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Effects of Inorganic Phosphorus and Nitrogen on the Growth of an Estuarine *Cladophora* in Culture

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Abstract

Response of *Cladophora* to different P and N regimes under otherwise non-limiting conditions in cultures of artifical seawater suggests that growth rates would be limited in the field by P and N concentrations in the water column above the algae, but not in the algal bed. Treatment with P over the range 0 to 0.25 mg ℓ^{-1} (with 5.0 mg ℓ^{-1} N) and N over the range 0 to 5.0 mg ℓ^{-1} (with 0.5 mg ℓ^{-1} P) produced yields proportional to increasing concentrations.

Growth rates were obviously saturated above 0.4 mg ℓ^{-1} N and 0.2 mg ℓ^{-1} P, and the response was most dramatic in substrate concentration ranges of 0 to 0.1 mg ℓ^{-1} N and 0 to 0.03 mg ℓ^{-1} P.

Uptake rates of phosphorus increased in proportion to substrate P concentrations, with the highest rates occurring immediately after conditioning in P-free medium. Rates over a combined light and dark period were typically lower than those measured in the light, which gave a v_{max} of 0.11 mg g^{-1} dry weight hr^{-1}. Ammonia and nitrate were taken up simultaneously at all concentrations, in the presence of 0.5 mg ℓ^{-1} P. There was a clear preference for ammonia, with suppression of nitrate uptake at the highest N substrate concentration. Uptake rates of both N species was highest in the light, with a v_{max} 1.82 for NH_4 and 0.59 mg g^{-1} dry weight hr^{-1} for NO_3.

Tissue concentrations of nitrogen and phosphorus increased in proportion to the substrate concentration; the relationship was linear except for high nitrogen substrate concentrations, where there was loss of organic nitrogen from the tissue.

Relating the growth-saturating N or P concentrations in the water with their corresponding tissue N or P concentrations suggests that the "critical" tissue concentrations for growth are about 21 mg g^{-1} N and 3.3 mg g^{-1} P. Tissue levels well above this "critical" concentration were obtained for those algae treated with very high N or P, suggesting that the alga has the capacity to build up its N and P reserves to very high levels in the field under non-limiting environmental conditions. The "minimum viable" tissue content was estimated for N at 12 mg g^{-1} dry weight, and for P at 0.5 mg g^{-1} dry weight. A comparison with tissue levels measured on field-grown material suggests that there is luxury storage of N and P.

Removal of either nutrient from solution, after growth in complete medium, resulted in a marked reduction in growth rate, with a concomitant decrease in levels of tissue N and P after 4 weeks, particularly for those algae without added nitrogen. Those which had been incubated in higher concentrations grew more vigorously on return to minimal medium. There was a strong correlation between tissue nitrogen levels and chlorophyll *a*, though this was less obvious with phosphorus. Removal of nitrogen from the medium resulted in distinct chlorosis of algal filaments after 5 weeks, suggesting that nitrogen availability may well be important in the interpretation of differences in thallus colour.

Introduction

This work is concerned with the nutrients which may control growth of the green alga *Cladophora* aff. *albida*. This alga is prominent in Peel Inlet, Western Australia, the larger of two linked estuarine basins (McComb *et al.* 1980). It grows as small spheres (1–3 cm diameter) of densely-branched, radiating filaments which lie unattached on the estuary floor, where they may form large beds, usually 1–10 cm deep. The spheres may drift shorewards and accumulate in the shallows, where they eventually decompose. The resulting deterioration of once clean beaches (Cross 1974) and, more significantly, the increased eutrophication of the water (of which the alga is a symptom) are a major concern in an estuary which supports a commercial fishing industry and is in demand for recreational purposes.

Rivers flowing into the Peel-Harvey estuarine system provide large inputs of nitrogen and phosphorus to the

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water in winter (McComb *et al.* 1980). This input, along with recycling from decomposing algae in the underlying layers of the beds, provides the most obvious supply of these nutrients for growth of *Cladophora*.

It is difficult to directly measure the interactions between nutrient uptake, environmental changes and growth in the estuary. Temperature falls to limiting levels in winter. Light is at limiting levels at the surface of the algal bed in winter and throughout the year for those algae beneath the bed surface (Gordon *et al.* 1980).

However, the main reason for accumulation of large populations of the alga, which has taken place since about 1966, has presumably been increased nutrient availability. Laboratory growth experiments were therefore undertaken to help interpret the roles of nitrogen and phosphorus in the control of *Cladophora* growth, by observing the effects of these nutrients while maintaining light and temperature at levels unlikely to be limiting.

Materials and Methods

Plant Material

Cladophora aff. *albida* (Huds.) Kütz. was collected from Falcon Bay, Peel Inlet in February 1979 and grown in artificial seawater (see below) at 25 °C for two weeks before the start of this study. Known fresh weights (20 mg) of algae were initially placed into 2 litre conical flasks filled with 1.5 litres of medium.

These cultures were essentially "unialgal", but there were always some epiphytes associated with the filaments. Where epiphyte loads were high, the alga was shaken in N- and P-free artificial seawater and examined under the microscope.

Medium

The artificial seawater was adapted from the ASP_{12} recipe of Provasoli (1964). This medium (Table I) has a wide spectrum of nutrients, and has been used successfully in cultures of marine macroalgae (e.g. Iwasaki 1967) including *Cladophora* (Wik-Sjöstedt and Nordqvist 1970). Modifications were the addition of an inorganic carbon source, as $NaHCO_3$, giving alkalinities similar to those of the estuary. Inorganic nitrogen was added as NH_4NO_3 rather than $NaNO_3$, as both N species are present in the estuary. Inorganic phosphorus was added as K_2HPO_4. No glycerophosphate and hence no nitrilotriacetic acid buffer was included. *Tris* buffer was deleted because its presence interfered with colour development during ammonia determinations; however, the pH of the medium during these experiments did not vary by more than 0.3 of a unit (8.0 ± 0.3). Two experiments were run, one in which inorganic P was varied and the second in which inorganic N was varied, in both cases maintaining all other nutrients at levels unlikely to be limiting. For logistical reasons, both experiments were conducted essentially as batch cultures, making possible the measurement of rates of uptake of inorganic N and P. Concentrations of N and P were measured daily, and regular solution changes were required, usually every third day. Where the treatment concentration required was low, only the particular nutrient required was added at intermediate times. Uptakes rates of PO_4-P, NH_4-N and NO_3-N were calculated as loss from solution.

Tab. I. Modified artificial seawater medium (ASP_{12}) (adapted from Provasoli 1964)

	Chemical	Amount (g ℓ^{-1})
Salt block	NaCl	28.0
	KCl	0.7
	$MgSO_4 \cdot 7H_2O$	7.0
	$MgCl_2 \cdot 6H_2O$	4.0
	Ca (as Cl^-)	0.4
Nutrient block	NH_4NO_3	1.430×10^{-2} (= 5,000 μgN ℓ^{-1})
	K_2HPO_4	1.145×10^{-3} (= 250 μgP ℓ^{-1})
	$NaHCO_3$ [a])	0.2
	$Na_2SiO_3 \cdot 9H_2O$	1.5×10^{-2}
Vitamin block	B_{12}	2×10^{-7}
	Biotin	1×10^{-6}
	Thiamine – HCl	1×10^{-4}
Buffer	TRIS	1.0
Trace metals	P_{II} [b])	10 ml
	S_{II} [c])	10 ml
pH	8.0	
Salinity	$33.7\,^0/_{00}$	

[a]) Bicarbonate added at levels similar to those indicated from alkalinity measurements from estuary water in Peel Inlet.
[b]) 1 ml of P_{II} contains: EDTA (Na_2), 1 mg; Fe (as Cl^-), 0.01 mg; $B(H_3BO_3)$, 0.2 mg; Mn (as Cl^-), 0.04 mg; Zn (as Cl^-), 5 μg; Co (as Cl^-), 1 μg.
[c]) 1 ml of S_{II} metals contains: Br (as Na), 1 mg; Sr (as Cl^-), 0.2 mg; Rb (as Cl^-), 0.02 mg; Li (as Cl^-), 0.02 mg; Mo (as Na salt), 0.05 mg; I (KI), 1 μg.

Nutrient Analyses

Phosphate-P was analysed by the single solution method (Major *et al.* 1972). Ammonia-N was determined using the phenolnitroprusside technique (Dal Pont *et al.* 1974), and nitrate-N using an autoanalyser (Technicon Industrial Method No. 100-70W, Technicon Industrial Systems, Terrytown, New York). pH and alkalinity were recorded initially and when solutions were changed, the latter by titration (Anon 1971). Total N and P in algal tissue were measured on acid digested samples (100% HNO_3 followed by 50% $HClO_4$); nitrogen was measured colorimetrically (Technicon Industrial Method No. 334-74W/B, Technicon Industrial Systems, Terrytown, New York) and phosphorus by the single solution method (Major *et al.* 1972).

Chlorophyll *a* was measured on 30 mg (fresh weight) samples which were ground with acid-washed sand containing basic $MgCO_3$, washed into centrifuge tubes to a volume of 8 ml with 90% w/w acetone, and kept in the dark for 24 hr. Solutions were centrifuged (3000 × g; 15 min) and read on a spectrophotometer at 665 and 770 nm using 1 cm cells (Series 634, Varian Techtron Pty. Ltd., Australia). Chlorophyll *a* concentrations were calculated (Parsons and Strickland 1963) and expressed as mg g^{-1} dry weight. Phaeophytins were measured in these samples following acidification with 0.1 N HCl (Lorenzen 1967).

Experimental design

Each experiment was run in three stages: growth in N or P depleted media ("pre-conditioning") was followed by growth in complete media, and finally a return to depleted media. Pre-conditioning was for 7 days during which algae to be grown under different P regimes were placed in P-free medium with 5.0 mg ℓ^{-1} inorganic N, while those to be grown under different N regimes were supplied with 0.5 mg ℓ^{-1} P, but no inorganic N. Three replicate flasks of 5 treatments were run for each experiment, covering a range of inorganic P and N concentrations similar to those at sites of growth in the estuary (McComb *et al.* 1980).

Phosphorus treatments were from 0 to 0.25 mg ℓ^{-1} and nitrogen from 0 to 5.0 mg ℓ^{-1}. Because nutrient levels fell between solution changes, concentrations used in calculations were the average of intermediate concentrations observed during each period. The levels were, for initial and averaged concentrations (in brackets), 0 (0); 0.010 (0.006); 0.025 (0.015); 0.050 (0.032); 0.250 (0.203) mg ℓ^{-1} P, each at 5.0 mg ℓ^{-1} N, and 0 (0.007); 0.10 (0.08); 0.50 (0.43); 1.0 (0.83); and 5.0 (4.56) mg ℓ^{-1} N each at 0.5 mg ℓ^{-1} P. P treatments were run for 3 weeks and N for 2 weeks, after which the algae were returned for 3–4 weeks to solutions identical to those in which they had been preconditioned.

Culture apparatus

Algae were grown in acid-washed, 2 litre Erlenmeyer flasks supported over a bank of 4 × 110 watt fluorescent lamps (Sylvania, Cool White, VHO 48″), with a day length of 12 hr, at 23 ± 1 °C. Photosynthetically-active radiation (PAR) was measured with a quantum sensor (LiCor, Lincoln, Nebraska, USA). Readings were taken with the sensor immersed in a flask containing 1.5 litres of medium. The light ranged from 270 to 450 μEm^{-2} sec^{-1} which is above saturation for this species (Gordon *et al.* 1980). All solutions were aerated continuously, the air bubbling through 25% H_2SO_4 into deionised, distilled water and finally into each flask.

Growth measurements

Growth was measured as change in fresh weight over approximately weekly intervals and converted to dry weight from previously determined fresh weight-dry weight ratios (2.56 ± 0.10).

Calculations

Relative growth rates were determined for each interval using the equation $\overline{RGR} = (\ln W_2 - \ln W_1)/(t_2 - t_1)$ where W_1 and W_2 are dry weight at times t_1 and t_2 respectively.

The Monod growth expression $\mu = \mu_{max} \cdot (s/K_s + s)$ was used to calculate equations relating the growth rate, μ, to the external nutrient concentration, s. The half-saturation constant, K_s, and the maximum growth rate, μ_{max}, were estimated from a linear regression of μ on s/μ. Similarly, uptake rates, v, were modelled using a linear transform of the Michaelis-Menton equation where $s/v = K_s/v_{max} + 1/v_{max} \cdot s$. This transform was chosen instead of the frequently used Lineweaver-Burk plot since the latter has been shown to give more biased results (Dowd and Riggs 1965). Transforms of this sort are usually performed on very short time-course depletion curves (e.g. D'Elia *et al.* 1978), where substrate concentration remains relatively unaffected. In the present study, where uptake was measured from depletion of nutrients over longer periods (up to 24 hr), substrate concentrations fell appreciably, and here s represents the average concentration of substrate during this time.

Results

Growth

Increases in dry weight are shown in Fig. 1 and Fig. 2. The greatest response for P-treated plants was obtained with the highest substrate concentration, representing some 17-fold increase in dry weight over 3 weeks. During the third week of treatment, yields were proportional to the substrate concentration supplied. Growth occurred by fragmentation of algal material, and the formation of new ball-like plants similar to those observed in the estuary (Fig. 3).

For nitrogen, there was no significant difference after 2 weeks amongst final weights attained by plants given 0.5, 1.0 or 5.0 mg ℓ^{-1}, which all gave more than a 5-fold increase in dry weight over the controls.

Relative growth rates (RGR) for each weekly interval, are shown in Tables II and III for P and N trials respectively, along with the estimated doubling times over each interval. A regression of the logarithm of growth increments against time showed that algae at each substrate concentration in these experiments were growing exponentially in complete medium. The lag phase of the

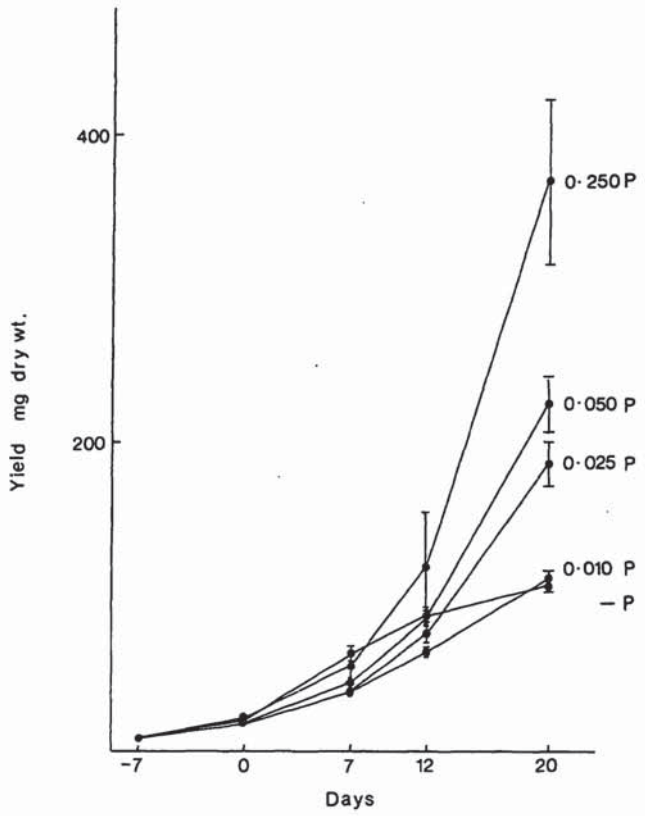

Fig. 1. Yields of *Cladophora* grown with different inorganic phosphorus substrate concentrations (0 to 0.25 mg ℓ^{-1} P) each in the presence of 5.0 mg ℓ^{-1} inorganic nitrogen. Data shown include pre-conditioning in P-depleted medium for 7 days (– 7 to 0) followed by 20 days in complete medium. Each point is the mean of 3 replicates ± S.E.

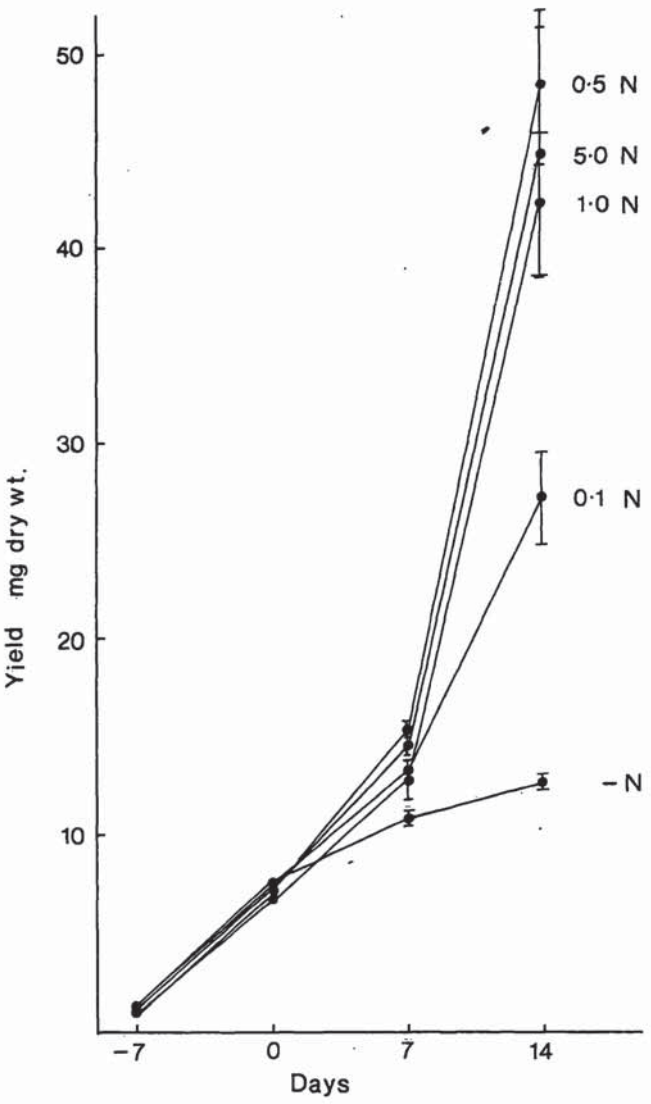

Fig. 2. Yields of *Cladophora* grown with different inorganic nitrogen substrate concentrations (0 to 5 mg ℓ^{-1} N) each in the presence of 0.5 mg ℓ^{-1} inorganic phosphorus. Data shown include pre-conditioning in N-depleted medium for 7 days (– 7 to 0) followed by 14 days in complete medium. Each point is the mean of 3 replicates ± S.E.

growth curve was most obvious during the first week, with lower RGR values, particularly in the N treatments (Tab. III).

Relative growth rates for both experiments during 7 days of pre-conditioning in depleted media suggest that the inocula were growing well even before treatments were started. During this period the algae in P-free media doubled their weight in 5 days (Tab. II) and in 8 days for those growing in N-free media (Tab. III). Growth rates fell steadily in control flasks during these experiments to 20% of rates measured over the week of preconditioning. These give estimated doubling times of 28 and 34 days after 4 and 3 weeks without P and N respectively. While these treatments showed steadily decreasing rates; those algae in higher substrates had increased their rates up to 0.17 day^{-1} by the end of the growth period in complete medium, this representing a doubling time of 4 days (Tab. III).

Equations relating growth during this final week of treatment in complete medium to the external substrate concentrations are shown in Figs. 4 and 5. Concentrations below about 0.02 to 0.03 mg ℓ^{-1} P strongly influenced the growth rate, and above these levels the effect was much reduced. For nitrogen, growth rates were saturated above 0.4 mg ℓ^{-1}, with the greatest response below 0.1 mg ℓ^{-1}.

Growth on return to minimal medium

Rates of growth following a return to either P-free or N-free media are shown in Tables II and III, and yields are given in Figs. 6 and 7. For both nutrients, better growth was obtained on return to depleted media, if the algae had been growing in high concentrations of nutrients. Though it was very difficult to entirely remove inorganic P from the P-free media, relative growth rates fell to 0.018 day^{-1} after 50 days without added P (though with N in high concentration; Tab. II). Similarly, those algae in N-free media (though with P in high concentration) showed little growth after 40 days

Tab. II. Relative growth rates, RGR (day^{-1}), and doubling times D (days) of *Cladophora* during each phase of growth with different substrate P concentrations, each in the presence of 5.0 mg ℓ^{-1} N. Growth rates are the means ± S. E. for 3 replicates at each substrate concentration

Substrate P ($mg\ \ell^{-1}$)		Pre-conditioning in depleted medium (– P)	Treatment with complete medium			Growth in depleted medium (– P)			
		Days – 7 – 0	0–7	7–12	12–20	0–6	6–18	18–23	23–30
0	RGR	0.130 ± 0.001	0.165 ± 0.009	0.065 ± 0.001	0.026 ± 0.002	0.027 ± 0.002	0.039 ± 0.002[1])	0.025 ± 0.004	0.018 ± 0.002
	D	5.3 – 5.4	4.0 – 4.4	10.5 – 10.8	24.7 – 28.9	23.9 – 27.7	16.9 – 18.7	23.9 – 33.0	34.6 – 43.3
0.010	RGR	0.109 ± 0.008	0.111 ± 0.001	0.102 ± 0.005	0.068 ± 0.001	0.092 ± 0.011	0.032 ± 0.002	0.073 ± 0.002[2])	0.028 ± 0.009
	D	5.9 – 6.7	6.2 – 6.3	6.5 – 7.1	10.0 – 10.3	6.7 – 8.5	20.4 – 23.1	9.2 – 9.8	18.7 – 36.5
0.025	RGR	0.112 ± 0.001	0.110 ± 0.001	0.138 ± 0.008	0.110 ± 0.001	0.152 ± 0.001	0.051 ± 0.001	0.079 ± 0.003	0.037 ± 0.001
	D	6.1 – 6.2	6.2 – 6.3	4.7 – 5.3	6.2 – 6.3	4.5 – 4.6	13.3 – 13.9	8.4 – 9.1	18.2 – 19.2
0.050	RGR	0.126 ± 0.004	0.118 ± 0.003	0.137 ± 0.006	0.119 ± 0.001	0.215 ± 0.001	0.078 ± 0.001	0.055 ± 0.001	0.042 ± 0.001
	D	5.3 – 5.7	5.7 – 6.0	4.8 – 5.3	5.8 – 5.9	3.2 – 3.3	8.8 – 9.0	12.4 – 12.8	16.1 – 16.9
0.250	RGR	0.134 ± 0.009	0.145 ± 0.016	0.151 ± 0.012	0.141 ± 0.015	0.219 ± 0.020	0.061 ± 0.004	0.047 ± 0.010	0.024 ± 0.005
	D	4.8 – 5.5	4.3 – 5.3	4.2 – 5.0	4.4 – 5.5	2.9 – 3.5	10.7 – 12.2	12.2 – 18.7	23.9 – 36.5

[1]), [2]) Higher rates, particularly in the low treatments, are presumably due to P contamination

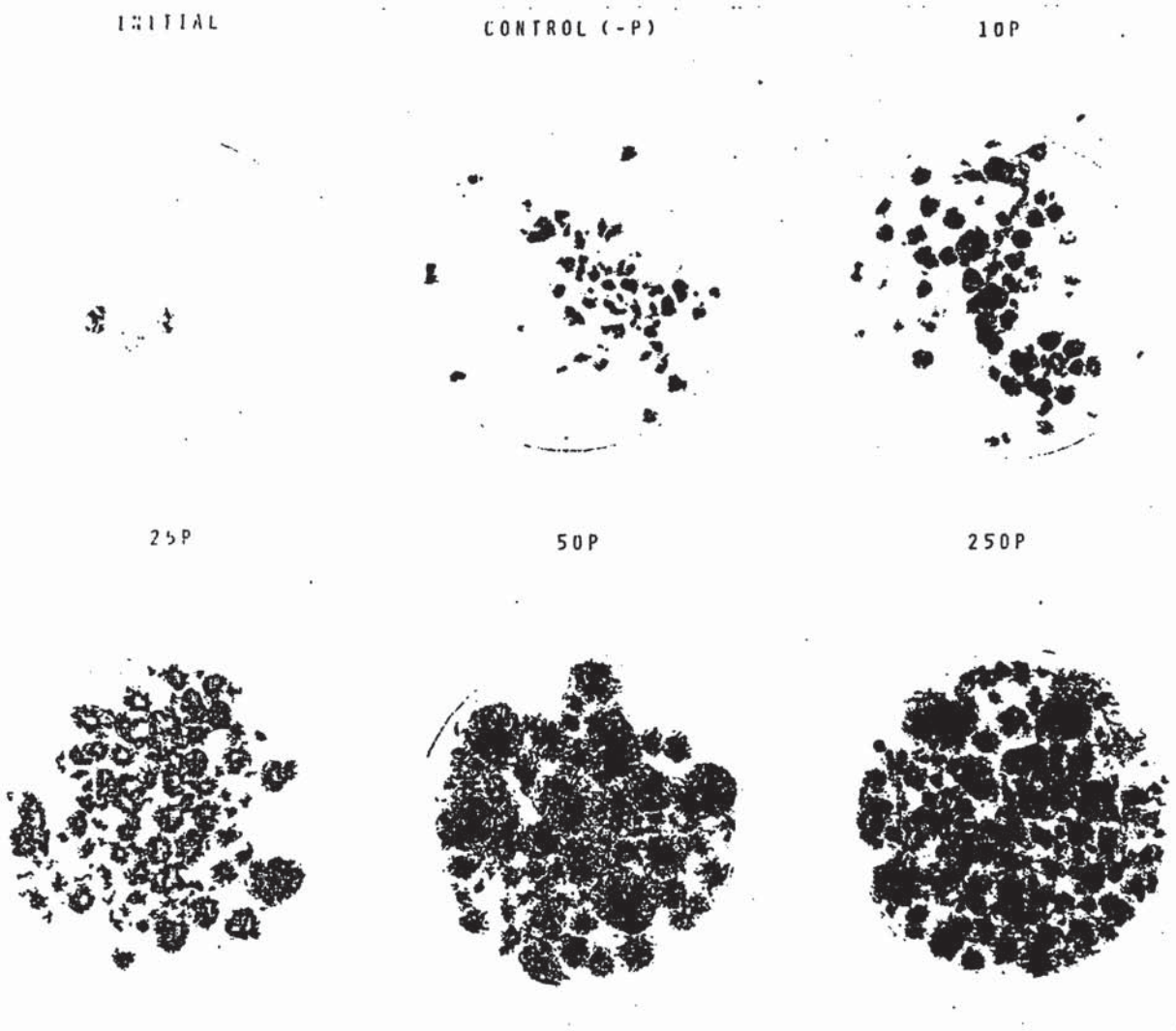

Fig. 3. Yields of *Cladophora* after 20 days growth in complete medium with control (P-free), 10P (0.010 mg ℓ^{-1} P), 25P (0.025 mg ℓ^{-1} P), 50P (0.050 mg ℓ^{-1} P) and 250P (0.250 mg ℓ^{-1} P), each in the presence of 5.0 mg ℓ^{-1} N, compared with initial inoculum. New plants are ball-like, densely branched, radiating filaments similar to those in beds in the estuary

Tab. III. Relative growth rates, RGR (day^{-1}), and doubling time D (days) of *Cladophora* during each phase of growth with different substrate N concentrations, each in the presence of 0.5 mg ℓ^{-1} P. Growth rates are the means ± S. E. for 3 replicates at each substrate concentration.

Substrate N (mg ℓ^{-1})		Pre-conditioning in depleted medium (– N)	Treatment with complete medium		Growth in depleted medium (– N)		
		Days – 7 – 0	0–7	7–14	0–6	6–14	14–20
0	RGR	0.087 ± 0.001	0.049 ± 0.005	0.022 ± 0.002	0.016 ± 0.005	0.008 ± 0.002[1])	
	D	7.9 – 8.1	12.8 – 15.7	28.9 – 34.6	33.0 – 63.0	69.3 – 115.5	
0.1	RGR	0.083 ± 0.013	0.078 ± 0.003	0.101 ± 0.004	0.038 ± 0.002	0.024 ± 0.007	
	D	7.2 – 9.9	8.5 – 9.2	6.6 – 7.1	17.3 – 19.2	22.3 – 40.8	
0.5	RGR	0.078 ± 0.003	0.108 ± 0.001	0.163 ± 0.009	0.120 ± 0.007	0.032 ± 0.002	0.013 ± 0.006
	D	8.5 – 9.2	6.3 – 6.5	4.0 – 4.5	5.4 – 6.1	20.4 – 23.1	36.5 – 99.0
1.0	RGR	0.078 ± 0.006	0.093 ± 0.007	0.170 ± 0.001	0.111 ± 0.002	0.043 ± 0.001	0.011 ± 0.001
	D	8.2 – 9.6	6.9 – 8.1	4.0 – 4.1	6.1 – 6.3	15.7 – 16.5	57.8 – 69.3
5.0	RGR	0.085 ± 0.008	0.099 ± 0.001	0.161 ± 0.014	0.083 ± 0.005	0.070 ± 0.02	–0.007 ± 0.001
	D	7.4 – 9.0	6.9 – 7.1	4.0 – 4.7	7.9 – 8.9	9.6 – 10.2	–

[1]) Rates low and erratic; data averaged for last 14 days

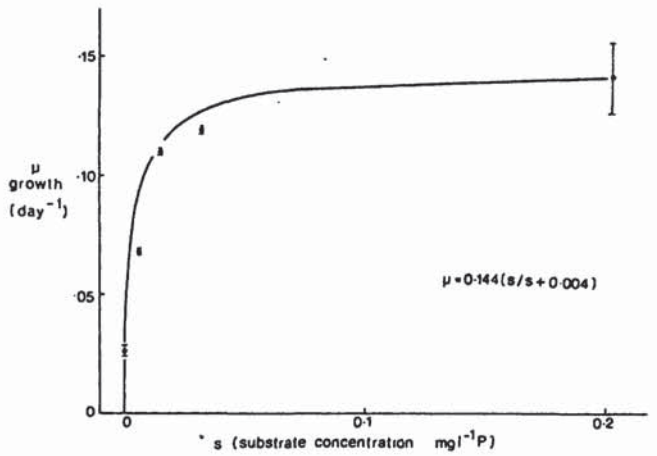

Fig. 4. Relative growth rates (day^{-1}) of *Cladophora* during the last week of growth in complete medium (0 to 0.25 mg ℓ^{-1} P; 5.0 mg ℓ^{-1} N) as a function of the substrate P concentration. Each point is the mean of 3 replicates ± S.E.

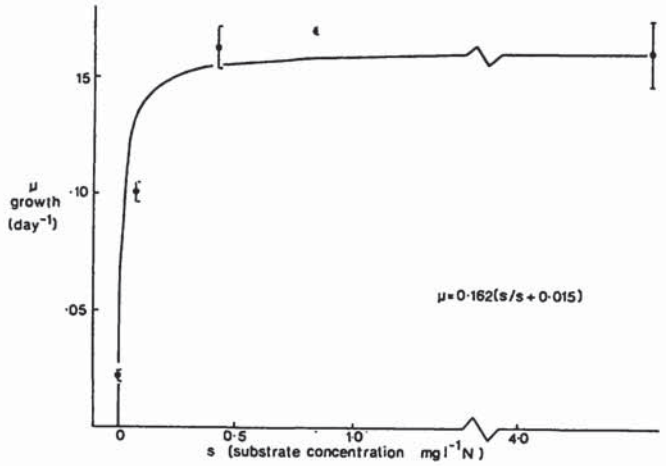

Fig. 5. Relative growth rates (day^{-1}) of *Cladophora* during the last week of growth in complete medium (0 to 5.0 mg ℓ^{-1} N; 0.5 mg ℓ^{-1} P) as a function of the substrate N concentration. Each point is the mean of 3 replicates ± S.E.

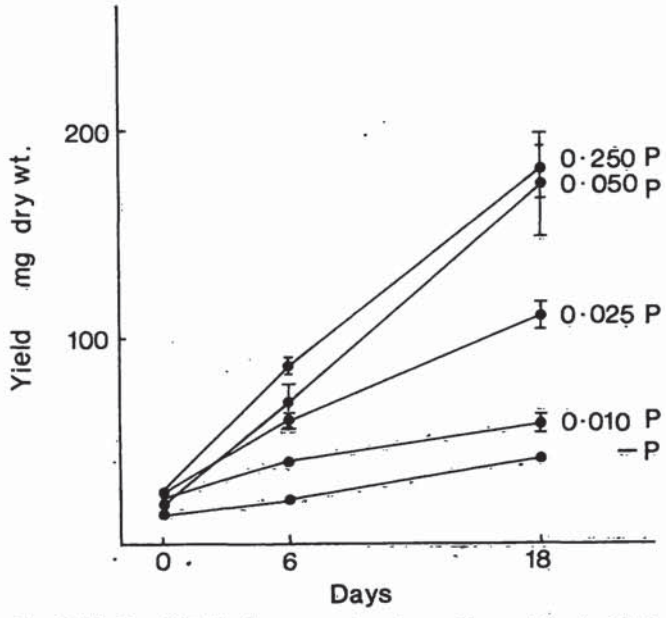

Fig. 6. Yields of *Cladophora* on return to medium without added P in the presence of 5.0 mg ℓ^{-1} N, following growth for 3 weeks in complete medium (0 to 0.25 mg ℓ^{-1} P; 5.0 mg ℓ^{-1} N). Each point is the mean of 3 replicates ± S.E.

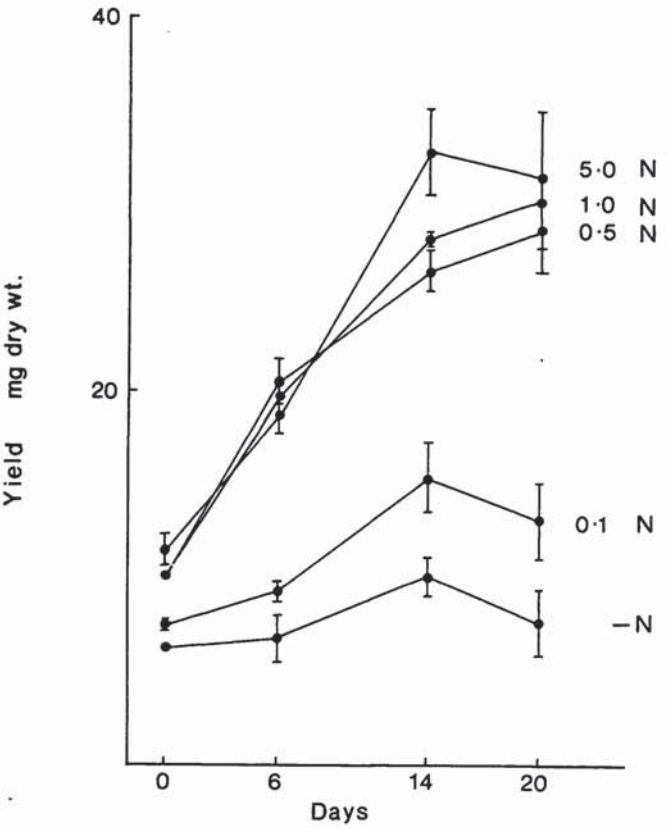

Fig. 7. Yields of *Cladophora* on return to medium without added N in the presence of 0.5 mg ℓ^{-1} P, following growth for 2 weeks in complete medium (0 to 5.0 mg ℓ^{-1} N; 0.5 mg ℓ^{-1} P). Each point is the mean of 3 replicates ± S.E.

(Tab. III). In contrast, those previously given high concentrations of N or P continued growing at high rates for a longer period. For example, following 3 weeks in high nutrients, *Cladophora* returned to P-free media was still growing well (up to 0.042 day^{-1}) 4 weeks later (Tab. II), with a doubling time up to 16 days. Those returned to N-free media for 3 weeks, after 2 weeks in high nutrients, showed lower growth rates (up to 0.013 day^{-1}) with a doubling time greater than 36 days (Tab. III).

Nutrient uptake

Rates of uptake of P for each substrate concentration are shown in Table IV. Despite the approximations outlined earlier, (see Materials and Methods), these data were fitted to a Michaelis-Menten transform and the resulting maximum uptake rates (v_{max}) and half-saturation constants (K_s) are shown at the beginning and near the end of growth in complete medium (Tab. V).

Uptake rates increased with increasing P substrate concentrations, particularly during the final week in complete medium. The affinity for P was more obvious in cultures supplied with the nutrient soon after pre-conditioning in P-free medium, with higher rates during the first week. This is also evident from the lower K_s values for the first day in complete medium compared with those measured 18 days later. Uptake rates were

Tab. IV. Uptake rates of phosphate-phosphorus by *Cladophora* growing in complete medium with different P-substrate concentrations. Data are weekly means (± S.E.) from daily uptake rates on 3 replicates at each substrate concentration. Rates were measured over a combined light and dark period over 13 ± 2, 22 ± 2 and 20 ± 2 hours for weeks 1, 2 and 3 respectively.

Substrate P (mg ℓ^{-1})	P uptake (mg g^{-1} dry weight hr^{-1}) Week 1	Week 2	Week 3
0.010	0.036 ± 0.009	0.006 ± 0.001	0.008 ± 0.002
0.025	0.071 ± 0.015	0.017 ± 0.003	0.015 ± 0.004
0.050	0.072 ± 0.013	0.031 ± 0.002	0.025 ± 0.007
0.250	0.078 ± 0.019	0.034 ± 0.006	0.056 ± 0.008

Tab. V. Maximum uptake rate (v_{max}) and half-saturation (K_s) of inorganic phosphorus by *Cladophora* growing with different P substrate concentrations (0–0.25 mg ℓ^{-1}), each in the presence of 5.0 mg ℓ^{-1} inorganic nitrogen. Data shown are at the start (day 1) and near the end (day 18) of growth in complete medium. Data were calculated from the mean of 3 replicates at each of 5 substrate concentrations in the light (L) and over a combined light and dark period (L + D)

	Illumination	Time (hrs)	v_{max} (mg g^{-1} dry wt. hr^{-1})	K_s (mg ℓ^{-1})
Day 1	L	4.5	0.127 ± 0.003	0.002 ± 0.003
Day 1	L + D	17.0	0.121 ± 0.001	0.011 ± 0.001
Day 18	L	3.5	0.112 ± 0.019	0.015 ± 0.001
Day 18	L + D	22.5	0.058 ± 0.012	0.055 ± 0.010

Tab. VI. Uptake rates of ammonia and nitrate-nitrogen by *Cladophora* growing in complete medium with different N substrate concentrations. Data are weekly means (± S.E.) from daily uptake rates on 3 replicates at each substrate concentration. Rates were measured over a combined light and dark period over 28 ± 6 and 23 ± 5 hours for weeks 1 and 2 respectively

Substrate N	N uptake (mg g^{-1} dry weight hr^{-1}) Week 1		Week 2	
(mg ℓ^{-1})	NH_4-N	NO_3-N	NH_4-N	NO_3-N
0.1	0.15 ± 0.06	0.09 ± 0.03	0.15 ± 0.05	0.11 ± 0.04
0.5	0.45 ± 0.12	0.15 ± 0.05	0.32 ± 0.06	0.23 ± 0.05
1.0	0.88 ± 0.18	0.15 ± 0.07	0.54 ± 0.06	0.26 ± 0.09
5.0	1.76 ± 0.46	0.22 ± 0.14	0.63 ± 0.17	0.05 ± 0.03

Tab. VII. Simultaneous uptake rates of ammonia and nitrate-nitrogen in *Cladophora* after 2 weeks growth in complete medium, measured over 5 hours in the light and 17 hours in the dark. Each datum is the mean of 3 replicates ± S.E.

Substrate N	N uptake (mg g^{-1} dry weight hr^{-1}) NH_4-N		NO_3-N	
(mg ℓ^{-1})	Light	Dark	Light	Dark
0.1	0.42 ± 0.03	0.10 ± 0.01	0.33 ± 0.02	0.11 ± 0.01
0.5	0.64 ± 0.12	0.12 ± 0.05	0.47 ± 0.04	0.26 ± 0.12
1.0	0.53 ± 0.06	0.23 ± 0.02	0.65 ± 0.13	0.07 ± 0.03
5.0	1.67 ± 0.49	0.33 ± 0.17	0	0.04 ± 0.03

typically lower when calculated over a combined light and dark period compared to those measured over shorter periods in the light.

The average weekly uptake rates of ammonia and nitrate-nitrogen are shown in Table VI. *Cladophora* was able to use both N species simultaneously in all treatments, though at the highest substrate concentration, uptake of nitrate was negligible compared to ammonia. Ammonia uptake rates were higher than those for nitrate at all treatment concentrations. Preferential uptake of ammonia during the last week of growth in complete medium was demonstrated here, where rates of simultaneous uptake of both species in the light and in the dark are compared (Tab. VII). Uptake rates were clearly lower in the dark compared with those in the light, though there was still preferential uptake of ammonia. These data give v_{max} and K_s values, in the light, of 1.82 ± 0.31 mg g^{-1} dry weight hr^{-1}; 0.29 ± 0.16 mg ℓ^{-1} for ammonia-nitrogen, and 0.59 ± 0.08 mg g^{-1} dry weight hr^{-1}; 0.02 ± 0.01 mg ℓ^{-1} for nitrate-nitrogen. The transform for NO_3-N was calculated excluding rates at the highest substrate N concentration, these having fallen close to zero. Over the substrate concentrations used, however, the fit is significant ($r = 0.99$).

Nutrient in the algal tissue

The relationship between the total amount of nutrient taken up from solution over the period of treatment and the corresponding change in total P and N in the tissue is shown for both experiments in Fig. 8. There is almost a 1:1 ($r = 0.99$) relationship between these for the P treatments. Those grown in the highest P concentration, however, have exaggerated tissue P concentrations, which suggests some contamination at these higher levels. In contrast, the relationship was not linear with nitrogen, where there was little further increase in nitrogen concentration in the tissue in the presence of large amounts of this nutrient. This has also been reported for aquatic angiosperms (Gerloff and Krombholz 1966) where most N added to the medium was recovered in the tissue, except in those plants growing with high external N concentrations (> 21 mg ℓ^{-1}). Nitrogen taken up but not incorporated may have been released back to solution in organic form at these high levels. At the time of the last harvest, organic nitrogen in the water was high (0.78 ± 0,01 to 1.03 ± 0.01 mg ℓ^{-1}), particularly in the two highest N treatments. Nevertheless, the relationship between the amount of nutrient taken up and corresponding incorporation into the tissue is linear for substrate P and N supplied at concentrations similar to those in estuary water.

Concentrations of nitrogen and phosphorus in the tissue and their corresponding N:P ratios during each phase of the experiment are summarised in Tab. VIII and IX, where increasing substrate concentrations result in in-

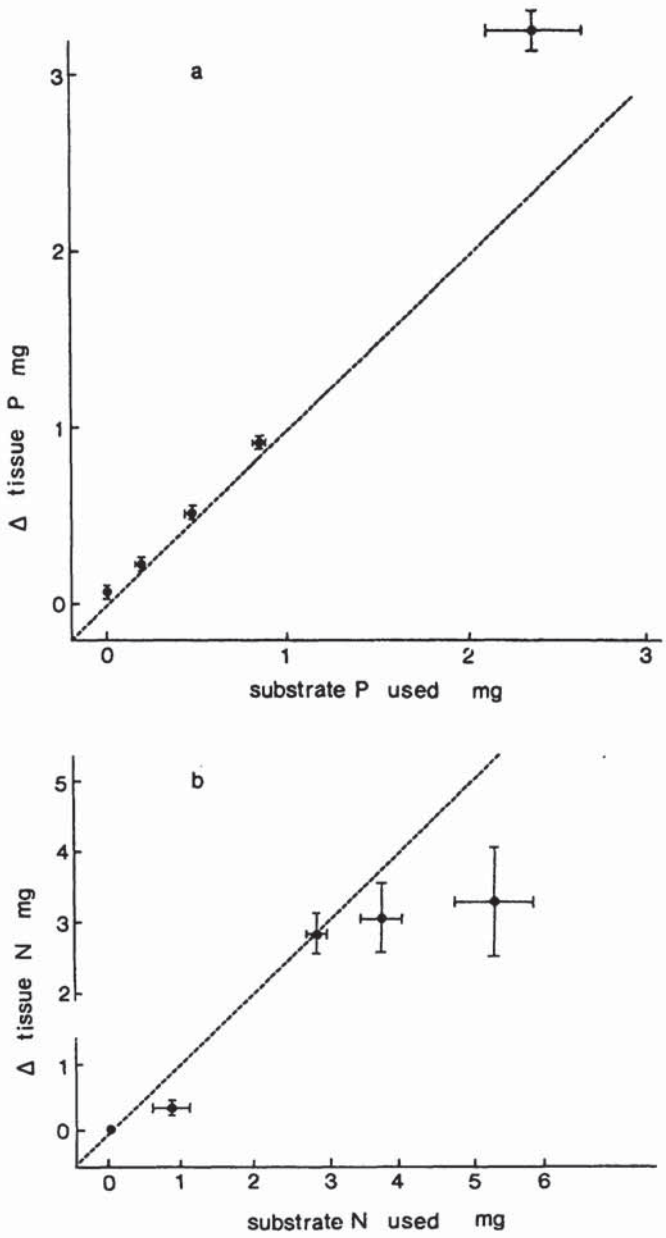

Fig. 8a. Increase in total phosphorus (mg) present in the tissue of *Cladophora* after 3 weeks growth in complete medium (0 to 0.25 mg ℓ^{-1} P; 5.0 mg ℓ^{-1} N) as a function of the amount of inorganic phosphorus (mg) taken up over this time

Fig. 8b. Increase in total nitrogen (mg) present in the tissue of *Cladophora* after 2 weeks growth in complete medium (0 to 5.0 mg ℓ^{-1} N; 0.5 mg ℓ^{-1} P) as a function of the amount of inorganic nitrogen (mg; NH_4-N + NO_3-N) taken up over this time

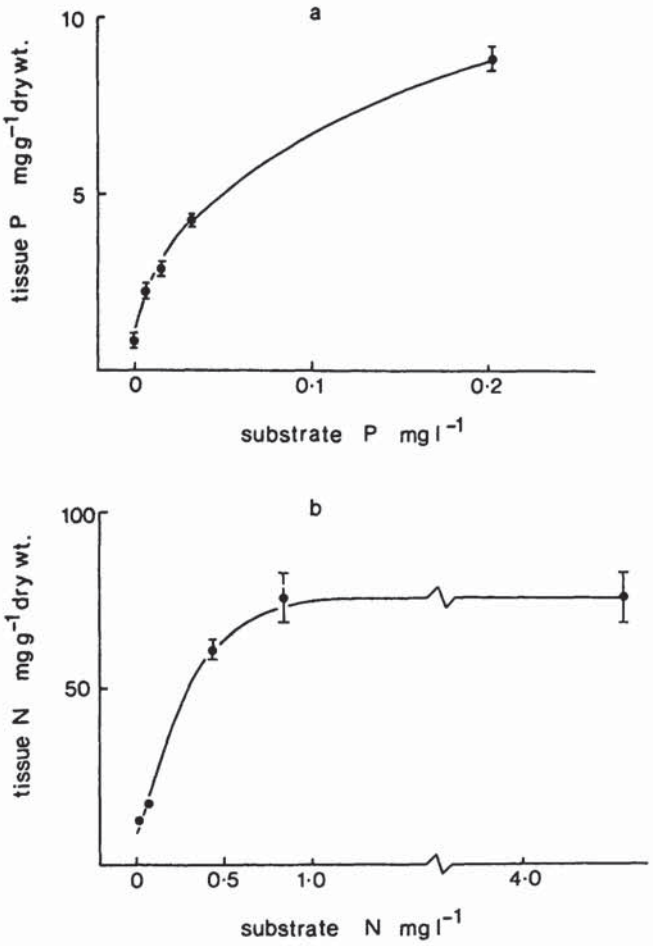

Fig. 9a. Concentration of total P in *Cladophora* tissue (mg g^{-1} dry wt.) during the last week of growth in complete medium (0 to 0.25 mg ℓ^{-1} P; 5.0 mg ℓ^{-1} N) as a function of the substrate P concentration (mg ℓ^{-1}) supplied

Fig. 9b. Concentration of total N in *Cladophora* tissue (mg g^{-1} dry wt.) during the last week of growth in complete medium (0 to 5.0 mg ℓ^{-1} N; 0.5 mg ℓ^{-1} P) as a function of the substrate N concentration (mg ℓ^{-1}) supplied

creases in tissue N and P in complete medium. Concentrations of nitrogen and phosphorus in the tissue following the period of growth in complete medium are shown as a function of P and N substrate concentrations in Fig. 9. Saturation of the tissue is evident when high concentrations of inorganic N are supplied, though this is not the case with phosphorus, where the high substrate concentration supplied did not saturate the tissue; higher concentrations of P may well have resulted in even higher tissue P levels. Similar trends have been observed with *Cladophora* in the field (Wallentinus 1979), where tissue nitrogen concentrations were saturated above 50 mg g^{-1} dry weight (*cf* 76 mg g^{-1} in culture in this study), these levels corresponding to growth with the highest external nitrogen concentration. For the alga growing without added P (controls) there was a reduction in tissue P of over 50% after 4 weeks (Tab. VIII). N in this tissue was initially high, though within the range encountered in the field, but was reduced by a similar amount over this period. At P substrate concentrations above 0.01 mg ℓ^{-1}, phosphorus in the tissue was maintained above 2 mg g^{-1} dry weight, Tab. VIII), and increased with corresponding increases in the substrate concentration. In these P treatments (with N in high supply), some incorporation of N into the tissue was evident after 3 weeks, particularly the two highest P treatments, with over 50 mg g^{-1} dry weight, this representing an increase over initials of up to 32%.

Nitrogen concentrations fell in tissue to which no N was supplied; the fall was almost 47% during the week of

pre-conditioning, and up to 34% over the following 2 weeks when tissue N concentrations were then about 12 mg g^{-1} dry weight (Tab. IX). Tissue N levels increased in those plants given a substrate concentration above 0.1 mg ℓ^{-1} N, reaching a plateau at high substrate concentrations, (Tab. IX), the maximum being 76 mg g^{-1} dry weight, which corresponds with an increase of almost 300% in internal nitrogen over 2 weeks. Some incorporation of P into these tissues was also evident, being most pronounced in the alga grown with the highest N treatments.

Concentrations of tissue P in those plants without added N did not fall markedly during pre-conditioning, though there was an 18% decrease over the following 2 weeks to 2.25 mg g^{-1} dry weight.

Nutrient levels on return to depleted medium

The change in tissue P and N on return to depleted medium is also shown in Tables VIII and IX. There was a significant reduction in final concentrations after 3–4 weeks, particularly those previously growing in high substrate concentrations of P or N. For example, those algae whose growth in high nutrients resulted in elevated internal P concentrations up to nearly 9 mg g^{-1} dry weight (Tab. VIII), were reduced by over 90% to 0.76 mg g^{-1} after 4 weeks in P-free medium. Similarly, those which had accumulated very high N levels (76 mg g^{-1} dry weight) were reduced by over 66% following return to N-free media for 3 weeks.

The loss of stored nitrogen in N-treated algae on return to depleted medium for 3 weeks was proportional to the concentration of substrate N under which they had been previously growing. Prolonging this period of growth in depleted medium beyond 3 weeks may have reduced tissue nitrogen concentrations in those algae which were previously growing in high N substrates to levels as low as those of the controls. The extent of losses of stored P on return to P-free medium for 4 weeks was similar for all treatments regardless of the substrate concentrations under which they had been previously growing.

Growth rates and nutrient levels

Growth rates during the last week of treatment in complete medium are shown as a function of the corresponding concentration of nitrogen and phosphorus in the tissue in Fig. 10. Extrapolation of the "limiting" portion of these curves with that portion saturating the growth rates results in a "critical" tissue nutrient concentration of 3.3 mg g^{-1} and 21 mg g^{-1} dry weight for P and N respectively. Here, "critical" tissue content refers to the minimum concentration of nitrogen or phosphorus in the tissue associated with maximum growth (Gerloff and Krombholz 1966). Extrapolating growth rates in Fig. 10 to zero gives some estimate of the "minimum viable" tissue content for *Cladophora*, below which the growth rate is negligible. For nitrogen this is about 12 mg g^{-1} dry weight, and 0.5 mg g^{-1} dry weight for phosphorus.

Tab. VIII. Concentration of P and N (mg g^{-1} dry weight) in *Cladophora* tissue and their ratios, initially and following each phase of growth with different P substrate concentrations, each in the presence of 5.0 mg ℓ^{-1} N. Each figure is the mean ± S.E. of 3 replicates

Substrate P (mg ℓ^{-1})	Concentration in tissue (mg g^{-1} dry weight) Initially			After 3 weeks growth in complete medium			Four weeks after return to medium without added P		
	P	N	N:P	P	N	N:P	P	N	N:P
0	2.04 ± 0.15	43.0 ± 0	21:1	0.99 ± 0.16	21.8 ± 1.5	22:1	0.53 ± 0.09	18.6 ± 1.8	35:1
0.010	2.04 ± 0.15	43.0 ± 0	21:1	2.21 ± 0.16	41.5 ± 1.6	19:1	0.86 ± 0.02	29.5 ± 1.6	34:1
0.025	2.04 ± 0.15	43.0 ± 0	21:1	2.92 ± 0.18	47.5 ± 2.2	16:1	0.74 ± 0.03	28.0 ± 1.9	38:1
0.050	2.04 ± 0.15	43.0 ± 0	21:1	4.24 ± 0.07	56.7 ± 2.6	13:1	0.61 ± 0.02	26.0 ± 0.7	42:1
0.250	2.04 ± 0.15	43.0 ± 0	21:1	8.88 ± 0.31	50.0 ± 2.1	6:1	0.76 ± 0.04	28.6 ± 0.6	38:1

Tab. IX. Concentration of P and N (mg g^{-1} dry weight) in *Cladophora* tissue and their ratios, initially and following each phase of growth with different N substrate concentrations, each in the presence of 0.5 mg ℓ^{-1} P. Each figure is the mean ± S.E. of 3 replicates

Substrate N (mg ℓ^{-1})	Concentration in tissue (mg g^{-1} dry weight) Initially			After 2 weeks growth in complete medium			Three weeks after return to medium without added N		
	P	N	N:P	P	N	N:P	P	N	N:P
0	2.64 ± 0.22	36.6 ± 2.9	14:1	2.25 ± 0.08	12.8 ± 0.9	6:1	–	14.5 ± 0.2	–
0.1	2.64 ± 0.22	36.6 ± 2.9	14:1	4.07 ± 0.07	17.1 ± 0.6	4:1	4.27 ± 0.13	16.9 ± 4.2	4:1
0.5	2.64 ± 0.22	36.6 ± 2.9	14:1	5.67 ± 0.90	61.4 ± 3.4	11:1	4.27[1])	18.0 ± 2.2	4:1
1.0	2.64 ± 0.22	36.6 ± 2.9	14:1	5.62 ± 0.22	75.9 ± 4.2	13:1	5.19 ± 0.15	22.3 ± 2.8	8:1
5.0	2.64 ± 0.22	36.6 ± 2.9	14:1	6.95 ± 0.83	76.4 ± 7.5	11:1	12.75 ± 1.27	25.6 ± 2.4	2:1

[1]) One value only

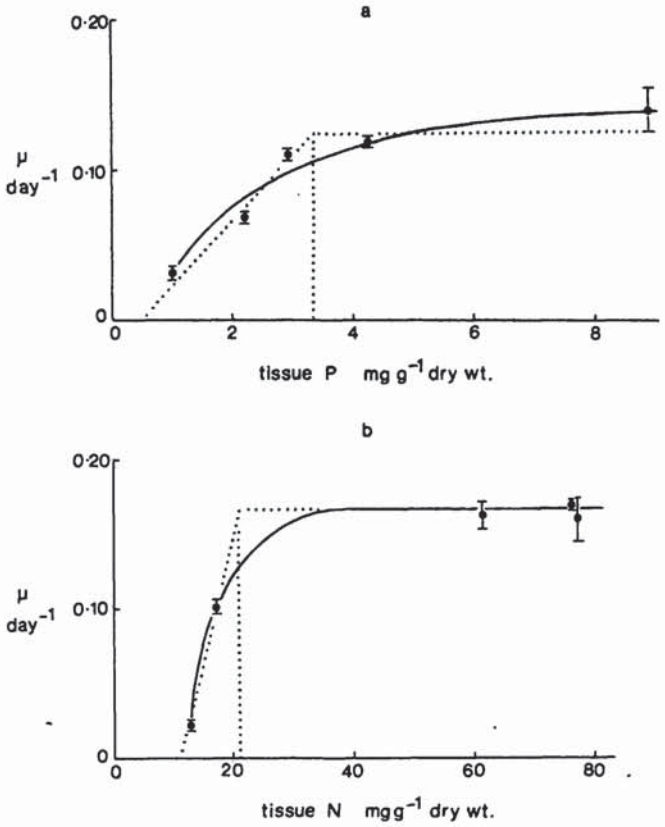

Fig. 10a. Relative growth rate (day^{-1}) of *Cladophora* during the last week in complete medium (0 to 0.25 mg ℓ^{-1} P; 5.0 mg ℓ^{-1} N) as a function of the total P concentration in the tissue (mg g^{-1} dry weight) during this time

Fig. 10b. Relative growth rate (day^{-1}) of *Cladophora* during the last week in complete medium (0 to 5.0 mg ℓ^{-1} N; 0.5 mg ℓ^{-1} P) as a function of the total N concentration in the tissue (mg g^{-1} dry weight) during this time

Chlorophyll a

There was a strong relationship between growth rates in these experiments during the final week of growth in complete medium and corresponding concentrations of chlorophyll *a* in the tissue (Fig. 11), these ranging from 3.6 to 12.0 and 0.6 to 9.1 mg g^{-1} dry weight in P and N experiments respectively. A close relationship between chlorophyll *a* and nitrogen was evident from the strong correlation between changes in tissue chlorophyll *a* and corresponding changes in tissue nitrogen (Fig. 12), though the relationship was not significant with phosphorus. This has been reported previously with *Cladophora* (Wallentinus 1975). Those algae growing in high substrate N concentrations were noticeably darker in colour, in contrast to controls where removal of nitroten resulted in a marked reduction in chlorophyll *a* in the tissue, with a concomitant rise in the levels of phaeophytins. For example those algae grown without N (controls) for 3 weeks had reduced their chlorophyll *a* from 3.73 mg g^{-1} to 0.69 mg g^{-1} dry weight, the latter associated with 3.82 mg g^{-1} phaeophytines. Following a further 3 weeks in N-depleted medium, the controls had obviously senesced, with no measurable chlorophyll *a*, nitrogen starvation producing chlorosis in these cells (Wallentinus 1975).

Chlorophyll *a* in the remaining treatments had similarly fallen at this time, the loss being less marked in those previously growing with high substrate nitrogen, though it is likely these would have fallen to low levels soon after this experiment was terminated.

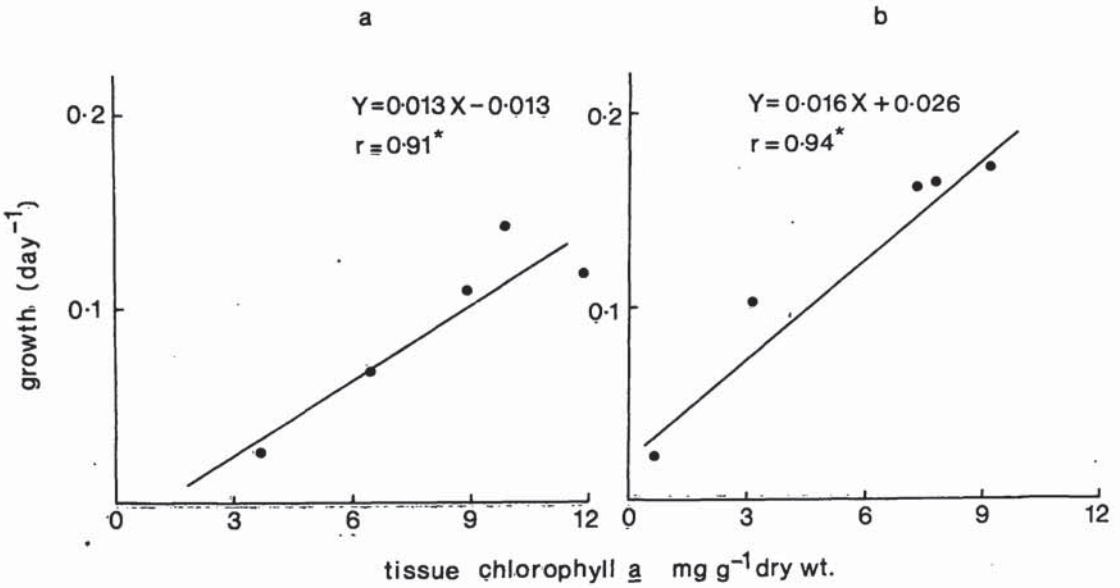

Fig. 11a. Regression of relative growth rate (day^{-1}) of *Cladophora* during the last week in complete medium (0 to 0.25 mg ℓ^{-1} P; 5.0 mg ℓ^{-1} N) with corresponding concentrations of chlorophyll *a* in the tissue (mg g^{-1} dry wt.)

Fig. 11b. Regression of relative growth rate (day^{-1}) of *Cladophora* during the last week in complete medium (0 to 5.0 mg ℓ^{-1} N; 0.5 mg ℓ^{-1} P) with corresponding concentrations of chlorophyll *a* in the tissue (mg g^{-1} dry wt.)

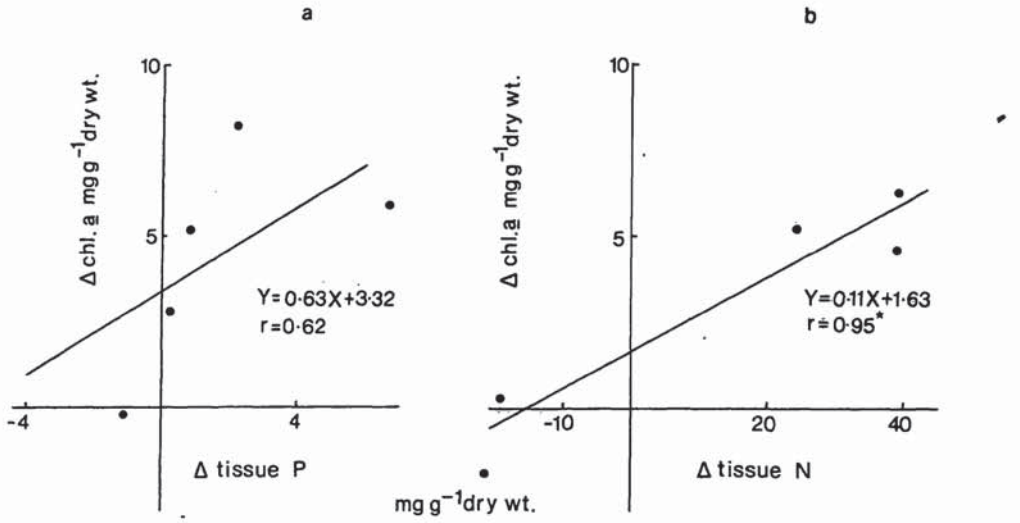

Fig. 12a. Regression of change in concentration of chlorophyll *a* in the tissue (mg g^{-1} dry wt.) with corresponding change in tissue P concentration (mg g^{-1} dry wt.) following growth in complete medium (0 to 0.25 mg ℓ^{-1} P; 5.0 mg ℓ^{-1} N)
Fig. 12b. Regression of change in concentration of chlorophyll *a* in the tissue (mg g^{-1} dry wt.) with corresponding change in tissue N concentration (mg g^{-1} dry wt.) following growth in complete medium (0 to 5.0 mg ℓ^{-1} N; 0.5 mg ℓ^{-1} P)

Discussion

Growth

Though there are few data available for comparison, yields of *Cladophora* in this study appear high when compared with culture studies of other *Cladophora* species (e.g. Pitcairn and Hawkes 1973). In contrast, reports of cellular doubling times of 1–2 days for a freshwater *Cladophora* growing exponentially under stagnant conditions in synthetic medium (Zuraw 1969), are higher than the maximum of 3–4 days obtained for whole plants in this study, with high concentrations of N and P supplied (Tab. II and III). A doubling time of between 5 and 6 days, has been recorded for the brown alga *Ectocarpus* growing under near-exponential conditions in medium supplemented with 3 mg ℓ^{-1} P and 7 mg ℓ^{-1} N (Boalch 1961). Comparisons of this sort are of limited use, however, because of the differing responses which may be obtained with different background media (e.g. Oza and Sreenivasa Rao 1977). Aeration and agitation would certainly have promoted growth in the present study by increasing diffusion rates of nutrients in solution (Conover 1968, Whitford and Schumacher 1961).

It is clear from Fig. 2 that under conditions where the P supply is plentiful, solution N:P ratios (by weight) of 10:1, 2:1 and 1:1 result in very similar yields. The response falls with higher ratios if N is limiting, as exemplified with yields obtained with either no added N (controls) or 0.1 mg ℓ^{-1} N.

If N is at high concentration the response is greatest with an N to P supply of 20:1 (by weight), this being the lowest tested in the P trials. Higher ratios produced successively poorer yields as P levels became lower (Fig. 1). At sites of growth within the algal bed, *Cladophora* is intimately associated with nutrient-rich water, resulting from mineralization of decomposing, underlying layers of the alga, which produces a fine, black mud (McComb *et al.* 1980). Under such chemically-reduced conditions, large amounts of nutrients may be released into the water column during decomposition (Gordon and McComb, unpublished). Concentrations of ammonia, nitrate and phosphate in this "interalgal water" are in fact some 4, 2 and 15 times, respectively, those of the overlying water, the resulting inorganic N:P ratio being typically 2:1 (by weight) (McComb *et al.* 1980). Comparing these concentrations with those producing the best yields in culture would suggest that N and P in the algal bed are unlikely to limit growth. In contrast, changes in the concentration of N and P in the overlying water may be significant to growth of *Cladophora* balls which are not associated with an algal bed. During the few weeks in winter when river flow results in a considerable input of phosphate and nitrogen (particularly nitrate; McComb *et al.* 1980), water column nutrients are very high, inorganic nitrogen reaching nearly 2 mg ℓ^{-1} at the bottom, while PO_4-P may reach 0.15 mg ℓ^{-1}. In the water column PO_4-P remains low (< 0.01 mg ℓ^{-1}), unless there has been river flow, and could limit the growth rate (Fig. 4), while nitrogen, predominantly as ammonia, is rarely above the concentration likely to saturate growth rates (Fig. 5). Consequently, in winter, nutrients are unlikely to limit growth, either in the water column or in the bed itself, controlling factors at this time being light and temperature (Gordon *et al.* 1980).

Uptake rates

The algae in these cultures were continuously agitated, promoting nutrient diffusion in contrast to those in the algal bed which remain relatively static. Maximum uptake rates of P observed in culture provide a basis for estimating the alga's best utilization of P under non-limiting conditions, and it is unlikely that rates in the field would exceed this, at least where nitrogen is not limiting.

Uptake rates of ammonia and nitrate, added together in nearly equal proportions, increased with all substrate concentrations except the highest (Tab. VI) where there was suppression of nitrate assimilation, presumably because of the inactivation of nitrate reductase in the presence of high levels of ammonia (Conway 1977; Eppley *et al.* 1977). Ammonia was taken up more rapidly than nitrate in this study though both were removed from solution simultaneously (D'Elia and De Boer 1978; Haines and Wheeler 1978; Hanisak and Harlin 1978). The ability of *Cladophpora* to assimilate both ammonia and nitrate is significant during the winter river flush when most allochthonous inorganic nitrogen is present as nitrate. However, recalling that inorganic nitrogen of "interalgal" water within the algal beds is present predominantly as ammonia throughout the year, and in view of the rapid uptake of ammonia-nitrogen in culture, this N species is the most likely major N source for *Cladophora* growth in the estuary.

Uptake rates in the dark, though lower than those in the light (Tab. VII), provide evidence that *Cladophora* in the underlying layers of the algal bed (where light is unavailable for growth) utilizes available nutrients when cellular energy reserves permit.

Tissue nutrients and growth rates

In this study increasing substrate concentrations resulted in successively higher final tissue nutrient concentrations (Fig. 9), to levels which are well above those recorded in the field. For example, for station 4, a growth site near the river mouth, internal N ranges typically between 16 and 40 mg g^{-1} N dry weight (mean 26 ± 5) and phosphorus between 0.8 and 3.0 mg g^{-1} P dry weight (mean 2.2 ± 0.6), which are similar to levels of 8 to 52 mg g^{-1} N and 0.73 to 5.42 mg g^{-1} P reported by Wallentinus (1979) for *Cladophora* growing in the Baltic.

Tissue N and P levels range, therefore, from above "critical" down to nearly "minimum viable" concentrations, and the higher concentrations suggest luxury uptake. The high tissue concentration found experimentally here, to levels well above those measured in the field, would suggest that the alga has the capacity under nonlimiting conditions to take up nutrients at concentrations well above those considered "critical".

"Critical" concentrations in culture here are high compared with those reported for other aquatic plants. For example, concentrations above 13 mg g^{-1} N dry weight and 1.3 mg g^{-1} P dry weight have been considered indicative of luxury tissue concentrations for aquatic angiosperms (Gerloff and Krombholz 1966), while a value of 1.6 to 1.7 mg g^{-1} P dry weight was reported for a riverine *Cladophora*, as the tissue P concentration above which there was little increase in photosynthetic rate (Wong and Clark 1976). An even lower "critical" value of 0.6 mg g^{-1} P has been reported for *Cladophora* in culture (Gerloff and Fitzgerald cited in Lin 1977) which is close to the typical "starved" level found for algae in this study following their growth for a number of weeks without phosphorus (Tab. VIII). Tissue N:P ratios during exponential growth in culture here ranged from 4:1 to 22:1 (by weight) Tab. VIII and IX). At the highest substrate concentrations, with the best growth rates, these ranged between 6:1 and 13:1 (by weight), rising to as much as 42:1 (by weight) following a return to depleted medium for 4 weeks. These high ratios, associated with low growth rates, particularly in those algae given no P for a number of weeks, clearly reflect P limitation and senescence of the alga. Ratios in those algae producing maximum growth rates in this study are similar to the range of 6:1 to 9:1 (by weight) reported for *Cladophora glomerata* producing best photosynthetic rates (Wallentinus 1979). A ratio of 7:1 (by weight) has been reported typical for growth of marine phytoplankton (Harris and Riley cited in Redfield *et al.* 1963) and this is close to the ratio of "critical" tissue N and P for *Cladophora* (6.4:1) calculated here from two independent experiments.

It is not the purpose in this paper to interpret growth rates of the alga in the field from the results observed in these culture studies. It is worth mentioning, however, that measurement of concentrations of N and P in algal tissue and their relation to external supply of these nutrients available for growth is useful in estimating the degree of nutrient deficiency of the alga (e. g. Healey 1978), particularly in view of the marked reduction in internal N and P concentration observed here following removal of either nutrient from solution. The data are consistent with the view that *Cladophora* has the ability to utilize its stored reserves of nitrogen and phosphorus when external supplies become depleted (Chapman and Craigie 1977, Hanisak 1979).

Conclusion

These laboratory data suggest that concentrations of inorganic nitrogen and phosphorus may limit growth of *Cladophora* in the water column during most of the

year, except for the brief period in winter, when there is substantial nutrient input from river flow. In the algal bed, however, nutrients are typically at higher concentrations, and exceed levels which saturate the growth rate.

Uptake of nitrogen and phosphorus into the cells, to levels well above those considered "critical" for growth, suggest the alga has the ability for luxury uptake. Removal of either nutrient from solution, in the presence of high concentrations of the other, results in a marked reduction in growth rates with a corresponding decrease in tissue nitrogen and phosphorus, approaching the minimum necessary for growth.

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