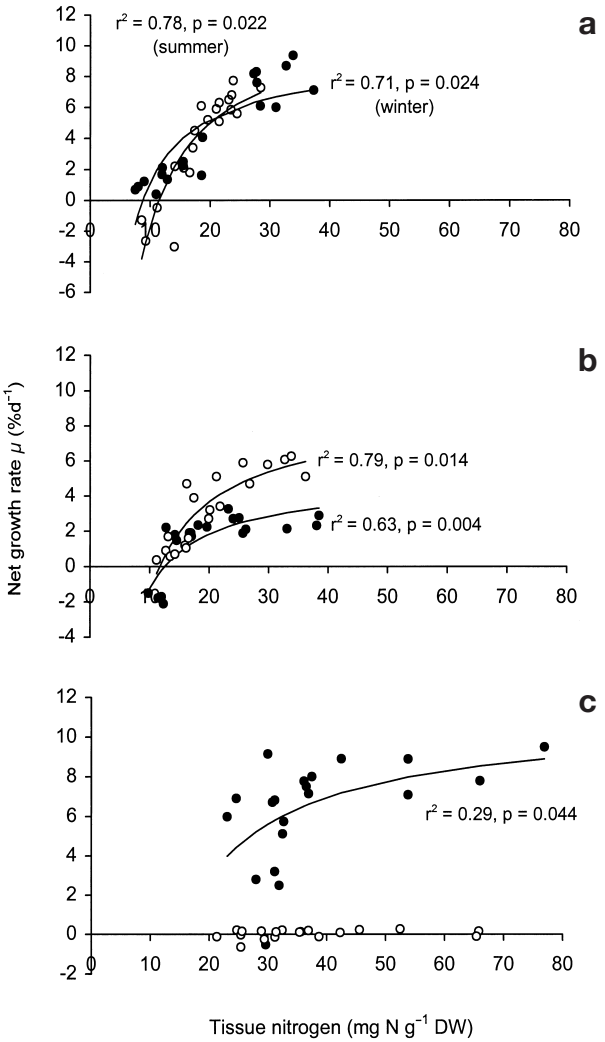

Fig. 2. (a) *Hincksia sordida*, (b) *Polysiphonia decipiens* and (c) *Ulva* sp. Net growth rates in relation to tissue-N content (mg N g^{-1} dry wt) in winter (●) and summer (○) , n = 20

Table 3. *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. Three-factor ANOVA comparing growth rates of the macroalgae after 14 d. Factors were season (winter vs summer), N treatments (<0.4 to 28.6 µM NH_4^+), and species. Data were log-transformed and fulfilled the Cochran test of homogeneity of variances

Source of variation	df	*F*	p
Season	1	6.12	0.015
Treatment	4	223.38	0.001
Species	2	66.58	0.001
Season × Treatment	4	4.71	0.002
Season × Species	2	50.79	0.001
Species × Season	8	15.97	0.001
Season × Species × Treatment	8	13.37	0.001
Error	90		

growth rates at the high ammonium concentrations than at the low ammonium concentrations for all species except summer *Ulva* sp.; higher growth of *Hincksia sordida* and *Ulva* sp. during winter than in summer but higher growth of *Polysiphonia decipiens* in summer compared to winter; and generally higher growth of *H. sordida* and *Ulva* sp. than of *P. decipiens*.

Critical tissue-N concentrations

Maximum tissue-N (N_{max}) represents the maximum field tissue-N contents of algae used in experiments and not the maximum achieved under experimental conditions (Table 4). This concentration is used as it attempts to represent what is achievable under field situations. Critical N contents (N_c) were highest during winter for *Polysiphonia decipiens* (28.8 mg N g^{-1} dry wt) but similar between winter and summer for *Hincksia sordida* (20.8 and 21.2 mg N g^{-1} dry wt respectively). Only winter values of N_c could be calculated for *Ulva* sp. (mean = 27.5 mg N g^{-1} dry wt) due to low summer growth (Table 4). The subsistence quota (N_q) of all the species investigated did not vary between seasons, but N_q was lower for *H. sordida* compared to *P. decipiens* and *Ulva* sp. in winter. N_q could not be calculated for *Ulva* sp. in summer because of the absence of a relationship between growth and tissue-N (Table 4).

Nitrogen storage and requirements

The amount of N stored in excess of the critical limit ($N_s = N_{max} - N_c$) varied among species, with the lowest values observed during summer in *Hincksia sordida* (4.2 mg N g^{-1} dry wt) and the highest in winter (31.5 mg N g^{-1} dry wt) (Table 4). Seasonal responses of N_s were the same for *H. sordida* and *Polysiphonia decipiens* with higher values found during winter. The tissue N-pool which could support N-limited growth (N_{red}) did not differ between seasons for *H. sordida* but was higher for *P. decipiens* in winter (Table 4).

The N required to sustain maximum algal growth ($N_{req} = \mu_{max} \times N_c$) varied seasonally for *Hincksia sordida* and *Polysiphonia decipiens* with higher values in summer (Table 5). N_{req} also varied among species with lowest values found in winter *P. decipiens* (1.41 mg N g^{-1} dry wt d^{-1}) and highest in winter *Ulva* sp. (3.03 mg N g^{-1} dry wt d^{-1}).

The storage capacity (t_{max}), defined as the number of days that excess N (N_s) could support maximum growth, was similar for *Hincksia sordida* and *Polysiphonia decipiens* in winter (9.9 and 9.1 d respectively) and in summer (1.6 and 3.9 d respectively). A relatively low t_{max} was observed in *Ulva* sp. (5.2 d) during winter

Table 4. Maximum tissue-N (N_{max}), critical N (N_c), subsistence quota (N_q), N pools in excess of that necessary for maximum growth (N_s), and N pools that allow reduced growth (N_{red}). All values were obtained from laboratory experiments, except for N_{max} values which were derived from tissue-N contents of algae collected from the field. Values are means (CI), n = 4, in mg N g^{-1} dry wt

Species	N_{max}		N_c		N_q		N_s (N_{max} – N_c)		N_{red} (N_c – N_q)	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
H. sordida	52.3 (10.6)	25.4 (5.3)	20.8 (3.8)	21.2 (1.7)	8.8 (1.4)	11.4 (1.2)	31.5	4.2	10.3	9.8
P. decipiens	45.0 (5.6)	33.2 (2.1)	28.8 (2.0)	23.5 (2.4)	12.5 (1.9)	11.7 (1.3)	16.2	9.7	16.3	11.8
Ulva sp.	48.5 (3.1)	23.2 (2.1)	27.5 (7.5)	–	15.7 (6.8)	–	21.0	–	12.8	–

Table 5. Nitrogen requirements at maximum growth rates ($N_{req} = \mu_{max} \times N_c$), storage capacity at non-limited growth (t_{max}), and the possible duration of reduced growth (t_{red}) based on N_{red} of 3 species of macroalgae from Port Phillip Bay. All values were obtained from laboratory experiments. Values are means, n = 4

Species	N_{max} (mg N g^{-1}dry wt d^{-1})		t_{max}		t_{red}	
	Winter	Summer	Winter	Summer	Winter	Summer
Hincksia sordida	1.93	2.46	9.9	1.6	18.6	10.7
Polysiphonia decipiens	1.41	2.06	9.1	3.9	34.1	15.9
Ulva sp.	3.03	–	5.2	–	11.4	–

(Table 5). The period of time over which algal growth could proceed at N-limited rates (t_{red}) was lowest in *H. sordida* and *P. decipiens* during summer (Table 5). During winter, N-limited or reduced growth rates could be supported for a relatively long period in *P. decipiens* (34.1 d) compared to *H. sordida* (18.6 d) and *Ulva* sp. (11.4 d). t_{red} was lower in summer than winter, in both *H. sordida* (10.7 d) and *P. decipiens* (15.9 d).

DISCUSSION

The key finding of the present study was that all 3 species of macroalgae exhibited a high requirement for ammonium nitrogen at high concentrations. The good relationship between growth and tissue N for *Hincksia sordida* and *Polysiphonia decipiens* indicates a utilisation of N for growth. The experimental enrichment studies here suggest that in moderate to high nitrogen-enriched waters (i.e. 7.1 to 28 mM NH_4-N) these fast-growing macroalgae attain nutrient sufficiency and, as a consequence are able to colonise large areas of reef. The dominance of these fast-growing macroalgae at nearshore reefs in Port Phillip Bay is likely to represent a shift from slower growing macroalgae that may have once inhabited these areas (Brown et al. 1980) to dominance by macroalgae with rapid nitrogen-uptake capacities, high surface to volume ratios and high turnover rates.

In most cases, ammonium-N was rarely depleted by the cultures of algae over the enrichment period, suggesting that only algae in the low-ammonium treatments were undersupplied. However, the absence of saturated growth of *Hincksia sordida* suggests that this species may be able to exploit even higher concentrations of N than those employed. The saturated growth (beyond 2 to 3% tissue-N content) in both *Ulva* sp. and *Polysiphonia decipiens* during winter suggests a capacity for N storage at high ammonium concentrations. Likewise, growth demands have been met at 2 to 3% tissue-N contents in other fast-growing ephemeral species (Pedersen & Borum 1996, Fong et al. 1998). Differences in N usage between the 3 species examined were evident, with internal N reserves supporting reduced growth of *H. sordida* and *Ulva* sp. for shorter periods than *P. decipiens*. The ability of these 2 fast-growing algae to store N during non-limited growth was low when compared with *P. decipiens*. From September to February, low DIN availability would be insufficient to saturate N-uptake rates of these macroalgae (Campbell 1999), suggesting that N could potentially limit macroalgal growth over this period if N uptake were directly coupled with growth. It would therefore appear that the rapid growth of these species under N-enriched conditions and their prevalence in nearshore waters at certain times of the year is a direct indication of coastal eutrophication.

Comparisons between the estimated critical and subsistence tissue-N levels with field tissue nutrient data are useful to evaluate the nutrient supply and

potential nutrient limitation of the 3 species. The relatively high tissue-N contents of *Hincksia sordida* and *Polysiphonia decipiens* collected from the field during winter, compared with N-enriched cultures in the laboratory, suggests that the growth of these 2 species may have been N-limited even at the highest N concentrations supplied. This may lead to an underestimation of critical N (N_c) and subsistence quotas (N_q) and may explain the comparatively low estimates calculated for *H. sordida*. The range of critical threshold limits of N (N_c) (20.8 to 28.8 mg g^{-1} dry wt) of all the species examined are, however, comparable to those recorded for other macroalgae (e.g. Fujita et al. 1989, Pedersen & Borum 1996). In winter, field tissue-N concentrations of the 3 species of macroalgae examined rarely fell below the critical or subsistence levels. Therefore, these algae are unlikely to be N-limited in winter. The relatively high winter N_c for *P. decipiens* is most likely associated with its reduced metabolism and growth during winter. During the austral summer, field tissue-N concentrations of *H. sordida* fell below the critical N_c values, hence any additional supply of N at this time may lead to enhanced growth of *H. sordida*. 'Bloom' events are therefore likely to be dependent on moderate to high N availability and provide a direct indication of high nutrient richness in these coastal waters. The high N_q of *Ulva* sp. relative to *P. decipiens* and *H. sordida* also implies a high requirement for N, and its prolific growth may also indicate high nutrient richness.

The lack of growth in N-replete *Ulva* sp. during summer is not entirely clear, but is likely to be a seasonal response whereby growth is uncoupled from physiological requirements and other factors such as temperature and light primarily control growth. Much of the N assimilated in this period may accumulate in non-photosynthetic components (inorganic storage pools of NH_4^+ and NO_3^-, nucleic acids and proteins) associated with cell regulation and respiration (McGlathery et al. 1996, McGlathery & Pedersen 1999). A minor proportion of N is also most likely to be used in pigment synthesis, and this has been demonstrated by increased photosynthetic maxima (P_{max}) and pigment contents in these algae during winter (S.C. unpubl. data). This, however, is likely to constitute a small proportion of the total tissue-N (i.e. 2 to 3%) as pigments represent only a small percentage of tissue-N in macroalgae (Rosenberg & Ramus 1982, Duke et al. 1986).

The relative potential for nitrogen limitation of these macroalgae were evaluated by examining their demands for N at maximum growth rates. In summer, the N demands (N_{req}) of *Hincksia sordida* and *Polysiphonia decipiens* were high because of their high growth rates during this time. In winter, the high requirements for N (N_{req}) by *H. sordida* and *Ulva* sp. relative to *P. decipiens* also reflect high growth rates. In summer, the N_{req} of *H. sordida* and *P. decipiens* would not be met by the nutrient supply through the uptake of ammonium; however, in winter, the N_{req} of *P. decipiens* and *Ulva* sp. would be met by N uptake (Campbell 1999). In conjunction with the finding that tissue contents in *H. sordida* were below critical tissue levels (in summer) these findings suggest that growth is N-limited during summer. The range in N_{req} for the 3 species in Port Phillip (1.4 to 3.0 mg g^{-1} dry wt d^{-1}) are about 3- to 10-fold higher than reported for slow-growing species such as *Sargassum baccularia* (0.08 to 0.21 mg g^{-1} dry wt d^{-1}) (Schaffelke & Klumpp 1998) and *Ecklonia radiata* (0.36 to 1.44 mg g^{-1} dry wt d^{-1}) (Paling 1991). Accordingly, the N demands and high uptake rates of fast-growing species are likely to be very competitive with the demands of slower-growing macroalgae during high nutrient availability. This is indicated by the prolific abundance of small, ephemeral species at the site of study and the absence of large, structurally complex, slow-growing taxa (e.g. *E. radiata*).

The potential importance of internally stored N for algal growth in Port Phillip Bay was estimated from the amount of time during which algal growth could proceed at non-nutrient limited and reduced rates, assuming that no uptake of DIN occurred from the water. These estimates allow inter-species comparisons to be made, useful for describing their potential responses to periods of low N supply. The capacity of *Hincksia sordida* and *Polysiphonia decipiens* to mobilise stored N reserves to support non-limited growth was limited to shorter periods in summer algae than in winter algae. In summer *P. decipiens* would have a lower demand for N than *H. sordida*, evidenced by its 2-fold higher excess-N pool (N_s = 9.7 mg N g^{-1} dry wt) compared to *H. sordida* (4.2 mg N g^{-1} dry wt). An opposite trend was apparent in winter, indicating of relatively fast growth and high N demands of *H. sordida*. Although this high demand for N may in part be due to higher laboratory temperatures (15°C) relative to field temperatures (10 to 12°C), these demands are consistent with field observations of rapid growth in winter under saturating light (i.e. > 150 mmol m^{-2} s^{-1}) (S.C. unpubl. data).

The N_{req} and storage capacities of *Ulva* sp. appeared to differ markedly from those of the other species. Elevated winter N requirements of *Ulva* sp. were reflected by relatively high N_c, N_{req}, N_s and N_{red} and a low t_{max} (5.2 d), indicating that most of the N pool in this species is used for growth, rather than being stored in winter. These findings are comparable to those of Fujita (1985), who found that stored N could support growth of *Gracilaria tikvahiae* for longer periods (14 d) than the faster-growing *Ulva* sp. (6 d) and *Enteromorpha intestinalis* (8 d), after which N-limited growth oc-

curred. Pedersen & Borum (1996) also reported similar time periods during which stores of N could support non-limited growth for *Ceramium rubrum* (2.3 d) and the chlorophytes *Ulva lactuca* (3.1d) and *Cladophora sericea* (3.4 d). In addition, *U. lactuca* has been shown to survive for shorter periods at reduced rates of growth (6.5 d) compared to other fast-growing algae such as *Ceramium rubrum*, *Cladophora sericea* and *Chaetomorpha linum* (8.7 to 10.1 d) (Pedersen & Borum 1996). The results of the present study also show that a shorter period of reduced growth could be supported by internal N reserves in *Hincksia sordida* and *Ulva* sp. (10.7 to 18.6 d) compared to *Polysiphonia decipiens* (15.9 to 34.1 d). In conjunction with findings on non-limited growth rates, it appears that of the 3 species examined, these 2 opportunistic species are most inclined to respond to high N availability with increased growth.

This study provides the first comparative account of N requirements and potential N storage capacities of fast-growing ephemeral macroalgae in south-eastern Australia. The high requirement for N and relatively low capacity for N storage by the opportunistic *Hincksia sordida* makes it susceptible to N-limitation in summer, when inputs of N to coastal waters are low. *Ulva* sp. also has a high requirement for N and a limited capacity to grow on stored reserves, its growth being dictated primarily by shifts in light and temperature. In contrast, *Polysiphonia decipiens* appears to have the capacity to store and survive on limited reserves of N for comparatively longer periods. The high nutrient demands and immediate use of N by these macroalgae suggests that they have a low capacity for buffering fluctuations in external nutrient concentrations. High nutrient inputs into nearshore waters sustain growth of these species and may increase their competitive potential at the expense of slower-growing macroalgae (e.g. *Sargassum* sp. and *Ecklonia radiata*), which grow in non-nutrient-enriched habitats in Port Phillip Bay. The growth and productivity of these fast-growing species are therefore important indicators of eutrophication in this nearshore marine system.

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Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: November 29, 1999; Accepted: June 29, 2000
Proofs received from author(s): November 27, 2000