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The development from kinetic coefficients of a predictive model for the growth of *Eichhornia crassipes* in the field. I. Generating kinetic coefficients for the model in greenhouse culture

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Keywords : *Eichhornia crassipes*, growth kinetics, model development

ABSTRACT

The kinetics of N- and P- limited growth of *Eichhornia crassipes* (Mart.) Solms were investigated in greenhouse culture with the object of developing a model for predicting population sizes, yields, growth rates and frequencies and amounts of harvest, under varying conditions of nutrient loading and climate, to control both nutrient inputs and excessive growth in eutrophied aquatic systems. The kinetic coefficients, maximum specific growth rate (Umax), half saturation coefficient (Ks) and yield coefficient (Yc) were measured under N and P limitation in replicated batch culture experiments. Umax values and Ks concentrations derived under N limitation ranged from 5,37 to 8,86% d^{-1} and from 400 to 1 506 µg N ℓ^{-1} respectively. Those derived under P limitation ranged from 4,51 to 10,89% d^{-1} and from 41 to 162 µg P ℓ^{-1} respectively. Yc values (fresh mass basis) determined ranged from 1 660 to 1 981 (87 to 98 dry mass basis) for N and from 16 431 to 18 671 (867 to 980 dry mass basis) for P. The reciprocals of Yc values (dry mass basis), expressed as percentages, adequately estimated the minimum limiting concentrations of N and P (% dry mass) in the plant tissues. Kinetic coefficients determined are compared with those reported for algae. The experimental method used and results obtained are critically assessed.

INTRODUCTION

Eutrophication, the enrichment of aquatic systems with inorganic nutrients (Stewart & Rohlich, 1967), is a world-wide water quality problem (Stumm, 1974). *Eichhornia crassipes* (Mart.) Solms (water hyacinth), a free-floating, aquatic plant (Penfound & Earle, 1948; Bock, 1966), which has a high growth rate (Penfound, 1956; Yount & Crossman, 1970; Boyd, 1976) and produces a large standing crop per unit area (Knipling *et al.*, 1970; Boyd & Scarsbrook, 1975), is the most promising floating, vascular aquatic plant species for removing nutrients from eutrophied aquatic systems (Boyd, 1970). This species absorbs large quantities of N and P, the nutrients generally associated with eutrophication (Mackenthun, 1964; 1965), from sewage effluents (Clock, 1968; Miner *et al.*, 1971; Cornwell *et al.*, 1977). In addition, it removes heavy metal and other chemical pollutants from secondary waste-water effluents (Wolverton, 1975; Wolverton & McDonald, 1975a; 1975b; 1976; Wolverton & McKown, 1976) and reduces levels of suspended solids, biochemical oxygen demand substances and other chemical factors in such effluents to levels below the standards set by some pollution control agencies (Wolverton & McDonald, 1975c; 1975d). Its cultivation and removal may, therefore, constitute an effective means of withdrawing nutrients from effluents prior to their release into natural waters (Yount & Crossman, 1970). Similarly, the removal of water hyacinths growing in eutrophied aquatic systems may also assist in controlling excessive growth of plants by reducing nutrient levels.

To achieve maximum nutrient removal efficiency by *E. crassipes* in a nutrient removal scheme, it is necessary to establish how much and how frequently to harvest the population. Clearly, if the population is continually over-harvested, the size of the population and its effectiveness in removing nutrients will be progressively reduced. Alternatively, if the population is under-harvested, nutrient removal may be ineffective and other adverse effects may arise.

Maintenance of a high growth rate and nutrient removal capacity by *E. crassipes* is facilitated if the size of the population required to maintain desirable nutrient concentrations in the water, under varying conditions of nutrient loading and climate, can be predicted. Since harvesting is required to control the population size, amounts and frequencies of harvest must also be predicted.

From the kinetic standpoint, it is theoretically feasible to construct a mathematical model for *E. crassipes* from which population sizes, yields, growth rates and frequencies and amounts of harvest, under varying conditions of nutrient loading and climate, can be predicted to control both nutrient inputs and excessive growth in eutrophied aquatic systems (Toerien, 1972; Musil & Breen, 1977). The following relationships, however, require mathematical formulation:

(i) The relationship between the yield of *E. crassipes*, i.e. the mass of plant material produced and the mass of a specific *limiting nutrient absorbed. The following mathematical expression describes this relationship:

$$Yc = \frac{Xt - Xo}{So - St}$$

where Yc = yield coefficient; Xo = initial biomass; Xt = final biomass; So = initial concentration of

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*Nutrient present at concentrations below that required for maximum plant growth and hence restricting the growth rate.

limiting nutrient; St = final concentration of limiting nutrient.

(ii) The relationship between the specific growth rate of *E. crassipes*, i.e. the increase in mass of plants, per unit mass of plant material, per unit time (Malek & Fencl, 1966; Radford, 1967) and the concentration of a specific limiting nutrient. Various models have been used to quantify this relationship in algae and bacteria (Shelef *et al.*, 1968; Toerien *et al.*, 1971; Goldman, 1972). The most important are Blackman's first order-zero order model, Teisser's exponential model and Monod's rectangular hyperbola model, the last defined as:

$$U = Umax \frac{S}{Ks + S}$$

where U = specific growth rate; Umax = maximum specific growth rate; S = concentration of limiting nutrient; Ks = half saturation coefficient = S when U = 0,5 Umax.

(iii) The relationship between the maximum specific growth rate of *E. crassipes* and temperature. Under a constant light intensity, the maximum specific growth rate (Umax) may be described solely as a function of temperature, as shown by Goldman (1972) and Goldman & Carpenter (1974) for various species of marine and fresh water algae, by an Arrhenius equation, defined as:

$$Umax = Ae^{-E/RT}$$

where A = constant day^{-1}; E = activation energy cal. $mole^{-1}$; R = universal gas constant cal. $mole^{-1}$ $°K^{-1}$; T = temperature on Kelvin scale °K.

Incorporating the Arrhenius equation into the Monod model, the following mathematical expression is obtained in which the specific growth rate (U) is related to both temperature and the limiting nutrient concentration:

$$U = Ae^{-E/RT} \times \frac{S}{Ks + S}$$

The predictive abilities of such models have been demonstrated in algae, for example, by Toerien & Huang (1973) where the P-limited growth rate of *Selenastrum capricornutum* in batch cultures was accurately predicted from its kinetic coefficients and by Bhagat *et al.* (1972) where the algal concentration of a Vancouver Lake was adequately predicted by a water quality simulation model also using kinetic coefficients.

A number of restrictions to the general use of the above equations, however, do exist. Firstly, for each plant species the Arrhenius equation is applicable only over a defined temperature range as shown by Sorokin (1960) for various algal species. Secondly, there is evidence of a strong interaction between light intensity and temperature. Sorokin (1960; 1971) found that for a given temperature the activation energy decreases with increasing light energy and Shelef (1968) has shown that the saturation light intensity is highly temperature dependent. Thirdly, the half saturation coefficient for nutrient uptake is also sensitive to changes in temperature (Shelef *et al.*, 1970). A further potential complication is the possible temperature dependency of the yield coefficient, since minor variations in the yield coefficient have been found with high and low temperature strains of *Chlorella* grown under NO_3–N limitation in continuous cultures (Shelef *et al.*, 1970) and in the bacterium *Aerobacter aerogenes* (Topiwala & Sinclair, 1971).

No attempts have, as yet, been made to model the effects of temperature on the half saturation (Ks) and yield coefficients (Yc), although in *Aerobacter aerogenes* and *Escherichia coli*, Topiwala & Sinclair (1971) and Sawada *et al.* (1978) demonstrated that Ks changes with temperature and that an Arrhenius plot of the change is linear. The difficult task of determining temperature dependent kinetic coefficients such as Ks and Yc in natural systems may restrict their application to well defined laboratory conditions. On a seasonal basis, however, it should be possible to assess the significance of these kinetic coefficients in modelling.

Numerous references exist in the literature on the nutrient uptake and growth characteristics of *E. crassipes*. Despite this, the necessary mathematical relationships required for the proper evaluation and potential design of a predictive model have not been adequately formulated. In a preliminary study, Musil & Breen (1977) measured the kinetic coefficients, Umax, Ks and Yc for *E. crassipes* in one NO_3–N-limited batch culture experiment. They illustrated how these coefficients could be used in a predictive model, although its validity was not tested under field conditions. Since both N and P are the nutrients most frequently limiting for *E. crassipes* under natural conditions (Wahlquist, 1972), this investigation was designed to generate kinetic coefficients for *E. crassipes* growing under N and P limitation with the objective of developing and validating a predictive model.

MATERIALS AND METHODS

The batch culture method or non steady-state approach (Toerien *et al.*, 1971) was used to measure kinetic coefficients for *E. crassipes* growing under specific nutrient limitation. Batch culture experiments were repeated, five times under N and three times under P limitation.

In each experiment, ca 120, vegetatively-propagated offsets (daughter plants) of uniform size (possessing two pseudolaminae with bulbous petioles and having a fresh mass ranging from ca 4 to 10 g) were sampled from a loosely crowded population in a sewage maturation pond. Plants were rinsed through three changes of deionised-distilled water, shaken to dislodge adhering water and their fresh masses recorded on an electric, top-loading balance. They were placed into 5ℓ capacity, inert polyethylene vessels (buckets) each containing 5ℓ of culture solution deficient in either N or P. One or two plants were used as an inoculum in each vessel (Table 1).

A modified culture solution based on that of Hamner *et al.* (1942) was used (Table 2) in which the concentrations of either of the anions, NO_3^- or PO_4^{2-}, could be varied independently with minimum influence on the concentrations of cations and other anions. Reduced cation concentrations, resulting

TABLE 1.—Treatment differences between experiments designed to measure kinetic coefficients for *E. crassipes* growing in N-and P-limited cultures

N-limited cultures				P-limited cultures			
Experiment No.	No. of plants as inoculum	N added $\times 10^3$ ug N ℓ^{-1}	No. of replicates per treatment	Experiment No.	No. of plants as inoculum	P added $\times 10^3$ ug N ℓ^{-1}	No. of replicates per treatment
1	2	0; 11,29; 22,58; 33,87; 45,16; 56,45	20	6	2	0; 1,30; 2,61; 3,91; 5,22	20
2	1	0; 4,52; 9,03; 18,06; 27,10; 36,13	16	7	1	0; 0,65; 1,63; 2,61; 3,91; 5,22	16
3	1	0; 4,52; 9,03; 18,06; 27,10; 36,13	16	8	1	0; 1,30; 3,26; 5,22; 7,83; 10,44	18
4	1	0; 9,03; 18,06; 28,10; 36,13; 45,16	18				
5	1	0; 9,03; 18,06; 28,10; 36,13; 45,16	18				

from the lowering in concentration of an anion in the culture solution, were restored by supplementing it with the appropriate additional cations. These were added predominantly as chlorides. The total salinity of the culture solution was 0,31‰. This is well below the salinity of 16,6‰ reported by Haller *et al.* (1974) to inhibit *E. crassipes* growth rate in culture. Ions were supplied to the culture solution in the inorganic form and in sufficient quantities not to be limiting for *E. crassipes* (Musil, 1982). Culture solutions were changed and adjusted to pH 7,0 weekly using 5% H_2SO_4 and 10% NaOH. Evaporation loss from cultures was replaced daily with deionised-distilled water.

Experiments were conducted in an air-conditioned greenhouse during summer when light intensities (radiant flux densities) and air temperatures were high. Maximum daytime air temperatures in the greenhouse were maintained at ca 30°C required for maximum growth of plants (Knipling *et al.*, 1970). Diurnal air temperature and relative humidity fluctuations in the greenhouse, recorded on a thermohydrograph, did not exceed the ranges 6 to 11°C

TABLE 2.—Chemical composition and ionic concentration of culture solution used for growing *E. crassipes*

Solution No.	Chemical	Ionic concentration					
		Cations			Anions		
			$\times 10^3$ ug ℓ^{-1}	$\times 10^3$ u eq ℓ^{-1}		$\times 10^3$ ug ℓ^{-1}	$\times 10^3$ u eq ℓ^{-1}
1	KNO_3	K	8,41	0,215	NO_3	13,33	0,215
	$Ca(NO_3)_2.4H_2O$	Ca	4,31	0,215	NO_3	13,33	0,215
	$Mg(NO_3)_2.6H_2O$	Mg	2,61	0,215	NO_3	13,33	0,215
2	KH_2PO_4	K	8,05	0,206	PO_4	20,00	0,206
3	K_2SO_4	K	8,14	0,208	SO_4	10,00	0,208
	$MgSO_4.7H_2O$	Mg	2,53	0,208	SO_4	10,00	0,208
4	KCl	K	15,40	0,394	Cl	13,97	0,394
5	$CaCl_2$	Ca	35,69	1,781	Cl	63,15	1,781
6	$MgCl_2.6H_2O$	Mg	34,86	2,867	Cl	101,63	2,867
7	NaCl	Na	20,00	0,869	Cl	30,84	0,869
		Total		7,718			7,718
8	FeEDTA	Fe	0,40				
9	$CuSO_4.5H_2O$	Cu	0,03				
	$MnSO_4.H_2O$	Mn	0,27				
	$ZnSO_4.7H_2O$	Zn	0,13				
	H_3BO_3	B	0,27				
	$(NH_4)_6Mo_7O_{24}.4H_2O$	Mo	0.01				

and 50 to 90% respectively, recommended by Robbins (1946) for culturing higher plants.

Every two to four days, plants were removed from culture, allowed to drain for two minutes above the culture vessels, shaken to dislodge adhering water, their fresh masses recorded and returned to culture. Plants were grown in either N- or P-deficient cultures until they showed a reduced growth rate, evident as a deviation from linearity in a plot of their fresh mass against time, indicating a N or P deficiency. They were then harvested from culture and necrotic or damaged leaves and roots removed. Culture solutions were changed, fresh masses of plants redetermined and plants returned to culture.

In each experiment, N- or P-deficient cultures were spiked, at this stage, with six different levels of N or P to obtain six treatments (16 to 20 replicates per treatment) in which N concentrations in N-limited cultures ranged from 0 to $11{,}29 \times 10^3$ μg N ℓ^{-1} and P concentrations in P-limited cultures ranged from 0 to $2{,}09 \times 10^3$ μg P ℓ^{-1} (Table 1). A randomized block design was adopted (Rayner, 1967).

After spiking, mass recordings, which included both fresh as well as dead mass of plants arising through necrosis of plant material during growth, continued every two to four days for all plants until no significant increase was recorded in the total fresh mass (fresh and dead mass) of all plants grown at each level of N or P supplied.

Culture solutions were not changed again. However, they were topped-up daily with deionised-distilled water and adjusted to pH 7,0 weekly, since the Ks may be influenced by pH (Goldman, 1972) and maximum growth of *E. crassipes* occurs at this pH in culture (Chadwick & Obeid, 1966). Concentrates of the culture solution deficient in either N or P were added to the cultures at two weekly intervals to ensure an adequate supply of nutrients, other than the specific limiting nutrient, to the plants. In P-limited culture experiments, additional N at a concentration of $9{,}03 \times 10^3$ μg N ℓ^{-1} was also added to cultures in the intervening weeks to ensure that N concentrations remained above those limiting for *E. crassipes* (Musil, 1982). The total nutrient additions after spiking, however, did not increase the salinity of cultures above 1,6‰, i.e. 10% of the inhibitory salinity value of 16,6‰ for *E. crassipes* (Haller *et al.*, 1974).

When mass recordings were terminated, plants, including their offsets, were harvested from culture allowing the culture solution retained by plants to drain back into each vessel. Plants were shaken to dislodge adhering water and reweighed. They were then dried in a forced draft oven at 60°C to a constant weight and their dry masses determined. The dry plant tissues were ground in a mill, redried at 60°C in a forced draft oven to a constant weight, and stored in sealed glass bottles for later chemical analysis.

After plants had been harvested, the culture solutions in three vessels taken at random from each treatment in each experiment were topped-up to the 5ℓ mark with deionised-distilled water and analysed for remaining N or P using published methods (Environmental Protection Agency, 1974; American Public Health Ass.: *Standard Methods*, 1975). Loss of the specific limiting nutrient (N or P) from cultures, resulting from shaking of plants at each weighing interval, could not be accounted for, but was considered to be small.

The minimum concentration or subsistence quota (Rhee & Gotham, 1981) of the specific limiting nutrient in harvested plants was analysed in three batches of dry, ground, harvested plant tissues chosen at random from each treatment in each experiment using published methods (Association of Official Agricultural Chemists, 1975).

In each experiment, specific growth rates were calculated according to Malek & Fencl (1966) and Radford (1967) for each plant between each weighing interval for a period of ca 21 days after spiking. The highest specific growth rate attained by each plant in each treatment during this period was taken as its specific growth rate at that particular N or P concentration. The Umax value and Ks concentration were extrapolated for *E. crassipes* in each experiment from the intercepts of a reciprocal plot of specific growth rates of plants against limiting nutrient concentrations (Lineweaver & Burk, 1934; Currie, 1982). The Yc value was derived in each experiment from the slope of the line relating total fresh mass yields of plants to quantities of limiting nutrient absorbed. Simple linear regressions were used to obtain the best straight lines through all points (Rayner, 1967). All linear regressions were subjected to an analysis of variance (Rayner, 1967).

RESULTS AND DISCUSSIONS

Growth in deficient culture

Plants with two pseudolaminae introduced into N- (Experiments 1 to 5) and P-(Experiments 6 to 8) deficient cultures showed an initial lag phase in growth lasting ca two to four days (Figs 1 & 2). Growth of plants in N- and P-deficient cultures then proceeded more or less linearly until they showed a reduced growth rate, at which stage the growth rate of plants was assumed to be N- or P-limited. No significant differences ($P \leq 0{,}05$) existed at this stage between the mean fresh masses of groups of plants that were to comprise each treatment in each experiment (Musil, 1982). In each experiment, a different growth period in deficient cultures was required to induce in plants a N- or P-limited state. This was attributed partly to different quantities of N and P stored in plants collected on different occasions from the field for each experiment. No correlation was evident between the duration of plant growth in deficient cultures, required to induce N or P limitation, and environmental conditions recorded in the greenhouse (Musil, 1982).

Growth after spiking

In each experiment, the addition of the limiting nutrient caused an increase in growth rate with a short (three to four day) period of maximum growth rate which was proportional to the level of N or P supplied. The periods of mean maximum growth rate of each group of plants for each treatment were

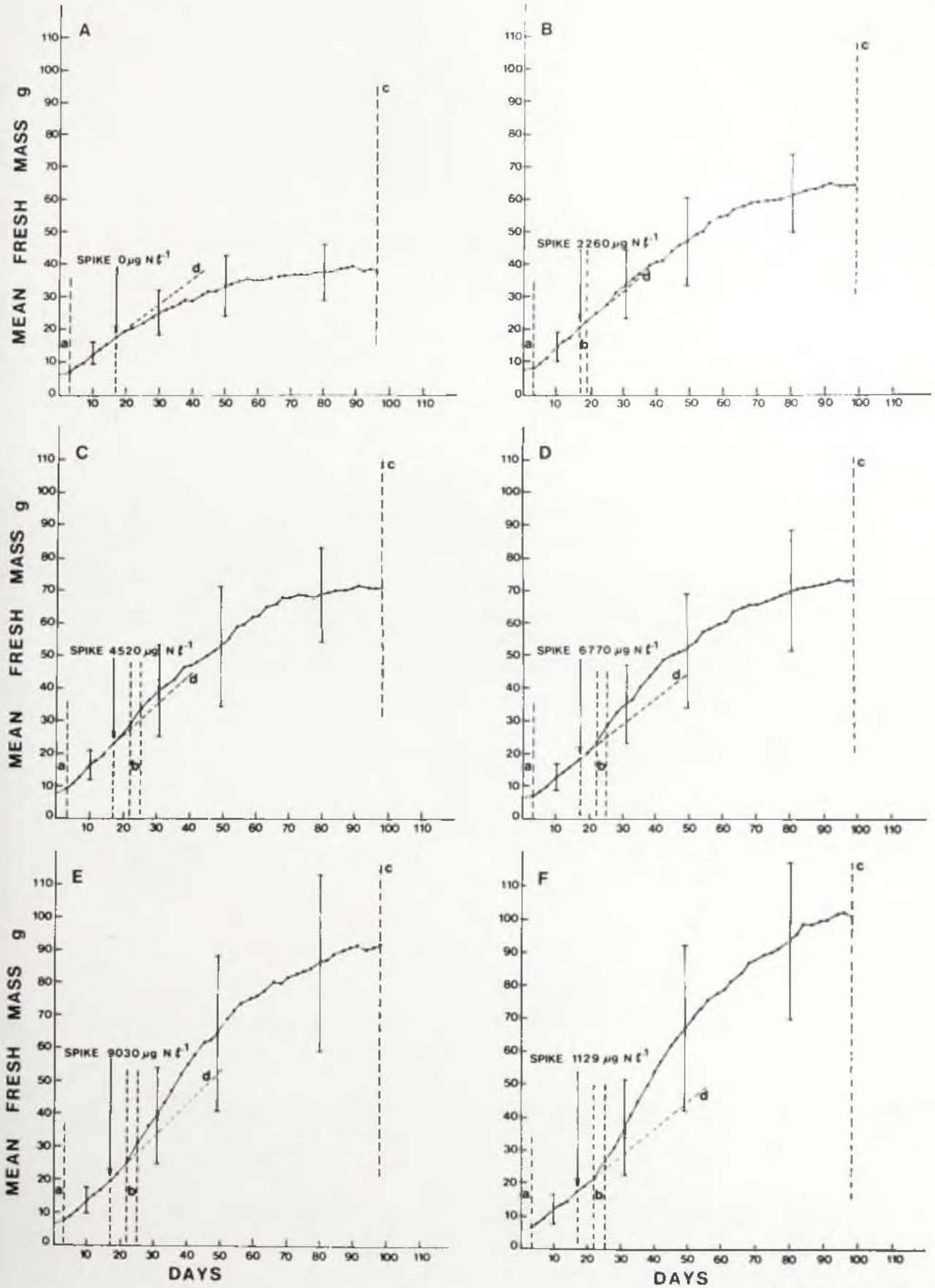

FIG. 1. — Experiment 1. Change in fresh mass (means of 20 plants/treatment) of *E. crassipes* grown under varying conditions of N supply. All treatments were grown under N-deficient conditions for 18 days before N was added. A, no N; B 11 290, µg N (2 260 µg N ℓ^{-1}); C, 22 580 µg N (4 520 µg N ℓ^{-1}); D, 33 870 µg N (6 770 µg N ℓ^{-1}); E, 45 160 µg N (9 030 µg N ℓ^{-1}); F, 56 450 µg N (11 290 µg N ℓ^{-1}). Standard deviations of means are shown by bars: a = lag phase of growth; b = period of maximum growth rate; c = termination of fresh mass recordings; d = projected growth in the absence of N.

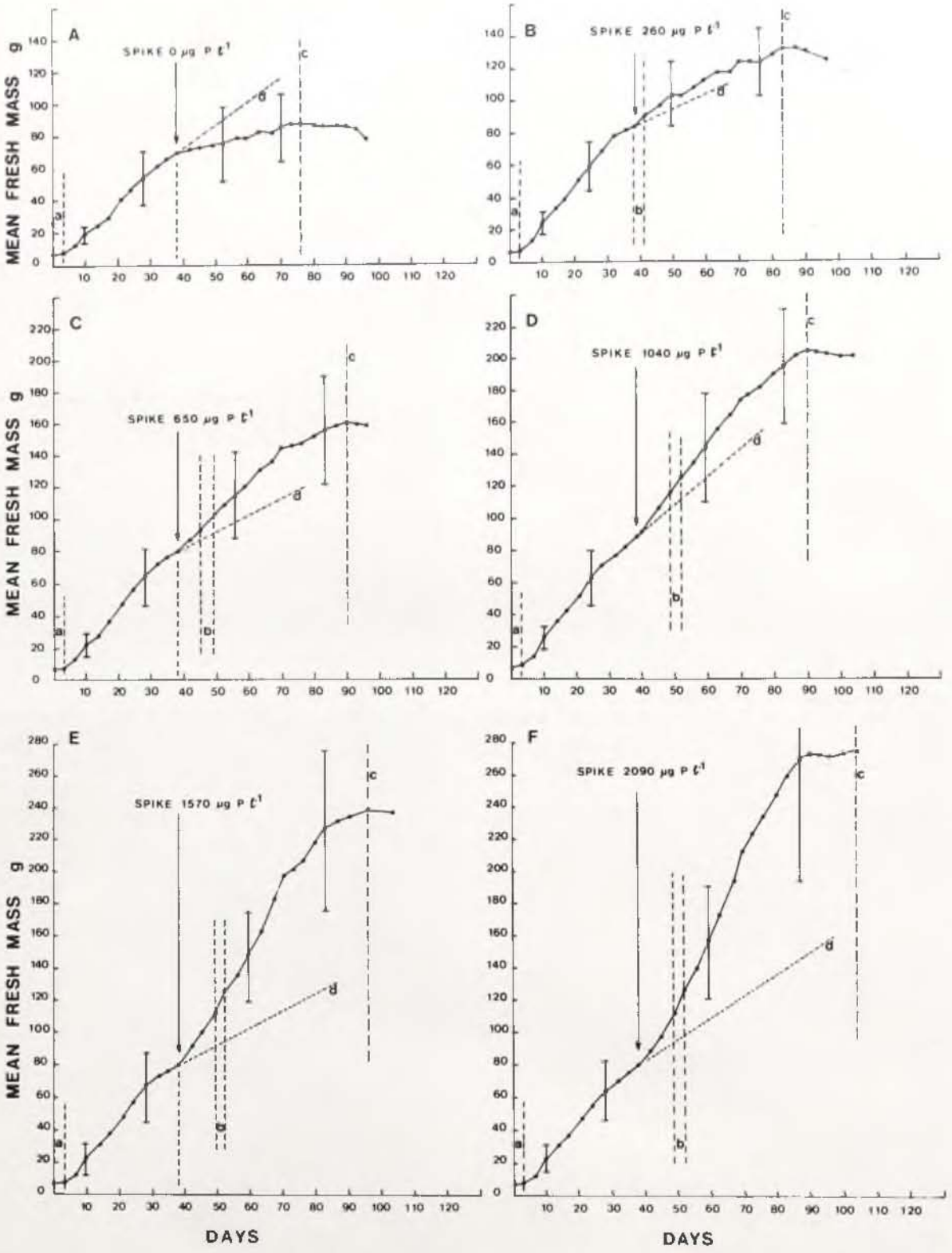

FIG. 2. — Experiment 8. Change in fresh mass (means of 18 plants/treatment) of *E. crassipes* grown under varying conditions of P supply. All treatments were grown under P-deficient conditions for 38 days before P was added. A, no P; B, 1 300 µg P (260 µg P ℓ^{-1}); C, 3 260 µg P (650 µg P ℓ^{-1}); D, 5 220 µg P (1 040 µg P ℓ^{-1}); E, 7 830 µg P (1 570 µg P ℓ^{-1}); F, 10 440 µg P (2 090 µg P ℓ^{-1}). Standard deviations of means are shown by bars: a = lag phase of growth; b = period of maximum growth rate; c = termination of fresh mass recordings; d = projected growth in the absence of P.

evident from the maximum slopes of curves relating growth (fresh mass) and time (Figs 1 & 2B, C, D, E, F). Thereafter, the growth rates of plants decreased progressively until there was no measurable increase in the total fresh mass (fresh including dead mass produced during growth) of plants. This required ca 75 to 95 days after the addition of N and ca 50 to 65 days after the addition of P, in those treatments where these limiting nutrients were supplied at the highest levels to cultures. In Experiments 4 and 5, mass recordings were terminated prior to cessation of plant growth, i.e. about 21 days after spiking.

Nitrogen- and P-limited plants responded differently to the different levels of limiting nutrient supplied to cultures. In Treatments 3 to 6, where the limiting nutrients were supplied at levels above 2 260 µg N ℓ^{-1} and 260 µg P ℓ^{-1} (Figs 1 & 2C, D, E, F), plants generally attained a maximum growth rate much later after the addition of N and P than in Treatment 2 (Figs 1 & 2B), where the limiting nutrients were supplied at lower levels. This could not be reasonably explained by a restricted uptake of N or P in *E. crassipes* due to a limited nitrate reductase or alkaline phosphate activity in plants resulting from their growth in deficient cultures (Schwoerbel & Tillmans, 1974). Oaks *et al.* (1972) in a study of the induction kinetics in the roots of *Zea mays* seedlings have shown that the induction of nitrate reductase is very rapid with maximum levels of nitrate reductase being achieved four to six hours after transference of seedlings from a NO_3–N-deficient medium to one containing NO_3–N. Fitzgerald & Nelson (1966) and Fitzgerald (1969), on the other hand, have reported that alkaline phosphatase activity increases in algal cells and higher aquatic plants such as *Ceratophyllum demersum* L. with increasing P deficiency. It would appear, therefore, that in those treatments where plants were exposed to high levels of NO_3–N and PO_4–P, these nutrients may have been accumulated in a pool and then reduced and incorporated into metabolism at a later stage, i.e. the assimilation of N and P by plants and their incorporation into new growth did not keep pace with their uptake in culture. Further research on the depletion of NO_3–N and PO_4–P in the culture solution, levels and location of nitrate reductase and alkaline phosphatase in the plant, however, will be required before any meaningful conclusions can be drawn from this problem.

TABLE 3.—Statistical analysis of regressions of 1/U against 1/limiting nutrient concentration for *E. crassipes* grown in N-(Experiments 1 to 5) and P-(Experiments 6 to 8) limited cultures

Experiment No.	Correlation coefficient (r)	Degrees of freedom (n–l)	Significance level %	Analysis of variance: Variance ratio (F value)	Analysis of variance: Significance level %
1	0,6877	99	0,1	78,19	0,1
2	0,4258	79	0,1	9,49	1
3	0,7018	79	0,1	79,06	0,1
4	0,5697	89	0,1	22,37	0,1
5	0,5578	89	0,1	20,51	0,1
6	0,3463	79	1	4,57	5
7	0,3799	79	0,1	9,73	1
8	0,4769	89	0,1	13,51	0,1

Maximum specific growth rate (Umax)

Lineweaver-Burk plots of the reciprocals of specific growth rates (1/U), i.e. the highest specific growth rate attained by each plant after the addition of N or P, against the reciprocals of limiting nutrient concentrations (Figs 3 & 4) showed that the relationship between 1/U and 1/N or 1/P was linear in each experiment with a high degree of correlation, significant at $P \leq 0,01$ (Table 3). An analysis of variance of the regressions showed that the slopes and intercepts

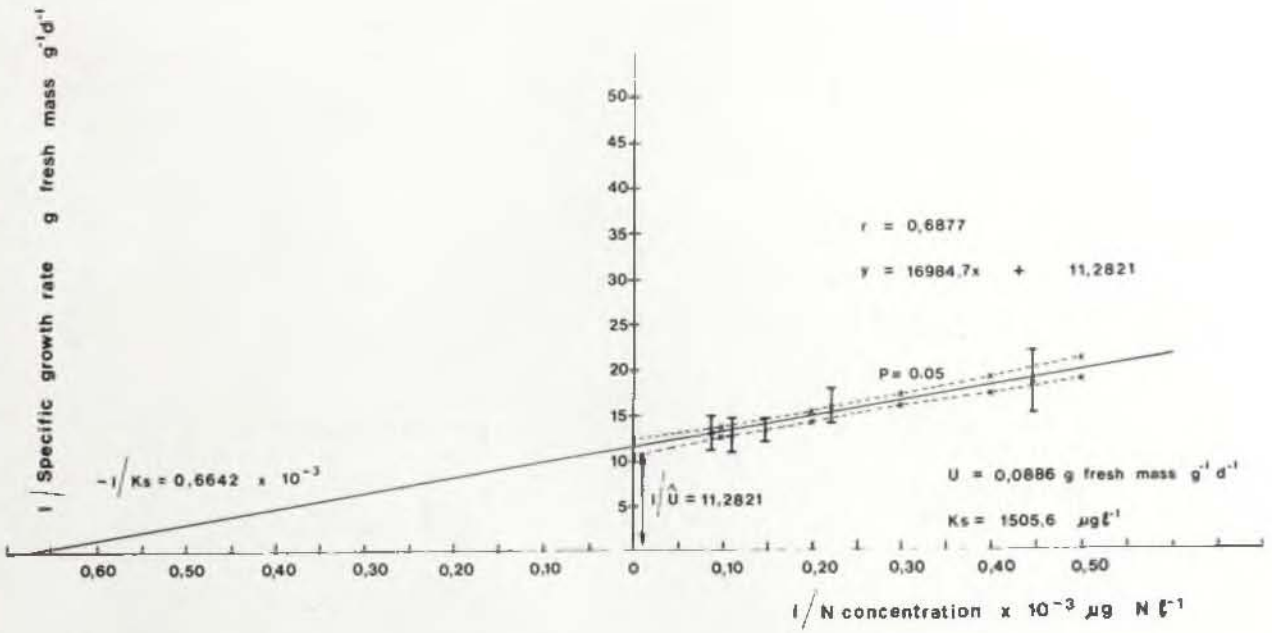

FIG. 3. — Experiment 1. A Lineweaver-Burk plot of specific growth rates of *E. crassipes* (means of 20 plants/treatment) against levels of N supplied in culture. Broken lines show 95% confidence limits on either side of the regression line. Standard deviations of means are shown by bars. U = Umax.

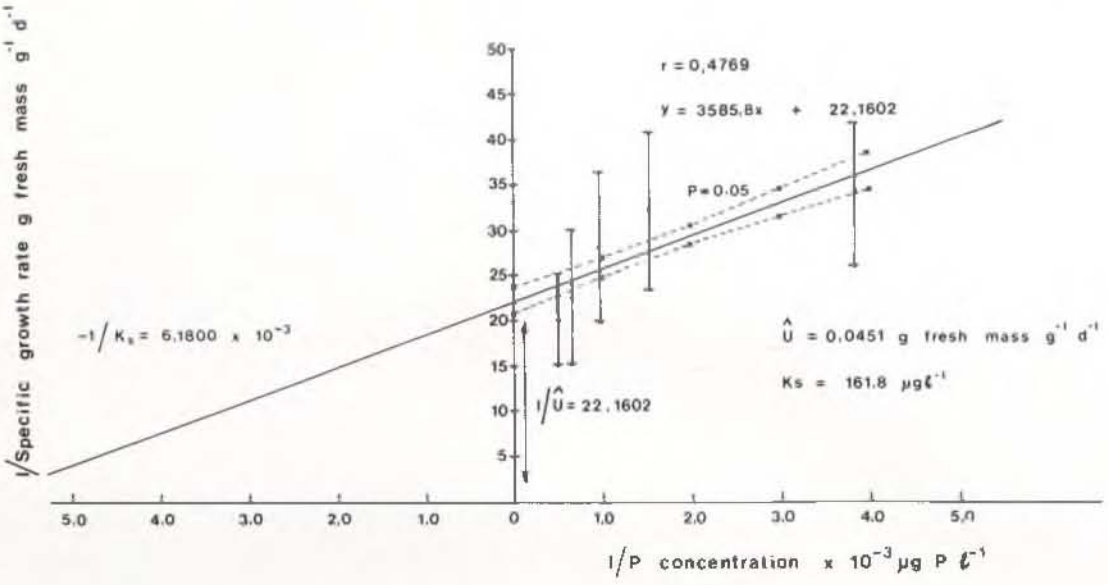

FIG. 4. — Experiment 8. A Lineweaver-Burk plot of specific growth rates of *E. crassipes* (means of 18 plants/treatment) against levels of P supplied in culture. Broken lines show 95% confidence limits on either side of the regression line. Standard deviations of means are shown by bars. U = Umax.

were significant at $P \leq 0,05$. The Umax value was extrapolated for *E. crassipes* in each experiment from the intercept of the regression line on the y axis, calculated from the regression equation.

The Umax values determined ranged from 0,0537 to 0,0886 g fresh mass g^{-1} d^{-1} (5,37 to 8,86% d^{-1}) in N-limited experiments and from 0,0451 to 0,1089 g fresh mass g^{-1} d^{-1} (4,51 to 10,89% d^{-1}) in P-limited experiments (Table 4). An exponential relationship was not evident between the Umax values derived under N and P limitation and the reciprocals of mean daily air temperatures (expressed as °K) recorded in the greenhouse (Arrhenius plot). In addition, no correlation was evident between the Umax values determined and mean daily relative humidities recorded in the greenhouse. Significantly lower Umax values, however, were obtained in Experiments 2, 3, 7 and 8 where longer growth periods in deficient cultures were required to induce N or P limitation (Table 4).

The latter observation could not be explained in terms of non-competitive inhibition, i.e. by a reduced uptake rate of the limiting nutrient by plants resulting from their longer growth periods in deficient cultures. Investigations of the uptake kinetics of higher plants have shown that growth of plants in starvation (deficient) media causes a subsequent increase in their nutrient uptake rate with a corresponding reduction in the half saturation coefficient (Km) for uptake. Glass (1978), for example, has shown that the uptake characteristics for K^+ of barley plants grown initially with or without K^+ are very different, the Km for K^+ uptake being reduced in the starved plant from 0,1 to 0,03 mM. The same occurs for other ions, as for NO_3^- (Smith, 1973) and PO_4^{2-} (Cartwright, 1972), and for other species. Doddema *et al.* (1979), for example, have shown a reduction in Km from 111 to 40 mM NO_3^- brought about by N starvation in *Arabidopsis thaliana*. It is suggested, therefore, that the different Umax values derived for

TABLE 4. — Maximum specific growth rates (Umax) and half saturation coefficients (Ks) derived for *E. crassipes* in N-(Experiments 1 to 5) and P-(Experiments 6 to 8) limited cultures

Experiment no.	Growth in deficient culture (days)	Umax		Ks µg ℓ⁻¹	Temperature °C			Relative humidity %		
		g fresh mass $g^{-1}d^{-1}$	95% confidence limits		Max	Mean	Min	Max	Mean	Min
1	18	0,0886	± 0,0064	1 505,6	25	24	23	80	67	55
2	39	0,0537	± 0,0028	399,8	31	28	25	71	62	53
3	57	0,0613	± 0,0089	1 085,3	30	26	23	73	63	54
4	21	0,0713	± 0,0042	914,0	28	24	21	76	66	56
5	17	0,0812	± 0,0050	975,5	30	26	22	72	63	55
6	17	0,1089	± 0,0045	79,5	31	28	25	70	61	52
7	39	0,0453	± 0,0016	41,1	30	27	25	72	62	53
8	38	0,0451	± 0,0030	161,8	30	26	22	73	64	55

E. crassipes under N and P limitation possibly reflect:

(i) the different physiological state of plants grown for different spans in deficient cultures and collected on different occasions from the field for each experiment;

(ii) differences in the ratio of plant mass at spiking to levels of limiting nutrient supplied to culture, since a larger plant mass resulted at spiking in those experiments where longer growth periods in deficient cultures were required to induce N or P limitation;

(iii) variations in light intensity in the greenhouse between experiments.

Half saturation coefficient (Ks)

The Ks was extrapolated for *E. crassipes* in each experiment from the intercept of the regression line of 1/U against 1/N or 1/P on the x axis, calculated from the regression equation (Figs 3 & 4). The Ks concentrations determined ranged from 399,8 to 1 505,6 μg N ℓ^{-1} in N-limited experiments and from 41,1 to 161,8 μg P ℓ^{-1} in P-limited experiments (Table 4.) They showed no correlation with mean daily air temperatures and relative humidities recorded in the greenhouse or with the duration of plant growth in deficient cultures required to induce N or P limitation. The same reasons given for the different Umax values determined may also partly explain the different Ks concentrations measured for *E. crassipes* under N and P limitation.

The Ks concentrations derived for *E. crassipes* under N limitation are in the range of those reported for various species of algae, whereas those derived under P limitation are much higher (Table 5). This indicates that *E. crassipes* has a potential similar to algae to produce a high growth rate in N-limited waters, but a potential lower than algae to produce a high growth rate in P-limited waters. Since P is the nutrient most frequently limiting algal growth rate in relatively oligotrophic waters (Toerien *et al.*, 1975), it would appear that in such waters P may also be the nutrient limiting for *E. crassipes*.

The mean Ksn concentration of 976 μg N ℓ^{-1} determined for *E. crassipes* from the five N-limited experiments falls in the range 500 to 1 000 μg N ℓ^{-1}, interpreted by Center & Spencer (1981) from the N/P uptake rates of *E. crassipes* of 5 to 10 ℓ^{-1} (Boyd, 1970; 1976; Dunigan *et al.*, 1975) as being the critical limiting N concentrations in the water for *E. crassipes* in the field, i.e. below which the growth rate of this plant is significantly influenced by the N concentration in the water. The mean Ksp concentration of 94,1 μg P ℓ^{-1} determined for *E. crassipes* from the three P-limited experiments compares favourably with 100 μg P ℓ^{-1} reported by Haller *et al.* (1970) and Knipling *et al.* (1970) as being the critical limiting P concentration in the water for *E. crassipes* in the field.

The ratio of the mean Ksn/Ksp concentrations, derived for *E. crassipes* under N and P limitation, suggest an optimal N/P ratio in the water for *E. crassipes* of ca 10, i.e. below which N and above which P concentrations in the water become growth rate limiting for this plant. This value is well below the optimal N/P ratio of 30 (cell and medium) reported by Rhee (1974, 1978) for algae. It should, however, be pointed out that, although the limiting nutrient can often be indicated from the N/P ratio in the water, in many instances the growth rate of phytoplankton is controlled by P even when the N/P ratio in the water is relatively low (Welch *et al.*, 1978).

Yield coefficient (Yc)

With the exception of Experiments 4 and 5, where mass recordings were terminated prior to cessation of plant growth, the quantities of limiting nutrient remaining in three culture solution samples taken at random from each treatment, after plants had been harvested, were below 0,1% of that initially added (Musil, 1982). It was assumed, therefore, that in all culture solutions, with the exception of Experiments 4 and 5, the N or P added had been absorbed by plants and incorporated into growth.

Plots of the total fresh mass yields of plants (fresh including dead mass produced during growth)

TABLE 5. — Half saturation coefficients (Ks) reported for various species of algae compared with those determined for *E. crassipes*

Organism	Limiting nutrient	Ks μg ℓ^{-1}	Reference
*Chlorella pyrenoidosa**	N	1400–3000	Shelef *et al.* (1968)
*Chlorella pyrenoidosa***	N	700–1400	Shelef *et al.* (1968)
Mixed algae	N	450	Shelef *et al.* (1968)
Selenastrum gracile	N	150	Middlebrooks *et al.* (1971)
Eichhornia crassipes	N	399,8–1 505,6 (mean: 976)	This study
*Chlorella pyrenoidosa**	P	55	Zabat *et al.* (1970)
*Chlorella pyrenoidosa**	P	12–29	Zabat *et al.* (1970)
Selenastrum gracile	P	10	Middlebrooks *et al.* (1971)
Selenastrum capricornutum	P	3,7–5,7	Toerien *et al.* (1971)
Eichhornia crassipes	P	41,1–161,8 (mean: 94,1)	This study

* High temperature strain
** Emersion strain

TABLE 6.— Statistical analysis of regressions relating total fresh mass yields to quantities of limiting nutrient supplied for *E. crassipes* grown in N-(Experiments 1 to 3) and P-(Experiments 6 to 8) limited cultures

Experiment no.	Correlation coefficient (r)	Degrees of freedom (n−1)	Significance level %	Analysis of variance	
				Variance ratio (F value)	Significance level %
1	0,7636	59	0,1	81,16	0,1
2	0,8141	95	0,1	184,72	0,1
3	0,7954	95	0,1	161,91	0,1
6	0,8259	49	0,1	49,34	0,1
7	0,8224	95	0,1	192,20	0,1
8	0,8458	107	0,1	196,25	0,1

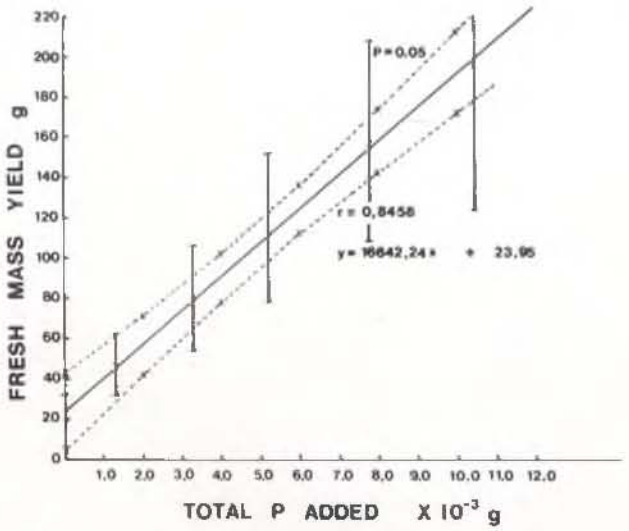

FIG. 5. — Experiment 1. The relationship between total fresh mass yields of *E. crassipes* (means of 20 plants/treatment) and quantities of N supplied in culture. Broken lines show 95% confidence limits on either side of the regression line. Standard deviations of means are shown by bars.

against the quantities of limiting nutrient added (Figs 5 & 6) showed that the relationship between these two factors, in each of the first three N- and P-limited experiments, was linear with a high degree of correlation, significant at $P \leq 0,001$ (Table 6). An analysis of variance of the regressions showed that the slopes and intercepts were significant at $P \leq 0,001$. The Yc value (fresh mass basis) was derived for *E. crassipes* in each experiment from the slope of the regression line given by the regression equation. The Yc values (fresh mass basis) determined ranged from 1 659,6 to 1 981,1 in N-limited experiments and from 16 431,2 to 18 670,6 in P-limited experiments (Table 7).

The mean water contents of plants, harvested from each of the first three N- and P-limited experiments, are given in Table 7. Water contents ranged from 94,72 to 95,05% and showed no significant differences ($P \leq 0,05$) between experiments. They compare favourably with the average water content of 94,75% derived from values reported by Penfound & Earle (1948), Westlake (1963) and Bock (1969). From the mean water contents of plants, the Yc values (fresh mass basis) were converted to a dry mass basis.

The Yc values (dry mass basis) determined ranged from 86,9 to 98,1 in N-limited experiments and from 867,1 to 980,2 in P-limited experiments (Table 7). Slightly higher Yc values (both fresh and dry mass basis) were obtained in Experiments 1 and 6 where plants were grown for the shortest spans in deficient cultures to induce N or P limitation.

In all experiments, some growth (yield in plant material) was produced by *E. crassipes* grown in the absence of N or P (Figs 5 & 6). This indicated that, although limiting N and P concentrations were existent in the plants, sufficient quantities were present to allow some growth. In fact, higher yields were produced by *E. crassipes* grown in the absence of the limiting nutrient in Experiments 1 and 6, where

TABLE 7.— Yield coefficients, Yc, (g of fresh mass yield of plant material per g of limiting nutrient absorbed by plants) derived for *E. crassipes* in N-(Experiments 1 to 3) and P-(Experiments 6 to 8) limited cultures. Yield coefficients (dry mass basis) are estimated from the mean water contents of plants determined in each experiment

Experiment no.	No. of plants inoculum	Growth in deficient culture (days)	Yc (fresh mass basis)	Mean water content plants		Yc (dry mass) basis)
				%	Standard deviation	
1	2	18	1 981,1	95,05	± 0,85	98,1
2	1	39	1 664,9	94,78	± 0,93	86,9
3	1	57	1 659,6	94,72	± 0,83	87,6
6	2	17	18 670,6	94,75	± 0,87	980,2
7	1	39	16 431,2	94,72	± 0,96	867,6
8	1	38	16 642,2	94,79	± 0,92	867,1
Analysis of variance						
Variance ratio (F value)				2,99		
Degrees of freedom (n−1)				615		
Significance level %				NS (P=0,05)		

NS = not significant

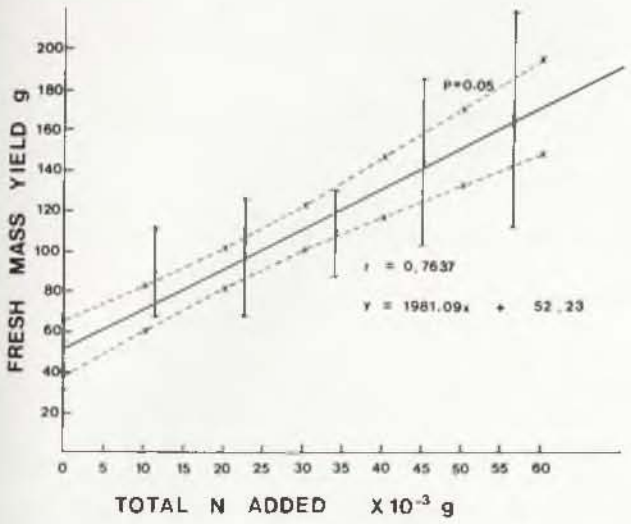

FIG. 6. — Experiment 8. The relationship between total fresh mass yields of *E. crassipes* (means of 18 plants/treatment) and quantities of P supplied in culture. Broken lines show 95% confidence limits on either side of the regression line. Standard deviations of means are shown by bars.

plants were grown for the shortest periods in deficent cultures to induce N or P limitation, than in other experiments (Musil, 1982). This suggests that the limiting nutrients (N or P) were present at higher concentrations in plants at spiking in these two experiments than in other experiments. In principle, however, higher limiting concentrations of N and P present in plants at spiking in Experiments 1 and 6 respectively should not have had an influence on the Yc values determined, since these were derived from the slopes of regression lines relating total fresh mass yields of plants to quantities of limiting nutrient (N or P) supplied in culture. Consequently, the slightly higher Yc values measured for N and P in Experiments 1 and 6 respectively could not be readily explained.

The Yc values (dry mass basis) derived for *E. crassipes* under P limitation are in the upper range of those reported for various species of diatoms and other algae, whereas those derived under N limitation are much higher (Table 8). This indicates that *E. crassipes* has the potential to produce a similar biomass per unit quantity of P absorbed, but a much larger biomass per unit quantity of N absorbed, than diatoms and other algae. Furthermore, the Yc values suggest that *E. crassipes* has the potential to remove similar quantities of P, but smaller quantities of N, per unit amount of plant mass than diatoms and other algae. Maximum specific growth rates reported for algae (Shelef *et al.*, 1968; Zabat *et al.*, 1970; Toerien *et al.*, 1971; Goldman, 1972), however, are considerably higher than those determined for *E. crassipes*. In eutrophic waters, therefore, in which the limiting nutrient concentrations are high and specific growth rates of both algae and *E. crassipes* approach their Umax values, *E. crassipes* would need to be present with a proportionately larger biomass than algae to compensate for its lower growth rate to ensure a potential similar to algae for removing nutrients. *E. crassipes* lower potential than algae to produce a high growth rate in waters where P is limiting, as evident from a comparison of its Ks concentrations for P with those of algae (Table 5), suggests that in relatively oligotrophic waters *E. crassipes* would also be less efficient in removing nutrients than algae, at least where both plants are present with the same biomass.

The minimum limiting concentrations of N and P (% dry mass) in plants harvested from each treatment (means of 3 batches), in each of the first three N- and P-limited experiments, are shown in Table 9. The minimum limiting concentrations of N and P (subsistence quotas) in the dry plant tissues ranged from 0,94 to 1,28% N in N-limited experiments and from 0,09 to 0,14% P in P-limited experiments. They

TABLE 8. — Yield coefficients (Yc) reported for various species of diatoms and other algae compared with those determined for *E. crassipes*

Organism	Limiting nutrient	Yc (dry mass basis)	Reference
Selenastrum capricornutum	N	35,0	Steyn (1973)
Microcystis aeruginosa	N	31,7	Gerloff & Skoog (1954)
Chlorella pyrenoidosa	N	20,0	Shelef *et al.* (1968)
Chlorella sorokiniana	N	17,9	Richardson *et al.* (1969)
Nitzschia perpusilla	N	23,6	Coetzer *et al.* (1977)
Nitzschia elliptica	N	20,0	Coetzer *et al.* (1977)
Nitzschia pelliculosa	N	15,6	Coetzer *et al.* (1977)
Nitzschia palea	N	15,0	Coetzer *et al.* (1977)
Eichhornia crassipes	N	86,9–98,1 (mean: 90,9)	This study
Selenastrum capricornutum	P	805	Toerien *et al.* (1971)
Microcystis aeruginosa	P	833–909	Gerloff & Skoog (1954)
Chlorella pyrenoidosa	P	312–374	Zabat *et al.* (1970)
Nitzschia elliptica	P	845	Coetzer *et al.* (1977)
Nitzschia perpusilla	P	455	Coetzer *et al.* (1977)
Nitzschia pelliculosa	P	177	Coetzer *et al.* (1977)
Nitzschia palea	P	171	Coetzer *et al.* (1977)
Eichhornia crassipes	P	867,1–980,2 (mean: 904,9)	This study

TABLE 9.—Minimum limiting concentrations of N and P (means of 3 batches) in *E. crassipes* harvested from culture

Treatment no.	Experiment no. 1	2	3	6	7	8
	N (% dry mass)			P (% dry mass)		
1	0,94	0,98	1,08	0,10	0,09	0,10
2	1,02	1,07	1,00	0,11	0,10	0,11
3	1,01	1,27	1,07	0,10	0,11	0,12
4	1,08	1,09	1,11	0,12	0,12	0,12
5	1,28	1,10	1,08	0,13	0,12	0,14
6	1,20	1,18	1,03		0,13	0,12
Means	1,09	1,11	1,06	0,11	0,11	0,12
Analysis of variance						
Variance ratio (F value)		0,48			0,73	
Degrees of freedom (n – 1)		53			50	
Significance level %		NS (P=0,05)			NS (P=0,05)	

NS = not significant

showed no significant differences ($P \leq 0,05$) between experiments (Table 9).

Toerien *et al.* (1971) and Coetzer *et al.* (1977) pointed out that the yield coefficient (dry mass basis) for a specific limiting nutrient, when expressed as a reciprocal and a percentage, should estimate the minimum concentration of the limiting nutrient in the dry plant tissue. The Yc values (dry mass basis) derived for *E. crassipes* under N and P limitation, when expressed as reciprocals and percentages ($1/Yc \times 100$), adequately estimated the minimum limiting concentrations of N and P in plants harvested from culture (Table 10). This suggests that the Yc values determined for *E. crassipes* are fairly reliable. The average minimum limiting concentrations of 1,10% N and 0,11% P, estimated in *E. crassipes* plant tissues from the mean Yc values for N and P respectively, also compare favourably with the minimum concentrations (% dry mass) of 1,33% N and 0,14% P reported by Boyd & Vickers (1971) in *E. crassipes* growing in the field, and with the minimum concentration (% dry mass) of 0,098% P reported by Hauer & Sutton (1973) in *E. crassipes* growing in the absence of P in culture.

Droop (1968) and Rhee (1973) showed that the minimum concentration of a specific limiting nutrient in algal cells is equal to, or not significantly different from, the intracellular half saturation coefficient (Kq) for the limiting nutrient. Consequently, if it is assumed that a similar situation exists in *E. crassipes*, then the ratio of the average minimum N/minimum P concentrations in *E. crassipes*, derived from the mean Yc values for these nutrients, give an optimal N/P ratio in *E. crassipes* of ca 10. This value compares favourably with the optimal N/P ratio in the water for *E. crassipes* of ca 10, estimated from the ratio of the mean Ksn/Ksp concentrations derived under N and P limitation in culture.

CONCLUSIONS

Maximum specific growth rates (Umax) and half saturation coefficients (Ks) were not adequately determined for *E. crassipes* growing in N- or P-limited batch cultures. In contrast, yield coefficients (Yc) were determined with sufficient accuracy. With better facilities, it is possible that the batch culture method used for measuring kinetic coefficients for *E. crassipes* growing under specific nutrient limitation in this investigation could be improved. For example, if plants for culture were collected from populations grown under controlled environmental conditions in a standardized culture medium, it is possible that a uniform growth period required to induce in plants a N- or P-limited state could be obtained. This might decrease the variability in Umax values and Ks concentrations determined. It is suggested, however, that precise measurements of Umax and Ks may only be obtained for *E. crassipes*, under specific nutrient limitation in culture, by growing plants under constant environmental conditions in some type of continuous flow culture system in which the limiting nutrient concentrations could be maintained at constant levels. In such a system, therefore, it would not be necessary to grow plants initially in deficient cultures to induce N or P limitation, since the specific growth rate of *E. crassipes* at

TABLE 10.—Minimum limiting concentrations of N and P in *E. crassipes* estimated from yield coefficients (Yc), derived under N and P limitation, compared with minimum limiting concentrations of N and P in plants harvested from culture

Experiment no.	Limiting nutrient	Yc (dry mass basis)	Minimum limiting nutrient concentration ($1/Yc \times 100$) % dry mass	Minimum limiting nutrient concentration in plants harvested from culture (means of 6 treatments) % dry mass
1	N	98,1	1,02	1,09
2	N	86,9	1,15	1,11
3	N	87,6	1,14	1,06
Means	N	90,9	1,10	1,09
6	P	980,2	0,10	0,11
7	P	867,6	0,11	0,11
8	P	867,1	0,11	0,12
Means	P	904,9	0,11	0,11

each limiting nutrient concentration could be established over a much longer growth period in culture. This would eliminate any adverse effects on the growth rate of *E. crassipes* arising through growth of plants in N- or P-deficient cultures.

Although Umax values and Ks concentrations derived for *E. crassipes* under N and P limitation varied considerably, it should be possible to evaluate their potential in modelling by using the most reliable values determined in culture in the Monod model to assess its predictive ability. This, in turn, may serve as a basis for refinement of the model. In this investigation, the Umax values measured for *E. crassipes* were adversely influenced by the duration of plant growth in deficient cultures required to induce N or P limitation. It is suggested, therefore, that, for purposes of testing the model and as a basis for its refinement, the values determined in those experiments where plants were grown for the shortest spans in N- and P-deficient cultures are possibly more reliable than those determined in other experiments. The mean Ks concentrations derived for *E. crassipes* under N and P limitation, on the other hand, are possibly more reliable than the individual concentrations determined, since they compare favourably with the critical limiting N and P concentrations in the water for *E. crassipes* in the field.

The Yc values derived for *E. crassipes* under N and P limitation showed little variation. They appear reliable, since their reciprocals (dry mass basis) expressed as percentages adequately estimated the minimum limiting concentrations of N and P in *E. crassipes* harvested from culture, i.e. when no further significant increase in the total fresh mass of plants at each level of N or P supplied was recorded. Since the minimum limiting concentrations of N and P in *E. crassipes* can be estimated from the respective Yc values for these nutrients, it should be feasible to predict the growth rate of *E. crassipes* in the field from the limiting N or P concentrations in plants using the Droop model (Droop, 1968; Rhee, 1973).

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UITTREKSEL

Die N- en P-beperkte groeikinetika van Eichhornia crassipes *(Mart.) Solms wat in 'n kweekhuis gekweek was, is ondersoek met die doel om 'n model te ontwerp waarvolgens populasiegroottes, opbrengs, groeitempo's en frekwensies en die hoeveelheid van die oes onder wisselende toestande van voedingslading en klimaat, vir hierdie plant voorspel kan word om beide die voedings-elementinvoer en buitensporige groei in eutrofiese waterstelsels te beheer. Die kinetiese koëffisiënte naamlik maksimum spesifieke groeitempo (Umax), halfversadigingskonstante (Ks) en opbrengskoëffisiënt (Yc) was van die N en P beperking in gerepliseerde lotkultuureksperimente gemeet. Umax waardes en Ks konsentrasies onder N beperking is van 5,37 tot 8,86% d^{-1} en van 400 tot 1 506 µg N ℓ^{-1} respektiewelik. Dié afgelei onder P beperking het gewissel van 4,51 tot 10,89% d^{-1} en van 41 tot 162 µg P ℓ^{-1} respektiewelik. Yc waardes (varsmassabasis) vir N bepaal, het gewissel van 1 660 tot 1 981 (87 tot 98 droëmassabasis) en van 16 431 tot 18 671 (867 tot 980 droëmassabasis) vir P. Die omgekeerdes van Yc waardes (droëmassabasis), as persentasies, gee 'n voldoende aanduiding van die minimum beperkende N en P konsentrasies (% droëmassa) in die plantweefsel. Die afgeleide kinetiese koëffisiënte word met dié van alge vergelyk. Die eksperimentele metodes gebruik en die resultate verkry, word krities beoordeel.*

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