Botanica Marina
Vol. XXIV, pp. 381–387, 1981

Nitrogen and Phosphorus Nutrition of *Cladophora* in the Peel-Harvey Estuarine System, Western Australia

P. B. Birch[1], D. M. Gordon and A. J. McComb

Botany Department, University of Western Australia, Nedlands, 6009, Australia

(Received September 15, 1980)

Abstract

Cladophora aff. *albida* (Huds.) Kütz. is a benthic alga which grows in nuisance proportions in the shallow Peel-Harvey Estuarine System of Western Australia. Tissue analyses revealed that although N and P contents varied seasonally, the alga was rarely below the minimum N tissue content necessary for maximum growth but always below this concentration for P. Lower tissue concentrations in summer and autumn suggested that the alga relied to some extent on stored N and P; at this time nutrient supply rates were insufficient to keep pace with higher growth rates. In winter and spring accumulation of N and P into the tissue occurred, and this can be related to high nutrient input from rivers. Other potentially important sources of nutrients were the surface sediments and decomposing *Cladophora* and phytoplankton. However, the full storage potential of *Cladophora* (demonstrated experimentally) was not reached at this time primarily because high river flow was confined to only a few weeks.

Overall, tissue analyses of *Cladophora*, when combined with laboratory experimentation, provided a useful tool for interpreting the role of nutrient storage and showed that P may be an important factor in the control of *Cladophora* growth in the estuary.

Introduction

This paper is the second (see Gordon *et al.* 1981) concerning the nitrogen (N) and phosphorus (P) nutrition of an estuarine *Cladophora*. It provides more information on the nutritional status of the alga in the field, and on uptake and storage of N and P during its annual growth cycle.

Cladophora aff. *albida* (Huds.) Kütz. is a green alga which is present in nuisance proportions in the shallow Peel-Harvey Estuarine System of Western Australia (Fig. 1). The plant grows as ball-like clumps of branched filaments about 1–3 cm in diameter. These balls form loose lying beds 1–10 cm thick, the lower sections of which decompose and form an anoxic black ooze over the bottom sediments. At frequent intervals large amounts of the alga can drift from these beds and foul the beaches, necessitating costly removal measures (Hodgkin and Lenanton 1981, McComb *et al.* 1981).

The relation between nutrient storage and growth was investigated because earlier work has shown that *Cladophora* grows best in summer when light and temperature are highest (Gordon *et al.* 1980). However, high nutrient concentrations are only found either in the "interalgal" water of the bed (which is effectively in darkness below 0.5 cm in the bed due to self shading) or briefly, in the overlying water in winter during river inflow (McComb *et al.* 1981; Fig. 2). The possibility that the alga can take up N and P under low light and/or temperature for later use under more favourable conditions is examined here by comparing tissue N and P changes in field populations with those obtained in laboratory experiments.

Materials and Methods

Tissue N and P contents of Cladophora *from the estuary*

Cladophora at the major growth sites (sites A, B and C; Fig. 1), were sampled at weekly intervals along with light, temperature and water nutrients from August 1977 to September 1979 (see McComb *et al.* 1981). Five core samples of *Cladophora*, each 50 cm^2 in surface area,

[1] Present address: Department of Conservation and Environment, 1 Mount Street, Perth, Western Australia, 6000.

0006-8055/81/0024-0381$02.00

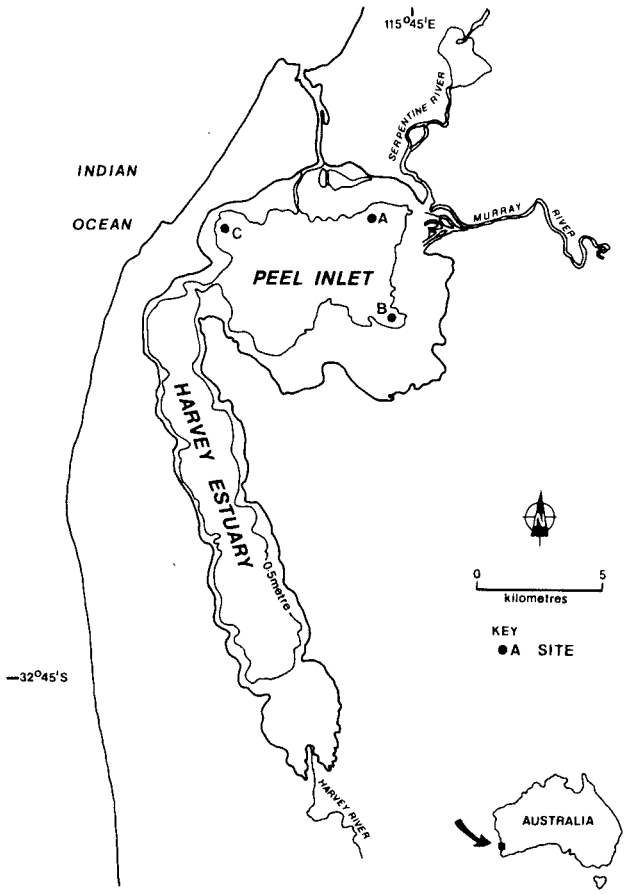

Fig. 1. Location of the Peel-Harvey Estuarine System of Western Australia, including *Cladophora* sampling sites A, B and C.

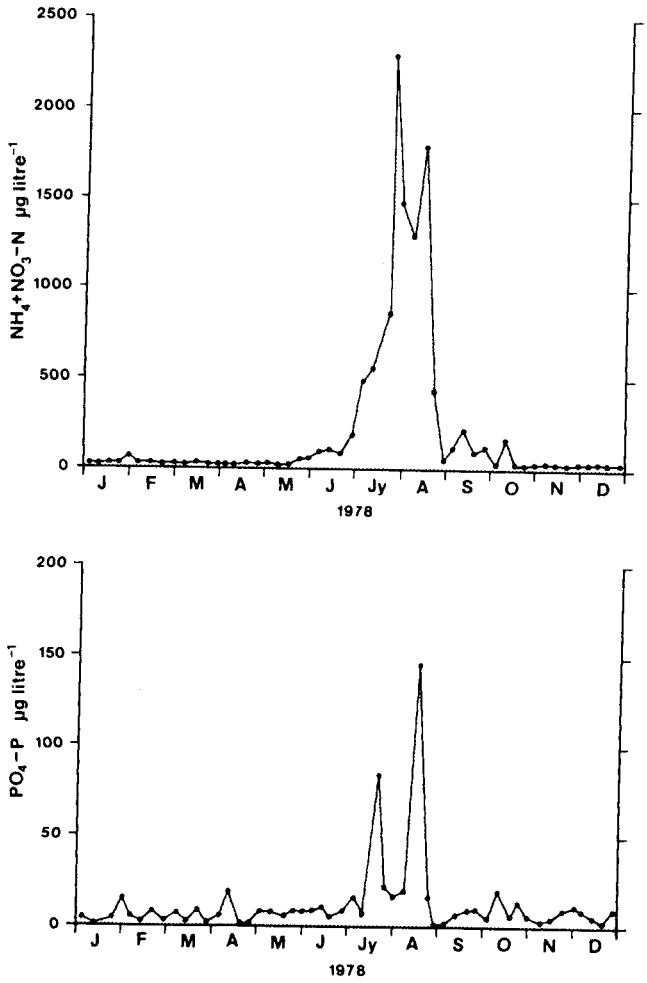

Fig. 2. Concentrations of (a) $NH_4 + NO_3 - N$ and (b) $PO_4 - P$ ($\mu g\ \ell^{-1}$) in bottom (1.9 m) estuary water at site A for 1978.

were collected from the algal bed at each site. In addition, 20 samples were also collected monthly at site A by diving and carefully scooping algae from the surface layer of the bed. Samples were washed in tap water then dried at 70 °C. The dried samples were milled and 200 mg subsamples assayed for total tissue P (Strickland and Parsons 1972) following digestion in $HClO_4$ and HNO_3 (Jackson 1958). Total tissue N was measured by an autoanalyzer (Technicon Corp, Tarrytown, N.Y., method 334–74 W/B) after digestion in Na_2SO_4 with an Hg catylist. Duplicates did not vary by more than 10%. Where sample size of the 5 combined replicates was less than 800 mg dry wt, samples from 2 or more weeks were composited.

N and P uptake

Rates of tissue N and P uptake by *Cladophora* under different levels of light and temperature were determined in the laboratory. Firstly, algal material was incubated for 12 days in modified ASP_{12} artificial seawater (Provasoli 1964, Gordon *et al.* 1981) with no added N or P to reduce tissue levels to a little below those typically found in the field. About 15 mg dry wt of fresh algae were weighed into each of 72, one litre Erlenmeyer flasks containing 1 litre of medium and incubated at 25 ± 1 °C or 16 ± 1 °C, approximating summer and winter water temperatures. At each temperature, one third of the flasks were placed in darkness, one third at 50 ± 10 $\mu E\ m^{-2}\ sec^{-1}$ (low light) and the remainder at 400 ± 70 $\mu E\ m^{-2}\ sec^{-1}$ (saturating light; Gordon *et al.* 1980). Each flask was bubbled with air which had been scrubbed in 25% H_2SO_4, rinsed in distilled water, then filtered through sintered glass. Nitrogen and P were added to the medium at 2 mg ℓ^{-1} $NH_4 + NO_3 - N$, and 0.2 mg ℓ^{-1} $PO_4 - P$. These levels approximated the highest observed during the winter of 1978 (Fig. 2).

Solutions were replaced every 1–4 days to maintain the desired concentrations, the more frequent changes being necessary at 25 ° in high light. Three replicates of each treatment were harvested on days 2, 4, 7 and 12, after which the samples were dried and analyzed for tissue N and P. Because sample size was small, samples of about 2 mg were weighed to ± 0.02 mg, placed in 20 ml de-ionized water and digested and analyzed by standard methods for water samples (Strickland and Parsons 1972; Technicon Autoanalyzer methods 376–74

W/B). Variability between replicates was usually less than 5%. A comparative test between this method and that used for the field samples showed good agreement.

Results

N and P contents of Cladophora *from the estuary*

The levels of tissue N and P in *Cladophora* at sites A, B and C are summarized in Figures 3 and 4. Sites A and B showed seasonal changes in tissue concentrations with tissue N and P lowest in summer and autumn and highest in winter and spring. Data from site C, which was collected for only one year, did not exhibit these seasonal trends, although values were generally highest in winter. While the seasonal trends were similar at sites A and B, the data collected weekly (e.g. site B) displayed marked weekly variations, sometimes as great as the seasonal range.

Figures 3 and 4 also illustrate the relationship between tissue concentrations and critical concentrations of N and P, where the critical concentration of a nutrient

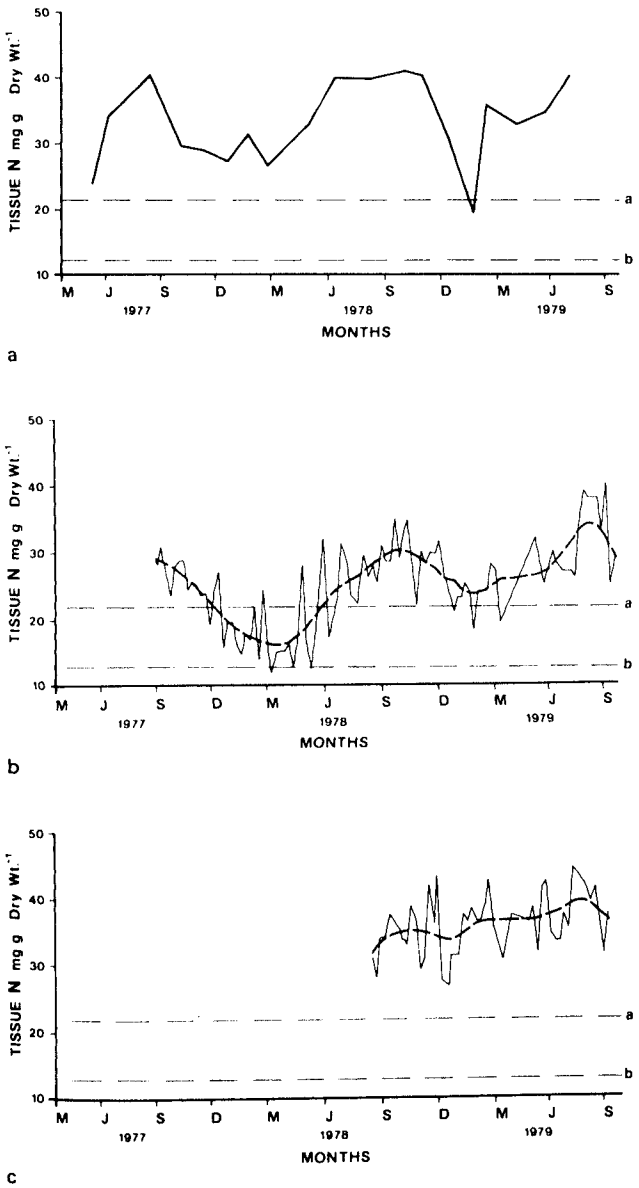

Fig. 3. Concentrations of tissue N (mg g dry wt^{-1}) in *Cladophora* at (a) site A (Top), (b) site B (middle) and (c) site C (Bottom) over the period 1977–79. The trend lines through the weekly collected data (sites B and C) represent the local mean at any point calculated by computer using a recursive least squares algorithm (Young 1980). The line (a) represents the critical concentration of P (3.3 mg P g dry wt^{-1}), the line (b) represents the minimum viable concentration of P (0.5 mg P g dry wt^{-1}; Gordon *et al.* 1981).

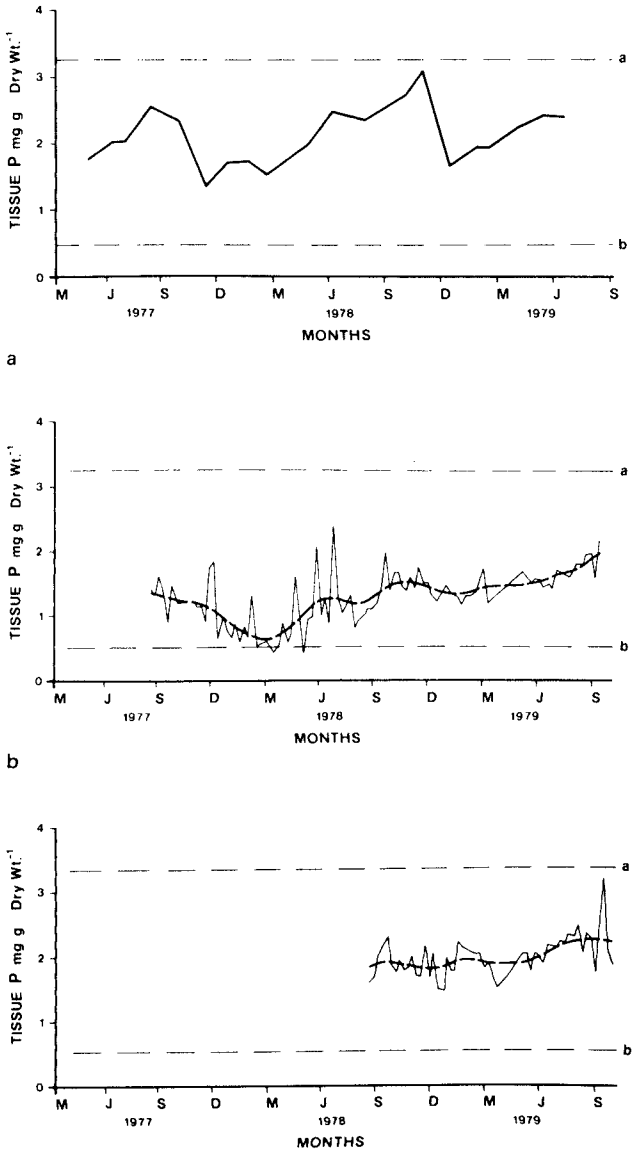

Fig. 4. Concentration of tissue P (mg g dry wt^{-1}) in *Cladophora* at (a) site A (Top), (b) site B (middle) and (c) site C (Bottom) over the period 1977–79. The trend lines through the weekly collected data represent the local mean at any point calculated by computer using a recursive least squares algorithm (Young 1980). The line (a) represents the critical concentration of N (21 mg N g dry wt^{-1}), the line (b) represents the minimum viable concentration of N (12 mg N g dry wt^{-1}; Gordon *et al.* 1981).

is defined as the minimum tissue content in a particular species that is necessary for maximum growth (Gerloff and Krombholz 1966). At most times, tissue N values were above the critical concentration of 21 mg g dry wt.$^{-1}$ except for early 1978 at site B. In contrast, tissue P was always below the critical concentration of 3.3 mg g dry wt^{-1} and during early 1978 values at site C approach concentrations associated with little or no growth, i.e. the "minimum viable" concentration (Gordon *et al.* 1980b).

The annual means of tissue concentrations for all sites are given in Table I. These were quite similar at sites A and C, both being higher than those recorded for site B, especially for tissue P. At all sites, average tissue P values were below the critical concentration, though this is not the case with tissue N where average concentrations were all above the critical level. This is reflected in the high N:P ratios at the sites (13–19:1, by weight).

Tab. I. Mean concentrations of nitrogen and phosphorus in water, sediment and *Cladophora* at sites A, B and C in the estuary[1]. The standard error is in brackets.

	Site A	B	C
Cladophora –		Nitrogen	
(total N, mg g dry wt^{-1})	32 (1)	28 (0.8)	36 (0.6)
Water-			
(NH_4 + NO_3 – N, $\mu g\ \ell^{-1}$)	67 (15)	84 (16)	110 (28)
Sediments[2]-			
(total N, mg g dry wt^{-1})	7.1	1.3	6.3
Cladophora –		Phosphorus	
(total P, mg g dry wt^{-1})	2.3 (0.08)	1.5 (0.04)	2.0 (0.04)
Water-			
(PO_4 – P, $\mu g\ \ell^{-1}$)	7.3 (0.96)	5.3 (0.6)	8.0 (1.4)
Sediments[2]-			
(total P, mg g dry wt^{-1})	0.73	0.088	0.72

[1] Tissue and water nutrient data collected weekly from August 22 1978–September 4 1979.

[2] Surface sediment including decaying plant material. Data from Gabrielson (1981).

N and P uptake

Uptake of N and P under different light and temperature is shown in Figures 5 and 6. In the light, tissue concentrations increased during the 12 days of incubation but

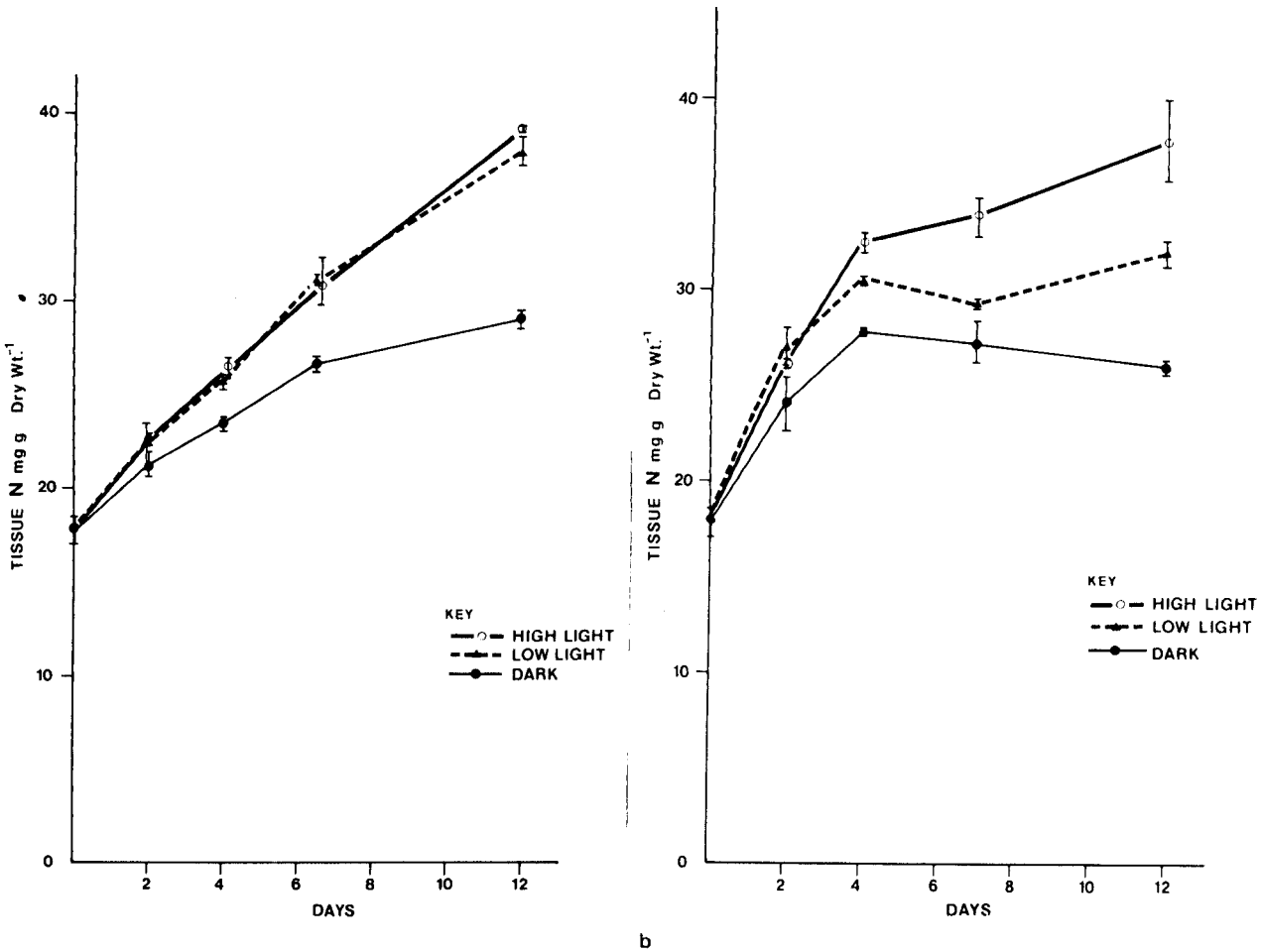

Fig. 5. Concentration of tissue N (mg g dry wt^{-1}) for *Cladophora* incubated in artificial seawater containing 0.2 mg ℓ^{-1} PO_4 – P and 2 mg ℓ^{-1} NH_4 + NO_3 – N at (left) 16 ± 1 °C and (right) 25 ± 1 °C at light levels of 0 (dark), 50 ± 10 (low light) and 400 ± 70 $\mu E\ m^{-2}$ sec^{-1} (high light).

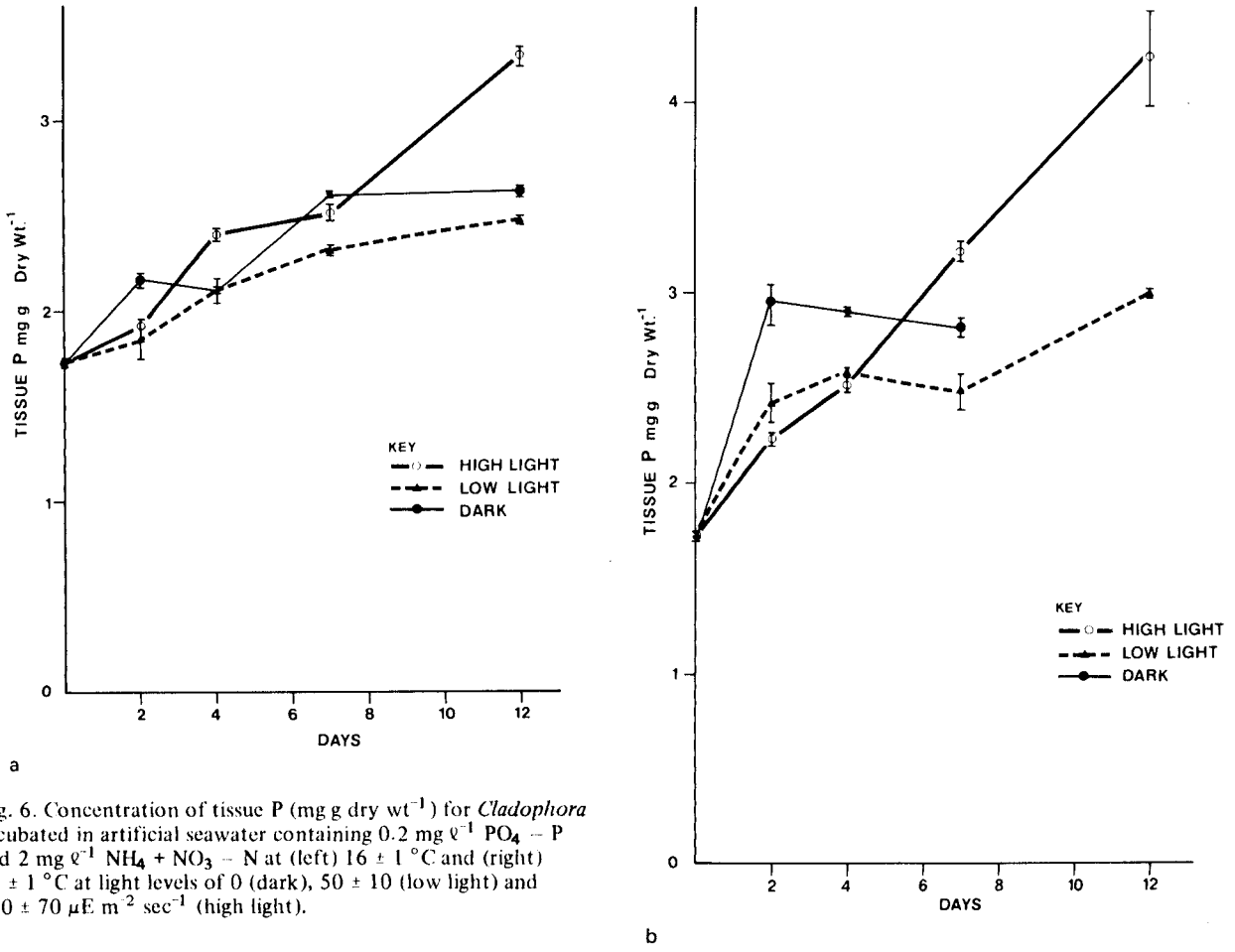

Fig. 6. Concentration of tissue P (mg g dry wt^{-1}) for *Cladophora* incubated in artificial seawater containing 0.2 mg ℓ^{-1} PO_4 – P and 2 mg ℓ^{-1} $NH_4 + NO_3$ – N at (left) 16 ± 1 °C and (right) 25 ± 1 °C at light levels of 0 (dark), 50 ± 10 (low light) and 400 ± 70 $\mu E\ m^{-2}\ sec^{-1}$ (high light).

in the dark there were increases of 40–60% in the first week, after which these treatments levelled off.

Rates of relative uptake of N and P into the tissue are compared with growth rates of *Cladophora* in Table II. Rates of uptake for each treatment were similar except at 16°

Tab. II. Effect of light and temperature on relative rate of increase of tissue nitrogen and phosphorus and dry weight of *Cladophora*[2]. The standard error is in brackets.

Light and Temperature regime[1]	Dry Weight day^{-1}	Tissue N day^{-1}	Tissue P day^{-1}
25 ± 1 °C			
high light	0.13 (0.02)	0.20 (0.008)	0.21 (0.008)
low light	0.037 (0.008)	0.087 (0.004)	0.085 (0.004)
dark	0.0014 (0.012)	0.033 (0.006)	0.032 (0.01)
16 ± 1 °C			
high light	0.035 (0.002)	0.10 (0.002)	0.090 (0.001)
low light	0.015 (0.002)	0.078 (0.001)	0.045 (0.001)
dark	– 0.0085 (0.005)	0.032 (0.003)	0.026 (0.002)

[1] Light levels were 400 ± 70, 50 ± 10 and 0 $\mu E\ m^{-2}\ sec^{-1}$ (high light, low light and dark, respectively).

[2] The experiment was for 12 days.

low light where N uptake was 1.6 times that for P. At high light, the Q_{10} between 15 ° and 25 ° for uptake was about 2. For growth, the Q_{10} was 3.7, in good agreement with the photosynthetic rate Q_{10} of 3.6 determined over a similar temperature range by Gordon *et al.* (1980). At each temperature, reduction of light to a low level decreased growth and uptake by approximately 60%, except at 16°, where N uptake was reduced by only 20% by reducing light.

Maximum tissue P concentrations attained here (4.2 mg g dry wt^{-1}) exceeded the highest recorded from the field (3.3 mg g dry wt^{-1}) while the maximum experimental N concentration (38 mg g dry wt^{-1}) was slightly less than the maximum observed in the field (43 mg g dry wt^{-1}).

Discussion

N and P limitation in the estuary

An important finding of this study is that tissue P concentrations, in contrast to tissue N concentrations, were always below the critical concentration. This suggests that *Cladophora* can acquire sufficient N but insufficient P for maximum growth rates and that P limits growth, depending on other factors such as light and temper-

ature. Earlier studies (Gordon *et al.* 1980, McComb *et al.* 1981) have found that light and temperature are sufficient for maximal growth in the photosynthesizing surface layer of *Cladophora* beds during summer, making this the time when P is most likely to limit growth. At other times of the year, and at depth in the algal bed, light and temperature are probably the most important factors controlling growth.

N and P uptake and storage

Although the tissue concentrations of N and P in the field varied seasonally there were also marked weekly variations in the data (Figs. 3 and 4). These short term variations are in part attributed to the size of samples taken, which were much smaller than those collected monthly. The small core samples would also be affected by including different proportions of senescing tissue from the lower parts of the beds. Shifts in the *Cladophora* bed surface, which continually expose or cover underlying algal layers, could have also contributed to this variation. Nevertheless tissue concentrations of N and P are generally highest in winter and spring and lowest in summer and autumn. This indicates that some summer growth takes place at the expense of stored reserves, meaning that nutrient supply rates are unable to keep pace with the relatively high growth rates of *Cladophora* at this time, at least at sites A and C.

The tendancy for tissue N and P concentrations to increase in the estuary from mid autumn is thought to be due to growth rates declining more quickly than uptake rates, growth rates being more sensitive to temperature (Tab. II). Since significant river inputs of nutrients do not begin until winter, the most obvious major source for the autumn increases is release from the sediment and decaying algae.

In winter, although the rivers contribute large amounts of N and P to the system, the full storage potential of *Cladophora* was not reached due to the few weeks in which there was high river flow. Maximum tissue concentrations, which were generally recorded in July–September, were well below those achieved in culture (Gordon *et al.* 1981). In 1978, concentrations of N and P in the water rose to levels similar to those in the present laboratory experiments (2 mg ℓ^{-1} $NH_4 + NO_3 - N$ and 0.2 mg ℓ^{-1} $PO_4 - P$), but only for about one week. For most of the remaining winter period $PO_4 - P$ was typically less than 0.02 mg ℓ^{-1}, although inorganic N remained above 0.5 mg ℓ^{-1} for 6 weeks (Fig. 2). In the laboratory (Fig. 6a) tissue increases of 0.8–1.6 mg P g dry wt^{-1} (depending on light conditions) occurred after 12 days incubation at 16 ° in 0.2 mg ℓ^{-1} PO_4 and this is similar to increases observed in the field populations in winter. Extrapolating the data from Figure 6a, suggestes that incubation periods of 7–14 weeks under winter light and temperature would be required to reach maximum levels achieved in earlier experiments (~ 9 mg P g dry wt^{-1}; Gordon *et al.* 1981). This is much longer than the observed period of high P concentrations in the overlying water. In the "interalgal" water within the bed, however, high concentrations are maintained throughout the year, but here uptake by shaded algae is probably limited by cellular energy reserves, as is indicated by lower uptake rates in the dark (Figs. 5 and 6).

Nitrogen uptake into the tissue in the algal beds during winter may also be limited by the few weeks of high river flow and, in addition, low inorganic P concentrations in the water. In the laboratory, *Cladophora* tissue increased from an initial 18 mg N g dry wt^{-1} to 38 mg N g dry wt^{-1} (~ the maximum field concentration) after 12 days incubation in medium containing 2 mg $NO_3 + NH_4 - N$ ℓ^{-1} (Fig. 5). Since winter inorganic N concentrations were between 1 and 2 mg ℓ^{-1} for as long as 4 weeks in the estuary, higher tissue concentrations could have been expected. However, inorganic P concentrations at this time were mostly around 0.02 mg ℓ^{-1} and earlier work indicates that the rate of N uptake is greatly reduced once inorganic P falls below about 0.05 mg ℓ^{-1} (Gordon *et al.* 1981).

During September and October 1978, following the main river flows, continued increases in tissue concentrations may have been due, in part, to recycling of nutrients from decomposing phytoplankton. In winter 1978, a phytoplankton bloom immediately followed the increase in nutrient concentrations (McComb *et al.* 1981). Part of this bloom would have settled onto the algal beds and decomposed. The amount of nutrients made available to *Cladophora* by this mechanism is not yet known, however, Gabrielson (1981) found high levels of extractable N and P in surface sediments of the estuary in August 1978 and suggested decomposing phytoplankton could have been partly the cause. The importance of a particulate source of nutrients, especially P, has been also emphasized by modelling studies of this system (e.g. Hornberger and Spear 1980, Spear and Hornberger 1980).

Differences between the sites

Variations in tissue N and P at the sites appear related to average concentrations of inorganic N and P in the water and total N and P in the surface sediments (Tab. I). Higher concentrations of P in the water and sediments at sites A and C are probably due to their proximity to sources of P; site A is near the mouths of the Murray and Serpentine Rivers while site C is close to the outflow from the Harvey Estuary, whose mean total P concentration is double that of Peel Inlet (Hodgkin and Lenanton 1981).

Lack of seasonality noted earlier in the data of site C when compared to the other sites may be due, in part, to sedimented phytoplankton from a massive bloom of *Nodularia spumigena*, which was recorded in the Harvey

Estuary in November 1978 (Huber 1980). Part of this bloom was carried by tidal exchange into Peel Inlet, and high levels of chlorophyll *a* were recorded at site C, due to its proximity to the Harvey Estuary. As this phytoplankton bloom decomposed in December 1978 and January 1979 it could have released significant quantities of N and P for uptake by *Cladophora* at site C. This hypothesis is supported by Gabrielson (1981) who measured increased levels of extractable N and P in the surface sediments at this time.

Conclusions

The data presented here illustrate that concentrations of nutrients in algal tissues can, when viewed against a background of laboratory-derived data, provide a valuable tool for interpreting the performance of the algae in the field. The data discussed here also highlight the importance of nutrient supply, and in particular show that P may be a key factor in controlling the biomass of *Cladophora* in the estuary.

Acknowledgements

We wish to thank fellow workers in the Peel-Harvey Estuarine System Study for helpful discussions and assistance. Special thanks are due to K. S. Hamel for her help with the uptake experiment. Financial support, typing and drafting facilities provided by the Department of Conservation and Environment, Western Australia and a University Research Studentship held by D. M. G. are gratefully acknowledged.

References

Gabrielson, J. O. 1981. The sediment contribution to nutrient cycling in the Peel/Harvey Estuarine System. *Technical report No. 96 to Dept. Conservation and Environment*, Perth, Western Australia. pp. 64.

Gerloff, G. C. and P. H. Krombholz. 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. *Limnol. Oceanogr. 11*: 529–537.

Gordon, D. M., P. B. Birch and A. J. McComb. 1980. The effect of light, temperature and salinity on photosynthetic rates of an estuarine *Cladophora*. *Bot. Mar. 23*: 749–755.

Gordon, D. M., P. B. Birch and A. J. McComb (1981). Effects of inorganic phosphorus and nitrogen on the growth of an estuarine *Cladophora* in culture. *Bot. Mar. 24*: 93–106.

Hodgkin, E. P. and R. C. Lenanton. 1981. Estuaries and coastal lagoons of south western Australia. *In*: (Nielson, B. and A. Cronin eds.) *Nutrient enrichment in estuaries.* Humana Press, New Jersey. (in press).

Hornberger, G. M. and R. C. Spear. 1980. Eutrophication in Peel Inlet – I. The problem solving behaviour and a mathematical model for the phosphorus scenario. *Wat. Res. 14*: 29–42.

Huber, A. L. 1980. Cyanobacteria in the Peel-Harvey Estuarine System. *Technical report No. 94 to Dept. Conservation and Environment*, Perth, Western Australia. pp. 124.

Jackson, M. L. 1958. *Soil Chemical Analysis.* Prentice Hall.

McComb, A. J., R. P. Atkins, P. B. Birch, D. M. Gordon and R. J. Lukatelich. 1981. Eutrophication in the Peel-Harvey Estuarine System, Western Australia. *In*: (Nielson, B. and A. Cronin eds.). *Nutrient Enrichment in Estuaries.* Humana Press, New Jersey. (in press).

Provasoli, L. 1964. Growing marine seaweeds. *Proc. Int. Seaweed Symp. 4*: 9–17.

Spear, R. C. and G. M. Hornberger. 1980. Eutrophication in Peel Inlet – II. Identification of critical uncertainties via generalized sensitivity analyses. *Wat. Res. 14*: 42–49.

Strickland, J. D. and T. R. Parsons. 1972. A practical handbook of seawater analysis. *Bull. Fisheries Res. Bd. Canada.* No. 167.

Young, P. C. 1980. *Recursive Estimation.* Springer Verlag. (in press).