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T THE USE OF GROWTH KINETICS IN THE DEVELOPMENT
OF A PREDICTIVE MODEL FOR THE GROWTH OF
EICHHORNIA CRASSIPES (MART.) SOLMS IN THE FIELD/

by

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The author hereby declares that this whole thesis, unless specifically indicated to the contrary in the text, is his own original work.

TABLE OF CONTENTS

		Page
CHAPTER 1	REVIEW OF LITERATURE	1
1.1	EUTROPHICATION AND ITS CONTROL BY PLANT HARVESTING	1
1.1.1	Choice of plant	2
1.1.2	Disposal of plants	3
1.2	THEORETICAL ASPECTS OF MODEL	4
1.2.1	Proposed model	4
1.2.2	Limitations of model	7
1.3	PREVIOUS RESEARCH	10
1.3.1	Culture investigations	10
1.3.2	Field investigations	11
1.4	OBJECTIVES OF STUDY	15
CHAPTER 2	METHODS	16
2.1	DEVELOPMENT OF MODEL IN CULTURE	16
2.1.1	Selection of plants	16
2.1.2	Collection of plants	16
2.1.3	Measurement of growth	18
2.1.4	Culture vessels	20
2.1.5	Culture solution	23
2.1.6	Air and water temperatures	25
2.1.7	Radiant flux density and photoperiod	27
2.1.8	Relative humidity	30
2.1.9	Determination of kinetic coefficients	31
2.2	COLLECTION OF FIELD DATA TO TEST THE MODEL	38
2.2.1	Introduction	38
2.2.2	Selection of plants	40
2.2.3	Collection of plants	40
2.2.4	Selection of sites	41

2.2.5	Enclosures	42
2.2.6	Measurement of growth	44
2.2.7	Chemical analyses	46
2.2.7.1	Plants	46
2.2.7.2	Water	47
2.2.8	Physical analyses	48
2.2.8.1	Environment	48
2.2.8.2	Water	49
CHAPTER 3	EXPERIMENTAL DETERMINATION OF KINETIC COEFFICIENTS	50
3.1	INTRODUCTION	50
3.2	RESULTS	50
3.2.1	Nitrogen growth rate limitation	50
3.2.1.1	Growth in N deficient culture	50
3.2.1.2	Growth after the addition of N	51
3.2.1.3	Maximum specific growth rate (U_{max})	51
3.2.1.4	Half saturation coefficient (K_{sn})	52
3.2.1.5	Yield coefficient (Y_{cn})	53
3.2.2	Phosphorus growth rate limitation	54
3.2.2.1	Growth P deficient culture	54
3.2.2.2	Growth after the addition of P	55
3.2.2.3	Maximum specific growth rate (U_{max})	55
3.2.2.4	Half saturation coefficient (K_{sp})	56
3.2.2.5	Yield coefficient (Y_{cp})	56
3.3	DISCUSSION	58
3.4	CONCLUSIONS	66
CHAPTER 4	TESTING THE MODEL UNDER FIELD CONDITIONS	68
4.1	INTRODUCTION	68
4.2	RESULTS	69
4.2.1	Field data	69

4.2.1.1	Specific growth rates (U)	69
4.2.1.2	Air and water temperatures	70
4.2.1.3	Nitrogen and phosphorus concentrations	72
4.2.2	Comparison of predicted and measured specific growth rates	74
4.2.2.1	Nitrogen growth rate limitation	74
4.2.2.1.1	Maturation Pond 3 (MP3) site	77
4.2.2.1.2	Isipingo Lake (IL), Isipingo Canal (IC) and Discharge Canal (DC) sites	78
4.2.2.2	Phosphorus growth rate limitation	79
4.2.2.2.1	Botanic Gardens Lake (BGL) site	81
4.2.2.2.2	Hartbeespoort Dam (HD) site	82
4.3	DISCUSSION	82
4.4	CONCLUSIONS	90
CHAPTER 5	REFINING THE MODEL UNDER FIELD CONDITIONS	92
5.1	GENERATING U MAX IN THE FIELD	92
5.1.1	Introduction	92
5.1.2	Results	93
5.1.2.1	Comparison of predicted and measured specific growth rates	93
5.1.2.1.1	Botanic Gardens Lake (BGL) site	94
5.1.2.1.2	Hartbeespoort Dam (HD) site	95
5.1.2.1.3	Isipingo Lake (IL), Discharge Canal (DC) and Isipingo Canal (IC) sites	96
5.1.3	Discussion	97
5.1.4	Conclusions	100
5.2	CORRECTION OF U MAX FOR RADIANT FLUX DENSITY	100
5.2.1	Introduction	103
5.2.2	Results	105
5.2.2.1	Field data	103
5.2.2.2	Comparison of predicted and measured specific growth rates	105

5.2.2.2.1	Botanic Gardens Lake (BGL) site	106
5.2.2.2.2	Hartbeespoort Dam (HD) site	107
5.2.2.2.3	Isipingo Lake (IL), Discharge Canal (DC) and Isipingo Canal (IC) sites	108
5.2.3	Discussion	109
5.2.4	Conclusions	110
5.3	CORRECTION OF U MAX FOR RELATIVE HUMIDITY	111
5.3.1	Introduction	111
5.3.2	Results	113
5.3.2.1	Field data	113
5.3.2.2	Comparison of predicted and measured specific growth rates	114
5.3.2.2.1	Botanic Gardens Lake (BGL) site	115
5.3.2.2.2	Hartbeespoort Dam (HD) site	115
5.3.2.2.3	Isipingo Lake (IL), Discharge Canal (DC) and Isipingo Canal (IC) sites	116
5.3.3	Discussion	117
5.3.4	Conclusions	121
CHAPTER 6	PREDICTING GROWTH RATES FROM THE NUTRIENT CONCENTRATIONS IN THE PLANTS	123
6.1	INTRODUCTION	123
6.2	RESULTS	124
6.2.1	Field data	124
6.2.2	Comparison of predicted and measured specific growth rates	127
6.2.2.1	Botanic Gardens Lake (BGL) site	128
6.2.2.2	Isipingo Lake (IL), Discharge Canal (DC) and Hartbeespoort Dam (HD) sites	129
6.2.2.3	Isipingo Canal (IC) site	129
6.3	DISCUSSION	129
6.4	CONCLUSIONS	136

CHAPTER 7	GENERAL CONCLUSIONS AND APPLICATION OF REFINED MODEL	138
	SUMMARY	148
	ACKNOWLEDGEMENTS	154
	LIST OF REFERENCES	155
APPENDIX I	PILOT STUDIES	185
I	THE INFLUENCE OF THE MACRONUTRIENT CONCENTRATIONS IN THE CULTURE MEDIUM ON THE SPECIFIC GROWTH RATE OF <i>E. CRASSIPES</i>	185
1	Introduction	185
IA	THE INFLUENCE OF THE N CONCENTRATION IN THE CULTURE MEDIUM ON THE SPECIFIC GROWTH RATE OF <i>E. CRASSIPES</i>	185
2	Methods	185
3	Results	186
4	Discussion	186
5	Conclusions	187
IB	THE INFLUENCE OF THE K, Ca AND Mg CONCENTRATIONS AND THEIR RELATIVE PROPORTIONS IN THE CULTURE MEDIUM ON THE SPECIFIC GROWTH RATE OF <i>E. CRASSIPES</i>	187
2	Methods	187
3	Results	188
4	Discussion	188
5	Conclusions	189
II	THE INFLUENCE OF AERATION OF THE CULTURE MEDIUM ON SPECIFIC GROWTH RATE OF <i>E. CRASSIPES</i>	190
1	Introduction	190
2	Methods	190
3	Results	191

4	Discussion	191
5	Conclusions	192
III	THE RELATIONSHIP BETWEEN THE NUTRIENT CONCENTRATIONS IN THE WATER ENVIRONMENT AND THE NUTRIENT DISTRIBUTION PATTERN IN <i>E. CRASSIPES</i>	192
1	Introduction	192
2	Methods	193
3	Results and Discussion	194
	(a) Water analysis	194
	(b) Plant analysis	196
	(i) Whole plants	196
	(ii) Plant parts	194
	(iii) Marginal and central plants	197
4	Conclusions	198

CHAPTER 1

REVIEW OF LITERATURE

1.1 EUTROPHICATION AND ITS CONTROL BY PLANT HARVESTING

Eutrophication, the enrichment of water systems with inorganic nutrients (Stewart and Rohlich, 1967), is a world wide water quality problem (Stumm, 1974). Nutrients originate from various sources such as industrial and municipal effluents (Wiebel et al., 1963) and from run off and drainage from agricultural operations (Weider et al., 1963). In South Africa, dissolved nutrient levels have increased in many water systems in recent years (Scott et al., 1977; Walmsley et al., 1978) greatly accelerating the process of eutrophication. Enhanced growth of algae (Toerien et al., 1975) and higher aquatic plants (Scarsbrook and Davis, 1971) can lead to the occurrence of a number of water quality problems such as increased water treatment costs, aesthetic problems, health problems and interferences with recreational water use (Penfound and Earle, 1948; Ferguson, 1968; Holm et al., 1969). Research into more effective techniques to remove nutrients from effluents prior to their release into natural waters is urgently needed (Amer. Chem. Soc., 1969; Bolitho, 1976). Just as relevant is the need for methods of nutrient removal from natural waters that have become excessively eutrophic.

Aquatic vegetation absorbs large quantities of nutrients during growth (Rogers and Davis, 1972; Shillinglaw and Pieterse, 1977; Sutton and Ornes, 1975) and its removal may constitute an effective means of stripping nutrients from effluents prior to their release into natural waters (Yount and Crossman, 1970). Similarly, the removal of aquatic vegetation growing in eutrophied water systems may also assist in controlling excessive growth of plants by reducing nutrient levels.

1.1.1 Choice of plant

Several studies have dealt with nutrient removal from sewage effluents by harvesting planktonic algae, but rather elaborate and expensive techniques are required to harvest phytoplankton (Golueke, 1964). Various authors (Mackenthun, 1964; 1965; Burgess, 1965; Boyd, 1968) have hinted at the possibility of using higher aquatic plants for nutrient abatement purposes. Rooted submerged and rooted floating-leafed, aquatic vascular plants are unsuitable for nutrient abatement purposes as they are difficult to harvest and most species do not produce large quantities of biomass per unit area (Boyd, 1968; Little, 1968). Boyd (1970) pointed out that *Eichhornia crassipes* (Mart.) Solms (water hyacinth) is the most promising higher aquatic plant species for removing nutrients from eutrophied waters as it possesses the following advantages:

- (i) it has a free-floating nature and can, therefore, be harvested by relatively simple means;
- (ii) it produces a large standing crop per unit area (Knipling *et al.*, 1970; Boyd and Scarsbrook, 1975);
- (iii) it has a high growth rate, exceeding that even of the most productive agricultural crops (Westlake, 1963);
- (iv) it is capable of removing large quantities of nitrogen and phosphorus, the nutrients generally associated with eutrophication (Mackenthun, 1964; 1965), from sewage effluents (Clock, 1968; Steward, 1970; Miner *et al.*, 1971). In addition, this plant is capable of removing heavy metal and chemical pollutants from secondary waste water effluents (Wolverton, 1975; Wolverton and McDonald, 1975a; 1975b; 1976; Wolverton and McKown, 1976) as well as reducing 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 and McDonald, 1975c; 1975d; Wolverton *et al.*, 1975b).

1.1.2 Disposal of plants

If water hyacinths are harvested for nutrient abatement purposes, a disposal problem may occur. The question that arises is what to do with the harvested plants to help defray or at least not add appreciably to the removal costs. Research in Florida employing commercial mechanical harvesters to remove water hyacinths has indicated that harvest costs vary widely with location; water way topography, geometry and growth pattern (Van Dyke, 1971; Touzeau, 1972; Phillippy and Perryman, 1972a; 1972b). On a dry mass basis, harvesting and processing costs (Bagnall et al., 1973) appear relatively expensive when compared with agricultural commodities (Akeson and Stahmann, 1966). The huge bulk and high water content, about 95% (Penfound and Earle, 1948; Westlake, 1963; Bock, 1966), and heterogeneous fibrous nature of *E. crassipes* are not conducive to conventional schemes of agricultural raw material handling and utilization (Bates and Hentges, 1976). Harvested water hyacinths, however, are being investigated for many possible uses:

- (i) as a compost or soil conditioner (Smith and Thornton, 1945; Kamal and Little, 1970; Parra, 1974);
- (ii) as a component in cattle and sheep (Hentges et al., 1972; Bagnall et al., 1973; Baldwin, 1973), swine (Combs and Wallace, 1973), poultry and fish diets (Liang and Lovell, 1971);
- (iii) as an ingredient in paper and pulp manufacture (Nolan and Kirmse, 1974);
- (iv) as a protein source for animal and human feeding (Boyd, 1969);
- (v) as a substrate for industrial fermentations to produce a wide variety of valuable chemicals and gases (Archana, 1971; Wolverton et al., 1975a).

Presently, it appears that there are few direct commercial possibilities of utilizing harvested water hyacinths in the more advanced nations. If the plants are removed for nutrient

abatement purposes, it is possible, however, that they could be utilized as a compost and soil conditioner or as feedstuffs for ruminant animals. This would offset the cost of harvesting to some extent.

1.2 THEORETICAL ASPECTS OF MODEL

A prime consideration in the concept of harvesting *E. crassipes* for nutrient abatement purposes is how much and how frequently to harvest the population to achieve maximum nutrient removal efficiency. Clearly, if the population is overharvested, the size of the population will be severely reduced and its efficiency in removing nutrients will be diminished. If, on the other hand, the population is underharvested, the larger population resulting will reduce the nutrient levels in the water to such low levels that its growth rate will eventually be impeded. To achieve a balance between nutrient input and nutrient uptake in a nutrient removal scheme utilizing water hyacinths, a model which allows for the prediction of the amounts and frequencies of harvest under varying conditions of growth rate and nutrient supply is required. Such a predictive model should also provide a basis for managing, through nutrient limitation by mechanical harvesting, excessive growth of water hyacinth in natural water systems that have become eutrophied. This approach would therefore deal directly with the problem, i.e. nutrient enrichment of water, rather than with just the excessive aquatic plant (weed) growth which is a manifestation of the problem.

1.2.1 Proposed model

Toerien (1972) pointed out that from the kinetic standpoint, it should be feasible to construct a model from which the amounts and frequencies of harvest of *E. crassipes* could be predicted to control both nutrient inputs in eutrophied water systems and excessive growth of this plant. Emphasis was placed on culturally eutrophied water systems where nutrient inputs originate from point sources such as sewage treatment plants and factory

effluents. In naturally eutrophied water systems, where nutrient inputs originate from diffuse sources, it would, however, be virtually impossible to control these inputs, although it should be possible to reduce them.

Under "ideal" conditions the growth of an organism such as *E. crassipes* should increase according to a geometrical progression in time, i.e. it multiplies itself by a constant factor in each successive unit of time. Hence, growth can be mathematically described by the so-called general growth equation (Malek and Fenc1, 1966; Radford, 1967).

$$X_t = X_0 e^{ut}$$

where: X_t = amount of biomass at time t ,

X_0 = amount of biomass at time 0,

u = specific growth rate,

t = time period between time t and $t = 0$.

The daily incremental factors calculated by Bock (1966, 1969) to describe the rates of increase in fresh and dry mass and plant numbers of *E. crassipes* grown under natural field conditions suggest that the growth rate of *E. crassipes* may be described or closely approximated by this equation.

The relationships between the growth rate of *E. crassipes* and the concentrations of specific growth rate limiting nutrients are not known. It can, however, be postulated that such relationships do exist and can be formulated in mathematical terms. Such a postulate is supported by the fact that the growth relationships of algae and bacteria can be described by first order-zero order equations relating growth rate to the concentration of the growth rate limiting nutrient. Various models have been used to quantify this relationship (Shelef et al., 1968; Toerien et al., 1971; Goldman, 1972). The most important are:

- (i) Blackman's first order-zero order model,
- (ii) Teisser's exponential model and

(iii) Monod's rectangular hyperbola model which is defined as follows:

$$U = U_{\max} \frac{S}{K_s + S}$$

where: U = specific growth rate,
 U_{max} = maximum specific growth rate,
 S = concentration of growth rate limiting nutrient,
 K_s = half saturation coefficient = S when U = 0,5 U_{max}.

The Monod equation is another form of the equation developed by Michaelis and Menten (1913) to describe enzyme reaction kinetics. The fact that a model describing enzyme reaction kinetics can be used in another form to describe the growth rate of unicellular organisms points to the feasibility of using it to relate the growth relationships of higher organisms such as **E. crassipes** to the concentration of a specific nutrient while it is growth rate limiting.

The Monod equation expresses the relationship between the concentration of a specific growth rate limiting nutrient and the rate of growth of an organism. However, in order to make predictive estimates of the harvest sizes required for **E. crassipes** growing in a nutrient removal scheme, the amount of growth that can be produced from a specific quantity of growth rate limiting nutrient absorbed must also be known. This is the yield coefficient and is defined as the mass of growth produced by plants per mass of growth rate limiting nutrient absorbed:

$$Y_c = \frac{X_t - X_o}{S_o - S_t}$$

where: Y_c = yield coefficient,
 X_o = initial biomass,
 X_t = final biomass,

So = initial concentration of growth rate limiting nutrient,

St = final concentration of growth rate limiting nutrient.

The predictive abilities of such models have been demonstrated in the algae, for example, by Toerien and Huang (1973) where the phosphorus 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 fairly accurately predicted by a water quality simulation model also using kinetic coefficients.

1.2.2 Limitations of model

A potential complication to the use of the various kinetic coefficients in a predictive model is their temperature dependency. Goldman and Carpenter (1974), however, pointed out that when the light intensity is held constant, it is possible to describe the maximum specific growth rate (U_{max}) solely as a function of temperature by applying the Arrhenius equation:

$$U_{max} = Ae^{-\frac{E}{RT}}$$

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,
 U_{max} = maximum specific growth rate.

Using an Arrhenius plot, Goldman (1972) and Goldman and Carpenter (1974) demonstrated that the effect of temperature on the U_{max} values reported for various species of marine and freshwater algae generally followed the van't Hoff rule, i.e. they approximately doubled for each 10°C rise in the temperature. Substituting the Arrhenius equation into the Monod equation,

the following relationship is obtained in which the specific growth rate is related to both temperature and the growth rate limiting nutrient concentration:

$$U = Ae^{-\frac{E}{RT}} \cdot \frac{S}{K_s + S}$$

where: U = specific growth rate,
 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,
 S = concentration of growth rate limiting nutrient,
 K_s = half saturation coefficient.

There are, however, a number of restrictions to the general use of the above equation. Firstly, for each plant species the Arrhenius equation is applicable only over a definite 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 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 sensitive to changes in temperature (Shelef et al., 1970). Consequently, a more generally applicable relationship than that described by the above equation would be:

$$U = Ae^{-\frac{E(L)}{RT}} \cdot \frac{S}{K_s(T) + S}$$

where: K_s(T) = temperature dependent half saturation coefficient,
 E(L) = light dependent activation energy cal. mole⁻¹.

A further potential complication in the effect of temperature on plant growth is the possible temperature dependency of at least another important parameter controlling nutrient uptake and net plant growth, i.e. the yield coefficient (Y_c). Minor variations in the yield coefficient have been found for a high and low temperature strain of *Chlorella* grown in nitrate-nitrogen growth rate limited continuous cultures (Shelef et al., 1970). These results have also been duplicated by Topiwala and Sinclair (1971) in a continuous culture study of the bacterium *Aerobacter aerogenes*. The yield coefficient may, therefore, be relatively insensitive to temperature changes although the precise effect of temperature on this kinetic coefficient is not yet known.

The perhaps impossible task of determining temperature dependent kinetic coefficients such as $K_s(T)$ and $Y_c(T)$ 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. It may be possible to determine the temperature dependency of these kinetic coefficients in culture experiments. In continuous culture with temperature held constant, Shelef (1968) and Goldman et al. (1974) described these coefficients for several growth rate limiting nutrients. Unfortunately, there have been no attempts to model temperature effects in continuous culture algal studies, although Topiwala and Sinclair (1971) were able to develop relationships for the maximum specific growth rate (U_{max}) and the half saturation coefficient (K_s) as functions of temperature for *A. aerogenes* while Shelef et al. (1970) showed that U_{max} and K_s increased with increasing temperatures for *Chlorella*.

Apart from the half saturation coefficient (K_s) which may be influenced by the pH (Goldman, 1972), the influence of other environmental factors on the various kinetic coefficients are not known. This, however, may not necessarily restrict their application in predicting yields and growth rates for *E. crassipes* growing under environmental conditions in the

field, provided that they do not deviate to any large extent from those in the laboratory situation under which these kinetic coefficients are determined.

1.3 PREVIOUS RESEARCH

Many references exist in the literature on the nutrient uptake and growth characteristics of *E. crassipes* growing under laboratory culture and field conditions. Despite this, the necessary parameters (yields and growth rates under varying climatic conditions and nutrient supply) required for the proper evaluation and potential design of a model, from which amounts and frequencies of harvest of *E. crassipes* can be predicted to control both nutrient inputs in culturally eutrophied water systems and excessive growth of this plant, are either not available or have been inadequately determined.

1.3.1 Culture investigations

In laboratory culture, for example, it has been demonstrated that the growth of *E. crassipes* increases with an increase in the air and water temperature (Bock, 1969; Knipling *et al.*, 1970; Freidel *et al.*, 1978) and with an increase in the level of N (Chadwick and Obeid, 1966) or P (Haller and Sutton, 1973) supplied. The relationships between the growth rate of *E. crassipes* and the air and water temperatures and the concentrations of specific growth rate limiting nutrients, such as N and P (Wahlquist, 1972), however, have not been mathematically formulated and cannot be derived from the results of these investigations. These relationships would need to be known so that the growth rate of *E. crassipes* could be predicted for various temperatures and levels of growth rate limiting nutrient supply.

Rates of uptake of N and P (Rogers and Davis, 1972; Dunigan *et al.*, 1975a; 1975b) as well as phytotoxic heavy metals (Wolverton, 1975; Wolverton and McDonald, 1975a; 1975b; Wolverton *et al.*, 1975b) by *E. crassipes* in culture have also been investigated. Rogers and Davis (1972), for example, reported that the

quantities of P absorbed by *E. crassipes* in static water in growth chamber experiments averaged 1,1; 2,1; 3,1 and 1,6 mg of P per plant per day in 10, 25 and 50% Hoaglands solution and sewage effluent. In flowing water values were 1,7; 2,5 and 3,3 mg of P per plant per day in 10, 25 and 50% Hoaglands solution. Quantities of N absorbed were 5,3; 11,4; 19,8 and 6,6 mg of N per plant per day from static water and 9,9; 18,4 and 20,8 mg of N per plant per day from flowing water. From these investigations, they concluded that the absorptive capacity of one hectare of water hyacinths would exceed 2500 kg N and 700 kg P per year, equivalent to the N and P contributed by ca 800 people, if maximum growth could be sustained. The results of these uptake studies, based on the quantities of nutrients removed per plant per day, however, are unsuitable for modelling purposes as they do not relate the quantities of nutrients removed by plants to their mass or establish relationships between the rate of uptake of growth rate limiting nutrients, such as N and P, by *E. crassipes* and the growth rate of plants. It is clear that the latter would be influenced by both the growth rate limiting nutrient concentrations in the water and by the climatic conditions. This in turn would have an influence on the rate of uptake of nutrients by plants. In addition, the yields of *E. crassipes*, i.e. the amount of biomass produced per unit quantity of specific growth rate limiting nutrient absorbed by plants, were not determined. Such information would be required to develop a predictive model.

1.3.2 Field investigations

Yields and growth rates have been determined for *E. crassipes* under field conditions. Penfound (1956), for example, reported a growth rate for *E. crassipes* in natural waters in Louisiana of 12,7 to 14,6 g m⁻² d⁻¹ (dry mass basis). Boyd (1970) suggested a theoretical annual yield of 54,7 MT ha⁻¹ (5470 g m⁻² dry mass basis) based on Penfound's data of standing crop of 12,8 MT ha⁻¹ (1280 g m⁻² dry mass basis) and a maximum growth rate of 14,6 g m⁻² d⁻¹. Considerably higher yields and growth rates, however, have been reported for *E. crassipes*

growing in nutrient enriched (eutrophied) waters. Yount and Crossman (1970) in one series of investigations reported growth rates for *E. crassipes* grown in Milorganite fertilized ponds of more than $54 \text{ g m}^{-2} \text{ d}^{-1}$ (dry mass basis), although growth rates of $40 \text{ g m}^{-2} \text{ d}^{-1}$ were more common. Wooten and Dodd (1976), on the other hand, observed an average seasonal growth rate of $29 \text{ g m}^{-2} \text{ d}^{-1}$ (dry mass basis) for *E. crassipes* grown in secondary treated waste-water effluent, whereas Boyd (1976) measured growth rates averaging $19,4 \text{ g m}^{-2} \text{ d}^{-1}$ (dry mass basis) over a 5 month growth period in fertilized ponds and estimated a yield, assuming a 12 month growing season, of 65 MT ha^{-1} (6500 g m^{-2} dry mass basis). These investigations demonstrate that *E. crassipes* will have high growth rates and produce high yields in eutrophied water systems which would make this plant suitable for nutrient abatement purposes. The investigations, however, were not designed to establish the relationships between the growth rates of plants and the growth rate limiting nutrient concentrations in the water and the climatic conditions. Consequently, the information is of little value in developing or testing a predictive model.

Growth rates determined for *E. crassipes* in the above investigations were also measured in populations of variable density. There is some evidence presented in the literature, however, that *E. crassipes* plants of different growth form found in populations of different density have different metabolisms, net C uptake and P/R ratios (Mitsch, 1977), which suggests they may have different growth rates. For example, in field populations *E. crassipes* plants may be divided into 2 distinct growth forms, viz. marginal and central forms, based on their morphology and habitat preference. Mitchell and Tur (1975) used similar criteria in classifying *Salvinia molesta* Mitch. into 3 distinct growth forms viz. a primary-invading-form, an open-water-colonising-form and a mat-form. *E. crassipes* plants of the marginal growth form (marginal plants), referred to as dwarf water hyacinths by Mitsch (1977), are small plants with short inflated (bulbous) petioles ca 10 to 20 cm in length (Plate 1.1). These are usually found in loosely crowded field populations or in open water at the margins of densely crowded

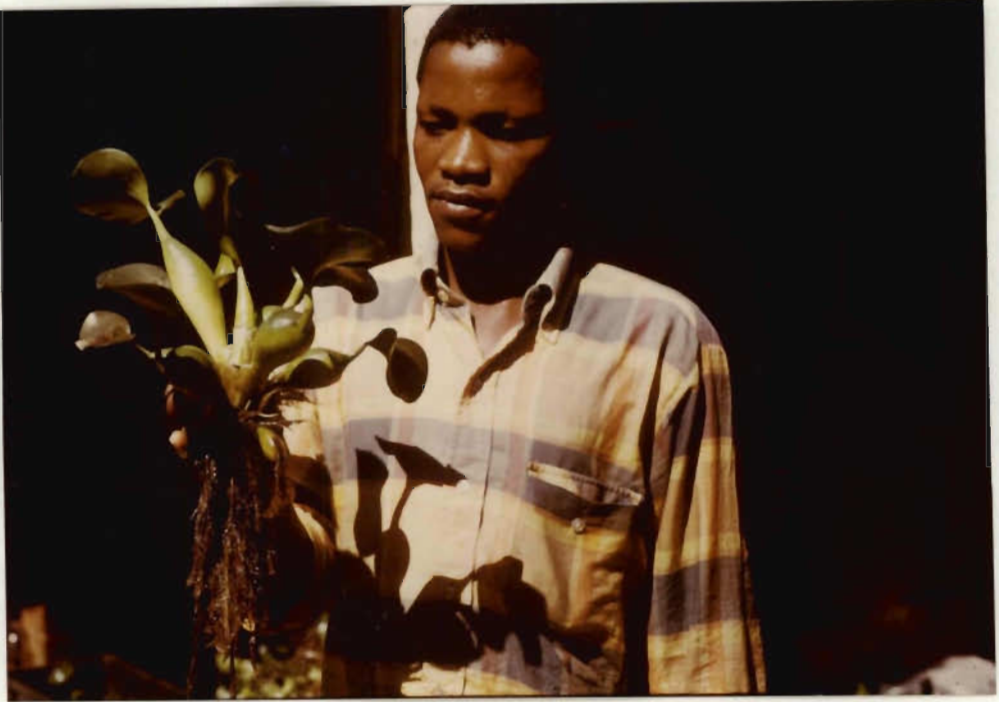


Plate 1.1 *E. crassipes* plant of the marginal growth form.



Plate 1.2 *E. crassipes* plant of the central growth form.

field populations (Ra0, 1920; Bruhl and Gupta, 1927; La Garde, 1930; Weber, 1950). *E. crassipes* plants of the central growth form (central plants), referred to as large water hyacinths by Mitsch (1977), are large plants with long, non-inflated (linear) petioles ca 60 to 100 cm in length (Plate 1.2). These are generally found in densely crowded field populations (McClellan, 1922; Lansdell, 1925; La Garde, 1930; Weber, 1950). The potential differences in growth rate between *E. crassipes* plants of the marginal and central growth forms, found in populations of different density, will need to be recognised in developing and applying a predictive model.

Several researchers (Furman and Gilcreas, 1965; Sheffield, 1966; Clock, 1968; Sheffield and Furman, 1969; Miner et al., 1971; Ornes and Sutton, 1975; Cornwell et al., 1977) have also investigated a variety of nutrient removal schemes using water hyacinths to remove nutrients from secondary treated waste-water effluents. Clock (1968), for example, reported that high removals of N and P could be obtained when secondary treated waste-water effluent was in contact with a dense mass of growing water hyacinths at a detention time of 5 days. Nitrate-nitrogen was reduced by 75% and orthophosphate by 61% from a mixture of extended aeration effluent and raw waste-water. Miner et al. (1971) in a study of treating swine manure found that water hyacinths removed 10,4 kg of ammonia-nitrogen, 7,72 kg of phosphate and 11,4 kg of total Kjeldahl nitrogen per acre (0,405 ha) with a 102 day detention time in ponds 460 mm deep. Most of these investigations, however, were designed to establish the depth of ponds and the detention time required by water hyacinths to remove nutrients effectively from secondary treated waste-water effluents. Cornwell et al. (1977), for example, showed that the nutrient removal capacity of water hyacinth was directly related to the pond surface area. They concluded that in designing a nutrient removal system using water hyacinths, the depth and the detention time in the pond must be set so as to provide a given amount of surface area per unit flow through the pond. In order to remove 80% of the nitrogen, 2,1 ha of water hyacinths were needed per $3800 \text{ m}^3 \text{ d}^{-1}$.

The corresponding phosphorus removal was about 44%. These investigations, however, do not relate the quantities of nutrients absorbed by plants to the size of the standing population, i.e. its total biomass, or to its growth rate. The latter would be influenced by the climatic conditions and by the growth rate limiting nutrient concentrations in the water. This, in turn, would influence the rate of uptake of nutrients by the plant population. Consequently, the information is of little value in developing a model from which the size of the standing population, that is required to remove nutrient inputs, could be derived and the rate at which the population would increase in size, under varying climatic conditions and nutrient supply, from which amounts and frequencies of harvest could be calculated.

1.4 OBJECTIVES OF STUDY

In view of the lack of suitable data present in the literature from which a model could be developed and tested, this study was, therefore, designed with the following objectives in mind:

- (i) To develop a model from kinetic coefficients generated for *E. crassipes* under N and P growth rate limitation in culture.
- (ii) To test the model generated in culture for predicting growth of *E. crassipes* in the field.
- (iii) To refine the model under field conditions.
- (iv) To illustrate the application of the refined model for predicting yields, growth rates and amounts and frequencies of harvest for *E. crassipes*, to control both nutrient inputs in culturally eutrophied water systems and excessive growth of this plant.

CHAPTER 2

METHODS

2.1 DEVELOPMENT OF MODEL IN CULTURE

2.1.1 Selection of plants

To reduce within treatment variation in growth and nutritional experiments, the plant material for all experiments was standardized. Various criteria: height, mass, numbers of pseudolaminae per plant and duration of growth in preliminary culture have been used by some researchers for selecting uniform *E. crassipes* plants for culture (Table 2.1).

For this study, *E. crassipes* plants of the central growth form were considered unsuitable, as being large, they require large containers for growth and tend to revert to the marginal growth form in culture. Plants of the marginal growth form, on the other hand, are considerably smaller, require smaller containers for growth and can be relatively easily collected as vegetatively propagated offsets from the field.

Marginal plants possessing 2 pseudolaminae and no offsets were selected as inocula. They appeared to be the youngest and smallest plants capable of growing in culture, and had a fresh mass ranging from ca 4 to 10 g (dry mass ca 0,20 to 0,60 g).

2.1.2 Collection of plants

Plants required for this study were those in which the nutrient concentrations were present in sufficient quantities not to be growth rate limiting, so that the subsequent growth of plants in N or P deficient cultures would ensure that only the specific deficient nutrient in culture would become growth rate limiting. Suitable facilities, however, were not available for

growing a large population of *E. crassipes* in culture supplemented with an excess supply of nutrients from which plants with high concentrations of nutrients in their tissues could be collected as required for culture.

Nitrogen and P are the nutrients most frequently limiting the growth rate of *E. crassipes* in the field (Wahlquist, 1972), whereas in secondary treated waste-water effluents and fertilized waters, water hyacinths do not exhibit nutrient deficiency symptoms, have high growth rates and high N and P concentrations in their tissues (Boyd, 1976; Wooten and Dodd, 1976). This suggests that nutrients, particularly N and P, are present in sufficient quantities not to be growth rate limiting in plants growing in such waters. Plants for culture were, therefore, collected from a population growing in secondary treated waste-water effluent. As the nutrient composition of *E. crassipes* may vary considerably with site (Boyd and Vickers, 1971), plants for culture were collected from one field site only, namely Maturation Pond 3 at the Northern sewage treatment works, Durban. To account for temporal variations in the nutrient concentrations in the water at this site, each batch of plants for each experiment was collected at the same time. Consequently, nutrient concentrations in the plant tissues within each batch were considered fairly uniform, although differences may have occurred between batches of plants collected on different occasions for different experiments.

No standardized procedures have been reported in the literature for the collection of *E. crassipes* for culture. Generally only sites of collection are reported. The following standardized procedure was, therefore, adopted for collecting plants:

Vegetatively propagated offsets (daughter plants) of the marginal growth form, possessing 2 pseudolaminae, were collected from a population growing in loosely crowded situations in the maturation pond. Stolons connecting the offsets to their respective parents were severed as close as possible to the offsets. Plants were picked free of debris,

washed thoroughly in site water to remove all extraneous particles, and placed into 50 x 50 x 25 cm polyethylene troughs (ca 30 plants per trough) containing site water. Plants in each trough were covered with thin, polyethylene sheeting to prevent dehydration and immediately transported in shade to the greenhouse in Pietermaritzburg. On arrival, the polyethylene sheeting covering the plants was removed and the plants were left in the troughs for 2 days, to allow them to acclimatise to the new environmental conditions in the greenhouse, before being introduced into culture.

2.1.3 Measurement of growth

In culture, growth of *E. crassipes* has been measured as the increase in dry or fresh masses of plants and numbers of offsets produced (Table 2.2). In general, researchers have used 3 to 4 replicates per treatment, with growth periods ranging from 3 to 4 weeks in culture. Significant differences between treatment means were established using either a Duncan's multiple range test or an analysis of variance.

In this study, it was necessary to measure growth of *E. crassipes* regularly, at short intervals, ca every 2 to 4 days. Consequently, a large number of replicates were required at each treatment to give an acceptable standard error in measurement at each measuring interval. A standard error in measurement of 10% of the mean was adopted (Snedecor and Cochran, 1967). Estimates of the sample sizes, or numbers of plants per treatment, required to give a standard error in measurement of mass of 10% of the mean were based on measurements of the fresh and dry masses of 20 marginal plants possessing 2 pseudolaminae. The following formula was used to estimate the sample sizes required to give a standard error in measurement of mass of 10% of the mean:

$$n = \frac{(SD)^2}{(0,1\bar{x})^2}$$

where: SD = standard deviation in fresh or dry mass,
 $0,1\bar{x}$ = 10% of the mean fresh or dry mass,
n = number of plants or sample size.

It was estimated that 8 and 14 plants respectively would be required at each treatment to give a standard error in measurement of the dry and fresh masses of plants of 10% of the mean (Table 2.3).

Growth of *E. crassipes*, in this study, was measured as a rate (specific growth rate) i.e. as the increase in a mass of plants, per unit mass of plant material, per unit time under specific atmospheric and nutritional conditions in culture. Two parameters were considered for determinations of mass viz: dry mass and fresh mass of plants.

Dry mass, as a parameter for measuring growth of *E. crassipes*, was considered unsuitable, as it has the disadvantage that plants need to be destroyed at each measuring interval. Consequently, a large initial population of plants needs to be grown at each treatment in culture, from which sufficient numbers of plants can be removed at each measuring interval, to give the required standard error in measurement. For example, over a hypothetical growth period of 90 days in culture using 6 treatments and 8 plants per treatment, to give a standard error in measurement of dry mass of 10% of the mean, and with dry mass measurements taken at 2 day intervals, an inoculum of 2160 plants would be required. Inadequate greenhouse facilities were available for growing such a large initial population of *E. crassipes* plants in culture.

Fresh mass, as a parameter for measuring growth of *E. crassipes*, on the other hand, has the advantage that a smaller population of plants needs to be grown at each treatment in culture as plants are not destroyed at each measuring interval, merely being temporarily removed from culture at required intervals, for determinations of their fresh mass. Consequently, regardless of the length of the

period of growth of plants in culture and the number of measuring intervals and where 6 treatments and 14 plants per treatment, to give a standard error in measurement of fresh mass of 10% of the mean, are used, an inoculum of only 84 plants would be required.

For practical reasons, therefore, change in fresh mass was chosen as the most suitable parameter for measuring growth of *E. crassipes* in culture. No evidence was found in the literature that frequent removal of free floating aquatic plants from culture, for fresh mass determinations, has an adverse effect on their rate of growth. It was assumed, however, that the treatments plants received, each time they were removed from culture for determinations of their fresh mass, were sufficiently gentle to prevent any adverse effects.

As the yield coefficient is generally expressed on a dry mass basis, dry masses were determined for all plants harvested at each treatment, after the completion of each experiment. The mass of dead plant material produced and subsequently lost into the culture medium, during growth, was recorded and added to the fresh mass produced by plants.

2.1.4 Culture vessels

E. crassipes has been grown in a wide variety of vessels differing considerably in capacity and composition (Table 2.4). No conclusions, however, can be drawn from the literature as to the most suitable vessels for growing this plant.

For this study, vessels suitable for growing *E. crassipes* were selected on the following criteria **viz:** their composition as sources of macro- and micro-nutrient contamination, particularly with regard to N and P, in water cultures; their availability; durability and cost. The advantages and disadvantages associated with vessels of different composition have been reviewed in detail by Hewitt (1966).

Polyethylene vessels were selected for growing *E. crassipes* as they possess the greatest number of advantages when compared with vessels of other composition (Hewitt, 1966). The most important, is their high degree of inertness as sources of both macro- and micro-nutrient contamination in water cultures. Wide, shallow vessels composed of white, translucent, polyethylene, apparently devoid of any mineral "fillers", were selected. They had a capacity of 5 litres and a depth of 20 cm. Haas (1932; 1937), Hoagland and Broyer (1936), Hoagland and Arnon (1938) recommended wide, shallow vessels with a depth of 20 to 25 cm for culturing smaller plants as they give better natural aeration if forced aeration is not employed.

Translucent or transparent vessels are normally coated to exclude light from culture solutions and prevent them from becoming severely contaminated by algae. Algae may interfere with nutritional studies by absorbing nutrients during growth and, in some cases, may severely interfere with the growth of higher plants (Hewitt, 1966), possibly through the production of antibiotic substances (Jorgensen, 1962) or toxic amino acids and carboxylic acids (Steinberg, 1947a; 1947b; Woltz and Jackson, 1961; Woltz, 1963).

Various methods have been used for coating transparent or translucent vessels to exclude light from culture solutions. These include: 2 or more layers of thick, brown paper (Arnon, 1938); black and white or aluminium paints (Stout and Arnon, 1939; Vanselow and Data, 1949); 2 or more layers of material, the inner black to exclude light and the outer white to reflect heat (Zinzadze, 1932; Löhnis, 1937); aluminium foil (Minshall and Scarth, 1952) and even boxes have been recommended for sheltering water cultures (Lehr, 1940).

Most of the methods listed for coating vessels had the disadvantage in that they could cause inconvenience or contamination with the rigorous acid treatments used for cleaning vessels. In this study, vessels were inserted into black, polyethylene bags to exclude light from the culture



Plate 2.1 Culture vessels used for growing *E. crassipes*. Vessels were inserted into black polyethylene bags to exclude light from the culture solutions.

solutions (Plate 2.1). Polyethylene bags had the advantage in that they could easily be removed during cleaning of vessels and replaced.

2.1.5 Culture solution

E. crassipes has been grown successfully in both tap and pond water, supplemented with nutrients, as well as in various culture solutions, differing considerably in composition and ionic concentration (Table 2.5). No conclusions, however, can be drawn from the literature as to the most suitable culture solution for growing this plant. *E. crassipes* grows well in all culture solutions, except in full strength standard Long Ashton solution (Chadwick and Obeid, 1966).

Ideally, the culture solution required for this study was one in which the concentrations of one ion, either nitrate or phosphate, could be varied independently, while the concentrations and relative proportions of all other ions in the culture solution remained unchanged and at levels not limiting to the growth rate of *E. crassipes*. Cation or anion concentrations in any culture solution, however, cannot be varied independently without influencing the concentrations of the other ions. A change in the concentration of any given ion must be accompanied either by a corresponding change for an ion of the opposite sign or a complementary change for other ions of the same sign or both (Hewitt, 1966).

In view of this, a modified culture solution based on that of Hamner, Lyon and Hamner (1942) was devised (Table 2.6), in which the concentrations of either of the anions, nitrate or phosphate, could be varied independently, with the minimum influence on the concentrations of the cations and other anions. Reduced cation concentrations, resulting from the lowering in the concentration of an anion in the culture solution, could, however, be restored by supplementing the culture solution with the appropriate additional cations, as these were added predominantly as salts. The total salinity of

the culture solution was 0,031%, which is well below the salinity of 1,66% reported by Haller et al. (1974) as significantly reducing the growth of *E. crassipes* in culture.

Phosphorus was supplied to the culture solution as inorganic phosphate ($\text{PO}_4\text{-P}$), as Jeschke and Simonis (1965) reported that the main source of P for growth of aquatic plants is in the form of inorganic phosphates, and at a concentration of $20 \times 10^3 \text{ ug PO}_4 \text{ l}^{-1}$ ($6,53 \times 10^3 \text{ ug P l}^{-1}$), as Haller and Sutton (1973) reported that maximum growth of *E. crassipes* occurs at this phosphate concentration in culture.

Although the growth of *E. crassipes* increases with an increase in the level of N supplied in culture (Chadwick and Obeid, 1966), the N concentration at which *E. crassipes* shows a maximum growth rate in culture is not reported in the literature. Gosset and Norris (1971) suggested that N as nitrate was limiting to the growth rate of *E. crassipes* at concentrations below $42 \times 10^3 \text{ ug NO}_3 \text{ l}^{-1}$ in culture. In this study, N as nitrate was supplied to the culture solution at a concentration of $40 \times 10^3 \text{ ug NO}_3 \text{ l}^{-1}$, i.e. $9,03 \times 10^3 \text{ ug N l}^{-1}$, (see Appendix I, Pilot study IA). Nitrogen was supplied solely as nitrate-nitrogen and not as both nitrate-nitrogen and ammonium-nitrogen, as it has been shown in the algae, for example, that in the presence of ammonium the rate of uptake of nitrate-nitrogen is depressed at all light intensities (Bates, 1976). In addition, fairly low ammonium-nitrogen concentrations ($0,15 \text{ ug NH}_4\text{-N l}^{-1}$ and above) have been reported to inhibit nitrate-nitrogen assimilation in the algae (Eppley et al., 1969; Packard and Blasco, 1973). In higher aquatic plants, the addition of ammonium to cultures has been shown to inhibit the nitrate promoted formation of nitrate reductase in *Lemna minor* L. with a resultant loss in extractable nitrate reductase activity (Orebamjo and Stewart, 1975a; 1975b).

The K, Ca and Mg concentrations at which *E. crassipes* shows a maximum growth rate in culture are also not reported in the literature. These ions were supplied to the culture solution

at concentrations of $40 \times 10^3 \text{ ug l}^{-1}$ and in equal relative proportions (see Appendix I, Pilot study IB). The concentrations at which these ions were supplied to the culture solution, however, were in the range of those supplied in various other culture solutions in which *E. crassipes* has been successfully grown by various researchers (Table 2.7).

Iron and trace elements (Cu, Mn, Zn, B, Mo) were supplied to the culture solution at concentrations similar to those in the culture solution used by Gerloff and Kromholz (1966) for growing higher aquatic plants. Iron was added as a chelate complex with ethylene-diamine-tetra-acetic acid (Fe EDTA) because of its stability and high availability at pH values of 7,0 to 7,8, where many iron compounds fail to be absorbed by plants (Hewitt, 1966).

2.1.6 Air and water temperatures

E. crassipes has been grown over a wide range of air and water temperatures in culture (Table 2.8). Growth of *E. crassipes* increases with an increase in the air and water temperature in culture (Bock, 1969; Freidel et al., 1978), with maximum growth occurring at air and water temperatures of 28 to 30°C (Knipling et al., 1970). Growth of *E. crassipes*, however, is high over the temperature range 22 to 35°C with no growth occurring at temperatures above 40°C (Knipling et al., 1970).

In this study, an air conditioned greenhouse only was available for growing *E. crassipes*, in which limited control could be exercised over the air temperatures. To provide conditions conducive for maximum growth rate of *E. crassipes*, experiments were conducted during the summer months (September to March) when the air temperatures were high. As far as possible, maximum daily air temperatures in the greenhouse were maintained at, or in close proximity to, 30°C so that these did not exceed the optimum of 28 to 30°C required for maximum growth of plants (Knipling et al., 1970). As diurnal temperature changes in a greenhouse have a more beneficial effect on the growth of

plants than constant day temperatures (Went, 1944; Hewitt, 1966), the air temperatures in the greenhouse were allowed to fluctuate diurnally. These diurnal temperature fluctuations in the greenhouse, however, did not exceed the range 6 to 11°C recommended by Robbins (1946) for growing plants. Air temperatures were recorded daily in the greenhouse on a thermohydrograph.

In determining the maximum specific growth rate (U_{max}), the half saturation coefficient (K_s) and the yield coefficient (Y_c) for *E. crassipes* growing under specific nutrient growth rate limitation, at different air and water temperatures in culture, the influence of temperature on these kinetic coefficients required consideration. The U_{max} 's of various species of marine and fresh water algae have been shown to follow the van't Hoff rule, i.e. they approximately double for each 10°C rise in the temperature and can be described by an Arrhenius equation (Goldman, 1972; Goldman and Carpenter, 1974). Consequently, if it is postulated that the U_{max} of *E. crassipes* also follows the van't Hoff rule, it should be possible to predict the U_{max} of *E. crassipes*, determined at a specific temperature in culture, for other temperatures. Suitable facilities, however, were not available to determine whether the same relationship existed between the U_{max} of *E. crassipes* and the temperature, as in the algae. For the purposes of this study, it was, therefore, assumed, and later confirmed in the field, that the U_{max} of *E. crassipes* followed the van't Hoff rule. The K_s for nutrient uptake is also sensitive to changes in temperature (Shelef et al., 1970), while minor variations in the Y_c have been found for a high and low temperature strain of *Chlorella* grown in N growth rate limited continuous cultures (Shelef et al., 1970). However, as yet, no attempts have been made to model the effects of temperature on the K_s and Y_c , although in *Aerobacter aerogenes* and *Escherichia coli*, Topiwala and Sinclair (1971) and Sawada et al. (1978) demonstrated that the K_s changes with temperature and that an Arrhenius plot of the change is linear. For the purposes of this study, the K_s and Y_c were assumed to remain constant with changes in tempera=

ture. This, however, may place some constraints on the predictive ability of the model.

Water temperatures may influence the rates of uptake of nutrients by plants, although the magnitude differs according to the nutrient or species investigated (Hewitt, 1966). This, in turn, may influence the growth rate of plants, specifically where the nutrients are growth rate limiting. The influence of water temperature on the rate of nutrient uptake by *E. crassipes* is not reported in the literature. Experiments conducted with other higher plants, however, have shown that there is often an increase in the rate of uptake of N and P and other nutrients with an increase in the water temperature, although there are exceptions (Hewitt, 1966). Van den Honert and Hooymans (1955), for example, reported that between 20°C and 40°C, nitrate absorption by maize seedling roots increases with water temperature by a constant Q_{10} value of 1.7. McEvoy (1960) observed that the rate of P absorption by tobacco plants is increased 3 fold as the water temperature is raised from 10°C to 35°C.

Within the constraints of the air temperatures, no control could be exercised over the water temperatures in the cultures. Water temperatures, however, were recorded daily, with the aid of a maximum and minimum thermometer in one culture vessel chosen at random in each experiment. In general, water temperatures were very similar to and closely followed the air temperatures in the greenhouse and, like the air temperatures, did not exceed the optimum of 28 to 30°C required for maximum growth of plants (Knipling et al., 1970). It was considered, from the evidence presented above, that the higher water temperatures in culture would enhance the rate of uptake of N and P by *E. crassipes*, where these nutrients were growth rate limiting.

2.1.7 Radiant flux density and photoperiod

E. crassipes has been grown both in daylight and under

artificial illumination at various daily photoperiods in culture (Table 2.9). *E. crassipes* grows more rapidly under an 8 hour than a 16 hour daily photoperiod in culture (Bock, 1969). However, the influence of the radiant flux density (light intensity) on the growth rate of this plant is not reported in the literature.

Blackman and Wilson (1951a) showed that the net assimilation rate or unit leaf rate (Hunt, 1978) in all 10 species of "sun" and "shade" plants investigated was linearly related to the logarithm of the light intensity up to maximum daylight except during late autumn when the relative growth rates were low. The relationship between the relative growth rate, the product of the unit leaf rate and the leaf area ratio, and the light intensity was curvilinear or hyperbolic (Blackman and Wilson, 1951b). This suggests that the growth rate of *E. crassipes* should also be hyperbolically related to the light intensity. From functions relating the unit leaf rate and leaf area ratios with the light intensity, Blackman and Wilson (1951b) calculated light intensities relative to daylight which would permit maximum relative growth rates for the species investigated. Species differed widely in the light level at which maximum relative growth rate was attained. However, in most of the species investigated, light intensities permitting maximum relative growth rate were below that of daylight. *E. crassipes* has a high growth rate in comparison with other species of higher plants (Westlake, 1963) and grows well under artificial illumination in culture (Bock, 1969; Freidel et al., 1978). This suggests that light intensities in a subtropical latitude, particularly during summer, should approach those saturating to the growth rate of this plant.

In this study, a greenhouse only was available for growing *E. crassipes* in which no control could be exercised over the light intensities and daily photoperiods. To provide conditions conducive for the maximum growth rate of this plant, experiments were conducted during the summer months (September to March) when the light intensities were high. The light

intensity in a greenhouse, however, may be reduced and its quality affected under glass to varying degrees according to the alignment of the greenhouse and cleanliness of the glaze (Stone, 1913). Atkins and Poole (1929), for example, pointed out that in temperate regions the direct vertical illumination in a greenhouse may be as little as 25% of the daylight. Observations of greenhouse light intensities under tropical conditions have also been reported by Bolle-Jones (1956) and Lockard and Hayward (1963). Bolle-Jones (1956), for example, found that sunlight as measured on an exposure meter was diminished to a small extent in a greenhouse at noon, however, at 08h00 and 15h30 respectively, it was reduced to ca 50% of that outside the greenhouse. Lockard and Hayward (1963) reported that in full sunlight, the light intensity inside their glasshouse was reduced by 37%, while under cloudy conditions it was reduced by 65% by a combined glass roof and Tygan gauze screen. The attenuation in the light intensity in the greenhouse used in this study as measured at midday in full sunlight was ca 37%. Light intensities measured outside the greenhouse ranged from 1 050 to 1 100 μ Einsteins $m^{-2} sec^{-1}$ with a mean value of 1 070 μ Einsteins $m^{-2} sec^{-1}$, whereas those measured inside the greenhouse ranged from 640 to 720 μ Einsteins $m^{-2} sec^{-1}$ with a mean value of 674 μ Einsteins $m^{-2} sec^{-1}$. Consequently, the light intensities in the greenhouse may have been growth rate limiting for *E. crassipes*. This presents a constraint on the model developed. It is possible, however, that a correction factor could be incorporated into the model to account for this. Although *E. crassipes* grows more rapidly under an 8 hour than a 16 hour daily photoperiod (Bock, 1969), it was considered that the kinetic coefficients generated for *E. crassipes* under the 12 to 14 hour daily photoperiods in greenhouse culture should accurately predict specific growth rate of this plant growing under similar daily photoperiods at field sites in a fairly local latitude.

Light intensities and the daily photoperiod may influence the rate of uptake of nutrients by plants (Hewitt, 1966) which in

turn may influence their growth rate specifically where the nutrients are growth rate limiting. The interaction between the light intensity and the daily photoperiod on the rate of uptake of nutrients by *E. crassipes* is not reported in the literature. Experiments conducted with other plant species, however, have shown that high light intensities and long days often increase the rate of uptake of nutrients by plants and accentuate nutrient deficiency effects, although there are exceptions (Hewitt, 1966). Bates (1976), for example, reported that in the algae the rate of uptake of nitrate-nitrogen in the absence of ammonium-nitrogen increases with an increase in the light intensity. In higher aquatic plant species such as *Fontinalis antipyretica* L., the rate of uptake of nitrate-nitrogen has been shown to be regulated by the nitrate reductase activity in the leaves (Schwoerbel and Tillmans, 1974) which in turn is dependent on light (Candela et al., 1957; Hageman and Flesher, 1960). Wallace (1943), on the other hand, reported that nitrogen deficiency effects in plants may be accentuated by higher light intensities.

In view of the evidence presented above, it was considered that the longer daily photoperiods and the higher light intensities in the greenhouse during summer would enhance the rate of uptake of N and P by *E. crassipes*, where these nutrients were growth rate limiting in culture, as well as accentuate nutrient deficiency effects in the plants where initially grown in N or P deficient cultures in the determination of their kinetic coefficients.

2.1.8 Relative humidity

At the commencement of this study, the influence of the relative humidity on the growth rate of *E. crassipes* was not known. Researchers do not usually report relative humidities at which *E. crassipes* was grown in culture. After the initiation of this study, however, Freidel et al. (1978) reported that the growth rate of *E. crassipes* in culture increases with an increase in the relative humidity.

Nothing is known of the effect of the relative humidity on responses to nutritional factors by *E. crassipes* and comparatively little is known of such effects in other higher plants (Hewitt, 1966). Nightingale and Mitchell (1934) found that the relative humidity modified the responses of tomato plants to nitrogen deficiency, which was accentuated at low relative humidities. Ahi and Powers (1938) and Magistad et al. (1943) observed that conditions leading to physiological drought, such as a high salt content of the solution, were more serious in their effects under conditions of low relative humidity.

In this study, no control could be exercised over the relative humidity in the greenhouse in which *E. crassipes* was grown. In any one experiment, however, all treatments were exposed to the same variation in relative humidity. Relative humidities in the greenhouse ranged between 50 and 80%. These were in the range of relative humidities 50 to 90% recommended by Robbins (1946) for culturing higher plants and were recorded daily in the greenhouse on a thermohydrograph. As Freidel et al. (1978) have shown that the growth rate of *E. crassipes* in culture increases with an increase in the relative humidity, it is possible that the relative humidities in the greenhouse may have been growth rate limiting for this plant. This presents a constraint on the model developed. It is possible, however, that a correction factor could be incorporated into the model to account for this.

2.1.9 Determination of kinetic coefficients

The continuous culture method or steady-state approach, as used for determining kinetic coefficients in the algae (Toerien et al., 1971), is impractical for determining kinetic coefficients in higher aquatic plants, such as *E. crassipes*, as it requires maintaining plant populations in culture at a constant size and controlled state of growth; a specific, constant physiological state and under constant environmental conditions. Consequently, the batch culture method was used to determine kinetic coefficients for *E. crassipes* growing under specific nutrient

growth rate limitation in culture. This is a non steady-state approach in which nutrient concentrations and plant mass change continuously with time. At any given time, however, the rate of increase in plant material should be proportional to the growth rate limiting nutrient concentration in the medium (Toerien et al., 1971).

Nitrogen and P are the nutrients generally associated with artificial eutrophication (Mackenthun, 1964; 1965) and limit the growth rate of *E. crassipes* under natural field conditions (Wahlquist, 1972). Consequently, experiments were conducted to determine kinetic coefficients for *E. crassipes* growing under N and also under P growth rate limitation in culture. To determine the variability in the kinetic coefficients measured, experiments were repeated 5 times under N growth rate limitation and 3 times under P growth rate limitation in culture. Similar methods were used in each experiment, although in some experiments cultures were spiked with slightly different levels of N or P (Table 2.10). It was assumed that these differences in treatments would have no significant influence on the values of the kinetic coefficients measured, particularly as the different levels of N and P added to culture were growth rate limiting.

In each experiment, ca 120 *E. crassipes* plants collected from the field were rinsed through 3 changes of deionised-distilled water, shaken to dislodge adhering water, and their fresh masses recorded on an electronic top loading balance. A spring balance, as used by Bock (1969), was considered unsuitable for fresh mass measurements to 0,01 g.

Plants were placed into culture vessels each containing 5 litres of culture solution deficient in either N or P. In the first 2 experiments, conducted under N and P growth rate limitation respectively, 2 plants were used as an inoculum in each culture vessel. Subsequently, to provide more space for growth of plants, only one plant was used as an inoculum in each culture vessel (Table 2.10). Plants were initially grown in N or

P deficient cultures to ensure that only the specific deficient nutrient in culture would become growth rate limiting for plants. In addition, plants were required with low concentrations of the specific growth rate limiting nutrient in their tissues to ensure that their growth rate was determined by the nutrient supply and not by the levels in the plant tissues, as well as to ensure a subsequent high rate of uptake of the specific growth rate limiting nutrient, when supplied to culture. In the algae, for example, it has been shown that the rate of uptake of nitrate and ammonium (Caperon and Meyer, 1972b; Eppley and Renger, 1974) and inorganic phosphate (Droop, 1973; Brown and Harris, 1978) are inversely related to the total cell contents of N and P respectively.

As the half saturation coefficient may be influenced by the pH (Goldman, 1972), culture solutions in each experiment were adjusted to pH 7.0, at which maximum growth of *E. crassipes* occurs in culture (Chadwick and Obeid, 1966). At the various field sites where rates of growth of *E. crassipes* were to be measured to test the model, the pH of the water generally ranged in close proximity to pH 7.0. pH adjustments of the culture solutions were made, at weekly intervals, by titrating with 5% H_2SO_4 and 10% NaOH using a micropipette. Culture solutions were changed weekly to ensure an adequate supply of nutrients, other than N or P, to the plants. Evaporation loss from the cultures was replaced daily with deionised-distilled water. Unlike Chadwick and Obeid (1966), culture solutions were not aerated by a steady stream of air bubbles during growth of plants (see Appendix I, Pilot study II).

Maximum and minimum daily air temperatures and relative humidities in the greenhouse were recorded on a thermohydrograph.

Every 2 to 4 days plants were removed from culture, allowed to drain for 2 minutes above the culture vessels, shaken to dislodge adhering water and their fresh masses recorded on an electronic top loading balance before being returned to their

respective culture vessels. Plants were grown in either N or P deficient cultures until they showed a reduced growth rate, as evident from a deviation from linearity in a plot of their fresh mass versus time. This suggested that plants had depleted their internal reserves of N or P and indicated that N or P were limiting to the growth rate of plants. At this stage, plants were removed from culture. Necrotic or damaged leaves and roots were removed from the plants, so that loss of these did not interfere with subsequent measurements of growth or, through decay, release additional quantities of N and P into the cultures. 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 6 different levels of N or P respectively to obtain 6 treatments (16 to 20 replicates per treatment), in which the N concentrations in the N growth rate limited cultures ranged from 0 to $11,29 \times 10^3 \text{ ug N l}^{-1}$ (0 to $50 \times 10^3 \text{ ug NO}_3 \text{ l}^{-1}$) and the P concentrations in the P growth rate limited cultures ranged from 0 to $2,09 \times 10^3 \text{ ug P l}^{-1}$ (0 to $6,4 \times 10^3 \text{ ug PO}_4 \text{ l}^{-1}$) (Table 2.10). A randomized complete block design was adopted in each experiment (Rayner, 1967).

After the addition of N or P to the cultures, total fresh mass recordings, which included both fresh mass as well as the dead mass of plants arising through loss of plant material during growth, continued every 2 to 4 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 during growth of plants, although they were topped-up daily with deionised-distilled water and adjusted to pH 7,0 weekly. Concentrates of the culture solution, deficient in either N or P, were, however, added to the cultures at 2 weekly intervals to ensure an adequate supply of nutrients, other than the growth rate limiting nutrients (N or P), to the plants. In experiments

conducted under P growth rate limitation, additional N at a concentration of $9,03 \times 10^3 \text{ ug N l}^{-1}$ ($40 \times 10^3 \text{ ug NO}_3 \text{ l}^{-1}$) was also added to the cultures in the intervening weeks to ensure that the N concentrations in these cultures remained at, or above, a concentration of $9,03 \times 10^3 \text{ ug N l}^{-1}$ ($40 \times 10^3 \text{ ug NO}_3 \text{ l}^{-1}$), below which N becomes limiting to the growth rate of *E. crassipes* (see Appendix I, Pilot study IA). The total nutrient additions during the entire growth period of plants in culture, after spiking, did not increase the salinity of the cultures above 0,16%, i.e. 10% of the salinity value of 1,66% reported by Haller *et al.* (1974) as significantly reducing the growth of *E. crassipes* in culture.

When fresh mass recordings were terminated, plants were harvested from each culture vessel, 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.

To relate the yield of *E. crassipes* to the quantities of the growth rate limiting nutrient absorbed by plants during growth, rather than to that supplied in culture, culture solutions in each experiment were analyzed for remaining N or P when fresh mass recordings were terminated. The culture solutions in 3 culture vessels, taken at random from each treatment in each experiment, were topped up to the 5 litre mark with deionised-distilled water, after the plants had been harvested, and analyzed for remaining N or P using methods published in the Environmental Protection Agency (1974) and Standard Methods (1975). Loss of growth rate limiting nutrients (N or P) from the cultures, resulting from shaking of plants at each weighing interval could not be accounted for, but was considered to be small. This, however, may place some constraints on the reliability of the yield coefficient values determined.

In order to obtain an estimate of the minimum concentrations of the growth rate limiting nutrient, subsistence quota (Rhee and Gotham, 1981b), remaining in the plant tissues when fresh mass recordings were terminated, i.e. when no further significant increase in the fresh mass of plants was recorded, N and P concentrations were analyzed in the plants harvested from culture. They were analyzed in 3 batches of dry ground plant tissues, chosen at random from each treatment in each experiment, using methods published in the Association of Official Agricultural Chemists (1975). Toerien *et al.* (1971) and Coetzer *et al.* (1977) pointed out that the reciprocals of the yield coefficient values (dry mass basis), when expressed as a percentage, should estimate the minimum concentrations of the specific growth rate limiting nutrient in the dry plant tissues. Consequently, this provides a basis for evaluating the reliability of the yield coefficient values determined.

In each experiment, specific growth rates were determined 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 the above period, was taken as its specific growth rate at that particular N or P concentration. Specific growth rates were calculated using the following form of the general growth equation (Malek and Fenc1, 1966; Radford, 1967):

$$U = \frac{\ln X_t - \ln X_0}{t}$$

where: X_t = fresh mass at time = t_2 (g),
 X_0 = fresh mass at time = t_1 (g),
 U = specific growth rate (g fresh mass $g^{-1} d^{-1}$),
 t = time period between time t_2 and t_1 (days),
 \ln = \log_e (natural logarithm).

The maximum specific growth rate (U_{max}) and the half saturation coefficient (K_s) were determined for *E. crassipes* in each expe=

riment from the intercepts of a reciprocal plot of the specific growth rates of plants against the growth rate limiting nutrient concentrations (Lineweaver and Burk, 1934). These are derived from the following from of the Monod equation:

$$\frac{1}{U} = \frac{K_s}{U_{max}} \cdot \frac{1}{S} + \frac{1}{U_{max}}$$

where: U = specific growth rate,
 U_{max} = maximum specific growth rate,
 S = growth rate limiting nutrient concentration,
 K_s = half saturation coefficient,
 $\frac{1}{U_{max}}$ = intercept on y axis,
 $\frac{1}{K_s}$ = intercept on x axis,
 $\frac{K_s}{U_{max}}$ = slope.

The best straight line through all points was obtained using a simple linear regression (Rayner, 1967).

The yield coefficient (Y_c) was determined for *E. crassipes* in each experiment from the slope of the line relating the total fresh mass yields of plants to the quantities of N or P absorbed, i.e. the quantities of N or P supplied at spiking minus the quantities remaining in the culture vessels after fresh mass recordings had been terminated. A simple linear regression was used to obtain the best straight line through all points (Rayner, 1967).

All linear regressions were subjected to an analysis of variance (Rayner, 1967).

2.2 COLLECTION OF FIELD DATA TO TEST THE MODEL

2.2.1 Introduction

In order to compare specific growth rates of *E. crassipes* predicted from kinetic coefficients generated under N and P growth rate limitation in culture with those in the field, it was necessary to measure specific growth rates of *E. crassipes* growing at different levels of N and P supply and at different air and water temperatures in the field. Apart from the levels of N and P in the water and the air and water temperatures, other environmental factors, such as the radiant flux density, the daily photoperiod, the relative humidity, the pH and the dissolved oxygen concentrations of the water, may have an influence on the specific growth rate of *E. crassipes*. These factors were also recorded in the field so that their effect on the specific growth rate of *E. crassipes* could be used to refine the predictive model. In addition, crowding of plants in field populations may have an influence on the specific growth rate of *E. crassipes*. Evidence supporting this suggestion is provided by the investigations of Mitsch (1977) who found that dwarf water hyacinths (marginal plants) and large water hyacinths (central plants), occurring in loosely crowded and densely crowded field populations respectively, have different metabolisms (net C uptake and P/R ratios) which suggests that they may have different growth rates. In order to establish whether kinetic coefficients generated for plants of the marginal growth form in culture could also predict specific growth rates of central plants, growing in densely crowded field populations, it was necessary to measure specific growth rates of *E. crassipes* growing in both loosely and densely crowded field populations respectively.

The chemical composition of *E. crassipes*, like its growth rate (Yount and Crossman, 1970; Wooten and Dodd, 1976; Boyd, 1976), is influenced by the level of nutrients in the water (Dymond, 1948; Boyd, 1969; Boyd, 1976).

Attempts, however, to correlate the chemical composition of *E. crassipes* with the water environment have yielded conflicting results (Boyd and Vickers, 1971; Gosset and Norris, 1971). In algal cells, on the other hand, constant trends have been found between the decrease in intracellular levels of nutrients, limiting to the growth rate, and increasing nutrient deficiency. This pattern has been found in growth rate limitation by N (Caperon, 1968; Caperon and Meyer, 1972a); P (Fuhs, 1969); Si (Paasche, 1973); Fe (Davies, 1970) and by vitamin B₁₂ (Droop, 1968). The wide-spread occurrence of this phenomenon has led many researchers to conclude that the internal nutrient concentration, rather than the external nutrient concentration, exerts a controlling influence on the growth rate. An internal nutrient reservoir, which contains the raw material for growth, has been proposed to explain this effect. The size of the proposed internal reservoir determines the rate of growth, by analogy to enzyme-substrate reactions (Caperon, 1968; Williams, 1971; Caperon and Meyer, 1972a). Laboratory investigations have shown that the growth rate of algal populations depends on cellular levels of growth rate limiting nutrients. Mackereth (1953), for example, observed that the growth rate of the fresh water diatom, *Asterionella formosa*, is related to its intracellular P level. Caperon (1968) showed that the growth rate of the marine alga *Isochrysis galbana* could be hyperbolically related to the inferred cellular nitrate pool size, while Senft (1978) found that specific rates of photosynthesis, at saturating irradiances, by laboratory populations of *Chlorella* and *Anabaena* also depend on intracellular P levels. The relationships between phytoplankton growth rate and intracellular growth rate limiting nutrient concentrations have been described by hyperbolic functions (Droop, 1973; Rhee, 1973; Tillman and Kilham, 1976).

Consequently, in order to determine whether specific growth rates of *E. crassipes* in the field could also be predicted from the growth rate limiting nutrient (N or P) concentrations in the plants, apart from those in the water environment, it was necessary to analyze the N and P concentrations in the plants growing in the field.

2.2.2 Selection of plants

The plant material used for all field investigations was standardized. Various criteria, such as the dry mass and area of plants, have been used by some researchers for selecting uniform *E. crassipes* plants for field investigations (Table 2.11).

For this study, marginal plants possessing 2 pseudolaminae were found to be unsuitable as inocula for the field, as being small, they tended to become damaged through entanglement in the mesh of enclosures, particularly during the early stages of their growth in the enclosures. This problem, however, was not encountered where slightly larger plants possessing 3 pseudolaminae were used as inocula.

Consequently, both marginal and central plants possessing 3 pseudolaminae and no offsets were selected as inocula for the field. As new pseudolaminae were produced by *E. crassipes* ca every 2 to 4 days, it was assumed that over a 12 to 14 day growing interval in the field, the growth rates of plants possessing 3 pseudolaminae would not be significantly different from those possessing 2 pseudolaminae.

Marginal plants possessing 3 pseudolaminae selected as inocula had a fresh mass ranging from ca 7,5 to 22,0 g (dry mass ca 0,40 to 1,20 g) whereas central plants possessing 3 pseudolaminae had a fresh mass ranging from ca 47,5 to 110,5 g (dry mass ca 2,5 to 6,0 g).

2.2.3 Collection of plants

No standardized procedures have been reported in the literature for the collection of *E. crassipes* for field investigations. Generally only sites of collection are reported. The following standardized procedure was, therefore, adopted for collecting plants:

Vegetatively propagated offsets (daughter plants) possessing 3 pseudolaminae, of the marginal and central growth form, were collected from loosely and densely crowded populations respectively, at each field site where their rates of growth and chemical composition were to be investigated (see 2.1.2). Plants were immediately transported in shade to the Natal Herbarium, Durban. On arrival, they were tagged using small, 5 x 5 mm, numbered, aluminium strips attached by thin copper wire. Plants were allowed to drain for 2 minutes, shaken to dislodge adhering water, and their fresh masses recorded on an electronic top loading balance. After weighing, plants were replaced into polyethylene troughs containing site water, covered with thin polyethylene sheeting, and transported in shade immediately back to their respective field sites.

2.2.4 Selection of sites

Six sites were selected to investigate the influence of various site conditions, particularly nutrient enrichment of water by N and P, on the growth rate and chemical composition of *E. crassipes*. All sites selected were those subject to nutrient enrichment originating either from point sources, such as sewage treatment plants and industrial effluents, or from diffuse sources. *E. crassipes* populations were present at all sites. To minimise the effect of climatic variations between sites on the growth rate and chemical composition of *E. crassipes*, all sites, apart from one, were selected in the same climatic region, i.e. the warm to hot and humid, subtropical climate of the Durban area of Natal (Schulze, 1965). These sites were in close proximity to the meteorological station at Louis Botha Airport, Durban, from where climatic data was obtained. The following sites in the Durban area were selected (Figure 2.1):

- (i) A lake in the Botanic Gardens.
- (ii) A maturation pond at the Northern sewage treatment works, enriched by secondary waste-water effluent.

- (iii) A discharge canal carrying secondary treated waste-water from the Northern sewage treatment works.
- (iv) The Isipingo Canal, enriched by industrial effluent.
- (v) A shallow lake on the lower flood plain of the Isipingo River, enriched by secondary treated waste-water.

One site, namely the Hartbeespoort Dam, occurring near Pretoria in the Transvaal (Figure 2.1) was selected for comparing the growth rate and chemical composition of *E. crassipes*, growing in a less humid, subtropical climate, with those in the Durban area of Natal. Scott et al. (1981) describe the Hartbeespoort Dam as a hypertrophic, warm, monomictic impoundment.

2.2.5 Enclosures

As with Bock (1969), the growth rate and chemical composition of *E. crassipes* was investigated by tagging plants and introducing them, for selected growing intervals, into field populations. *E. crassipes*, however, is a free-floating aquatic plant and relatively easily dispersed by water movement, wind or wave action. Consequently, plants, including their offsets, may be lost or difficult to relocate in field populations unless they are contained within an enclosure. Various types of enclosures, differing considerably in size, construction and composition, have been used by various researchers for containing *E. crassipes* plants in the field (Table 2.12).

Floating enclosures, as described by Boyd and Scarsbrook (1975) and Boyd (1976) (Table 2.12), were unsuitable for this study, as plants within, particularly those of the marginal growth form, were relatively easily dispersed from these by mild wind or wave action and were also susceptible to destruction by water fowl.

For this study, the most reliable enclosures for containing *E. crassipes* plants in the field were those constructed of plastic coated wire mesh, ca 1,5 m high, held in place by metal fencing posts driven into the sediment.



Plate 2.2 Enclosures used for containing *E. crassipes* plants of the marginal growth form.



Plate 2.3 A population of *E. crassipes* plants of the central growth form enclosed by wire mesh.

As central plants are considerably larger than marginal plants and need to be confined in fairly large, densely crowded populations to maintain them in the central growth form, 2 different types of enclosures were used for containing marginal and central plants respectively. Both types of enclosures were of similar construction, but of different size.

Cylindrical enclosures, with a diameter of ca 1,0 m and a height of 1,5 m, were used for containing plants of the marginal growth form in the field (Plate 2.2). The water area, ca 0,8 m², contained within each of these enclosures, was large enough to accommodate an increase, over a 12 to 14 day growing interval, in the size of the marginal plant population introduced into each enclosure, without allowing the plants to become unduly crowded.

Densely crowded populations of the central growth form, areas of ca 6 m² extending from the shore into open water, were enclosed by wire mesh, ca 1,5 m high, at each site (Plate 2.3). The enclosure of the central plant populations ensured that the plants within were kept in densely crowded situations and maintained in the central growth form.

To minimise the disturbing effects of wind and wave action, both types of enclosures were located at each field site, on the leeward side of each water body in water ca 0,5 to 1,0 m deep.

2.2.6 Measurement of growth

Growth of *E. crassipes* in the field has generally been measured as the increase in fresh mass and numbers of plants produced, usually over 2 week growing intervals (Table 2.13). Dry masses of plants have mostly been estimated from subsamples of the fresh mass.

In this study, growth of *E. crassipes* was measured as a rate (specific growth rate) so that comparisons could be made be=

tween specific growth rates predicted for *E. crassipes*, from kinetic coefficients generated in culture, with those measured for plants in the field. As the growth rate of *E. crassipes* was determined by introducing tagged plants for selected growing intervals into field populations, change in fresh mass was chosen as the most practical parameter for measuring growth. A 12 to 14 day growing interval was selected over which to measure the growth rate of *E. crassipes* at field sites. Bock (1966) found that this growing interval was adequate for differences in the growth rate of *E. crassipes* to become apparent with the minimum influence of season. In order to obtain an acceptable standard error in measurement (less than 10% of the mean) and to account for possible losses of plants from enclosures or their damage during growth in enclosures, a large number of replicates, 40 marginal and 30 central plants, were used.

E. crassipes plants of the marginal and central growth form, collected from the field, were introduced into their respective enclosures at each field site. Marginal plants were introduced into 4 enclosures located at each field site. Ten marginal plants were introduced into each enclosure. Central plants were introduced into 2 densely crowded populations of the central growth form enclosed at each field site. Fifteen central plants were inserted at random into each population.

After a 12 to 14 day growing interval, marginal and central plants, including their offsets, were harvested from the enclosures at each field site. Considerable care was taken not to separate the offsets from their respective parents. Marginal and central plants were placed separately into 50 x 50 x 25 cm polyethylene troughs containing site water, covered with thin polyethylene sheeting to prevent dehydration, and immediately transported in shade to the Natal Herbarium, Durban. On arrival, plants were picked free of debris and washed thoroughly in tap water to remove all extraneous particles. They were allowed to drain for 2 minutes, shaken to dislodge adhering water, and their fresh masses (parents and offsets) recorded on an electronic top loading balance.

Specific growth rates were calculated for marginal and central plants, over each 12 to 14 day growing interval, using the general growth equation (Malek and Fenc1, 1966; Radford, 1967) as defined in section 2.1.9.

2.2.7 Chemical analyses

2.2.7.1 Plants

E. crassipes plants harvested, after each 12 to 14 day growing interval, at each field site were chemically analyzed. Chemical analyses were performed on samples comprising young and old plants (parents and offsets) as the elemental concentrations in *E. crassipes* may decline with the age of plants (Boyd, 1969). Plants of the marginal and central growth form were analyzed separately and analyses were performed on whole plants (see Appendix I, Pilot study III).

After fresh mass determinations had been completed, 10 *E. crassipes* plants, together with their respective offsets, were sampled at random from each batch of marginal and central plants harvested at each field site. Marginal and central plants were rinsed separately through 3 changes of deionised-distilled water and dried in a forced draft oven at 60°C. The dry tissues were separated into 3 batches and ground in a mill. The ground tissues were redried at 60°C in a forced draft oven to a constant weight and stored in sealed glass bottles.

Nitrogen and P were determined in each batch of dry, ground plant tissues using methods published in the Association of Official Agricultural Chemists (1975). Nitrogen was determined by the micro Kjeldahl method and P was determined colorimetrically using ammonium molybdate, after digestion of the samples with nitric and perchloric acids (Johnson and Ulrich, 1959).

2.2.7.2 Water

Water samples, for the chemical analysis of N and P, were collected from the immediate vicinity of the marginal and central plant populations enclosed at each field site, at the commencement and termination of each 12 to 14 day growing interval of plants.

To account for possible diurnal fluctuations in the nutrient levels in the water at each field site, water samples were collected at approximately the same time of the day at each respective field site.

Water samples were collected ca 20 cm below the water surface, to avoid surface contamination, in 500 ml plastic bottles (Golterman, 1969) previously cleaned with conc. HCl and rinsed thoroughly in deionised-distilled water. As a precaution against their contamination, bottles were sealed at each field site by placing a strip of Parafilm (American Can Company, Greenwich, Connecticut) over the aperture of the bottles before replacing the screw tops. Water samples were immediately transported in the dark in an insulated container to the laboratory. On arrival, water samples were filtered through a 2,5 μm pore, Whatmann No 5 filter paper, prewashed with deionised-distilled water, to remove the particulate fraction. Owing to the very high particulate fraction present in the water samples, it was not practically feasible to filter these through the recommended 0,5 μm pore membrane filter (Olsen, 1967, Golterman, 1969). Consequently, this may place a constraint on the accuracy with which the soluble P fractions were measured in the water samples (Rigler, 1964). To minimise chemical changes in the water samples, prior to analysis, the filtered water samples were preserved by the addition of 2,5 ml of CHCl_3 and frozen to -4°C (Golterman, 1969).

The following N and P fractions were analyzed in each water sample using methods published in the Environmental Protection Agency (1974) and Standard Methods (1975): nitrate-nitrogen by

colorimetry after reduction to nitrite; Kjeldahl nitrogen as ammonium ($\text{NH}_4\text{-N}$) after digestion of the samples by conc. H_2SO_4 in the presence of a mercury catalyst; soluble reactive phosphorus (SRP) (Twinn and Breen, 1980) by colorimetry using the molybdenum blue method and total phosphorus as soluble reactive phosphorus after digestion with H_2SO_4 and persulphate. Total nitrogen (total N) was calculated as the sum of Kjeldahl nitrogen and nitrate plus nitrite ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N} + \text{NO}_2\text{-N}$).

2.2.8 Physical analyses

2.2.8.1 Environment

The radiant flux density (diffuse component of the radiant flux) was recorded hourly each day during growth of *E. crassipes* in the field. The diffuse component of the radiant flux was chosen as a measure of the light as this includes a greater proportion of the photosynthetically active radiation. About one-third of the direct solar radiation, often referred to as the global component, is photosynthetically active as compared to over two-thirds for the diffuse component (Ross, 1975; Fitter and Hay, 1981). Theoretical calculations have shown that even under cloudless skies, the diffuse radiation (D) may account for between one-third and three-quarters of the total irradiance (T), and in a series of measurements in Cambridge the ratio D/T was always greater than 0,5 (Szeicz, 1974).

Other factors recorded during growth of *E. crassipes* in the field were the daily photoperiod, taken as the total hours of diffuse radiation per day, the maximum, minimum and mean daily air temperatures and relative humidities.

For plants growing at field sites in the Durban area, measurements were obtained from the meteorological station at Louis Botha airport, Durban. For plants growing at the Hartbeespoort Dam site, they were obtained from the meteorological station in Pretoria.

2.2.8.2 Water

The pH and the dissolved oxygen concentrations of the water and the water temperatures were recorded daily between 11h00 and 14h00 at the various field sites. They were recorded from the immediate vicinity of the marginal and central plant populations enclosed at each field site. pH measurements were made with a portable pH meter. Water temperatures and dissolved oxygen concentrations were determined with a portable oxygen meter fitted with a thermistor probe.

CHAPTER 3

EXPERIMENTAL DETERMINATION OF KINETIC COEFFICIENTS

3.1 INTRODUCTION

Eight experiments were conducted to determine kinetic coefficients for *E. crassipes* growing under N (Experiments 1 to 5) and P (Experiments 6 to 8) growth rate limitation in culture. Maximum specific growth rates (U_{max}) and half saturation coefficients (K_s) were determined for *E. crassipes* in each experiment. Yield coefficients (Y_c), however, were only determined for *E. crassipes* in each of the first 3 experiments conducted under N and P growth rate limitation respectively.

3.2 RESULTS

3.2.1 Nitrogen growth rate limitation

3.2.1.1 Growth in N deficient culture

In each of the 5 experiments conducted (Figs. 3.1 to 3.5), plants with 2 pseudolaminae introduced into N deficient cultures showed an initial lag phase in growth lasting ca 2 to 4 days. Growth of plants then proceeded more or less linearly until they showed a reduced growth rate, evident from a deviation from linearity in a plot of their fresh mass against time (Figures 3.1 to 3.5). At this stage, the growth rate of plants was assumed to be N limited. In each experiment, a different growth period in N deficient cultures was required to obtain plants in a N growth rate limited state (Figures 3.1 to 3.5). These different growth periods in N deficient cultures, however, showed no correlation with the average daily air and water temperatures and relative humidities recorded in the greenhouse (Table 3.1). Also noteworthy, is that at that stage when the growth rate of plants became N limited, i.e. at spik=

ing, there were no significant differences ($P = 0,05$) between the mean fresh masses (excluding dead mass) of groups of plants that were to comprise each treatment in each experiment (Table 3.2).

3.2.1.2 Growth after the addition of N

In each experiment, the addition of N effected an increase in growth rate with a short (3 to 4 day) period of maximum growth rate which was proportional to the level of N supplied. The periods of mean maximum growth rate of each group of plants for each treatment were evident from the maximum slopes of the curves relating growth (fresh mass) and time (Figures 3.1 to 3.5, B, C, D, E, F). Thereafter, the growth rates of plants decreased progressively in each treatment until there was no measurable increase in the total fresh mass (fresh mass, including dead mass produced during growth) of plants. This required ca 75 to 95 days after the addition of N, in those treatments (Figures 3.1 to 3.3, F) where N was supplied at the highest levels to the cultures. In Experiments 4 and 5 (Figures 3.4 and 3.5) measurements of growth were terminated ca 21 days after spiking. In those treatments where N was supplied at the lowest levels to the cultures (Figures 3.1 to 3.5, B), except Experiment 3 (Figure 3.3, B), plants generally attained a maximum growth rate 2 to 3 days after the addition of N. In all other treatments (Figures 3.1 to 3.5, C, D, E, F), plants attained a maximum growth rate 7 to 17 days after the addition of N. In Experiment 3, plants attained a maximum growth rate 10 days after the addition of N at all levels of N supplied (Figure 3.3, B, C, D, E, F).

3.2.1.3 Maximum specific growth rate (U_{max})

Lineweaver - Burk plots of the reciprocals of the specific growth rates ($1/U$), i.e. the highest specific growth rate attained by each plant after the addition of N (Table 3.3), against the reciprocals of the N ($1/N$) concentrations (Figures 3.6 to 3.10) showed that the relationship between $1/U$ and $1/N$ was linear in each experiment with a high degree of correla-

tion, significant at $P = 0,001$ (Table 3.4). An analysis of variance of the regressions showed they were significant at or less than $P = 0,01$ (Table 3.4). The maximum specific growth rate (U_{max}) was determined for *E. crassipes* in each experiment from the intercept of the regression line on the y axis, calculated from the regression equation (Figures 3.6 to 3.10). The variation about each U_{max} value determined was estimated from the fiducial band limits (95% confidence limits) to the regression line projected onto the y axis (Figures 3.6 to 3.10).

The U_{max} values determined in the 5 experiments (Table 3.5) ranged from 0,0537 to 0,0886 g fresh mass $g^{-1} d^{-1}$ (5,37 to 8,86% d^{-1})*. They showed no correlation with the average daily air and water temperatures and relative humidities recorded in the greenhouse during maximum growth rate of plants in each experiment (Table 3.5). Significantly lower values, however, were obtained in Experiments 2 and 3 (Table 3.5) where plants were grown for longer periods in N deficient cultures before they were obtained in a N growth rate limited state.

3.2.1.4. Half saturation coefficient (K_{sn})

The half saturation coefficient (K_{sn}) for N was determined for *E. crassipes* in each experiment from the intercept of the regression line of $1/U$ against $1/N$ on the x axis, calculated from the regression equation (Figures 3.6 to 3.10). The K_{sn} concentrations determined in the 5 experiments (Table 3.5) ranged from 399,8 to 1 505,6 $\mu g N l^{-1}$. They showed no correlation with the average daily air and water temperatures and relative humidities recorded in the greenhouse during maximum

*Measured and predicted growth rates in this study are given to 4 decimal places to facilitate conversion to % d^{-1} . Growth rates reported for *E. crassipes* and other higher aquatic plants in the literature are often expressed as % d^{-1} to 1 or 2 decimal places, eg. Mitchell and Tur (1975); Scott et al. (1979); Center and Spencer (1981).

growth rate of plants in each experiment or with the length of the period of growth of plants in N deficient cultures before they were obtained in a N growth rate limited state (Table 3.5).

3.2.1.5 Yield coefficient (Y_{cn})

In each of the first 3 experiments conducted (Experiments 1 to 3), the quantities of N analyzed in 3 culture solution samples taken at random from each treatment, after fresh mass determinations had been terminated, were very low (Table 3.6). In each case, the quantities of N remaining in the culture solutions were below 0,1% of that initially added (Table 3.6). It was assumed, therefore, that in all culture solutions, the N added had been absorbed by plants and incorporated into growth.

Plots of the total fresh mass yields (fresh mass including dead mass produced during growth) of plants against the quantities of N added (Figures 3.11 to 3.13) showed that the relationship between the total fresh mass yields of plants and the quantities of N supplied was linear in each of the first 3 experiments with a high degree of correlation, significant at $P = 0,001$ (Table 3.7). An analysis of variance of the regressions showed they were significant at $P = 0,001$ (Table 3.7). The yield coefficient (Y_{cn}) for N (fresh mass basis) was determined for *E. crassipes* in each experiment from the slope of the regression line given by the regression equation (Figures 3.11 to 3.13). The Y_{cn} values (fresh mass basis) determined in the 3 experiments ranged from 1 659,6 to 1 981,1 (Table 3.8).

The mean water contents of plants, harvested from each of the first 3 experiments, are shown in Table 3.8. Water contents ranged from 94,72 to 95,05% and showed no significant differences ($P = 0,05$) between experiments (Table 3.8). From the mean water contents of plants, the Y_{cn} values (fresh mass basis), determined in each experiment, were converted to a dry mass basis.

The Ycn values (dry mass basis) determined in the 3 experiments ranged from 86,9 to 98,1 (Table 3.8). Slightly higher Ycn values (both fresh and dry mass basis) were obtained in Experiment 1 where plants were grown for the shortest period in N deficient cultures before they were obtained in a N growth rate limited state (Table 3.8).

The minimum N concentrations (% dry mass) analyzed in 3 batches of plants harvested from each treatment in the first 3 experiments, when no further significant increase in the fresh mass of plants was recorded, are shown in Table 3.9. The minimum N concentrations (subsistence quotas) remaining in the dry plant tissues ranged from 0,94 to 1,28% N and showed no significant differences ($P = 0,05$) between experiments (Table 3.9).

3.2.2. Phosphorus growth rate limitation

3.2.2.1 Growth in P deficient culture

In each of the 3 experiments conducted (Figures 3.14 to 3.16), plants with 2 pseudolaminae introduced into P deficient cultures showed an initial lag phase in growth lasting ca 3 to 4 days. Growth of plants then proceeded more or less linearly until they showed a reduced growth rate, evident from a deviation from linearity in a plot of their fresh mass against time (Figures 3.14 to 3.16). At this stage, the growth rate of plants was assumed to be P limited. In each experiment, a different growth period in P deficient cultures was required to obtain plants in a P growth rate limited state (Figures 3.14 to 3.16). These different growth periods in P deficient cultures, however, showed no correlation with the average daily air and water temperatures and relative humidities recorded in the greenhouse (Table 3.10). Also noteworthy, is that at that stage when the growth rate of plants became P limited, i.e. at spiking, there were no significant differences ($P = 0,05$) between the mean fresh masses of groups of plants that were to comprise each treatment in each experiment (Table 3.11).

3.2.2.2. Growth after the addition of P

In each experiment, the addition of P effected an increase in growth rate with a short (3 to 4 day) period of maximum growth rate which was proportional to the level of P supplied. The periods of mean maximum growth rate of each group of plants for each treatment were evident from the maximum slopes of the curves relating growth (fresh mass) and time (Figures 3.14 to 3.16, B, C, D, E, F). Thereafter, the growth rates of plants decreased progressively in each treatment until there was no measurable increase in the total fresh mass (fresh mass, including dead mass produced during growth) of plants. This required ca 50 to 65 days after the addition of P, in those treatments (Figure 3.14, E; Figures 3.15 and 3.16, F) where P was supplied at the highest levels to the cultures. In those treatments where P was supplied at the lowest levels to the cultures (Figures 3.14 to 3.16, B), plants attained a maximum growth rate 3 to 7 days after the addition of P, whereas in all other treatments (Figures 3.14 to 3.16, C, D, E, F), except Experiment 6 (Figure 3.14, C, D, E), plants attained a maximum growth rate 10 to 14 days after the addition of P. In Experiment 6, plants attained a maximum growth rate 7 days after the addition of P at all levels of P supplied (Figure 3.14, B, C, D, E).

3.2.2.3 Maximum specific growth rate (U_{max})

Lineweaver - Burk plots of the reciprocals of the specific growth rates ($1/U$), i.e. the highest specific growth rate attained by each plant after the addition of P (Table 3.12), against the reciprocals of the P ($1/P$) concentrations (Figures 3.17 to 3.19) showed that the relationship between $1/U$ and $1/P$ was linear in each experiment with a high degree of correlation, significant at or less than $P = 0,01$ (Table 3.13). An analysis of variance of the regressions showed they were significant at or less than $P = 0,05$ (Table 3.13). The maximum specific growth rate (U_{max}) was determined for *E. crassipes* in each experiment from the intercept of the regression line on

the y axis, calculated from the regression equation (Figures 3.17 to 3.19). The variation about each U_{max} value determined was estimated from the fiducial band limits (95% confidence limits) to the regression line projected onto the y axis (Figures 3.17 to 3.19).

The U_{max} values determined in the 3 experiments (Table 3.14) ranged from 0,0451 to 0,1089 g fresh mass $g^{-1} d^{-1}$ (4,51 to 10,89% d^{-1}). They showed no correlation with the average daily air and water temperatures and relative humidities recorded in the greenhouse during maximum growth rate of plants in each experiment (Table 3.14). Significantly lower values, however, were obtained in Experiments 7 and 8 (Table 3.14) where plants were grown for longer periods in P deficient cultures before they were obtained in a P growth rate limited state.

3.2.2.4 Half saturation coefficient (K_{sp})

The half saturation coefficient (K_{sp}) for P was determined for *E. crassipes* in each experiment from the intercept of the regression line of $1/U$ against $1/P$ on the x axis, calculated from the regression equation (Figures 3.17 to 3.19).

The K_{sp} concentrations determined in the 3 experiments (Table 3.14) ranged from 41,1 to 161,8 $\mu g P l^{-1}$. They showed no correlation with the average daily air and water temperatures and relative humidities recorded in the greenhouse during maximum growth rate of plants in each experiment or with the length of the period of growth of plants in P deficient cultures before they were obtained in a P growth rate limited state (Table 3.14).

3.2.2.5 Yield coefficient (Y_{cp})

In each experiment conducted, the quantities of P analyzed in 3 culture solution samples taken at random from each treatment, after fresh mass determinations had been terminated, were very low (Table 3.15). In each case, the quantities of P remaining

in the culture solutions were below 0,1% of that initially added (Table 3.15). It was assumed, therefore, that in all culture solutions, the P added had been absorbed by plants and incorporated into growth.

Plots of the total fresh mass yields (fresh mass including dead mass produced during growth) of plants against the quantities of P added, (Figures 3.20 to 3.22) showed that the relationship between the total fresh mass yields of plants and the quantities of P supplied was linear in each experiment with a high degree of correlation, significant at $P = 0,001$ (Table 3.16). An analysis of variance of the regressions showed they were significant at $P = 0,001$ (Table 3.16). The yield coefficient (Y_{cp}) for P (fresh mass basis) was determined for *E. crassipes* in each experiment from the slope of the regression line given by the regression equation (Figures 3.20 to 3.22). The Y_{cp} values (fresh mass basis) determined in the 3 experiments ranged from 16 431,2 to 18 670,6 (Table 3.17).

The mean water contents of plants, harvested from each experiment, are shown in Table 3.17. Water contents ranged from 94,72 to 94,79% and showed no significant differences ($P = 0,05$) between experiments (Table 3.17). From the mean water contents of plants, the Y_{cp} values (fresh mass basis) determined in each experiment were converted to a dry mass basis.

The Y_{cp} values (dry mass basis) determined in the 3 experiments ranged from 867,1 to 980,2 (Table 3.17). Slightly higher Y_{cp} values (both fresh and dry mass basis) were obtained in Experiment 6 where plants were grown for the shortest period in P deficient cultures before they were obtained in a P growth rate limited state (Table 3.17).

The minimum P concentrations (% dry mass) analyzed in 3 batches of plants harvested from each treatment in each experiment, when no further significant increase in the fresh mass of plants was recorded, are shown in Table 3.18. The minimum P concentrations (subsistence quotas) remaining in the dry plant

tissues ranged from 0,09 to 0,14% P and showed no significant differences ($P = 0,05$) between experiments (Table 3.18).

3.3

DISCUSSION

In each experiment, a different growth period in deficient cultures was required to induce plants into a N or P growth rate limited state. This was attributed partly to different quantities of N and P stored in the plants collected on different occasions from the field for each experiment. There appeared to be no correlation between the length of the period that plants had to be grown in deficient cultures to induce them in a N or P growth rate limited state and the average daily air and water temperatures and relative humidities recorded in the greenhouse.

Nitrogen growth rate limited plants responded differently to the different levels of N supplied to culture. In those treatments where N was supplied at the lowest levels to the cultures, except Experiment 3, plants generally attained a maximum growth rate 2 to 3 days after the addition of N, whereas in all other treatments plants attained a maximum growth rate only 7 to 17 days after the addition of N. If it is assumed that in *E. crassipes* the uptake of nitrate-nitrogen is regulated by nitrate reductase in the leaves, as for example, in *Fontinalis antipyretica* L. (Schwoerbel and Tillmans, 1974), the limited availability of this enzyme may have restricted the N uptake in the plants. As the induction of nitrate reductase is roughly proportional to the amount of nitrate-nitrogen present in the plant tissues (Beevers et al., 1965), one may suggest that the nitrate-nitrogen concentrations in the tissues of *E. crassipes* grown under N deficient conditions would probably have been relatively low and so would have been their nitrate reductase activity. Consequently, this may have restricted the N uptake in the plants and delayed their attainment of a maximum growth rate in those treatments where N was supplied at the higher levels to the cultures. This suggestion, however, is not entirely plausible. 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 4 to 6 hours after transference of seedlings to a nitrate medium. It would appear, therefore, that the assimilation of N by *E. crassipes* and its incorporation into growth, in those treatments where N was supplied at higher levels to the cultures, probably did not keep pace with its uptake.

Phosphorus growth rate limited plants also responded differently to the different levels of P supplied to culture. In those treatments where P was supplied at the lowest levels to the cultures, plants attained a maximum growth rate 3 to 7 days after the addition of P, whereas in all other treatments, except Experiment 6, plants attained a maximum growth rate 10 to 14 days after the addition of P. It does not seem reasonable to explain the delayed attainment of a maximum growth rate by *E. crassipes*, in those treatments where P was supplied at higher levels to cultures, by a restricted uptake of P in the plants resulting from a limited availability of alkaline phosphatase in the plant tissues. Fitzgerald and Nelson (1966) and Fitzgerald (1969) have shown 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, as with N, the assimilation of P by plants and its incorporation into growth, in those treatments where P was supplied at higher levels to the cultures, probably did not keep pace with its uptake.

Maximum specific growth rates (U_{max}) determined for *E. crassipes*, under N and P growth rate limitation respectively, varied considerably between experiments. The error with which the specific growth rate of *E. crassipes* would be predicted as estimated from the range in variation is shown in Table 3.19. For example, in eutrophic waters where the N and P concentrations in the water are very high and approach those saturating to the growth rate of *E. crassipes*, i.e. where the ratio $S/(K_s + S)$ for either N or P approaches unity and the growth rate is

limited in a zero-order manner, the specific growth rate of *E. crassipes* would be predicted with an error of ca 2,60% d⁻¹ for plants growing under N growth rate limitation and with an error of ca 6,06% d⁻¹ for plants growing under P growth rate limitation (Table 3.19). In waters with a much lower N or P concentration, i.e. where the ratio $S/(K_s + S)$ for either N or P is 0,5 or less and the growth rate is limited in a first-order manner, the specific growth rate of *E. crassipes* would be predicted with a much lower error, ca 1,37% d⁻¹ for plants growing under N growth rate limitation and ca 3,19% d⁻¹ for plants growing under P growth rate limitation (Table 3.19). With increasing N or P growth rate limitation, the specific growth rate of *E. crassipes* would be predicted with a decreasing error. It would appear, therefore, that the U_{max} values determined for *E. crassipes* under N and P growth rate limitation in this study, when incorporated into the Monod model, may fairly accurately predict specific growth rates of *E. crassipes* in oligotrophic waters, where the growth rate limiting N or P concentrations in the water are very low, but not in eutrophic waters where these concentrations are high. Consequently, the values determined will probably be of little use in a model for predicting amounts and frequencies of harvest for *E. crassipes* growing in eutrophic waters.

The different U_{max} values determined for *E. crassipes*, under N and P growth rate limitation respectively, showed no correlation with the average daily air and water temperatures and relative humidities recorded in the greenhouse during maximum growth rate of plants in each experiment. They appeared to be predominantly influenced by the length of the period that plants had to be grown in deficient cultures to induce them into a N or P growth rate limited state. The significantly lower values obtained in those experiments where plants were grown for longer periods in N and P deficient cultures, however, could not be explained in terms of non-competitive inhibition, i.e. in a reduced rate of uptake of the growth rate limiting nutrient by plants resulting from a longer growth period in deficient cultures. Investigations of the uptake kinetics of higher plants have shown that the growth of plants

in starvation (deficient) media prior to experiments results in a subsequent increase in the rate of uptake of nutrients by plants with a corresponding reduction in the half saturation coefficient (K_m) for uptake. Glass (1978), for example, has shown that the uptake characteristics for potassium of barley grown initially with or without potassium are very different. The K_m for potassium uptake is reduced in the starved plant from 0,1 to 0,03 mM, and the same occurs for other ions, as for nitrate (Smith, 1973) or phosphate (Cartwright, 1972), and for other species; e.g. Doddema et al. (1979) show a reduction in K_m from 111 to 40 mM NO_3 brought about by N starvation in *Arabidopsis thaliana*. It is suggested, therefore, that the different U_{max} values determined for *E. crassipes* in the various experiments were probably the result of errors inherent in the batch culture method used, e.g.

- (i) The different physiological state of plants grown for different periods in N or P deficient cultures and collected on different occasions from the field for each experiment.
- (ii) To differences in the ratio of biomass of plants present at spiking to the levels of N or P supplied to culture. A larger biomass of plants resulted at spiking in those experiments where plants were grown for longer periods in deficient cultures.
- (iii) To variations in the light intensity in the greenhouse between experiments.

As the U_{max} values determined for *E. crassipes* in this study were adversely influenced by the length of the period that plants had to be grown in deficient cultures to induce them into a N or P growth rate limited state, it is suggested that, for purposes of testing the model and as a basis for its refinement, the U_{max} values determined for *E. crassipes* in those experiments where plants were grown for the shortest period in N and P deficient cultures respectively are possibly more reliable than those determined in other experiments.

Half saturation coefficients (K_s) determined for *E. crassipes*, under N and P growth rate limitation respectively, also varied

considerably between experiments. The error with which the specific growth rate of *E. crassipes* would be predicted as estimated from the range in variation is shown in Table 3.20. For example, in water with a high N and P concentration, i.e. where the growth rate of *E. crassipes* approaches its U_{max} and is limited in a zero-order manner, the specific growth rate of *E. crassipes* would be predicted with an error of ca 0,45% d^{-1} for plants growing under N growth rate limitation and with an error of ca 0,60% d^{-1} for plants growing under P growth rate limitation (Table 3.20). These estimates are based on the highest U_{max} values of 8,86% d^{-1} and 10,89% d^{-1} determined for *E. crassipes* under N and P growth rate limitation respectively, in culture. In waters with a lower N or P concentration, i.e. where the growth rate of *E. crassipes* is less than 50% of its U_{max} and is limited in a first-order manner, the specific growth rate of *E. crassipes* would be predicted with a much higher error, ca 1,91% d^{-1} for plants growing under N growth rate limitation and ca 2,36% d^{-1} for plants growing under P growth rate limitation (Table 3.20). With increasing N or P growth rate limitation, the specific growth rate of *E. crassipes* would be predicted with an increasing error. It would appear, therefore, that the K_s concentrations determined for *E. crassipes* under N and P growth rate limitation in this study, when incorporated into the Monod model, may not accurately predict specific growth rates of *E. crassipes* in oligotrophic waters, where the growth rate limiting N or P concentrations in the water are very low, but should fairly accurately predict specific growth rates of *E. crassipes* in eutrophic waters where these concentrations are high. Consequently, the K_s concentrations determined should be of some value in a model for predicting amounts and frequencies of harvest for *E. crassipes* growing in eutrophic waters.

The different K_s concentrations determined for *E. crassipes*, under N and P growth rate limitation respectively, could not be readily explained. They showed no correlation with the average daily air and water temperatures and relative humidities recorded in the greenhouse during maximum growth rate of plants

in each experiment or with the length of the period that plants had to be grown in deficient cultures to induce them into a N or P growth rate limited state. It is suggested, however, that as with the U_{max} values, the different K_s concentrations determined for *E. crassipes* in the various experiments were probably the result of errors inherent in the batch culture method used, as already outlined.

The K_s concentrations determined for *E. crassipes* under N growth rate limitation are in the range of those reported for various species of algae (Table 3.21), whereas those determined under P growth rate limitation are much higher (Table 3.22). This indicates that *E. crassipes* has a similar potential to the algae to produce a high growth rate in waters in which N is growth rate limiting, but has a lower potential than the algae to produce a high growth rate in waters where P is growth rate limiting. As P is the nutrient frequently limiting the growth rate of algae in relatively oligotrophic waters (Toerien et al., 1975), it would appear that in such waters P may be the nutrient limiting the growth rate of *E. crassipes*.

The mean K_{sn} concentration of 976 ug N l^{-1} , determined for *E. crassipes* in the 5 experiments conducted under N growth rate limitation, falls in the range of concentrations 500 to 1 000 ug N l^{-1} interpreted by Center and Spencer (1981) from the N/P uptake rates of *E. crassipes* of 5 to 10 l^{-1} (Boyd, 1970; 1976; Dunigan et al., 1975b) as being the critical growth rate limiting N concentrations in the water for *E. crassipes* in the field, i.e. below which the growth rate of *E. crassipes* is significantly influenced by the N concentration in the water. The mean K_{sp} concentration of $94,1 \text{ ug P l}^{-1}$, determined for *E. crassipes* in the 3 experiments conducted under P growth rate limitation, compares favourably with the concentration of 100 ug P l^{-1} reported by Haller et al. (1970) and Knipling et al. (1970) as being the critical growth rate limiting P concentration in the water for *E. crassipes* in the field. This suggests that for the purposes of testing the model and as a basis for its refinement, the mean K_s concentrations determined for

E. crassipes under N and P growth rate limitation respectively are possibly more reliable than the individual concentrations determined.

The mean water contents of **E. crassipes**, harvested from culture in the various experiments, ranged from 94,72 to 95,05%. These values compare favourably with the water content of 94,75% which is based on an average value of Penfound and Earle (1948), Westlake (1963) and Bock (1969).

Yield coefficients (Y_c) determined for **E. crassipes**, under N and P growth rate limitation respectively, showed little variation between experiments. The range in variation would give rise to an error in predicting the yield of **E. crassipes** from the growth rate limiting N or P concentrations in the water of ca 12% for both N and P. Consequently, the Y_c values determined in this study should be applicable in a model for predicting amounts and frequencies of harvest for **E. crassipes** in the field.

In all experiments, some growth (yield in plant material) was produced by **E. crassipes** grown in the absence of N or P. This indicates that, although the N and P concentrations in the plants were growth rate limiting, sufficient quantities were present to produce some growth. In fact, more growth, i.e. a greater yield in plant material, was produced by **E. crassipes** grown in the absence of N and P in Experiments 1 and 6 respectively, where plants were grown for the shortest period in deficient cultures to induce them in a N or P growth rate limited state, than in other experiments. This indicates that the growth rate limiting nutrients (N or P) were present in higher concentrations in the plant tissues at spiking in these 2 experiments than in other experiments. In principle, however, higher growth rate limiting N or P concentrations present in the plants at spiking in Experiments 1 and 6 should not have had an influence on the Y_c values determined as these were derived from the slopes of the regression lines relating the total fresh mass yields of plants to the quantities of the

growth rate limiting nutrient (N or P) supplied in culture. Consequently, the slightly higher Y_c values determined for *E. crassipes*, for N and P respectively, in these 2 experiments could not be readily explained.

The Y_c values (dry mass basis) determined for *E. crassipes* under P growth rate limitation are in the upper range of those reported for various species of diatoms and other algae (Table 3.23), whereas those determined under N growth rate limitation are much higher (Table 3.24). This indicates that *E. crassipes* has the potential to produce a similar biomass per unit quantity of P absorbed as the diatoms and algae, but has the potential to produce a much higher biomass than the diatoms and algae, per unit quantity of N absorbed. Maximum specific growth rates reported for algae (Shelef et al., 1968; Zabat et al., 1970; Toerien et al., 1971; Goldman, 1972) are considerably higher than those determined for *E. crassipes*. However, the potential of *E. crassipes* to produce a much greater yield in plant material per unit quantity of N absorbed may compensate for its lower growth rate and give it a similar potential to the algae for removing nutrients from eutrophic waters, at least in those in which P is not growth rate limiting. *E. crassipes* lower potential than the algae to produce a high growth rate in waters where P is growth rate limiting suggests that, in relatively oligotrophic waters, *E. crassipes* would be less efficient in removing nutrients than the algae, i.e. where both plants are present with the same biomass.

Toerien et al. (1971) and Coetzer et al. (1977) pointed out that the yield coefficients (dry mass basis), when expressed as a reciprocal and a percentage, should estimate the minimum concentrations of the specific growth rate limiting nutrient in the dry plant tissues. The Y_c values (dry mass basis) determined for *E. crassipes* under N and P growth rate limitation respectively, when expressed as a reciprocal and a percentage ($1/Y_c \times 100$), fairly accurately estimated the minimum concentrations of N (Table 3.25) and P (Table 3.26) analyzed in the plants harvested from culture. This suggests that the Y_c values

determined for *E. crassipes* in this study are fairly reliable. The average minimum concentrations of 1,10% N and 0,11% P estimated in *E. crassipes* plant tissues from the mean Y_c values, determined for N and P respectively in the various experiments, also compare favourably with the minimum N and P concentrations (% dry mass) of 1,33% N and 0,14% P reported by Boyd and Vickers (1971) in *E. crassipes* growing in the field, and with the minimum P concentration (% dry mass) of 0,098% P reported by Haller and Sutton (1973) in *E. crassipes* growing in the absence of P in culture.

As the minimum concentrations of N and P in *E. crassipes* were fairly accurately estimated from the respective Y_c values for these nutrients, it should be feasible to predict the growth rate of *E. crassipes* in the field from the growth rate limiting N or P concentrations in the plants using the simplified hyperbolic equation reported by Droop (1968) and Rhee (1973).

3.4 CONCLUSIONS

It is concluded that maximum specific growth rates (U_{max}) and half saturation coefficients (K_s) were not consistently determined for *E. crassipes* growing under N or P growth rate limitation in culture using a batch culture approach. In contrast, yield coefficients (Y_c) were determined with sufficient accuracy. With better facilities, however, it is possible that the batch culture method used for determining kinetic coefficients for *E. crassipes* growing under specific nutrient growth rate limitation in this study 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 obtain plants in a N or P growth rate limited state could be obtained. This might decrease the variability in the U_{max} values and the K_s concentrations determined. It is suggested, however, that precise measurements of the U_{max} and K_s may only be obtained for *E. crassipes*, under specific

nutrient growth rate limitation in culture, by growing plants under constant environmental conditions in some type of continuous flow culture system in which the growth rate limiting nutrient concentrations could be maintained at constant levels. In such a system it would, therefore, not be necessary to grow plants initially in deficient cultures to induce them into a N or P growth rate limited state as the specific growth rate of *E. crassipes* at each growth rate 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 U_{max} values and K_s concentrations determined for *E. crassipes* under N and P growth rate limitation in this study varied considerably, it should, however, 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.

The Y_c values determined for *E. crassipes*, under N and P growth rate limitation respectively, appear reliable as their reciprocals (dry mass basis) expressed as a percentage fairly accurately estimate the minimum N and P concentrations in *E. crassipes*, as determined in plants harvested from culture when no further significant increase in fresh mass of plants was recorded, and as reported in the literature. As the minimum N and P concentrations in *E. crassipes* can be derived from the respective Y_c values for these nutrients, it should be feasible to predict the growth rate of *E. crassipes* in the field from the growth rate limiting N or P concentrations in the plants using a simplified hyperbolic equation reported in the literature.

CHAPTER 4

TESTING THE MODEL UNDER FIELD CONDITIONS

4.1 INTRODUCTION

In testing kinetic coefficients, generated for *E. crassipes* under N and P growth rate limitation in culture, for predicting specific growth rates of *E. crassipes* growing at different air and water temperatures and under varying conditions of N and P supply in the field, 2 assumptions were made:

- (i) That the maximum specific growth rates (U_{max}) determined for *E. crassipes*, under N and P growth rate limitation respectively, follow the van't Hoff rule, i.e. they approximately double for each 10°C rise in the temperature. This assumption was based on the fact, and later confirmed for *E. crassipes* in the field, that the U_{max} 's of various species of marine and fresh water algae generally follow the van't Hoff rule (Goldman, 1972; Goldman and Carpenter, 1974).

- (ii) That the specific growth rate (U) of *E. crassipes* was limited not in a multiplicative or additive manner, but in a threshold pattern by the single nutrient in shorter supply. This assumption was based on the principle formulated by von Liebig in the mid-nineteenth century, that the maximum population size or maximum yield in plant material is controlled by a single factor in shorter supply (Blackman, 1905). Brandt (cited in Gran, 1912) observed that this principle also applied to the regulation of phytoplankton organic production by soluble nutrients. A more recent interpretation of "Liebig's law of the minimum" extends this classical concept to include the regulation or control of phytoplankton growth rate by the limiting nutrient (O'Brien, 1972). Droop (1974), for

example, showed that the growth rate of *Monochrysis lutherii* growing under P and B₁₂ growth rate limitation in culture was not limited in a multiplicative pattern, but by the single nutrient in shorter supply. Rhee (1978) also summarized that the growth rate of *Scenedesmus* was limited in a threshold pattern.

The evaluation and validation of models has been reviewed by Dent and Blackie (1979). Bell (1981) pointed out that, by analogy with the correlation coefficient (r), one can calculate a correlation factor R², to test model outputs compared to data, defined as:

$$R^2 = 1 - \frac{\text{sum of squares of residuals}}{nSD^2y}$$

where: n is the number of data points and SD²y is the variance of the predicted values. If R² = 1, then the fit is good; if R² = 0, the fit is poor. For R² in between, the situation is unclear. Dent and Blackie (1979) recommended a simple regression analysis between model outputs and the data as paired observations. This will produce the same value of R² as defined.

4.2 RESULTS

4.2.1 Field data

4.2.1.1 Specific growth rates (U)

Specific growth rates (means of 40 replicates) determined at ca 2 week intervals for *E. crassipes* plants of the marginal growth form (marginal plants), growing in loosely crowded populations, at 6 field sites during 1977 and 1978 are summarized in Tables I to III, Appendix II. Specific growth rates measured for marginal plants at the Maturation Pond 3 (MP3) and Botanic Gardens (BGL) sites, where field data was obtained over a 12 month period or longer, are graphically illustrated in Figure 4.1.

At both sites, specific growth rates of marginal plants followed a distinct seasonal pattern with values decreasing progressively after summer (September to March) through to winter (May to August). During 1978, the highest specific growth rates, 0,1698 and 0,1227 g fresh mass $g^{-1} d^{-1}$ (16,98 and 12,27% d^{-1}) at the MP3 and BGL sites respectively, were measured during summer in February with the lowest specific growth rates, 0,0526 and 0,0305 g fresh mass $g^{-1} d^{-1}$ (5,26 and 3,05% d^{-1}) at these 2 sites respectively, being measured during midwinter in June. Specific growth rates of marginal plants at the MP3 site were significantly higher (at or less than $P = 0,01$) throughout 1978, except in December, 1978, than those at the BGL site (Table 4.1).

Specific growth rates (means of 30 replicates) determined at **ca** 2 week intervals for **E. crassipes** plants of the central growth form (central plants), growing in densely crowded populations, at the MP3 and Discharge Canal (DC) sites during 1977 and 1978 are summarized in Table IV, Appendix II. Specific growth rates measured for central plants at the MP3 site are graphically illustrated in Figure 4.1. Unlike those of marginal plants, specific growth rates of central plants at the MP3 site did not follow any distinct seasonal pattern during 1978. No plants of the central growth form were produced during the midwinter months of June, July and August. The highest specific growth rate, 0,0659 g fresh mass $g^{-1} d^{-1}$ (6,59% d^{-1}), was measured for central plants at the MP3 site during summer in December, 1977 with the lowest specific growth rate, 0,0202 g fresh mass $g^{-1} d^{-1}$ (2,02% d^{-1}), being measured during winter in May, 1978. Throughout 1977 and 1978, specific growth rates of central plants at both the MP3 and DC sites were significantly lower ($P = 0,001$) than those of marginal plants (Table 4.2). Specific growth rates of central plants at these 2 sites ranged from 2 to 6 times lower than those of marginal plants (Table 4.2).

4.2.1.2 Air and water temperatures

The mean, maximum and minimum daily air temperatures and the water temperatures recorded over each growing interval of

marginal plants at 6 field sites during 1977 and 1978 are summarized in Tables I to III, Appendix II. The air and water temperatures are expressed in these tables as a daily average over each growing interval of plants.

Air and water temperatures recorded over each growing interval of marginal plants at the MP3 and BGL sites are graphically illustrated in Figures 4.2 and 4.3 respectively. At both sites, the air and water temperatures followed a similar seasonal pattern to the specific growth rates measured for marginal plants at these 2 sites (Figure 4.1). Mean daily air temperatures at the MP3 and BGL sites ranged from 25,2°C during summer to 16,1°C during winter. Water temperatures ranged from 27,8°C during summer to 15,1°C during winter.

Arrhenius plots of the specific growth rates of marginal plants, expressed as a natural logarithm (Log_e), against the reciprocals of the Absolute mean daily air (Figures 4.4 and 4.5) and the water temperatures (Figures 4.6 and 4.7) at the MP3 and BGL sites respectively, over the period February to December, 1978, in each case yielded a linear relationship with the correlation coefficients being high and significant at $P = 0,001$ (Table 4.3). At both sites, the highest correlation coefficient was obtained for the exponential relationship between the specific growth rates of marginal plants and the water temperatures. Marginal plants, however, showed a proportionally smaller increase in growth rate, at both sites, with a 10°C rise in the water temperature than with a similar increase in the mean daily air temperature. This is evident from the Q_{10} values calculated for each relationship (Table 4.3). In addition, at the BGL site, where the N and P concentrations in the water (Table II, Appendix II) were much lower than at the MP3 site (Table I, Appendix II), marginal plants showed a proportionally larger increase in growth rate with a 10°C rise in the mean daily air and water temperatures than at the MP3 site.

Air and water temperatures recorded over each growing interval of central plants at the MP3 and DC sites during 1977 and 1978

are summarized in Table IV, Appendix II. Water temperatures recorded from the vicinity of the central plant populations (Table IV, Appendix II) were not different from those recorded in the vicinity of the marginal plant populations (Tables I and III, Appendix II) enclosed at each of these sites.

Arrhenius plots of the specific growth rates of central plants, expressed as a natural logarithm (Log_e), against the reciprocals of the Absolute mean daily air (Figure 4.8) and water temperatures (Figure 4.9) at the MP3 site, over the period February to December, 1978, in each case yielded a linear relationship with the correlation coefficients being high and significant at or less than $P = 0,05$ (Table 4.3). The highest correlation coefficient was obtained for the exponential relationship between the specific growth rates of central plants and the mean daily air temperatures and not the water temperatures as with marginal plants. Like marginal plants, central plants at this site also showed a proportionally smaller increase in growth rate with a 10°C rise in the water temperature than with a similar increase in the mean daily air temperature. This is evident from the Q_{10} values calculated for each relationship (Table 4.3).

4.2.1.3 Nitrogen and phosphorus concentrations

The N ($\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and total N) and P (SRP and total P) concentrations analyzed in the water during 1977 and 1978 from the vicinity of the marginal plant populations enclosed at 6 field sites are summarized in Tables I to III, Appendix II. The N and P concentrations in the water are expressed in these tables as an average over each growing interval of plants.

Nitrogen and P concentrations analyzed in the water over each growing interval of marginal plants at the MP3 and BGL sites are graphically illustrated in Figures 4.10 and 4.11 respectively. Total N, $\text{NH}_4\text{-N}$, SRP and total P concentrations were very much higher in the water at the MP3 site than at the BGL site.

These higher N and P concentrations in the water at the MP3 site were reflected in the significantly higher specific growth rates measured for marginal plants at this site (Table 4.1). Air and water temperatures at both sites were very similar during the year (Figures 4.2 and 4.3). Although total N concentrations in the water at the BGL site were much lower than those at the MP3 site, $\text{NO}_3\text{-N}$ concentrations in the water were generally higher, whereas $\text{NH}_4\text{-N}$ concentrations in the water were very low. These higher $\text{NO}_3\text{-N}$, but low $\text{NH}_4\text{-N}$ concentrations in the water at the BGL site were reflected in the higher dissolved oxygen concentrations recorded in the water at the BGL site compared with those recorded in the water at the MP3 site (Figure 4.12), and was possibly indicative of a higher rate of nitrification (Keeny, 1973).

The N and P concentrations analyzed in the water during 1977 and 1978 from the vicinity of the central plant populations enclosed at the MP3 site and DC sites are summarized in Table IV, Appendix II. Nitrogen and P concentrations determined in the water from the vicinity of the central plant populations (Table IV, Appendix II) were not different from those determined in the water from the vicinity of the marginal plant populations (Tables I and III, Appendix II) enclosed at each of these sites.

The average total N and total P concentrations determined in the water at each of the 6 field sites during 1977 and 1978 are given in Table 4.4. Incorporating these average total N and total P concentrations in the water and the mean K_s concentrations of 976 ug N l^{-1} and $94,1 \text{ ug P l}^{-1}$, determined for *E. crassipes* under N and P growth rate limitation respectively in culture, into the Monod model, the percentage of the U_{max} that *E. crassipes* would achieve at the average total N and total P concentrations in the water at each of these sites was estimated. This may be illustrated by the following example:

The average total N and total P concentrations determined in the water at the BGL site during 1978 were $10\ 206 \text{ ug N l}^{-1}$ and

150 ug P l⁻¹ respectively (Table 4.4). The percentage of the U_{max} that **E. crassipes** would achieve at (i) the average total N and (ii) the average total P concentrations in the water at this site was calculated using the Monod model, as follows:

$$U = U_{\max} \frac{10\ 206}{976 + 10\ 206} \times 100$$

$$= 91,3\ \% U_{\max} \dots\dots\dots (i)$$

$$U = U_{\max} \frac{150}{94,1 + 150} \times 100$$

$$= 61,4\ \% U_{\max} \dots\dots\dots (ii)$$

Estimates were based on the total N and total P concentrations in the water rather than on the soluble N and P fractions in the water as it is subsequently shown, particularly in Chapter 5, that the specific growth rates of **E. crassipes** are more accurately predicted from the total N and P concentrations in the water than from the other N or P fractions in the water.

The results (Table 4.4) show that at the MP3, DC, Isipingo Lake (IL) and Isipingo Canal (IC) sites **E. crassipes** would achieve a lower percentage of the U_{max} at the average total N concentrations in the water than at the average total P concentrations in the water. This suggests that the growth rate of **E. crassipes** at these sites was limited by the N concentrations in the water. At the BGL and Hartbeespoort Dam (HD) sites, on the other hand, the results (Table 4.4) show that **E. crassipes** would achieve a lower percentage of the U_{max} at the average total P concentrations in the water than at the average total N concentrations in the water. This suggests that the growth rate of **E. crassipes** at these 2 sites was limited by the P concentrations in the water.

4.2.2 Comparison of predicted and measured specific growth rates

4.2.2.1 Nitrogen growth rate limitation

The U_{max} value determined for **E. crassipes** under N growth rate

limitation in culture at a mean daily air and water temperature of 24°C (Experiment 1, Table 3.5) was 0,0886 g fresh mass g⁻¹ d⁻¹. Using this value, the U_{max} of *E. crassipes* was predicted for other temperatures according to the van't Hoff rule (Table 4.5). The Arrhenius equation for the exponential relationship between the U_{max} and temperature in Table 4.5 is as follows:

$$U_{\max} = 3,9151 \times 10^7 e^{-\frac{5916}{T}}$$

where U_{max} = maximum specific growth rate under N growth rate limitation (g fresh mass g⁻¹ d⁻¹),
 T = Absolute mean daily temperature (°K).

Using this equation, U_{max}'s were predicted for *E. crassipes*, over each growing interval, at the IL, IC, DC and MP3 sites from the mean daily air temperatures. As shown (Table 4.4), the N concentrations in the water at these sites were estimated to be growth rate limiting for *E. crassipes*. Maximum specific growth rates were predicted for *E. crassipes* from the mean daily air temperatures rather than from the water temperatures as both marginal and central plants in the field showed a proportionally larger increase in growth rate with a 10°C rise in the mean daily air temperature than with a similar increase in the water temperature (Table 4.3).

Using the U_{max}'s predicted for *E. crassipes* from the mean daily air temperatures at the 4 mentioned sites and the mean K_{sn} concentration of 976 ug N l⁻¹, determined for *E. crassipes* under N growth rate limitation in culture, in the Monod model, specific growth rates were predicted for *E. crassipes*, over each growing interval, at each of these sites from the NO₃-N, NH₄-N and total N concentrations in the water. This may be illustrated by the following example:

The mean daily air temperature at the MP3 site over the growing interval 1/2 to 16/2/78 was 24,9°C (Table I, Appendix II). The

U_{max} predicted for *E. crassipes*, according to the van't Hoff rule, for this temperature was calculated as follows:

$$U_{\max} = 3,9151 \times 10^7 e^{-\frac{5916}{24,9 + 273,2}}$$

$$= 0,0941 \text{ g fresh mass g}^{-1} \text{ d}^{-1}$$

The NO₃-N, NH₄-N and total N concentrations determined in the water over this growing interval of plants at this site were 4 740, 11 230 and 15 970 ug N l⁻¹ respectively (Table I, Appendix II). Specific growth rates (U) predicted for *E. crassipes* from (i) the NO₃-N, (ii) the NH₄-N and (iii) the total N concentrations in the water were calculated using the Monod model as follows:

$$U = 0,0941 \frac{4\ 740}{976 + 4\ 740}$$

$$= 0,0780 \text{ g fresh mass g}^{-1} \text{ d}^{-1} \dots\dots\dots(i)$$

$$U = 0,0941 \frac{11\ 230}{976 + 11\ 230}$$

$$= 0,0866 \text{ g fresh mass g}^{-1} \text{ d}^{-1} \dots\dots\dots(ii)$$

$$U = 0,0941 \frac{15\ 970}{976 + 15\ 970}$$

$$= 0,0887 \text{ g fresh mass g}^{-1} \text{ d}^{-1} \dots\dots\dots(iii)$$

Specific growth rates (U) predicted for *E. crassipes* from the various N fractions in the water at the MP3, IC, IL and DC sites during 1977 and 1978 are compared with those measured for marginal plants, growing in loosely crowded populations at each of these sites, in Tables 4.6 and 4.7. Specific growth rates predicted for *E. crassipes* at the MP3 and DC sites are also compared with those measured for central plants growing in densely crowded populations at each of these sites in Table 4.8. Predicted specific growth rates and those measured for marginal and central plants at the MP3 sites are graphically illustrated in Figure 4.13.

4.2.2.1.1 Maturation Pond 3 (MP3) site

Specific growth rates predicted for *E. crassipes* from the total N, $\text{NH}_4\text{-N}$, and to a lesser extent from the $\text{NO}_3\text{-N}$ concentrations in the water at the MP3 site (Figure 4.13) followed a fairly similar seasonal pattern to those measured for marginal plants with values decreasing progressively after summer (September to March) through to winter (May to August). In general, however, specific growth rates predicted from the various N fractions in the water were significantly lower than those measured for marginal plants. For example, of the 29 specific growth rates predicted from the total N concentrations in the water, only 3 (during April and June) fell within the standard deviations of the measured values (Figure 4.13, Table 4.6). The smallest differences between the measured specific growth rates and those predicted from the total N concentrations in the water occurred during midwinter in June, when the relative humidities and diffuse radiant fluxes recorded at this site were at their lowest levels (Table I, Appendix II). Only 2 of the specific growth rates predicted for *E. crassipes* from both the $\text{NO}_3\text{-N}$ and the $\text{NH}_4\text{-N}$ concentrations in the water fell within the standard deviations of those measured for marginal plants. The differences between the measured specific growth rates and those predicted from the $\text{NO}_3\text{-N}$ and the $\text{NH}_4\text{-N}$ concentrations in the water were larger than the differences between the measured specific growth rates and those predicted from the total N concentrations in the water.

Specific growth rates predicted for *E. crassipes* from the various N fractions in the water at this site were generally significantly higher than those measured for central plants (Figure 4.13, Table 4.8). For example, of the 18 specific growth rates predicted from the total N concentrations in the water, only 2 fell within the standard deviations of the measured values. In the latter case, however, the differences between the measured and predicted values were fairly large. Two of the specific growth rates predicted for *E. crassipes* from the $\text{NH}_4\text{-N}$ concentrations in the water and 3 of those

predicted from the $\text{NO}_3\text{-N}$ concentrations in the water fell within the standard deviations of those measured for central plants. The differences between the measured specific growth rates and those predicted from the $\text{NO}_3\text{-N}$ and the $\text{NH}_4\text{-N}$ concentrations in the water were smaller than the differences between the measured specific growth rates and those predicted from the total N concentrations in the water.

In general, specific growth rates predicted for *E. crassipes* from the various N fractions in the water at the MP3 site ranged between those measured for marginal and central plants, growing in loosely and densely crowded populations, respectively.

4.2.2.1.2 Isipingo Lake (IL), Isipingo Canal (IC) and Discharge Canal (DC) sites

Specific growth rates predicted for *E. crassipes* from the various N fractions in the water at the IL, IC and DC sites were generally significantly lower than those measured for marginal plants (Table 4.7). For example, of the 10 specific growth rates predicted from the total N concentrations in the water at all 3 sites, only 3 fell within the standard deviations of the measured values. In the latter case, however, the differences between the predicted and measured values were fairly large. Only 1 of the specific growth rates predicted for *E. crassipes* from the $\text{NO}_3\text{-N}$ concentrations in the water and 2 of those predicted from the $\text{NH}_4\text{-N}$ concentrations in the water at all 3 sites fell within the standard deviation of those measured for marginal plants. The differences between the measured specific growth rates and those predicted from the $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations in the water were larger than the differences between the measured specific growth rates and those predicted from the total N concentrations in the water.

Specific growth rates predicted for *E. crassipes* from the $\text{NH}_4\text{-N}$ concentrations in the water at the DC site were not significantly different from those measured for central plants (Table

4.8). However, specific growth rates predicted from the $\text{NO}_3\text{-N}$ and total N concentrations in the water were generally significantly different from those measured for central plants. For example, all 3 of the specific growth rates predicted from the $\text{NH}_4\text{-N}$ concentrations in the water fell without the standard deviations of the measured values. The differences between the measured and predicted values, however, were fairly large. Only 1 of the specific growth rates predicted for *E. crassipes* from both the $\text{NO}_3\text{-N}$ and total N concentrations in the water at this site fell within the standard deviations of those measured for central plants. The differences between the measured specific growth rates and those predicted from the $\text{NO}_3\text{-N}$ and total N concentrations in the water were generally larger than the differences between the measured specific growth rates and those predicted from the $\text{NH}_4\text{-N}$ concentrations in the water.

In general, specific growth rates predicted for *E. crassipes* from the various N fractions in the water at the DC site ranged between those measured for marginal and central plants respectively.

4.2.2.2 Phosphorus growth rate limitation

The U_{max} value determined for *E. crassipes* under P growth rate limitation in culture at a mean daily air and water temperature of 28°C (Experiment 6, Table 3.14) was $0,1089 \text{ g fresh mass g}^{-1} \text{ d}^{-1}$. Using this value the U_{max} of *E. crassipes* was predicted for other temperatures according to the van't Hoff rule (Table 4.9). The Arrhenius equation for the exponential relationship between the U_{max} and temperature in Table 4.9 is as follows:

$$U_{\text{max}} = 6,5292 \times 10^7 e^{-\frac{6088}{T}}$$

where U_{max} = maximum specific growth rate under P growth rate limitation ($\text{g fresh mass g}^{-1} \text{ d}^{-1}$),

T = Absolute mean daily temperature ($^\circ\text{K}$).

Using this equation, U_{max} 's were predicted for **E. crassipes**, over each growing interval, at the BGL and HD sites from the mean daily air temperatures. As shown (Table 4.4), the P concentrations in the water at these sites were estimated to be growth rate limiting for **E. crassipes**.

Using the U_{max} 's predicted for **E. crassipes** from the mean daily air temperatures at the 2 mentioned sites and the mean K_{sp} concentration of 94,1 $\mu\text{g P l}^{-1}$, determined for **E. crassipes** under P growth rate limitation in culture, in the Monod model, specific growth rates were predicted for **E. crassipes**, over each growing interval, at each of these sites from the SRP and total P concentrations in the water. This may be illustrated by the following example:

The mean daily air temperature at the BGL site over the growing interval 1/2 to 15/2/78 was 24,9°C (Table II, Appendix II). The U_{max} predicted for **E. crassipes**, according to the van't Hoff rule, for this temperature was calculated as follows:

$$\begin{aligned} U_{max} &= 6,5292 \times 10^7 e^{-\frac{6088}{24,9 + 273,2}} \\ &= 0,0883 \text{ g fresh mass g}^{-1} \text{ d}^{-1} \end{aligned}$$

The SRP and total P concentrations determined in the water over this growing interval of plants at this site were 5 and 183 $\mu\text{g P l}^{-1}$ respectively (Table II, Appendix II). Specific growth rates (U) predicted for **E. crassipes** from (i) the SRP and (ii) the total P concentrations in the water were calculated using the Monod model as follows:

$$\begin{aligned} U &= 0,0883 \frac{5}{94,1 + 5} \\ &= 0,0044 \text{ g fresh mass g}^{-1} \text{ d}^{-1} \dots\dots\dots(i) \end{aligned}$$

$$\begin{aligned} U &= 0,0883 \frac{183}{94,1 + 183} \\ &= 0,0583 \text{ g fresh mass g}^{-1} \text{ d}^{-1} \dots\dots\dots(ii) \end{aligned}$$

Specific growth rates (U) predicted for *E. crassipes* from the various P fractions in the water at the BGL and HD sites during 1977 and 1978 are compared with those measured for marginal plants, growing in loosely crowded populations at each of these sites, in Tables 4.10 and 4.11 respectively. Specific growth rates predicted for *E. crassipes* and those measured for marginal plants at the BGL site are graphically illustrated in Figure 4.14.

4.2.2.2.1 Botanic Gardens Lake (BGL) site

Specific growth rates predicted for *E. crassipes* from the total P concentrations in the water at the BGL site (Figure 4.14) followed a fairly similar seasonal pattern to those measured for marginal plants with values decreasing progressively after summer (September to March) through to winter (May to August). Specific growth rates predicted from the SRP concentrations in the water, however, were extremely variable and did not follow any recognizable seasonal pattern. In general, specific growth rates predicted for *E. crassipes* from the various P fractions in the water were significantly lower than those measured for marginal plants. For example, of the 22 specific growth rates predicted from the total P concentrations in the water, only 6 (during March, April, June and July) fell within the standard deviations of the measured values (Figure 4.14, Table 4.10). The smallest differences between the measured specific growth rates and those predicted from the total P concentrations in the water occurred during midwinter in June, when the relative humidities and diffuse radiant fluxes recorded at this site were at their lowest levels (Table II, Appendix II). None of the specific growth rates predicted for *E. crassipes* from the SRP concentrations in the water fell within the standard deviations of those measured for marginal plants. The differences between the measured specific growth rates and those predicted from the SRP concentrations in the water were very much larger than the differences between the measured specific growth rates and those predicted from the total P concentrations in the water.

4.2.2.2.2 Hartbeespoort Dam (HD) site

Specific growth rates predicted for *E. crassipes* from the various P fractions in the water at the HD site in the Transvaal, where relative humidities (Table III, Appendix II) were much lower than at other sites occurring in the Durban area of Natal (Tables I to III, Appendix II), were generally not significantly different from those measured for marginal plants (Table 4.11). For example, of the 5 specific growth rates predicted from the SRP and total P concentrations in the water, 4 of each fell within the standard deviations of the measured values. The correlation factor (R^2) calculated between the measured specific growth rates and those predicted from the total P concentrations in the water, however, was not very high, $R^2 = 0,4805$. The differences between the measured specific growth rates and those predicted from the SRP concentrations in the water were generally only slightly smaller than the differences between the measured specific growth rates and those predicted from the total P concentrations in the water.

4.3 DISCUSSION

Although specific growth rates as high as 19,91% d^{-1} were measured for *E. crassipes* growing in secondary treated wastewater effluent in the maturation pond, where the levels of N and P in the water were extremely high, specific growth rates measured for *E. crassipes*, particularly plants of the marginal growth form, at most other field sites were in the range of specific growth rates (3,0 to 12,5% d^{-1}) reported by various authors (Seaman and Porterfield, 1964; Bock, 1969; Knipling et al., 1970; Morris, 1974; Boyd, 1976) for *E. crassipes* growing under subtropical to tropical climates in other parts of the world.

E. crassipes grew more rapidly in loosely crowded than in densely crowded field populations. Specific growth rates of marginal plants growing in loosely crowded field populations

ranged from 2 to 6 times higher than those of central plants growing under similar environmental conditions at the same sites in densely crowded field populations. The significantly lower specific growth rates measured for *E. crassipes* in densely crowded field populations may be partly related to the adverse effects of self shading and overcrowding by plants in such populations, as well as to the intrinsic morphological limitations of *E. crassipes* plants of the central growth form occurring in such populations. Center and Spencer (1981) have shown that the production of *E. crassipes* plants with elongate petioles (central plants) is responsible for a decline in the lamina area ratio (LAR), i.e. in relatively less photosynthetic area per unit of plant weight. The ratio of the lamina area to plant weight is similar to the leaf area ratio of other authors (e.g. Beever and Cooper, 1964; Radford, 1967). Consequently, if it is assumed that photosynthesis is proportional to the lamina area and respiration to weight, the LAR should be an index of the P/R ratio and an indicator of the growth potential, i.e. net photosynthesis. Under these assumptions central plants with elongate petioles should have the smallest potential for growth and consequently the lowest growth rate.

In the field, specific growth rates of marginal and central plants were related exponentially to the reciprocals of the Absolute mean daily air and water temperatures. Both marginal and central plants showed a proportionally larger increase in growth rate with a 10°C rise in the mean daily air temperature than with a similar increase in the water temperature. Higher Q_{10} values in the temperature range 15 to 25°C, for example, were calculated from the regressions (Arrhenius plots) relating the specific growth rates of marginal and central plants exponentially to the reciprocals of the Absolute mean daily air temperatures, than to the reciprocals of the Absolute water temperatures. This suggests that air temperatures have a greater effect on the specific growth rate of *E. crassipes* than the water temperatures. Although *E. crassipes* floats freely on the water surface with a submerged root system, its vegetative parts and inflorescence rise above the water surface. Because of its growth habit, it behaves more like a terrestrial than a

true water plant (Den Hartog and Segal, 1964). Consequently, its growth rate may be influenced to a greater extent by the air rather than the water temperature.

At the BGL site, where the N and P concentrations in the water were much lower than at the MP3 site, marginal plants, however, showed a proportionally larger increase in growth rate with a 10°C rise in the mean daily air and water temperatures than at the MP3 site. Higher Q_{10} values in the temperature range 15 to 25°C, for example, were calculated from the regressions relating the specific growth rates of marginal plants exponentially to the reciprocals of the Absolute mean daily air and water temperatures at the BGL site than at the MP3 site. This suggests that with increasing nutrient growth rate limitation, temperature exerts a greater influence on the specific growth rate of *E. crassipes*. This may place a constraint on the model developed as in principle temperature should exert a smaller influence on the specific growth rate with increasing nutrient growth rate limitation.

It should, however, be pointed out that recently it has been found that plant growth in the field can respond linearly rather than exponentially to environmental temperature which suggests that the asymmetric bell-shaped response to temperature may not be as widely applicable as was formerly considered. Gallagher and Biscoe (1979), for example, have demonstrated that, in the absence of water stress, the expansion rate of barley leaves is directly proportional to the temperature of the stem apex. It remains to be seen, however, what the theoretical interpretation of such linear responses can be.

The nutrient limiting the growth rate of *E. crassipes* at each field site was estimated from the average total N and total P concentrations in the water using the mean K_s concentrations determined for this plant, under N and P growth rate limitation respectively, in the Monod model. At the MP3, DC, IL and IC sites, where the N concentrations in the water were estimated to be growth rate limiting for *E. crassipes*, the average total

N/total P ratios in the water ranged well below the optimal N/P ratio of 30 (cell and medium) below which Rhee (1974, 1978) reported that N becomes limiting to the growth rate in the algae. At the BGL site, where the P concentrations in the water were estimated to be growth rate limiting for *E. crassipes*, the average total N/total P ratio in the water ranged well above the optimal N/P ratio of 30 (cell and medium) above which Rhee (1974, 1978) reported that P becomes limiting to the growth rate in the algae. At the HD site, however, where the P concentrations in the water were also estimated to be growth rate limiting for *E. crassipes*, the average total N/total P ratio in the water ranged below this optimal N/P ratio. The ratio of the mean K_s (K_{sn}/K_{sp}) concentrations determined for *E. crassipes*, under N and P growth rate limitation respectively, suggest an optimal N/P ratio in the water for *E. crassipes* of ca 10, i.e. below which the N and above which the P concentrations in the water become growth rate limiting for this plant. It should, however, be pointed out that, although the nutrient that limits the growth rate can often be indicated from the N/P ratio in the water, in many instances the growth rate of phytoplankton has been shown to be controlled by P even when the N/P ratio in the water is relatively low (Welch et al., 1978). Consequently, this may place a constraint on the method used to estimate which nutrient was growth rate limiting for *E. crassipes* in the field.

At the MP3 site, where the N and P concentrations in the water were very high, it was estimated from the K_s concentrations determined for *E. crassipes* under N and P growth rate limitation in culture, that *E. crassipes* would achieve ca 95,5 and 98,6% of its U_{max} at the average total N and total P concentrations in the water respectively at this site. It is evident, therefore, that, even though the N concentrations were estimated to be growth rate limiting for *E. crassipes* at this site, the N and P concentrations in the water approached those saturating to the growth rate of this plant. Consequently, one may assume that the specific growth rates measured for marginal and central plants at specific temperatures at the MP3 site

closely approximated the U_{max} 's of marginal and central plants respectively. The Q_{10} value of 2,14, in the temperature range 15 to 25°C calculated from the regression relating the specific growth rate (assumed U_{max} 's) of marginal plants at the MP3 site exponentially to the reciprocals of the Absolute mean daily air temperatures, compares favourably with the Q_{10} value of 2,12 reported by Goldman (1972) for the exponential relationship between the U_{max} 's of various species of fresh water algae and temperature, in the range 19 to 39°C. It also demonstrates that the effect of air temperature on the U_{max} of **E. crassipes** (marginal plants) follows the van't Hoff rule, and confirms the initial hypothesis made. The activation energy of 12,978 calories mole⁻¹ calculated for marginal plants from the above regression compares favourably with the activation energy of 13,356 calories mole⁻¹ reported by Goldman (1972) for the algae. Central plants growing in densely crowded populations at the MP3 site, however, showed a proportionally smaller increase in their specific growth rate (assumed U_{max}) with a 10°C rise in the mean daily air and water temperature than did marginal plants growing in loosely crowded populations at this site. A Q_{10} value of only 1,71, in the temperature range 15 to 25°C, was calculated from the regression relating the specific growth rates (assumed U_{max} 's) of central plants at the MP3 site exponentially to the reciprocals of the Absolute mean daily air temperatures. The lower Q_{10} value obtained for central plants may be attributed to their lower specific growth rate.

Specific growth rates predicted for **E. crassipes** from the total N and total P concentrations in the water at the MP3 and BGL sites respectively, using kinetic coefficients in the Monod model generated for this plant under N and P growth rate limitation in culture, followed a fairly similar seasonal pattern to those measured for marginal plants growing in loosely crowded populations at these 2 sites. This suggests that the mean K_s concentrations determined for **E. crassipes**, under N and P growth rate limitation respectively, were fairly reliable. In general, however, specific growth rates predicted for **E. crassipes** from the growth rate limiting N or P concen=

trations in the water at field sites in the Durban area were significantly lower than those measured for marginal plants, except during winter (May to August) when the specific growth rates predicted from the total N or total P concentrations in the water occasionally fell within the standard deviations of the measured values. The differences between the specific growth rates predicted from the total N or total P concentrations in the water and those measured for marginal plants were generally smaller than the differences between the measured specific growth rates and those predicted from the $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ or SRP concentrations in the water. This suggests that specific growth rates of *E. crassipes* in the field may be more accurately predicted from the total N or total P concentrations in the water than from the other N or P fractions in the water. At the MP3 and BGL sites, the smallest differences between the specific growth rates predicted from the total N and total P concentrations in the water respectively and those measured for marginal plants occurred during midwinter in June, when the diffuse radiant fluxes and relative humidities recorded at these sites were at their lowest levels. At the HD site in the Transvaal, where relative humidities were much lower than at sites in the Durban area of Natal, specific growth rates predicted for *E. crassipes* from the P concentrations in the water were not significantly different from those measured for marginal plants. These results suggest that the U_{max} values determined for *E. crassipes*, under N and P growth rate limitation respectively in culture, may have been limited by the light intensities and relative humidities in the greenhouse. This may explain why the specific growth rates predicted for *E. crassipes* from the total N or total P concentrations in the water were significantly lower than those measured for marginal plants except when the diffuse radiant fluxes and relative humidities in the field were low.

Relative humidities recorded in the greenhouse during the experimental determination of kinetic coefficients for *E. crassipes* were lower than those recorded during summer at field sites in the Durban area. Mean daily relative humidities in the

greenhouse ranged from 61 to 67%, whereas those recorded in the field ranged from 74 to 85%. As Freidel *et al.* (1978) have shown that the growth rate of *E. crassipes* in culture decreases with a decrease in the relative humidity, it would appear that the U_{max} values determined for this plant in culture were limited by the lower relative humidities in the greenhouse. Radiant flux densities, however, were not recorded in the greenhouse during the experimental determination of kinetic coefficients for *E. crassipes*. It is possible that these were also growth rate limiting for *E. crassipes*, particularly as it was pointed out in Chapter 2, 2.1.7 that the light intensities in the greenhouse were attenuated by *ca* 37%.

It appears unlikely that a correction factor could be introduced into the model to amend the U_{max} values generated for *E. crassipes* under the lower relative humidities and light intensities in the greenhouse, as the ratios between the measured and predicted specific growth rates varied considerably during the year. For example, at the MP3 site, the ratios between the specific growth rates measured for marginal plants and those predicted for *E. crassipes* from the total N concentrations in the water ranged from *ca* 2,5 during summer to *ca* 1,1 during winter. At the BGL site, the ratios between the measured specific growth rates and those predicted from the total P concentrations in the water ranged from *ca* 2,6 during summer to *ca* 1,1 during winter. Maximum specific growth rates, however, may be derived for *E. crassipes* from the field data. As pointed out, specific growth rates measured for *E. crassipes* at specific temperatures at the MP3 site, where the N and P concentrations in the water approached those saturating to the growth rate of this plant, may be assumed to closely approximate the U_{max} 's of *E. crassipes*. Consequently, it should be possible to use the specific growth rates (assumed U_{max} 's) measured for marginal plants at specific temperatures at the MP3 site in the Monod model, in place of those generated in culture, to predict specific growth rates of marginal plants from the growth rate limiting N or P concentrations in the water at other field sites.

In general, specific growth rates predicted for *E. crassipes* from the growth rate limiting N concentrations in the water at the MP3 and DC sites were significantly higher than those measured for central plants growing in densely crowded populations at these 2 sites. This may be attributed to the fact that the specific growth rate of *E. crassipes* was detrimentally affected in densely crowded field populations and suggests that, unless a correction factor is introduced into the model to amend the U_{max} for the density of the plant population, the U_{max} values generated for marginal plants in culture cannot be used in the Monod model to predict specific growth rates of central plants growing in densely crowded field populations. In general, however, specific growth rates predicted for *E. crassipes* from the total N concentrations in the water ranged between those measured for marginal and central plants respectively. As De Busk et al. (1981) have recently shown that the specific growth rate of *E. crassipes* in the field decreases, more or less linearly, with an increase in the density of the plant population, it is possible that the U_{max} values generated for *E. crassipes* in culture may be used in the Monod model to predict specific growth rates of *E. crassipes* growing in populations of intermediate density in the field. It should, however, also be possible to use the specific growth rates (assumed U_{max} 's) measured for central plants at specific temperatures at the MP3 site in the Monod model to predict specific growth rates of central plants growing in densely crowded populations at other field sites.

No significance was attached to the observation that the specific growth rates of central plants at the DC site were fairly accurately predicted from the NH_4-N concentrations in the water or to the observation that the differences between the specific growth rates predicted for *E. crassipes* from the NH_4-N and NO_3-N concentrations in the water at the MP3 and DC sites and those measured for central plants were generally smaller than the differences between the measured specific growth rates and those predicted from the total N concentrations in the water.

4.4 CONCLUSIONS

It is concluded that the specific growth rate of *E. crassipes* in the field is related exponentially to the reciprocals of the Absolute mean daily air and water temperatures and is detrimentally affected in densely crowded field populations. Air temperatures appear to have a greater effect on the specific growth rate of *E. crassipes*, both plants of the marginal and central growth form, than do the water temperatures. At field sites where the N and P concentrations in the water approach those saturating to the growth rate of *E. crassipes* and the specific growth rate of *E. crassipes* may be assumed to closely approximate its maximum specific growth rate (U_{max}), specific growth rates (assumed U_{max} 's) of marginal plants follow the van't Hoff rule, i.e. they approximately double with a 10°C rise in the mean daily air temperature. This confirms the initial hypothesis made. Central plants, however, show a proportionally smaller increase in their assumed U_{max} with a 10°C rise in the mean daily air or water temperatures than do marginal plants.

Using kinetic coefficients generated for *E. crassipes* under N and P growth rate limitation in culture in the Monod model, specific growth rates of marginal plants, growing in loosely crowded field populations, are not accurately predicted from the growth rate limiting N or P concentrations in the water, except when the diffuse radiant fluxes and/or relative humidities in the field are low. In the latter case, specific growth rates of marginal plants are fairly accurately predicted from the total N or total P concentrations in the water. It is suggested that the U_{max} values determined for *E. crassipes*, under N and P growth rate limitation respectively, were limited by the lower light intensities and relative humidities in the greenhouse. No correction factor, however, can be introduced into the model to amend the U_{max} values generated for *E. crassipes* under the lower light intensities and relative humidities in the greenhouse, as the ratios between the measured and predicted specific growth rates in the field varied considerably during the year.

Specific growth rates of central plants, growing in densely crowded field populations, are also not accurately predicted from the growth rate limiting nutrient concentrations in the water using kinetic coefficients in the Monod model generated for *E. crassipes* in culture. Predicted specific growth rates were significantly higher than the measured specific growth rates. This may be attributed to the fact that the specific growth rate of *E. crassipes* is detrimentally affected in densely crowded field populations and suggests that, unless a correction factor is introduced into the model to amend the U_{max} for the density of the plant population, the U_{max} values generated for marginal plants in culture cannot be used in the Monod model to predict specific growth rates for central plants growing in densely crowded field populations. As the specific growth rates predicted for *E. crassipes* generally ranged between those measured for marginal and central plants respectively, it may be possible to use the U_{max} values generated for *E. crassipes* in culture to predict specific growth rates for this plant growing in populations of intermediate density in the field.

Finally, it is suggested that U_{max} 's may be derived for marginal and central plants respectively at one field site where the N and P concentrations in the water were high and approached those saturating to the growth rate of *E. crassipes*. Consequently, it should be possible to use these assumed U_{max} 's of marginal and central plants, derived from the field, in the Monod model, in place of those generated in culture, to predict specific growth rates of marginal and central plants respectively, from the growth rate limiting N or P concentrations in the water, at other field sites. This may improve the predictive ability of the model.

CHAPTER 5

REFINING THE MODEL UNDER FIELD CONDITIONS

5.1 GENERATING U MAX IN THE FIELD

5.1.1 Introduction

As pointed out in Chapter 4, 4.3, the N and P concentrations in the water at the MP3 site approached those saturating to the growth rate of *E. crassipes*. Consequently, one may assume that the specific growth rates measured for marginal and central plants at specific temperatures at this site closely approximated the U_{max}'s of marginal and central plants respectively.

The regression equation relating the specific growth rates (assumed U_{max}'s) of marginal plants exponentially to the reciprocals of the Absolute mean daily air temperatures at the MP3 site (Figure 4.4) was as follows:

$$U = 5,2631 \times 10^8 e^{-\frac{6540}{T}}$$

where U = specific growth rate (assumed maximum specific growth rate) g fresh mass g⁻¹ d⁻¹,

T = Absolute mean daily temperature °K.

The regression equation relating the specific growth rates (assumed U_{max}'s) of central plants exponentially to the reciprocals of the Absolute mean daily air temperatures at the MP3 site (Figure 4.8) was as follows:

$$U = 1,9932 \times 10^5 e^{-\frac{4661}{T}}$$

where U = specific growth rate (assumed maximum specific growth rate) $g \text{ fresh mass } g^{-1} d^{-1}$,
 T = Absolute mean daily temperature $^{\circ}K$.

Using the above regression equations, it should, therefore, be possible to predict U_{max} 's for marginal and central plants, growing in loosely crowded and densely crowded populations respectively, at other sites in the field from the mean daily air temperatures.

5.1.2 Results

5.1.2.1 Comparison of predicted and measured specific growth rates

Specific growth rates were measured for central plants at the MP3 and DC site. At the latter site, however, a few measurements only were obtained. Consequently, no reliable comparisons could be made between specific growth rates measured for central plants at the DC site and those predicted from the growth rate limiting N concentrations in the water using U_{max} values, derived for central plants at the MP3 site, in the Monod model.

Maximum specific growth rates (U_{max}), however, were predicted for marginal plants from the mean daily air temperatures, over each growing interval, at the BGL, HD, IL, IC and DC sites. The regression equation relating the assumed U_{max} 's of marginal plants in the field exponentially to the reciprocals of the Absolute mean daily air temperatures was used. This may be illustrated by the following example:

The mean daily air temperature recorded at the BGL site, over the growing interval 1/2 to 15/2/78 was $24,9^{\circ}C$ (Table II, Appendix II). The U_{max} predicted for marginal plants for this temperature was calculated as follows:

$$\begin{aligned}
 U_{\max} &= 5,2631 \times 10^8 e^{-\frac{6540}{273,2 + 24,9}} \\
 &= 0,1560 \text{ g fresh mass g}^{-1} \text{ d}^{-1}.
 \end{aligned}$$

Using the U_{\max} values predicted for marginal plants from the mean daily air temperatures at the 5 mentioned sites and the mean K_s concentrations of 976 ug N l^{-1} and $94,1 \text{ ug P l}^{-1}$, determined for *E. crassipes* under N and P growth rate limitation in culture, in the Monod model, specific growth rates (U) were predicted for marginal plants, over each growing interval, at each of these sites from the growth rate limiting N or P concentrations in the water. Predicted specific growth rates were compared with those measured for marginal plants, growing in loosely crowded field populations, at each of these sites.

At the BGL and HD sites, specific growth rates were predicted for marginal plants from the P (SRP and total P) concentrations in the water, whereas at the IL, IC and DC sites, they were predicted from the N ($\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and total N) concentrations in the water. As shown (Table 4.4), the P concentrations in the water were estimated to be growth rate limiting for *E. crassipes* at the BGL and HD sites, whereas the N concentrations in the water were estimated to be growth rate limiting for this plant at the IL, IC and DC sites.

5.1.2.1.1 Botanic Gardens Lake (BGL) site

Specific growth rates predicted for marginal plants from the total P concentrations in the water at the BGL site generally followed the same seasonal pattern as the measured specific growth rates (Figure 5.1), with values decreasing progressively after summer (September to March) through to winter (May to August). Of the 22 specific growth rates predicted from the total P concentrations in the water, 14 (ca 64%) fell within the standard deviation of the measured specific growth rates (Figure 5.1, Table 5.1). The correlation factor (R^2) calculated between the measured specific growth rates and those

predicted from the total P concentrations in the water, however, was not very high ($R^2 = 0,5321$). In general, the largest differences between the measured specific growth rates and those predicted from the total P concentrations in the water occurred during the summer months when the diffuse radiant fluxes recorded at this site were high (Table II, Appendix II). Specific growth rates predicted from the SRP concentrations in the water were extremely variable and did not follow any recognizable seasonal pattern. Only 1 of the specific growth rates predicted from the SRP concentrations in the water fell within the standard deviations of the measured specific growth rates. The differences between the measured specific growth rates and those predicted from the SRP concentrations in the water were very much larger than the differences between the measured specific growth rates and those predicted from the total P concentrations in the water.

5.1.2.1.2 Hartbeespoort Dam (HD) site

Specific growth rates predicted for marginal plants from the various P fractions in the water at the HD site were generally significantly higher than the measured specific growth rates (Table 5.2). For example, of the 5 specific growth rates predicted from the SRP concentrations in the water, only 2 fell within the standard deviations of the measured specific growth rates. In the latter case, however, the differences between the predicted and measured values were fairly large. Only 1 of the specific growth rates predicted from the total P concentrations in the water fell within the standard deviations of the measured specific growth rates. The differences between the measured specific growth rates and those predicted from the total P concentrations in the water were not very much different from the differences between the measured specific growth rates and those predicted from the SRP concentrations in the water.

5.1.2.1.3 Isipingo Lake (IL), Discharge Canal (DC) and Isipingo Canal (IC) sites

Specific growth rates predicted for marginal plants from the total N concentrations in the water at the IL and DC sites (Table 5.3) were generally not significantly different from the measured specific growth rates. For example, of the 7 specific growth rates predicted from the total N concentrations in the water at both sites, 5 fell within the standard deviations of the measured specific growth rates. The correlation factor, (R^2) calculated between the measured specific growth rates and those predicted from the total N concentrations in the water for both sites, however, was low, ($R^2 = 0,2547$). Only 4 of the specific growth rates predicted from the $\text{NH}_4\text{-N}$ concentrations in the water and 3 of those predicted from the $\text{NO}_3\text{-N}$ concentrations in the water at these 2 sites fell within the standard deviations of the measured specific growth rates. The differences between the measured specific growth rates and those predicted from the $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations in the water at the IL and DC sites respectively, were generally much larger than the differences between the measured specific growth rates and those predicted from the total N concentration in the water. The differences between the measured specific growth rates and those predicted from the $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations in the water at the IL and DC sites respectively, however, were not very much different from the differences between the measured specific growth rates and those predicted from the total N concentrations in the water.

Specific growth rates predicted for marginal plants from the various N fractions in the water at the IC site were all significantly lower than the measured specific growth rates (Table 5.3). None of the specific growth rates predicted from the $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ or total N concentrations in the water fell within the standard deviations of the measured specific growth rates.

5.1.3 Discussion

Specific growth rates of marginal plants growing in loosely crowded populations at the BGL, IL and DC sites were fairly accurately predicted from the growth rate limiting total N or P concentrations in the water using U_{max} values corrected for the mean daily air temperature, derived for marginal plants in the field, and K_s concentrations generated for *E. crassipes* under N and P growth rate limitation in culture in the Monod model. Approximately .65% of the specific growth rates predicted from the total N or total P concentrations in the water at all 3 sites fell within the standard deviations of the measured specific growth rates. The correlation factor (R^2) calculated between the measured specific growth rates and those predicted from the total N or total P concentrations in the water for all 3 sites, however, was not very high, $R^2 = 0,4478$. Consequently, the degree of similarity between the predicted and measured values is uncertain.

At the BGL site, the largest differences between the measured specific growth rates and those predicted from the total P concentrations in the water generally occurred during the summer months (September to March), when the diffuse radiant fluxes recorded at this site were high. This suggests that if the U_{max} 's derived for marginal plants in the field are also corrected for the radiant flux density, it may improve the predictive ability of the model.

In general, specific growth rates of marginal plants at the 3 above mentioned sites were more accurately predicted from the total N or total P concentrations in the water than from the other N or P fractions in the water. This suggests that *E. crassipes* may utilize both inorganic and organic forms of N and P for growth.

With respect to N, Sculthorpe (1967) suggested that NH_4-N does not serve as a N source for growth of aquatic plants, however, several authors (Von Schwoerbel and Tillmans, 1972; Toetz,

1971; 1973) have subsequently shown a preference by aquatic plants for $\text{NH}_4\text{-N}$ as a N source for growth. Best (1980) demonstrated that, although $\text{NH}_4\text{-N}$ supplied at low concentrations for a short period stimulated the growth of *Ceratophyllum demersum* L. in culture, higher concentrations (in excess of $45 \times 10^3 \text{ ug NH}_4\text{-N l}^{-1}$) applied for a prolonged period were toxic. The ammonium induced inhibition of growth has also been reported for several other aquatic macrophytes by Mulligan et al. (1976). High $\text{NH}_4\text{-N}$ concentrations in the water may repress the nitrate induced formation of nitrate reductase as shown by Orebamjo and Stewart (1975a, 1975b) in *Lemna minor* L. A reduced nitrate reductase activity would mean a reduced N uptake and consequently a reduced growth rate.

With respect to P, Jeschke and Simonis (1965) reported that the main source of P for growth of aquatic plants is in the form of inorganic phosphate. However, as the specific growth rates of marginal plants at the BGL site were more accurately predicted from the total P than from the SRP concentrations in the water, it is suggested that the total P concentrations in the water at this site possibly reflected the total amount of P available to plants during growth, some of the P for growth of plants possibly being provided by release of that bound to the sediments as well as to other soluble and insoluble fractions in the water. For example, when P is added to lakes, it is rapidly removed from solution by adsorption onto the sediments (Hepher, 1958; Hayes and Phillips, 1968). This P is not rendered entirely unavailable since sediment P and dissolved P exist in equilibrium (Hepher, 1958; Pomeroy et al., 1965). The equilibrium concentration increases with increased P content in the sediment (Pomeroy et al., 1965). Consequently, removal of P from the water by *E. crassipes* during growth would displace the P equilibrium allowing additional P to be released from the sediments into the water. It should, however, be pointed out that many zooplankton and phytoplankton species excrete alkaline phosphatases which may accelerate phytoplankton growth by supplying orthophosphate from suitable organic esters (Berman, 1969; 1970; Jansson, 1976; Wynne and Gophen, 1981).

The excretion of dissolved organic compounds by higher aquatic plants has been reported by Wetzel (1969a, 1969b). Consequently, it is possible that higher aquatic plants such as *E. crassipes* may excrete alkaline phosphatases which would allow them to utilize normally unavailable organic forms of P for growth.

In general, specific growth rates of marginal plants at the IC and HD sites were not accurately predicted from the growth rate limiting N or P concentrations in the water using U_{max} values corrected for the mean daily air temperature, derived for marginal plants in the field, and K_s concentrations generated for *E. crassipes* under N and P growth rate limitation in culture in the Monod model.

At the IC site, the predicted specific growth rates were significantly lower than the measured specific growth rates. It is suggested that the quantities of N and P available to plants for growth at this site may have been much larger than actually determined from the N and P concentrations in the water. Periodic discharges of industrial effluent into the Isipingo Canal during growth of plants at this site may partly explain this.

At the HD site, on the other hand, the predicted specific growth rates were generally significantly higher than the measured specific growth rates. A possible explanation may lie in the fact that the U_{max} values used in the Monod model were derived for marginal plants at the MP3 site in the Durban area of Natal, where relative humidities were much higher than at the HD site in the Transvaal. As Freidel et al. (1978) have shown that the growth rate of *E. crassipes* decreases with a decrease in the relative humidity, it is evident that the U_{max} 's predicted for marginal plants from the mean daily air temperatures at the HD site, using the regression equation relating the assumed U_{max} 's of marginal plants at the MP3 site exponentially to the reciprocals of the Absolute mean daily air temperatures, were too high. Consequently, when these were

incorporated into the Monod model they overpredicted the specific growth rates of marginal plants at the HD site. This suggests that if the U_{max} 's derived for marginal plants in the field are also corrected for the relative humidity, it may improve the predictive ability of the model.

5.1.4 Conclusions

It is concluded that specific growth rates of marginal plants growing in loosely crowded field populations are fairly accurately predicted from the growth rate limiting total N or total P concentrations in the water using U_{max} values corrected for the mean daily air temperature, derived for marginal plants in the field, and K_s concentrations generated for *E. crassipes* under N and P growth rate limitation in culture in the Monod model. The largest differences between the predicted and measured specific growth rates occur during summer (September to March), when the diffuse radiant fluxes in the field are high. It is suggested that if the U_{max} 's derived for marginal plants in the field are also corrected for the radiant flux density, it may improve the predictive ability of the model.

In general, specific growth rates of marginal plants are more accurately predicted from the total N or total P concentrations in the water than from the other N or P fractions in the water. This suggests that *E. crassipes* may utilize both inorganic and organic forms of N and P for growth.

5.2 CORRECTION OF U_{MAX} FOR RADIANT FLUX DENSITY

5.2.1 Introduction

The growth responses of nutrient satiated algal cells is a complex function of temperature, light intensity and an interaction between the two. In general, growth rate increases exponentially with temperature up to an optimum temperature ($T_{opt.}$) and then declines rapidly as temperature exceeds this optimum (Sorokin and Krauss, 1962; Smayda, 1969; Eppley, 1972;

Park *et al.*, 1975). Similar responses to temperature are found among higher plants (Pisek *et al.*, 1973; Cooper, 1973; Sutcliffe, 1977). Growth rate also increases hyperbolically with increasing irradiance up to an optimum or saturating light intensity (I_{opt}). As irradiance exceeds this optimum, the growth rate either plateaus or declines (Maddux and Jones, 1964; Harris and Lott, 1973) depending upon the proximity of temperature to T_{opt} . At low temperature, algal growth is inhibited by high light intensities (Sorokin, 1960; Sorokin and Krauss, 1962; Smayda, 1969), but as temperature increases a higher light intensity is required for optimum growth rate (Sorokin, 1960; Smayda, 1969). The interaction between temperature and light intensity has also been reported in higher plants. Pisek *et al.* (1973), for example, showed that in alpine species increasing the light intensity results in an increase in the rate of net photosynthesis and an upward shift in the optimum temperature range. Billings (1974) reported that photosynthesis in arctic and alpine species is saturated by lower light intensities at low temperatures than at higher.

Steele (1965) proposed an empirical relationship between growth rate and irradiance defined as:

$$U = U_{max} \cdot I/I_{opt} \cdot \exp. (1 - I/I_{opt}.)$$

where: U is the observed specific growth rate at light intensity I ; U_{max} is the maximum specific growth rate; I_{opt} is the light intensity at which $U = U_{max}$. Assuming that the 2 parameters U_{max} and I_{opt} both vary with temperature, the above model describes the complex response to irradiance and temperature outlined. Cloern (1977) in a study of the kinetics of light intensity and temperature on the growth rate of *Cryptomonas ovata*, however, found that the above model did not give accurate estimates of the growth rate over a wide range of temperatures and irradiances.

Several environmental modellers (Di Toro *et al.*, 1971; Chen and Orlob, 1972; Park *et al.*, 1975; Kiefer and Enns, 1976) have

used a multiplication of independent light and temperature functions in phytoplankton population/productivity models, in many instances without experimental evidence. Rodhe (1948, 1978) suggested that the combined effects of factors such as temperature, light and daylength on the population dynamics of phytoplankton may be more important than the effect of any single one. In view of this, 2 hypothetical multiplicative expressions were investigated in this study for correcting the U_{max} of *E. crassipes* in the field for the radiant flux density:

The first assumed that the effect of the radiant flux density on the U_{max} of *E. crassipes* could be considered independent of the effect of temperature. Consequently, if the U_{max} 's of *E. crassipes* in the field were limited by both the temperature and radiant flux density, they may be corrected for both using the following expression:

$$U_{max} (I \text{ opt.}) = A e^{-\frac{E}{RT}} \cdot \frac{K_I + I}{I} \dots\dots\dots (i)$$

where $U_{max} (I \text{ opt.})$ = maximum specific growth rate at an optimum or saturating radiant flux density
g fresh mass $g^{-1} d^{-1}$,

I = radiant flux density (diffuse component of the radiant flux) megajoules $m^{-2} hr^{-1}$,

K_I = half saturation coefficient = I when U_{max} is $0,5 U_{max}(I \text{ opt.})$,

T = Absolute mean daily air temperature $^{\circ}K$,

A = constant day^{-1} ,

E = activation energy $cal. mole^{-1}$,

R = universal gas constant $cal. mole^{-1} ^{\circ}K^{-1}$.

The second assumed that the effect of the radiant flux density on the U_{max} of *E. crassipes* could not be considered independent of the effect of temperature because of the strong interaction between temperature and light intensity on the growth rate.

Consequently, the U_{max} 's of *E. crassipes* in the field may be corrected for the temperature and radiant flux density using the following expression:

$$U_{max} = Ae^{-\frac{E}{R(T \times I)}} \dots\dots\dots (ii)$$

- where U_{max} = maximum specific growth rate g fresh mass $g^{-1} d^{-1}$,
 T = Absolute mean daily air temperature $^{\circ}K$,
 I = radiant flux density (diffuse component of the radiant flux) megajoules $m^{-2} hr^{-1}$,
 A = constant day^{-1} ,
 E = activation energy cal. $mole^{-1}$,
 R = universal gas constant cal. $mole^{-1} ^{\circ}K$.

5.2.2 Results

5.2.2.1 Field data

The diffuse radiant fluxes and the daily photoperiods recorded over each growing interval of marginal plants at 6 field sites during 1977 and 1978 are summarized in Tables I to III, Appendix II. To account for variations in the length of the daily photoperiod (total hours of diffuse radiation per day) during the year, the diffuse radiant fluxes are expressed in these tables as an hourly average over each growing interval of plants.

The diffuse radiant fluxes recorded over each growing interval of marginal plants at the MP3 and BGL sites are graphically illustrated in Figure 5.2. At both sites, the diffuse radiant fluxes followed a similar seasonal pattern to the specific growth rates measured for marginal plants at these 2 sites (Figure 4.1). Diffuse radiant fluxes at the MP3 and BGL sites ranged from 0,7215 megajoules $m^{-2} hr^{-1}$ during summer to 0,3024 megajoules $m^{-2} hr^{-1}$ during winter.

The effect of the radiant flux density (diffuse component of the radiant flux) on the specific growth rates (assumed U_{max} 's) of marginal plants at the MP3 site was investigated by first normalizing the specific growth rates measured for marginal plants at different air temperatures at this site to one temperature, 15°C (Table 5.4). Specific growth rates (U) were normalized to 15°C ($U_{15°C}$) using the regression equation relating the specific growth rates (assumed U_{max} 's) of marginal plants at the MP3 site exponentially to the reciprocals of the Absolute mean daily air temperatures. The following formula was used:

$$\text{Normalized } U_{15°C} = \frac{\text{Predicted } U \text{ at } t°C}{\text{Predicted } U \text{ at } 15°C} \times \text{Measured } U \text{ at } t°C$$

This may be illustrated by the following example: The specific growth rate measured for marginal plants at the MP3 site over the growing interval 1/2 to 16/2/78, at a mean daily air temperature of 24,9°C, was 0,1498 g fresh mass $g^{-1} d^{-1}$ (Table I, Appendix II). This specific growth rate (U) was normalized to 15°C ($U_{15°C}$) as follows:

$$U_{15°C} = \frac{5,2631 \times 10^8 e^{-\frac{6540}{273,2 + 24,9}}}{5,2631 \times 10^8 e^{-\frac{6540}{273,2 + 15,0}}} \times 0,1498$$

$$= 0,0704 \text{ g fresh mass } g^{-1} d^{-1}.$$

A Lineweaver - Burk plot of the reciprocals of the specific growth rates (assumed U_{max} 's) of marginal plants normalized to 15°C ($1/U_{15°C}$) against the reciprocals of the diffuse radiant fluxes ($1/DRF$) at the MP3 site, over the period February to December, 1978 (Figure 5.3), yielded a linear relationship with the correlation coefficient ($r = 0,4230$) being significant at $P = 0,05$.

The intercept on the y axis yielded a maximum specific growth rate at an optimum diffuse radiant flux (U_{max} I opt.) at 15°C of 0,0967 g fresh mass $g^{-1} d^{-1}$. The intercept on the x axis yielded a half saturation coefficient (K_I) of 0,1464 megajoules $m^{-2} hr^{-1}$. The fiducial band limits (95% confidence limits) to the regression line, however, do not indicate a meaningful relationship between the normalized specific growth rates (assumed U_{max} 's) of marginal plants and the diffuse radiant fluxes. Consequently, the half saturation coefficient (K_I) for the diffuse radiant flux derived was considered of little value in the first multiplicative expression proposed for correcting the U_{max} of *E. crassipes* in the field for the radiant flux density.

An Arrhenius plot of the specific growth rates (assumed U_{max} 's) of marginal plants, expressed as a natural logarithm (Log_e), against the products of the reciprocals of the Absolute mean daily air temperatures and diffuse radiant fluxes at the MP3 site, over the period February to December, 1978 (Figure 5.4), yielded a linear relationship with the correlation coefficient ($r = 0,8329$) being high and significant at $P = 0,001$. The regression equation obtained for the above exponential relationship was as follows:

$$U = 0,2574 e^{-\frac{107}{T \times DRF}}$$

where U = specific growth rate (assumed maximum specific growth rate) g fresh mass $g^{-1} d^{-1}$,
 T = Absolute mean daily air temperature °K,
 DRF = diffuse component of the radiant flux megajoules $m^{-2} hr^{-1}$.

5.2.2.2 Comparison of predicted and measured specific growth rates

Maximum specific growth rates (U_{max}) were predicted for marginal plants from the mean daily air temperatures and diffuse

radiant fluxes, over each growing interval, at the BGL, HD, IL, IC and DC sites. The regression equation relating the assumed U_{max} 's of marginal plants in the field exponentially to the products of the reciprocals of the Absolute mean daily air temperatures and the diffuse radiant fluxes was used. This may be illustrated by the following example:

The mean daily air temperature and diffuse radiant flux recorded at the BGL site over the growing interval 1/2 to 15/2/78 were 24,9°C and 0,6127 megajoules $m^{-2} hr^{-1}$ respectively (Table II, Appendix II). The U_{max} predicted for marginal plants for this temperature and diffuse radiant flux was calculated as follows:

$$U_{max} = 0,2574 e^{-\frac{107}{(273,2 + 24,9) \times 0,6127}}$$

$$= 0,1433 \text{ g fresh mass } g^{-1} d^{-1}.$$

Using the U_{max} values predicted for marginal plants from the mean daily air temperatures and diffuse radiant fluxes at the 5 mentioned sites and the mean K_s concentrations of 976 $\mu g N l^{-1}$ and 94,1 $\mu g P l^{-1}$, determined for *E. crassipes* under N and P growth rate limitation in culture, in the Monod model, specific growth rates (U) were predicted for marginal plants, over each growing interval, at each of these sites from the growth rate limiting N or P concentrations in the water. Predicted specific growth rates were compared with those measured for marginal plants, growing in loosely crowded field populations, at each of these sites.

5.2.2.2.1 Botanic Gardens Lake (BGL) site

Specific growth rates predicted for marginal plants from the total P concentrations in the water at the BGL site closely followed the same seasonal pattern as the measured specific growth rates (Figure 5.5). Of the 22 specific growth rates predicted from the total P concentrations in the water, 15

(ca 68%) fell within the standard deviations of the measured specific growth rates (Figure 5.5, Table 5.5). The correlation factor (R^2) calculated between the measured specific growth rates and those predicted from the total P concentrations in the water was fairly high ($R^2 = 0,7973$). The largest differences between the measured specific growth rates and those predicted from the total P concentrations in the water occurred during the midsummer months (November to February), when the relative humidities recorded at this site were high (Table II, Appendix II). Specific growth rates predicted from the SRP concentrations in the water were extremely variable and did not follow any recognizable seasonal pattern. Only 1 of the specific growth rates predicted from the SRP concentrations in the water fell within the standard deviations of the measured specific growth rates. The differences between the measured specific growth rates and those predicted from the SRP concentrations in the water were very much larger than the differences between the measured specific growth rates and those predicted from the total P concentrations in the water.

5.2.2.2.2 Hartbeespoort Dam (HD) site

Specific growth rates predicted for marginal plants from the various P fractions in the water at the HD site were generally significantly higher than the measured specific growth rates (Table 5.6). For example, of the 5 specific growth rates predicted from the SRP and total P concentrations in the water, only 1 of each fell within the standard deviations of the measured specific growth rates. In the latter case, however, the differences between the predicted and measured values were fairly large. The differences between the measured specific growth rates and those predicted from the total P concentrations in the water were not very much different from the differences between the measured specific growth rates and those predicted from the SRP concentrations in the water.

5.2.2.2.3 Isipingo Lake (IL), Discharge Canal (DC) and Isipingo Canal (IC) sites

Specific growth rates predicted for marginal plants from the total N concentrations in the water at the IL and DC sites (Table 5.7) were generally not significantly different from the measured specific growth rates. For example, of the 7 specific growth rates predicted from the total N concentrations in the water at both sites, 6 fell within the standard deviations of the measured specific growth rates. The correlation factor (R^2) calculated between the measured specific growth rates and those predicted from the total N concentrations in the water for both sites, however, was low ($R^2 = 0,2807$). Five of the specific growth rates predicted from the $\text{NH}_4\text{-N}$ concentrations in the water and 3 of those predicted from the $\text{NO}_3\text{-N}$ concentrations in the water at these 2 sites fell within the standard deviations of the measured specific growth rates. The differences between the measured specific growth rates and those predicted from the $\text{NO}_3\text{-N}$ concentrations in the water at the IL site were much larger than the differences between the measured specific growth rates and those predicted from the total N concentrations in the water. The differences between the measured specific growth rates and those predicted from the $\text{NH}_4\text{-N}$ concentrations in the water at the IL site and from the $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations in the water at the DC site, however, were generally not very much different from the differences between the measured specific growth rates and those predicted from the total N concentrations in the water.

Specific growth rates predicted for marginal plants from the various N fractions in the water at the IC site were all significantly lower than the measured specific growth rates (Table 5.7). None of the specific growth rates predicted from the $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ or total N concentrations in the water fell within the standard deviations of the measured specific growth rates.

5.2.3 Discussion

The Lineweaver - Burk plot of the assumed U_{max} 's of marginal plants, normalized to a temperature of 15°C, against the diffused radiant fluxes at the MP3 site suggested that the U_{max} 's of marginal plants in the field were influenced by the radiant flux density. As pointed out in the results, however, the K_I for the diffuse radiant flux derived from the above relationship was considered of little value in the first multiplicative expression proposed for correcting the U_{max} of *E. crassipes* in the field for the radiant flux density.

The Arrhenius plot of the assumed U_{max} 's of marginal plants against the products of the reciprocals of the Absolute mean daily air temperatures and diffuse radiant fluxes at the MP3 site, on the other hand, showed that the U_{max} 's of marginal plants in the field could be related exponentially to the products of the reciprocals of these 2 factors. In fact, a higher correlation coefficient was obtained from the regression relating the assumed U_{max} 's of marginal plants in the field exponentially to the products of the reciprocals of the Absolute mean daily air temperatures and diffuse radiant fluxes (Figure 5.4) than to the reciprocals of the Absolute mean daily air temperatures only (Figure 4.4).

In general, specific growth rates of marginal plants growing in loosely crowded populations at the BGL, IL and DC sites were more accurately predicted from the growth rate limiting total N or total P concentrations in the water using U_{max} values, in the Monod model, corrected for the mean daily air temperature and diffuse radiant flux in the field, than for the mean daily air temperature only. In the former case ca 72% of the specific growth rates predicted from the total N or total P concentrations in the water at all 3 sites fell within the standard deviations of the measured specific growth rates compared with ca 65% in the latter. The correlation factor (R^2) calculated between the measured specific growth rates and those predicted from the total N or total P concentrations in

the water at all 3 sites was also higher in the former case ($R^2 = 0,5972$) than in the latter ($R^2 = 0,4478$). In addition, the differences between the predicted and measured specific growth rates at the 3 sites were generally much smaller in the former case than in the latter (Table 5.8). In both cases, specific growth rates of marginal plants were more accurately predicted from the total N or total P concentrations in the water than from the other N or P fractions in the water.

At the BGL site, the largest differences between the measured specific growth rates and those predicted from the total P concentrations in the water occurred during the midsummer months (November to February), when the relative humidities recorded at this site were at their highest levels. This suggests that if the U_{max} 's derived for marginal plants in the field are also corrected for the relative humidity, it may improve the predictive ability of the model.

In general, specific growth rates of marginal plants at the IC and HD sites were not accurately predicted from the growth rate limiting N or P concentrations in the water using U_{max} values, in the Monod model, corrected for the mean daily air temperature and diffuse radiant flux in the field. Similar reasons as given in section 5.1.3 may partly explain this.

5.2.4 Conclusions

It is concluded that maximum specific growth rates (U_{max}) derived for marginal plants in the field can be related exponentially to the products of the reciprocals of the Absolute mean daily air temperatures and diffuse radiant fluxes.

Specific growth rates of marginal plants growing in loosely crowded field populations are more accurately predicted from the growth rate limiting total N or total P concentrations in the water using U_{max} values, in the Monod model, corrected for the mean daily air temperature and diffuse radiant flux in the field, than for the mean daily air temperature only. In both

cases, specific growth rates of marginal plants are more accurately predicted from the total N or total P concentrations in the water than from the other N or P fractions in the water. The largest differences between the predicted and measured specific growth rates occur during midsummer (November to February) when the relative humidities in the field are at their highest levels. It is suggested that if the U_{max} 's derived for marginal plants in the field are also corrected for the relative humidity, it may improve the predictive ability of the model.

5.3 CORRECTION OF U_{MAX} FOR RELATIVE HUMIDITY

5.3.1 Introduction

Freidel et al. (1978) showed that the growth rate of *E. crassipes* in culture increases with an increase in the relative humidity, however, the manner in which the relative humidity influences the growth rate of *E. crassipes* is not known. The relative humidity may influence the transpiration rate of plants which in turn may influence the hydration or water potential (Slayter, 1967; Meidner and Sheriff, 1976) of the leaf cells. Cell and leaf growth are highly sensitive to a reduced water potential, particularly as cell expansion is caused by the action of turgor pressure upon "softened" cell walls (Greacen and Oh, 1972). Slavik (1963) reported that the intensity of photosynthesis and respiration is related directly to differences in hydration of leaf cells. A diminished metabolic activity resulting from a reduction in the water potential of the leaf cells would, therefore, mean a diminished growth rate. In fact, Hsiao et al. (1976) show that even mild water stress in mesophytic leaves, i.e. where the water potential of the leaf cells is reduced by only a few bars, can result in a reduction in the growth rate and the disruption of several metabolic processes, including protein and chlorophyll biosyntheses.

The rate of transpiration, on the other hand, is a function of the vapour pressure deficit or evaporating power of the atmosphere which fluctuates as a result of humidity and temperature changes (Crafts et al., 1949). Coupled with this, is the influence of light intensity on transpiration. This may act directly to increase the temperature of leaves above that of the atmosphere or physiologically to alter the permeability of the protoplasm or the movement of stomata (Crafts et al., 1949). Transpiration, however, is also subject to the effects of structural and functional features peculiar to the plant so that evaporation rates cannot be assumed to indicate transpiration rates although the two curves correspond closely under certain conditions (Briggs and Shantz, 1916). The interaction between temperature, light intensity and relative humidity in influencing the transpiration rate and consequently the growth rate suggests that the effect of the relative humidity on the growth rate of *E. crassipes* cannot be considered independent of the temperature and light intensity. In view of this, the following hypothetical multiplicative expression was investigated in this study for correcting the U_{max} of *E. crassipes* in the field for the temperature, radiant flux density and relative humidity:

$$U_{max} = Ae^{-\frac{E}{R(T \times I \times RH)}}$$

- where U_{max} = maximum specific growth rate g fresh mass $g^{-1} d^{-1}$,
 T = Absolute mean daily air temperature $^{\circ}K$,
 I = radiant flux density (diffuse component of the radiant flux) megajoules $m^{-2} hr^{-1}$,
 RH = mean daily relative humidity %,
 A = constant day^{-1} ,
 E = activation energy cal. $mole^{-1}$,
 R = universal gas constant cal. $mole^{-1} ^{\circ}K$.

5.3.2 Results

5.3.2.1 Field data

The mean, maximum and minimum daily relative humidities recorded over each growing interval of marginal plants at 6 field sites during 1977 and 1978 are summarized in Tables I to III, Appendix II. The relative humidities are expressed in these tables as a daily average over each growing interval of plants.

Relative humidities recorded over each growing interval of marginal plants at the MP3 and BGL sites are graphically illustrated in Figure 5.6. At both sites, the relative humidities followed a similar seasonal pattern to the specific growth rates measured for marginal plants at these 2 sites (Figure 4.1). Mean daily relative humidities at the MP3 and BGL sites ranged from 85% during summer to 64% during winter.

An Arrhenius plot of the specific growth rates (assumed U_{max} 's) of marginal plants, expressed as a natural logarithm (Log_e), against the products of the reciprocals of the Absolute mean daily air temperatures, diffuse radiant fluxes and mean daily relative humidities at the MP3 site, over the period February to December, 1978 (Figure 5.7), yielded a linear relationship with the correlation coefficient ($r = 0,8567$) being high and significant at $P = 0,001$. The regression equation obtained for the above exponential relationship was as follows:

$$U = 0,2247 e^{-\frac{7000}{T \times DRF \times RH}}$$

where U = specific growth rate (assumed maximum specific growth rate)g fresh mass $g^{-1} d^{-1}$,
 T = Absolute mean daily air temperature °K,
 DRF = diffuse component of the radiant flux megajoules $m^{-2} hr^{-1}$,
 RH = mean daily relative humidity %.

5.3.2.2 Comparison of predicted and measured specific growth rates

Maximum specific growth rates (U_{max}) were predicted for marginal plants from the mean daily air temperatures, diffuse radiant fluxes and mean daily relative humidities, over each growing interval, at the BGL, HD, IL, IC and DC sites. The regression equation relating the assumed U_{max} 's of marginal plants in the field exponentially to the products of the reciprocals of the Absolute mean daily air temperatures, diffuse radiant fluxes and mean daily relative humidities was used. This may be illustrated by the following example:

The mean daily air temperature, diffuse radiant flux and mean daily relative humidity recorded at the BGL site over the growing interval 1/2 to 15/2/78 were 24,9°C, 0,6127 megajoules $m^{-1} hr^{-1}$ and 83,0 % respectively (Table II, Appendix II). The U_{max} predicted for marginal plants for this temperature, diffuse radiant flux and relative humidity was calculated as follows:

$$U_{max} = 0,2247 e^{-\frac{7000}{(273,2 + 24,9) \times 0,6127 \times 83,0}}$$

$$= 0,1416 \text{ g fresh mass } g^{-1} d^{-1}.$$

Using the U_{max} values predicted for marginal plants from the mean daily air temperatures, diffuse radiant fluxes and mean daily relative humidities at the 5 mentioned sites and the mean K_s concentrations of 976 $\mu g N l^{-1}$ and 94,1 $\mu g P l^{-1}$, determined for *E. crassipes* under N and P growth rate limitation in culture, in the Monod model, specific growth rates (U) were predicted for marginal plants, over each growing interval, at each of these sites from the growth rate limiting N or P concentrations in the water. Predicted specific growth rates were compared with those measured for marginal plants, growing in loosely crowded populations, at each of these sites.

5.3.2.2.1 Botanic Gardens Lake (BGL) site

Specific growth rates predicted for marginal plants from the total P concentrations in the water at the BGL site closely followed the same seasonal pattern as the measured specific growth rates (Figure 5.8). Of the 22 specific growth rates predicted from the total P concentrations in the water, 17 (ca 77%) fell within the standard deviations of the measured specific growth rates (Figure 5.8, Table 5.9). The correlation factor (R^2) calculated between the measured specific growth rates and those predicted from the total P concentrations in the water was high ($R^2 = 0,7870$). The largest differences between the measured specific growth rates and those predicted from the total P concentrations in the water occurred during the midsummer months (November to February), when the highest specific growth rates were measured for marginal plants at this site. Specific growth rates predicted from the SRP concentrations in the water were extremely variable and did not follow any recognizable seasonal pattern. Only 1 of the specific growth rates predicted from the SRP concentrations in the water fell within the standard deviations of the measured specific growth rates. The differences between the measured specific growth rates and those predicted from the SRP concentrations in the water were very much larger than the differences between the measured specific growth rates and those predicted from the total P concentrations in the water.

5.3.2.2.2 Hartbeespoort Dam (HD) site

Of the 5 specific growth rates predicted for marginal plants from both the SRP and the total P concentrations in the water at the HD site, 3 of each fell within the standard deviations of the measured specific growth rates (Table 5.10). In both cases, however, the differences between the predicted and measured values were fairly large. The correlation factor (R^2) calculated between the measured specific growth rates and those predicted from the total P concentrations in the water was not very high ($R^2 = 0,3989$). The differences between the measured

specific growth rates and those predicted from the total P concentrations in the water were not very much different from the differences between the measured specific growth rates and those predicted from the SRP concentrations in the water.

5.3.2.2.3 Isipingo Lake (IL), Discharge Canal (DC) and Isipingo Canal (IC) sites

Specific growth rates predicted for marginal plants from the total N concentrations in the water at the IL and DC sites (Table 5.11) were generally not significantly different from the measured specific growth rates. For example, of the 7 specific growth rates predicted from the total N concentrations in the water at both sites, 6 fell within the standard deviations of the measured specific growth rates. The correlation factor, (R^2) calculated between the measured specific growth rates and those predicted from the total N concentrations in the water for both sites, however, was low, ($R^2 = 0,2927$). Five of the specific growth rates predicted from the $\text{NH}_4\text{-N}$ concentrations in the water and 3 of those predicted from the $\text{NO}_3\text{-N}$ concentrations in the water at these 2 sites fell within the standard deviation of the measured specific growth rates. The differences between the measured specific growth rates and those predicted from the $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations in the water at the IL and DC sites respectively, were generally larger than the differences between the measured specific growth rates and those predicted from the total N concentration in the water. The differences between the measured specific growth rates and those predicted from the $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations in the water at the IL and DC sites respectively, however, were not very much different from the differences between the measured specific growth rates and those predicted from the total N concentrations in the water.

Specific growth rates predicted for marginal plants from the various N fractions in the water at the IC site were all significantly lower than the measured specific growth rates

(Table 5.11). None of the specific growth rates predicted from the $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ or total N concentrations in the water fell within the standard deviations of the measured specific growth rates.

5.3.3 DISCUSSION

The Arrhenius plot of the assumed U_{max} 's of marginal plants against the products of the reciprocals of the Absolute mean daily air temperatures; diffuse radiant fluxes and mean daily relative humidities at the MP3 site showed that the U_{max} 's of marginal plants in the field could be related exponentially to the products of the reciprocals of these 3 factors. In fact, a higher correlation coefficient was obtained from the regression relating the assumed U_{max} 's of marginal plants in the field exponentially to the products of the reciprocals of the Absolute mean daily air temperatures, diffuse radiant fluxes and mean daily relative humidities (Figure 5.7), than to the products of the reciprocals of the Absolute mean daily air temperatures and diffuse radiant fluxes (Figure 5.4) or to the reciprocals of the Absolute mean daily air temperatures only (Figure 4.4). This suggests that the U_{max} 's of marginal plants in the field were influenced by the relative humidity.

In general, specific growth rates of marginal plants growing in loosely crowded populations at the BGL, IL and DC sites were as, but not more accurately predicted from the growth rate limiting total N or total P concentrations in the water using U_{max} values, in the Monod model, corrected for the mean daily air temperature, diffuse radiant flux and mean daily relative humidity in the field, than for the mean daily air temperature and diffuse radiant flux only. In the former case ca 79% of the specific growth rates predicted from the total N or total P concentrations in the water at all 3 sites fell within the standard deviations of the measured specific growth rates compared with ca 72% in the latter. The correlation factor (R^2) calculated between the measured specific growth rates those predicted from the total N or total P concentrations in

the water at all 3 sites, however, was not very much higher in the former case ($R^2 = 0,6100$) than in the latter ($R^2 = 0,5972$). In addition, the differences between the predicted and measured specific growth rates at the 3 sites were generally not much different in the former case than in the latter (Table 5.12). In both cases, specific growth rates of marginal plants were more accurately predicted from the total N or total P concentrations in the water than from the other N or P fractions in the water.

At the BGL site, the largest differences between the measured specific growth rates and those predicted from the total P concentrations in the water still occurred during the midsummer months (November to February). It appears unlikely, however, that the pH of the water had any significant influence on the specific growth rate of *E. crassipes* at this site or at the MP3 site from where the U_{max} 's of marginal plants were derived. The pH of the water at these 2 sites (Figure 5.9) ranged at or in close proximity to pH 7.0 at which maximum growth of *E. crassipes* occurs (Chadwick and Obeid, 1966). In addition, only minor variations in the pH of the water (0,3 and 0,5 pH units at the BGL and MP3 sites respectively) occurred at these 2 sites during the year. Chadwick and Obeid (1966) showed that a variation in the pH of the water of 1,3 pH units or greater was required to have a significant influence on the growth of *E. crassipes*. In view of this, 2 possible reasons may be given to explain the poor correlation between the predicted and measured specific growth rates at the BGL site during the midsummer months:

- (i) That the K_s concentrations determined for *E. crassipes*, under N and P growth rate limitation respectively, were temperature dependent. In *Chlorella pyrenoidosa* and *Oscillatoria agardhii*, Shelef et al. (1970) and Ahlgren (1978) showed that K_s varied with temperature, whereas in *Aerobacter aerogenes* and *Escherichia coli*, Topiwala and Sinclair (1971) and Sawada et al. (1978) also demonstrated that K_s changes with temperature and an Arrhenius plot of the change is linear.

Different K_s concentrations in the water for *E. crassipes* during the midsummer months when the air and water temperatures were at their highest levels may have brought the predicted specific growth rates closer to the measured specific growth rates. Consequently, it might be possible to more accurately describe the effects of the growth rate limiting nutrient and temperature on the growth rate of *E. crassipes* by expressing both the U_{max} and K_s as temperature functions. This may improve the predictive ability of the model.

- (ii) That the specific growth rates (assumed U_{max} 's) measured for marginal plants at the MP3 site, particularly during the midsummer months, may have been depressed by some toxic factor in the water. High phytoplankton population densities in the Maturation Pond, particularly during the midsummer months when the temperatures were at their highest levels, may have resulted in the excretion by the phytoplankton of some toxic factor in sufficiently high concentrations to be inhibitory to the growth rate of *E. crassipes*. Algae in some instances may severely interfere with the growth of higher plants (Hewitt, 1966), possibly through the production of antibiotic substances (Jorgensen, 1962) or toxic amino and carboxylic acids (Steinberg, 1947a; 1947b; Woltz and Jackson, 1961; Woltz, 1963). In addition, the presence of phenolic acids, which are common bi-products of decomposition, in the secondary treated waste-water effluent in the Maturation Pond may also have had an inhibitory effect on the growth rate of *E. crassipes*. Glass (1973, 1974) has shown that phenolic acids massively inhibit uptake of phosphorus and potassium by barley.

It is possible, therefore, that the U_{max} 's predicted for marginal plants during the midsummer months at the BGL site, using the regression equation relating the assumed U_{max} 's of marginal plants at the MP3 site exponentially to the products of the reciprocals of the Absolute mean daily air temperatures, diffuse radiant fluxes and mean daily relative humidities, may

have been too low. Consequently, when these were incorporated into the Monod model they underpredicted the specific growth rates of marginal plants at the BGL site, at least during the midsummer months.

At the HD site, where relative humidities were substantially lower than at sites in the Durban area of Natal, specific growth rates of marginal plants were more accurately predicted from the growth rate limiting P concentrations in the water using U_{max} values, in the Monod model, corrected for the mean daily air temperature, diffuse radiant flux and mean daily relative humidity in the field, than for the mean daily air temperature and diffuse radiant flux or the mean daily air temperatures only. The differences between the predicted and measured specific growth rates were generally much smaller in the former case than in the two latter (Table 5.13). This indicates that at sites where relative humidities are substantially different from that from which the U_{max} 's of *E. crassipes* are derived, the correction of the U_{max} for the relative humidity, as well, improves the predictive ability of the model.

It should, however, be pointed out that, although specific growth rates of marginal plants at the HD site were more accurately predicted from the growth rate limiting P concentrations in the water using U_{max} values, in the Monod model, corrected for all 3 above mentioned environmental factors, the differences between the predicted and measured values were generally fairly large. The correlation factor (R^2) calculated between the measured specific growth rates and those predicted from the total P concentrations in the water was not very high ($R^2 = 0,3989$). In general, the predicted values were higher than the measured values. It is suggested that the specific growth rates measured for marginal plants at this site may have been partly inhibited by a toxic factor in the water, resulting from the extensive chemical control measures (Scott et al., 1979) conducted on the water hyacinth populations on other parts of this dam during the course of these measurements.

Specific growth rates of marginal plants at the IC site, were not accurately predicted from the growth rate limiting N concentrations in the water using U_{max} values, in the Monod model, corrected for the mean daily air temperature, diffuse radiant flux and mean daily relative humidity in the field. Predicted specific growth rates were significantly lower than the measured specific growth rates. A similar reason as given in section 5.1.3 may partly explain this.

5.3.4 Conclusions

It is concluded that maximum specific growth rates (U_{max}) derived for marginal plants in the field can be related exponentially to the products of the reciprocals of the Absolute mean daily air temperatures, diffuse radiant fluxes and mean daily relative humidities.

At field sites where relative humidities do not differ substantially from that at which the U_{max} 's of marginal plants are derived, specific growth rates of marginal plants, growing in loosely crowded field populations, are as, but generally not more accurately predicted from the growth rate limiting total N or total P concentrations in the water using U_{max} values, in the Monod model, corrected for the mean daily air temperature, diffuse radiant flux and mean daily relative humidity in the field, than for the mean daily air temperature and diffuse radiant flux only. In both cases, specific growth rates of marginal plants are more accurately predicted from the total N or total P concentrations in the water than from the other N or P fractions in the water. The largest differences between the predicted and measured specific growth rates occur during midsummer (November to February). It is suggested that the K_s concentrations generated for *E. crassipes* under N and P growth rate limitation respectively in culture were temperature dependent and/or that the U_{max} 's derived for marginal plants in the field, particularly during the midsummer months, may have been limited by some toxic factor in the water. This may partly explain the poor correlation between the predicted and measured specific growth rates during the midsummer months.

At field sites where relative humidities do differ substantially from that at which the U_{max} 's of marginal plants are derived, specific growth rates of marginal plants are generally more accurately predicted from the growth rate limiting nutrient concentrations in the water using U_{max} values, in the Monod model, corrected for the mean daily air temperature, diffuse radiant flux and mean daily relative humidity in the field, than for the mean daily air temperature and diffuse radiant flux or the mean daily air temperature only.

CHAPTER 6

PREDICTING GROWTH RATES FROM THE NUTRIENT
CONCENTRATIONS IN THE PLANTS

6.1 INTRODUCTION

The specific growth rate of phytoplankton is a direct function of the cellular levels of growth rate limiting nutrients (Caperon, 1968; Droop, 1968; Fuhs, 1969; Davies, 1970; Rhee, 1973; Paasche, 1973).

Muller (1970) reported that the specific growth rate (U) is related to the internal growth rate limiting nutrient concentration in the form of:

$$U = U_{\max} \frac{q - q_0}{Kq + (q - q_0)}$$

where q_0 = minimum intracellular concentration of growth rate limiting nutrient or subsistence quota,
 q = measured intracellular concentration of growth rate limiting nutrient,
 U_{\max} = maximum specific growth rate,
 Kq = half saturation coefficient.

Droop (1968) and Rhee (1973) showed that the half saturation coefficients (Kq) for specific growth rate limiting nutrients in algal cells are equal to, or at least not significantly different from, the minimum intracellular concentrations of the specific growth rate limiting nutrients. If $Kq = q_0$ or $q = 2q_0$ when $U = 0,5 U_{\max}$, the above equation assumes the following simplified hyperbolic form (Droop, 1968; Rhee, 1973):

$$U = U_{\max} \frac{q - q_0}{q}$$

If it is postulated, therefore, that the half saturation coefficients (K_q) for specific growth rate limiting nutrients in *E. crassipes* are also equal to the minimum concentrations of the specific growth rate limiting nutrients in the plants, then the above simplified hyperbolic equation can be used to predict the specific growth rates of *E. crassipes* from the growth rate limiting N or P concentrations in the plants. As shown in Chapter 3, 3.3, the minimum concentrations of N and P in *E. crassipes* may be derived from the yield coefficient (Y_c) values (dry mass basis), when expressed as a reciprocal and a percentage ($1/Y_c \times 100$), determined for this plant under N and P growth rate limitation in culture.

6.2 RESULTS

6.2.1 Field data

The N and P concentrations (means of 3 batches) analyzed in marginal plants, harvested after each growing interval, at 6 field sites during 1977 and 1978 are summarized in Tables I to III, Appendix II. The N and P concentrations analyzed in marginal plants at the MP3 and BGL sites are graphically illustrated in Figures 6.1 and 6.2 respectively. Nitrogen and P concentrations were significantly higher ($P = 0,001$) in marginal plants at the MP3 site than at the BGL site (Table 6.1). These significantly higher N and P concentrations in marginal plants at the MP3 site were reflected in both the significantly higher specific growth rates measured for marginal plants at this site (Table 4.1) and in the higher N and P concentrations analyzed in the water (Figures 4.10 and 4.11).

Plots of the N and P concentrations in marginal plants against the N ($\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and total N) and P (SRP and total P) concentrations in the water at the MP3 and BGL sites, over the

period February to December, 1978, showed that the N and P concentrations in marginal plants were significantly correlated with, at or less than $P = 0,05$, and linearly related to the $\text{NH}_4\text{-N}$, total N and SRP concentrations in the water respectively at the MP3 site (Figures 6.3 to 6.5), but not to those at the BGL site (Table 6.2). These linear relationships, however, are only valid in the range of concentrations shown. Nitrogen and P concentrations in marginal plants showed no significant correlation ($P = 0,05$) with the $\text{NO}_3\text{-N}$ and total P concentrations in the water respectively at both sites (Table 6.2). The fiducial band limits (95% confidence limits) to the regression lines do not indicate a meaningful correlation between the P concentrations in marginal plants and the SRP concentrations in the water at the MP3 site (Figure 6.5).

The N and P concentrations (means of 3 batches) analyzed in central plants, harvested after each growing interval, at the MP3 and DC sites during 1977 and 1978 are summarized in Table IV, Appendix II. The N and P concentrations analyzed in central plants at the MP3 site are graphically illustrated in Figures 6.1 and 6.2 respectively. Nitrogen and P concentrations were significantly lower, at or less than $P = 0,05$, in central plants at the MP3 site than in marginal plants (Table 6.3).

Plots of the N and P concentrations in central plants against the N($\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and total N) and P(SRP and total P) concentrations in the water at the MP3 site, over the period February to December, 1978, showed that the N and P concentrations in central plants, like those of marginal plants, were significantly correlated with, at or less than $P = 0,01$ (Table 6.2), and linearly related to the $\text{NH}_4\text{-N}$, total N and SRP concentrations in the water respectively (Figures 6.6 and 6.7). These linear relationships, however, are only valid in the range of concentrations shown. Nitrogen and P concentrations in central plants, like those of marginal plants, showed no significant correlation ($P = 0,05$) with the $\text{NO}_3\text{-N}$ and total P concentrations in the water respectively at this site (Table 6.2).

The average N and P concentrations determined in marginal and central plants at each of the 6 field sites during 1977 and 1978 are given in Table 6.4. Incorporating these average N and P concentrations in marginal and central plants and the average minimum N and P concentrations of 1,10% N and 0,11% P in *E. crassipes*, derived from the mean Y_c values (dry mass basis) for these nutrients (see Chapter 3, 3.3), in Droop's simplified hyperbolic equation, the percentage of the U_{max} that marginal and central plants would achieve at the average N and P concentrations in the plants at each of these sites was estimated. This may be illustrated by the following example:

The average N and P concentrations determined in marginal plants at the BGL site during 1978 were 3,34% N and 0,51% P respectively (Table 6.4). The percentage of the U_{max} that marginal plants would achieve at (i) the average N and (ii) the average P concentrations in the plants at this site was calculated using Droop's simplified hyperbolic equation as follows:

$$U = U_{max} \frac{3,34 - 1,10}{3,34} \times 100$$

$$= 67,1\% U_{max} \dots\dots\dots (i)$$

$$U = U_{max} \frac{0,51 - 0,11}{0,51} \times 100$$

$$= 78,4\% U_{max} \dots\dots\dots (ii)$$

The results (Table 6.4) show that at the MP3, BGL, IL, DC and HD sites, marginal and central plants would achieve a lower percentage of the U_{max} at the average N concentrations in the plants than at the average P concentrations in the plants. This suggests that the growth rates of marginal and central plants at these sites were limited by the N concentrations in the plants. At the IC site, on the other hand, the results (Table 6.4) show that marginal plants would achieve a lower percentage of the U_{max} at the average P concentrations in the plants than at the average N concentrations in the plants.

This suggests that the growth rate of marginal plants at this site was limited by the P concentrations in the plants.

6.2.2 Comparison of predicted and measured specific growth rates

As pointed out in Chapter 5, 5.1.2.1, only a few measurements of the specific growth rates of central plants were obtained at the DC site. Consequently, no reliable comparisons could be made between specific growth rates measured for central plants at the DC site and those predicted from the growth rate limiting N concentrations in the plants using U_{max} values, derived for central plants at the MP3 site, in Droop's simplified hyperbolic equation.

On the assumption that the specific growth rates measured for marginal plants at specific temperatures, diffuse radiant fluxes and relative humidities at the MP3 site closely approximated the U_{max} 's of marginal plants, maximum specific growth rates (U_{max}) were predicted for marginal plants from the mean daily air temperatures, diffuse radiant fluxes and mean daily relative humidities, over each growing interval, at the BGL, HD, IL, IC and DC sites. The regression equation relating the assumed U_{max} 's of marginal plants in the field exponentially to the products of the reciprocals of the Absolute mean daily air temperatures, diffuse radiant fluxes and mean daily relative humidities was used (see Chapter 5, 5.3.2.1).

Using the U_{max} values predicted for marginal plants from the mean daily air temperatures, diffuse radiant fluxes and mean daily relative humidities at the 5 mentioned sites and the average minimum N and P concentrations of 1,10% N and 0,11% P in *E. crassipes*, derived from the mean Y_c values (dry mass basis) for these nutrients, in Droop's simplified hyperbolic equation, specific growth rates (U) were predicted for marginal plants, over each growing interval, at each of these sites from the growth rate limiting N or P concentrations in the plants. This may be illustrated by the following example:

The U_{max} predicted for marginal plants from the mean daily air temperature of $24,9^{\circ}\text{C}$, the diffuse radiant flux of $0,6127$ megajoules $\text{m}^{-2} \text{hr}^{-1}$ and the mean daily relative humidity of $83,0\%$ over the growing interval $1/2$ to $15/2/78$ at the BGL site was $0,1416$ g fresh mass $\text{g}^{-1} \text{d}^{-1}$ (see Chapter 5, 5.3.2.2). The N concentration analyzed in marginal plants over this growing interval at this site was $2,33\%$ N (Table II, Appendix II). The specific growth rate (U) predicted for marginal plants from the N concentration in the plants was calculated using Droop's simplified hyperbolic equation as follows:

$$U = 0,1416 \frac{2,33 - 1,10}{2,33}$$

$$= 0,0747 \text{ g fresh mass } \text{g}^{-1} \text{d}^{-1}.$$

Specific growth rates predicted for marginal plants from the growth rate limiting N or P concentrations in the plants were compared with those measured for marginal plants, growing in loosely crowded populations, at each site. At the BGL, HD, IL and DC sites specific growth rates were predicted for marginal plants from the N concentrations in the plants, whereas at the IC site they were predicted from the P concentrations in the plants. As shown (Table 6.4), the N concentrations in the plants were estimated to be growth rate limiting for marginal plants at the BGL, HD, IL and DC sites, whereas the P concentrations in the plants were estimated to be growth rate limiting at the IC site.

6.2.2.1 Botanic Gardens Lake (BGL) site

Specific growth rates predicted for marginal plants from the N concentrations in the plants at the BGL site closely followed the same seasonal pattern as the measured specific growth rates (Figure 6.8). Of the 22 predicted specific growth rates, 13 (ca 59%) fell within the standard deviations of the measured specific growth rates (Figure 6.8, Table 6.5). The correlation factor (R^2) calculated between the measured and predicted values was fairly high ($R^2 = 0,6866$). The largest differences

between the predicted and measured specific growth rates occurred during the midsummer (November to February) and midwinter (June and July) months, when the highest and lowest air temperatures and diffuse radiant fluxes respectively were recorded at this site (Table II, Appendix II).

6.2.2.2 Isipingo Lake (IL), Discharge Canal (DC) and Hartbeespoort Dam (HD) sites

Specific growth rates predicted for marginal plants from the N concentrations in the plants at the IL and DC sites (Table 6.6) were generally not significantly different from the measured specific growth rates. For example, of the 7 predicted specific growth rates, 6 fell within the standard deviations of the measured specific growth rates. The differences between the measured and predicted values, however, were generally fairly large. The correlation factor (R^2) calculated between the measured and predicted specific growth rates for both sites was low ($R^2 = 0,2527$).

Specific growth rates predicted for marginal plants from the N concentrations in the plants at the HD site (Table 6.6) were generally significantly higher than the measured specific growth rates. Of the 5 predicted values, only 2 fell within the standard deviations of the measured values.

6.2.2.3 Isipingo Canal (IC) site

Specific growth rates predicted for marginal plants from the P concentrations in the plants at the IC site (Table 6.7) were all significantly lower than the measured specific growth rates. None of the predicted values fell within the standard deviations of the measured values.

6.3 DISCUSSION

Nitrogen and P concentrations differed significantly in marginal and central plants growing in loosely and densely

crowded field populations respectively at the MP3 site. It does not seem reasonable to explain these differences in terms of the N and P concentrations analyzed in the water from the vicinity of the marginal and central plant populations enclosed at this site, as these were similar in both situations. One is, therefore, left to postulate that these differences reflect some aspect of the physiology of plants growing under different degrees of crowding. This postulate is supported by the investigations of Mitsch (1977) who found that large water hyacinths (central plants) had a lower P/R ratio than dwarf water hyacinths (marginal plants). This author concluded that large water hyacinths must, therefore, put more of their captured energy into the maintenance of their structures and consequently would not be as efficient nutrient traps per unit amount of biomass as dwarf water hyacinths. In fact, the significantly higher N and P concentrations analyzed in marginal plants would seem to support the conclusions of Mitsch (1977).

At the MP3 site, where the N and P concentrations in the water were very high, the N and P concentrations in both marginal and central plants were significantly correlated with and linearly related to the $\text{NH}_4\text{-N}$, total N and SRP concentrations in the water respectively, but not to the $\text{NO}_3\text{-N}$ or total P concentrations in the water. These linear relationships, however, are only valid in the range of concentrations shown. No projections can be made beyond the limits of the regression lines, particularly as at the BGL site, where the N and P concentrations in the water were much lower than at the MP3 site, the N and P concentrations in marginal plants showed no significant correlation with the various N and P fractions in the water respectively. These results suggest that the N and P concentrations in the water can only be reliably estimated from those in the plants in eutrophic waters, where the N and P concentrations in the water are very high. It is possible that the relationship between the N and P concentrations in *E. crassipes* and those in the water environment over a much wider range of concentrations may be hyperbolic. In addition,

the results suggest that *E. crassipes* absorbs $\text{NH}_4\text{-N}$ and inorganic phosphate preferentially as N and P sources for growth.

With respect to N, it is possible that high $\text{NH}_4\text{-N}$ concentrations in the water may repress the uptake of $\text{NO}_3\text{-N}$ in *E. crassipes* by inhibiting nitrate reductase formation. Bates (1976) found that in the presence of nitrate and ammonium, ammonium was preferentially taken up by the algae and nitrate uptake was depressed at all light intensities. The repression or inhibition of nitrate induced synthesis of nitrate reductase enzymes in the presence of ammonium has been reported by Joy (1969) and Orebamjo and Stewart (1975a, 1975b) in *Lemna minor* L. This is a widespread phenomenon having been reported in many species of fungi (Morton, 1956; Kinsky, 1961; Cove, 1966; Sims et al., 1968; Lewis and Fincham, 1970) in several species of algae (Morris and Syrett, 1963, Losada et al., 1970, Rigano, 1971; Thacker and Syrett, 1972) as well as in higher plant species other than the Lemnaceae (Smith and Thompson, 1971; Stewart et al., 1974). The precise mechanism through which ammonium exerts its influence has not been unequivocally determined. Orebamjo and Stewart (1975a) in a study of the kinetics of ammonium inhibition of nitrate reductase formation in *Lemna minor* L. indicated that this is not a direct effect of ammonium. They suggested that inhibition may result from the build up of a product of ammonium assimilation or an ammonium induced regulatory protein.

With respect to P, the significant correlation obtained between the P concentrations in marginal and central plants and the SRP, but not the total P, concentrations in the water supports the findings of Jeschke and Simonis (1965) who reported that the main source of P for growth of aquatic plants is in the form of inorganic phosphates.

The nutrient in the plants limiting the growth rate of *E. crassipes* at each field site was estimated from the average N and P concentrations in the plants using the average minimum

N and P concentrations of 1,10% N and 0,11% P in *E. crassipes*, derived from the mean Y_c values (dry mass basis) for these nutrients, in Droop's simplified hyperbolic equation. At the MP3, BGL, IL, DC and HD sites where the N concentrations in the plants were estimated to be growth rate limiting for *E. crassipes*, the average N/P ratio in the plants ranged well below the optimal N/P ratio of 30 (cell and medium) below which Rhee (1974, 1978) reported that N becomes limiting to the growth rate in the algae. At the IC site, where the P concentrations in the plants were estimated to be growth rate limiting for *E. crassipes*, the average N/P ratio in the plants, however, ranged below the optimal N/P ratio of 30 (cell and medium) above which Rhee (1974, 1978) reported that P becomes limiting to the growth rate in the algae. If it is assumed that the minimum N and P concentrations in *E. crassipes* are equal to, or at least not significantly different from, its K_q concentrations for these nutrients, then the ratio of the average minimum N/minimum P concentrations suggest an optimal N/P ratio in *E. crassipes* of ca 10, i.e. below which the N and above which the P concentrations in this plant become growth rate limiting. This value compares favourably with the suggested optimal N/P ratio in the water of ca 10 (see Chapter 4, 4.3) estimated from the ratio of the mean K_{sn}/K_{sp} concentrations determined for *E. crassipes* under N and P growth rate limitation respectively in culture.

At the MP3 site, where it was assumed that the specific growth rates measured for marginal plants closely approximated their U_{max} 's, it was estimated that marginal plants would achieve ca 77,0 and 90,8% of the U_{max} at the average N and P concentrations in the plants respectively. These values are lower than those estimated from the average total N and total P concentrations in the water respectively at this site (Table 4.4). In this connection, it should be pointed out that the theoretical maximum specific growth rate (U'_{max}), at an infinite intracellular concentration of the growth rate limiting nutrient, is always larger than the true maximum specific growth rate (U_{max}), since in reality the intracellular

concentration of the growth rate limiting nutrient (q) has a finite maximum value at q_m and cannot be infinite. The difference between U_{max} and U'_{max} is related to the storage capacity of cells of the growth rate limiting nutrient (q_m) in relation to the minimum intracellular concentration of the growth rate limiting nutrient (q_0) or the q_0/q_m ratio (Droop, 1973; 1974; Goldman and McCarthy, 1978).

Specific growth rates of marginal plants growing in loosely crowded populations at the BGL, IL and DC sites were fairly accurately predicted from the growth rate limiting nutrient (N or P) concentrations in the plants using U_{max} values corrected for the mean daily air temperature, diffuse radiant flux and mean daily relative humidity, derived for marginal plants in the field, and the minimum N and P concentrations in *E. crassipes*, derived from the Y_c values for these nutrients, in Droop's simplified hyperbolic equation. Approximately 65% of the specific growth rates predicted from the growth rate limiting nutrient concentrations in the plants at all 3 sites fell within the standard deviations of the measured specific growth rates. The correlation factor (R^2) calculated between the predicted and measured specific growth rates for all 3 sites, however, was not very high ($R^2 = 0,4946$). Consequently, the degree of similarity between the predicted and measured values is uncertain.

At the BGL site, the largest differences between the predicted and measured specific growth rates occurred during the midsummer (November to February) and midwinter (June and July) months, when the highest and lowest air temperatures and diffuse radiant fluxes respectively were recorded at this site. It is suggested that the minimum N and P concentrations in *E. crassipes* may be dependent on the temperature and radiant flux density. This may partly explain the poor correlation between the predicted and measured specific growth rates during the midsummer and midwinter months. Rhee and Gotham (1981a, 1981b) have shown that the minimum intracellular concentrations, minimum cell quotas (q_0), of N and P in algal cells

increase with decreasing temperatures and irradiances indicating that at lower temperatures and irradiances algal cells require more of the growth rate limiting nutrient to grow at the same rate as they do at optimal temperatures and irradiances. Goldman (1979) also reported an increase of q_0 at a low temperature in P growth rate limited *Monochrysis lutheri*, whereas Davis (1976) noticed that the intracellular concentrations of growth rate limiting nutrients in the marine diatom *Skeletonema costatum* were greater at suboptimal light levels than those at light-saturated growth. The greater requirement for nutrients at lower temperatures may reflect the cells' need for more RNA to synthesize the same amount of protein, as suggested by Tempest and Hunter (1965). Chohji et al. (1976) showed that the efficiency of protein synthesis, measured as the rate of protein synthesis per unit weight of RNA, decreases at lower temperatures. Higher minimum N and P concentrations in *E. crassipes* during the midwinter months, when the temperatures and diffuse radiant fluxes were at their lowest levels, would have brought the higher predicted specific growth rates closer to the measured specific growth rates. Similarly, lower minimum N and P concentrations in *E. crassipes* during the midsummer months, when the temperatures and diffuse radiant fluxes were at their highest levels, would also have brought the lower predicted specific growth rates closer to the measured specific growth rates. In addition, it is possible that the U_{max} 's derived for marginal plants in the field, particularly during the midsummer months, may have been depressed by some toxic factor in the water, as suggested in Chapter 5, 5.3.3. This may also partly explain why the predicted specific growth rates were significantly lower than the measured specific growth rates during the midsummer months.

In general, specific growth rates of marginal plants at the BGL, IL and DC sites were less accurately predicted from the growth rate limiting nutrient (N or P) concentrations in the plants than from the growth rate limiting nutrient (total N or total P) concentrations in the water. In the former case, only ca 65% of the predicted specific growth rates at all 3 sites

fell within the standard deviations of the measured specific growth rates compared with ca 79% in the latter. The correlation factor (R^2) calculated between the predicted and measured values for all 3 sites was also much lower in the former case ($R^2 = 0,4946$) than in the latter ($R^2 = 0,6100$). In addition, the differences between the predicted and measured values were generally much larger in the former case than in the latter (Table 6.8).

Specific growth rates of marginal plants at the HD and IL sites were generally not accurately predicted from the growth rate limiting N or P concentrations in the plants using U_{max} values corrected for the mean daily air temperature, diffuse radiant flux and mean daily relative humidity, derived for marginal plants in the field, and the minimum N and P concentrations in *E. crassipes*, derived from the Y_c values for these nutrients, in Droop's simplified hyperbolic equation.

At the HD site, the predicted specific growth rates were generally significantly higher than the measured specific growth rates. As suggested in Chapter 5, 5.3.3, it is possible that the specific growth rates measured for marginal plants at this site may have been inhibited partly by a toxic factor in the water.

At the IC site, on the other hand, the predicted specific growth rates were significantly lower than the measured specific growth rates. In Chapter 5, 5.1.3, it was suggested that the quantities of N and P available to plants for growth at this site may have been much larger than actually determined from the N and P concentrations in the water. This explanation, however, is not entirely plausible, particularly as the suggested larger quantities of N and P available to plants for growth at this site should have been reflected in the plants and consequently in the specific growth rates predicted from the growth rate limiting P concentrations in the plants. It is possible the the U_{max} 's derived for marginal plants in the field, particularly during the midsummer months, may have been

depressed by some toxic factor in the water, as suggested in Chapter 5, 5.3.3. Consequently, when these were incorporated into Droop's simplified hyperbolic equation, they underpredicted the specific growth rates of marginal plants at the IC site. This may partly explain why the predicted specific growth rates were significantly lower than the measured specific growth rates at this site.

6.4 CONCLUSIONS

It is concluded that the N and P concentrations differ significantly in marginal and central plants growing in loosely crowded and densely crowded field populations respectively. It is suggested that these differences reflect some aspect of the physiology of plants growing under different degrees of crowding. Nitrogen and P concentrations in marginal and central plants are correlated with the $\text{NH}_4\text{-N}$, total N and SRP concentrations in the water respectively, but not with the $\text{NO}_3\text{-N}$ or total P concentrations in the water. This suggests that *E. crassipes* absorbs $\text{NH}_4\text{-N}$ and inorganic phosphate preferentially as N and P sources for growth. It would appear, however, that the N and P concentrations in the water can only be reliably estimated from those in the plants in eutrophic waters where the N and P concentrations in the water are high.

Specific growth rates of marginal plants growing in loosely crowded field populations are fairly accurately predicted from the growth rate limiting nutrient (N or P) concentrations in the plants using U_{max} values corrected for the mean daily air temperature, diffuse radiant flux and mean daily relative humidity, derived for marginal plants in the field, and the minimum N and P concentrations in *E. crassipes*, derived from the Y_c values for these nutrients, in Droop's simplified hyperbolic equation. The largest differences between the predicted and measured specific growth rates occur during midsummer (November to February) and midwinter (June and July), when the air temperatures and diffuse radiant fluxes in the field are at their highest and lowest levels respectively. It

is suggested that the minimum N and P concentrations in *E. crassipes* are dependent on the temperature and radiant flux density. This may partly explain the poor correlation between the predicted and measured specific growth rates during the midsummer and midwinter months.

In general, specific growth rates of marginal plants are less accurately predicted from the growth rate limiting nutrient (N or P) concentrations in the plants than from the growth rate limiting nutrient (total N or total P) concentrations in the water.

CHAPTER 7

GENERAL CONCLUSIONS AND APPLICATION OF

REFINED MODEL

Detailed discussions and conclusions on various aspects of the model in its development and progressive refinement and of other results obtained in this study are included at the end of each relevant chapter. Consequently, this chapter is restricted to some of the general implications of the results obtained, to suggest some possible further refinements to the model and to illustrate the application of the refined model for predicting yields, growth rates and amounts and frequencies of harvest for *E. crassipes*, to control both nutrient inputs in culturally eutrophied water systems and excessive growth of this plant.

The results of this study demonstrate that, apart from the yield coefficient, kinetic coefficients were not consistently determined for *E. crassipes* growing under N or P growth rate limitation in culture using a batch culture approach. With more adequate facilities, however, it is possible that the batch culture method used in this study for determining kinetic coefficients for *E. crassipes* growing under specific nutrient growth rate limitation could be improved. The growth rates of plants in culture, for example, could be enhanced by growing plants at much higher relative humidities and with the daylight intensities (radiant flux densities) in the greenhouse supplemented with an artificial light source. Furthermore, if plants for culture were collected from a population grown under controlled environmental conditions in a standardized culture medium, it is possible that a uniform growth period required to induce plants into a N or P growth rate limited state could be obtained. This might decrease the variability in the U_{max}

values and K_s concentrations determined. Precise measurements of the U_{max} and K_s will probably only be obtained for *E. crassipes*, under specific nutrient growth rate limitation, by growing plants under constant environmental conditions in a continuous flow culture system, as suggested in Chapter 3, 3.4. The dependence of the U_{max} of *E. crassipes* on the air temperature and possibly on the radiant flux density and relative humidity as well would, however, restrict the application of kinetic coefficients determined under constant environmental conditions to predicting growth rates of plants under well defined environmental conditions in the field. It is possible that the relationships between the U_{max} of *E. crassipes* and each of the above mentioned environmental factors could be established independently for plants growing under different air temperatures, radiant flux densities or relative humidities in a continuous flow culture system and their combined effects on the U_{max} of *E. crassipes* modelled. This, however, would require a considerable amount of experimentation.

As shown in this study, U_{max} 's may be derived for *E. crassipes* in the field in a water body in which the nutrient concentrations are high and approach those saturating to the growth rate of this plant, i.e. provided other environmental factors such as the pH of the water or toxic factors in the water are not growth rate limiting for plants. An exponential relationship can be established over an entire growing season, for a defined climatic area, between the assumed U_{max} 's of *E. crassipes* in the field and the products of the air temperatures, radiant flux densities (diffuse component of the radiant flux) and relative humidities. The regression equation obtained from this exponential relationship can then be incorporated into either the Monod model or Droop's simplified hyperbolic equation to fairly accurately predict specific growth rates of *E. crassipes* in the field from the growth rate limiting nutrient (total N or total P) concentrations in the water or the growth rate limiting nutrient (N or P) concentrations in the plants respectively.

In general, specific growth rates of marginal plants growing in loosely crowded field populations were more accurately predicted from the growth rate limiting nutrient concentrations in the water than from those in the plants using the refined model, i.e. where U_{max} 's corrected for the mean daily air temperature, diffuse radiant flux and mean daily relative humidity, derived for marginal plants in the field, were used in the Monod model and Droop's simplified hyperbolic equation respectively. The largest differences between the measured specific growth rates and those predicted from the growth rate limiting nutrient concentrations in the water occurred during midsummer (November to February). The largest differences between the measured specific growth rates and those predicted from the growth rate limiting nutrient concentrations in the plants occurred both during midsummer and midwinter (June and July), when the air temperatures and diffuse radiant fluxes in the field were at their highest and lowest levels respectively. It is possible that the K_s concentrations determined for *E. crassipes* under N and P growth rate limitation in culture and the minimum growth rate limiting nutrient (N and P) concentrations in *E. crassipes*, derived from the Y_c values for these nutrients, are dependent on the temperature and possibly on the radiant flux density as well, as suggested in Chapter 5, 5.3.3 and Chapter 6, 6.3. Consequently, it may be possible to more accurately describe the effects of the growth rate limiting nutrient in the water and in the plants on the growth rate of *E. crassipes* by expressing both the K_s concentrations and the minimum growth rate limiting nutrient concentrations in *E. crassipes* as the functions of the temperature and radiant flux density. The influence of the temperature and radiant flux density on the K_s concentrations and the minimum growth rate limiting nutrient concentrations in *E. crassipes* will, therefore, need to be established under controlled environmental conditions in a continuous flow culture system and mathematically formulated. This may improve the predictive ability of the model, at least during the midsummer and midwinter months. Although specific growth rates of marginal plants were not accurately predicted from the growth rate

limiting nutrient concentrations in the water during midsummer and from the growth rate limiting nutrient concentrations in the plants during midwinter and midsummer, on a seasonal (summer or winter) basis, it should, however, be possible to make fairly accurate predictions of the average specific growth rate of *E. crassipes*, from the growth rate limiting nutrient concentrations in the water or in the plants, using the refined model.

Insufficient field data was collected in this study to test whether specific growth rates of central plants growing in densely crowded field populations could be accurately predicted from the growth rate limiting nutrient concentrations in the water or in the plants using the refined model, i.e. by incorporating U_{max} 's corrected for the mean daily air temperature, diffuse radiant flux and mean daily relative humidity, derived for central plants in the field, in the Monod model and Droop's simplified hyperbolic equation respectively. Crowding of *E. crassipes* in any nutrient removal scheme to produce larger plants of the central growth form and hence improve the standing crop, however, would be inadvisable as this would have a detrimental influence on the growth rate and on the N and P concentrations in the plants. The latter would also adversely affect the value of the harvested plants from the standpoint of their use as feedstuffs and fertilizer. The nutrient removal capacity of *E. crassipes* would be a function of both its specific growth rate and the density of the plant population. De Busk et al. (1981) showed that the productivity of *E. crassipes*, the product of the specific growth rate and density, defines a bell shaped curve with maximum productivities being achieved at intermediate densities. Because of the practical problems in managing water hyacinth populations in large canals or ponds, the strict control of plant density, however, would probably only be of interest in small scale experimentation. Consequently, to achieve a maximum rate of growth and nutrient removal efficiency by *E. crassipes* in any nutrient removal scheme, the plant populations would, therefore, need to be harvested regularly to maintain them in loosely crowded situations and in the marginal growth form.

The application of the refined model for predicting yields, growth rates and amounts and frequencies of harvest for *E. crassipes* growing in loosely crowded field populations, to control both nutrient inputs in a culturally eutrophied water system and excessive growth of this plant, is illustrated by means of the following example:

In a hypothetical, culturally eutrophied water body occurring in the Durban area of Natal, it has been established, for example, that N is the growth rate limiting nutrient. If the N is derived from a single point source which annually yields 80 million litres of water, then the annual water hyacinth growth potential of the effluent in which the total N concentration is 10 000 ug N l⁻¹ would be:

$$80 \times 10^6 \times 10\ 000 \times 10^6 \times 1768,5$$

$$= 1414,816 \text{ metric tonnes of fresh water hyacinth plant material.}$$

where: 1768,5 = mean yield coefficient (Yc) value (fresh mass basis) determined for *E. crassipes* under N growth rate limitation in culture.

The average daily water hyacinth growth potential of the effluent in which the total N concentration is 10 000 ug N l⁻¹ would be:

$$\frac{1414,816}{365} = 3,8762 \text{ metric tonnes of fresh water hyacinth plant material.}$$

Annual and average daily water hyacinth growth potentials of the effluent as predicted for different N concentrations of the effluent are shown in Table 7.1.

During summer (September to March) in the Durban area of Natal, the average mean daily air temperature is 22,0°C, the average diffuse radiant flux is 0,5586 megajoules m⁻² hr⁻¹ and the

average mean daily relative humidity is 81%. During winter (April to August), they are 18,7°C, 0,3587 megajoules m⁻² hr⁻¹ and 76% respectively. The average specific growth rate of *E. crassipes* at a given N concentration of the effluent can be predicted for summer and winter respectively by incorporating the regression equation relating the assumed U_{max}'s of marginal plants in the field exponentially to the products of the reciprocals of the Absolute mean daily air temperatures, diffuse radiant fluxes and mean daily relative humidities into the Monod model as follows:

$$U = 0,2247 e^{-\frac{7000}{T \times DRF \times RH}} \cdot \frac{S}{K_{sn} + S}$$

where U = specific growth rate g fresh mass g⁻¹ d⁻¹,
 T = Absolute mean daily air temperature °K,
 DRF = diffuse radiant flux megajoules m⁻² hr⁻¹,
 RH = mean daily relative humidity %,
 S = N concentration of effluent ug N l⁻¹,
 K_{sn} = mean half saturation coefficient value determined for *E. crassipes* under N growth rate limitation in culture ug N l⁻¹.

The average specific growth rate of *E. crassipes* during summer at a N concentration of the effluent of 10 000 ug N l⁻¹ would be:

$$U = 0,2247 e^{-\frac{7000}{(273,2 + 22,0) \times 0,5586 \times 81}} \cdot \frac{10\ 000}{976 + 10\ 000}$$

$$= 0,1212 \text{ g fresh mass g}^{-1} \text{ d}^{-1}.$$

The average specific growth rate of *E. crassipes* during winter at a N concentration of the effluent of 10 000 ug N l⁻¹ would be:

$$U = 0,2247 e^{-\frac{7000}{(273,2 + 18,7) \times 0,3587 \times 76}} \cdot \frac{10\ 000}{976 + 10\ 000}$$

$$= 0,0849 \text{ g fresh mass g}^{-1} \text{ d}^{-1}.$$

Average specific growth rates predicted for *E. crassipes* for summer and winter for different N concentrations of the effluent are shown in Table 7.1. The results show that the higher the N concentration of the effluent, the higher the specific growth rate of *E. crassipes*. Specific growth rates of *E. crassipes* at given N concentrations of the effluent are higher during summer than during winter.

The minimum standing crop required to produce the average daily water hyacinth growth potential of the effluent can be solved from the general growth equation (Malek and Fencl, 1966; Radford, 1967).

$$X_t = X_0 e^{ut}$$

where: X_t = average daily water hyacinth growth potential of the effluent + minimum standing crop metric tonnes,
 X_0 = minimum standing crop metric tonnes,
 u = specific growth rate g fresh mass g⁻¹ d⁻¹,
 t = time interval between X_0 and X_t days.

The minimum standing crop required to produce the average daily water hyacinth growth potential of the effluent during summer at a N concentration of the effluent of 10 000 ug N l⁻¹ would be:

$$X_0 + 3,8762 = X_0 e^{0,1212 \times 1}$$

$$X_0 = 30,0830 \text{ metric tonnes of fresh water hyacinth plant material.}$$

Minimum standing crops required to produce the average daily water hyacinth growth potentials of the effluent predicted for summer and winter for different N concentrations of the effluent are shown in Table 7.1. The results show that the higher the N concentration of the effluent, the greater the size of the minimum standing crop required to produce the average daily water hyacinth growth potential of the effluent. The minimum standing crops at given N concentrations of the effluent are smaller during summer than during winter.

Van Dyke (1971) using a mechanical harvester prototype (Sarasota Weed and Feed Incorporation) removed 2 584,5 tons of fresh water hyacinths covering 20,64 acres over 64 days operating time. This is equivalent to 2 605,17 metric tonnes of water hyacinths covering some 8,353 hectares. From this information, one hectare of water hyacinths would weigh 311,854 metric tonnes. The data of Van Dyke (1971) was obtained from water hyacinths growing in densely crowded field populations. Stand densities (dry mass basis) reported for water hyacinths growing in loosely crowded and densely crowded field populations respectively are shown in Table 7.2. The results show that stand densities of water hyacinths growing in loosely crowded field populations are ca 10 times smaller than of those growing in densely crowded field populations. Consequently, a given mass of water hyacinths growing in loosely crowded field populations may be assumed to cover an area ca 10 times larger than a similar mass of water hyacinths growing in densely crowded field populations. Using these values, the approximate area of each minimum standing crop required to produce the average daily water hyacinth growth potential of the effluent at a given N concentration of the effluent may be estimated. For example, the minimum standing crop required to produce the average daily water hyacinth growth potential of the effluent during summer at a N concentration of the effluent of 10 000 $\mu\text{g N l}^{-1}$ was calculated as 30,0830 metric tonnes of fresh water hyacinth plant material. The approximate area of this minimum standing crop would be:

$$\frac{30,0830}{311,854} \times 10 = 0,9646 \text{ ha (} \times 10^4 \text{ m}^2\text{)}$$

In addition, Van Dyke (1971) reported that the harvester removed 15,51 tons of water hyacinths per hour of operating time, but since the harvester was in the actual process of loading trucks 38% of the time, it only removed 5,68 tons per hour, i.e. ca 5,9 metric tonnes per hour of total time. Assuming an 8 hour working day, the harvester would, therefore, remove ca 47 metric tonnes per day. The time required for each minimum standing crop to produce an additional 47 metric tonnes of water hyacinths at each growth rate can be solved from the following form of the general growth equation (Malek and Fenc1, 1966; Radford, 1967) and is referred to as the harvesting interval:

$$\text{Log}_e X_t = \text{Log}_e X_0 + ut$$

where: X_t = final biomass ($X_0 + 47,0$) metric tonnes,
 X_0 = initial biomass or minimum standing crop metric tonnes,
 u = specific growth rate g fresh mass $\text{g}^{-1} \text{d}^{-1}$,
 t = harvesting interval, i.e. time interval between X_0 and X_t days.

For example, the minimum standing crop of 30,0830 metric tonnes of water hyacinths will produce the average daily water hyacinth growth potential of the effluent, in which the N concentration is 10 000 $\mu\text{g N l}^{-1}$, at an average specific growth rate during summer of 0,1212 g fresh mass $\text{g}^{-1} \text{d}^{-1}$. The time required for 30,0830 metric tonnes of water hyacinths to produce an additional 47 metric tonnes would be:

$$\begin{aligned} \text{Log}_e (30,0830 + 47,0) &= \text{Log}_e 30,0830 + 0,1212 t \\ t &= 7,8 \text{ days.} \end{aligned}$$

The harvesting interval in this case would be 7,8 days. After this period, one days removal could then be initiated and this

could be repeated after a further 7,8 days growth. Harvesting intervals predicted for *E. crassipes* for summer and winter for different N concentrations of the effluent are shown in Table 7.1. The results show that the higher the N concentration of the effluent, the shorter the interval between each harvest. Harvesting intervals at given N concentrations of the effluent are shorter during summer than during winter. The N concentration of the effluent appears to have a greater influence on the harvesting interval than season.

In closing, it may be pointed out that in culturally eutrophied water systems, in which natural water hyacinth populations already occur in densely crowded situations and in the central growth form, it should be possible to predict the amounts and frequencies of harvest, necessary to control excessive growth of plants, using the refined model, provided that the U_{max} of *E. crassipes* is corrected for the density of the plant population. In this study, U_{max} 's were derived for central plants growing in densely crowded populations of unknown density in the field. These, however, are of little use in the model for predicting specific growth rates of *E. crassipes* growing in crowded field populations of different density, particularly as De Busk et al. (1981) have shown that the specific growth rate of *E. crassipes* in the field decreases more or less linearly with an increase in the density of the plant population. The relationship between the U_{max} of *E. crassipes* and the density of the plant population will, therefore, need to be established in the field before the specific growth rates, and consequently, the amounts and frequencies of harvest of *E. crassipes* growing in crowded field populations of different density, can be predicted using the refined model.

SUMMARY

This study was designed to develop a model to predict yields and growth rates for *E. crassipes*, growing under varying climatic conditions and nutrient supply, from which amounts and frequencies of harvest could be calculated to control both nutrient inputs in culturally eutrophied water systems and excessive growth of this plant.

The following kinetic coefficients were determined for *E. crassipes* growing under N and also under P growth rate limitation in culture: maximum specific growth rate (U_{max}), half saturation coefficient (K_s) and yield coefficient (Y_c). U_{max} values and K_s concentrations determined for *E. crassipes* under N and P growth rate limitation in culture varied considerably. Y_c values, however, showed little variation. The reciprocals of the Y_c values (dry mass basis) when expressed as a percentage ($1/Y_c \times 100$) fairly accurately estimated the minimum N and P concentrations analyzed in *E. crassipes* grown in culture and as reported in the literature.

In the field, the specific growth rate of *E. crassipes* was related exponentially to the reciprocals of the Absolute mean daily air and water temperatures and was detrimentally affected in densely crowded field populations. Air temperatures appeared to have a greater effect on the specific growth rate of *E. crassipes*, both plants of the marginal and central growth forms, than did the water temperatures. At field sites where the N and P concentrations in the water approached those saturating to the growth rate of *E. crassipes* and the specific growth rate of *E. crassipes* was assumed to closely approximate its U_{max} , specific growth rates (assumed U_{max} 's) of marginal plants followed the van't Hoff rule, i.e. they approximately doubled with a 10°C rise in the mean daily air temperature. Central plants, however, showed a proportionally smaller increase in their assumed U_{max} with a 10°C rise in the mean daily air or water temperatures than did marginal plants.

Using kinetic coefficients generated for *E. crassipes* under N and P growth rate limitation in culture in the Monod model, it was shown that

specific growth rates of marginal plants, growing in loosely crowded field populations, were not accurately predicted from the growth rate limiting nutrient (N or P) concentrations in the water, except when the radiant flux densities (diffuse component of the radiant flux) and/or relative humidities in the field were low. In the latter case, specific growth rates of marginal plants were fairly accurately predicted from the total N or total P concentrations in the water. It was suggested that the U_{max} values generated for *E. crassipes* under N and P growth rate limitation in culture were limited by the lower light intensities and relative humidities in the greenhouse. No correction factor, however, could be introduced into the model to amend the U_{max} values generated for *E. crassipes* under the lower light intensities and relative humidities in the greenhouse, as the ratio's between the measured and predicted specific growth rates in the field varied considerably during the year.

Specific growth rates of central plants, growing in densely crowded field populations, were also not accurately predicted from the growth rate limiting nutrient concentrations in the water using kinetic coefficients in the Monod model generated for *E. crassipes* in culture. Predicted specific growth rates were significantly higher than the measured specific growth rates. This was attributed to the fact that the specific growth rate of *E. crassipes* was detrimentally affected in densely crowded field populations and suggested that, unless a correction factor was introduced into the model to amend the U_{max} for the density of the plant population, the U_{max} values generated for marginal plants in culture could not be used in the Monod model to predict specific growth rates of central plants growing in densely crowded field populations.

U_{max} 's were derived for marginal and central plants at one field site where the N and P concentrations in the water were high and approached those saturating to the growth rate of *E. crassipes*. Using U_{max} values corrected for the mean daily air temperature, derived for marginal plants in the field, and K_s concentrations generated for *E. crassipes* under N and P growth rate limitation in culture in the Monod model, it was shown that specific growth rates of marginal plants in the field were fairly accurately predicted from the growth rate limiting nutrient (total N or total P) concentrations in the water. The largest differences between

the predicted and measured specific growth rates occurred during summer (September to March), when the diffuse radiant fluxes in the field were high. Specific growth rates of marginal plants were generally more accurately predicted from the total N or total P concentrations in the water than from the other N or P fractions in the water. This suggested that *E. crassipes* may utilize both inorganic and organic forms of N and P for growth.

Insufficient field data was collected in this study to test whether specific growth rates of central plants in the field could be accurately predicted from the growth rate limiting nutrient concentrations in the water by incorporating U_{max} values corrected for the mean daily air temperature, derived central plants in the field, and K_s concentrations generated for *E. crassipes* under N and P growth rate limitation in culture in the Monod model.

The model was refined for marginal plants by expressing the U_{max} 's derived for marginal plants in the field as a function of the temperature and diffuse radiant flux. Two hypothetical multiplicative expressions were investigated. U_{max} 's derived for marginal plants in the field were shown to be related exponentially to the products of the reciprocals of the Absolute mean daily air temperatures and diffuse radiant fluxes.

Specific growth rates of marginal plants were more accurately predicted from the growth rate limiting nutrient (total N or total P) concentrations in the water using U_{max} values, in the Monod model, corrected for the mean daily air temperature and diffuse radiant flux in the field, than for the mean daily air temperature only. In both cases, specific growth rates of marginal plants were more accurately predicted from the total N or total P concentrations in the water than from the other N or P fractions in the water. The largest differences between the predicted and measured specific growth rates occurred during midsummer (November to February), when the relative humidities in the field were at their highest levels.

The model was refined further for marginal plants by expressing the U_{max} 's derived for marginal plants in the field as a function of the

temperature, diffuse radiant flux and relative humidity using a hypothetical multiplicative expression. U_{max} 's derived for marginal plants in the field were shown to be related exponentially to the products of the reciprocals of the Absolute mean daily air temperatures, diffuse radiant fluxes and mean daily relative humidities.

At field sites where relative humidities did not differ substantially from that at which the U_{max} 's of marginal plants were derived, specific growth rates of marginal plants were as, but generally not more accurately predicted from the growth rate limiting nutrient (total N or total P) concentrations in the water using U_{max} values, in the Monod model, corrected for the mean daily air temperature, diffuse radiant flux and mean daily relative humidity in the field, than for the mean daily air temperature and diffuse radiant flux only. In both cases, specific growth rates of marginal plants were more accurately predicted from the total N or total P concentrations in the water than from the other N or P fractions in the water. The largest differences between the predicted and measured specific growth rates, however, still occurred during midsummer (November to February). It was suggested that the K_s concentrations generated for *E. crassipes* under N and P growth rate limitation in culture were temperature dependent and/or that the U_{max} 's derived for marginal plants in the field, particularly during the midsummer months, may have been depressed by some toxic factor in the water. This may partly explain the poor correlation between the predicted and measured specific growth rates during the midsummer months.

At field sites where relative humidities did differ substantially from that at which the U_{max} 's of marginal plants were derived, specific growth rates of marginal plants were generally more accurately predicted from the growth rate limiting nutrient concentrations in the water using U_{max} values, in the Monod model, corrected for the mean daily air temperature, diffuse radiant flux and mean daily relative humidity in the field, than for the mean daily air temperature and diffuse radiant flux only.

In the field, N and P concentrations differed significantly in marginal and central plants growing in loosely and densely crowded populations respectively. It was suggested that these differences reflected some

aspect of the physiology of plants growing under different degrees of crowding. Nitrogen and P concentrations in marginal and central plants were correlated with the $\text{NH}_4\text{-N}$, total N and SRP concentrations in the water respectively, but not with the $\text{NO}_3\text{-N}$ or total P concentrations in the water. This suggested that *E. crassipes* absorbs $\text{NH}_4\text{-N}$ and inorganic phosphate preferentially as N and P sources for growth. It was pointed out, however, that the N and P concentrations in the water can only be reliably estimated from those in the plants in eutrophic waters where the N and P concentrations in the water are high.

Using U_{max} values corrected for the mean daily air temperature, diffuse radiant flux and mean daily relative humidity, derived for marginal plants in the field, and the minimum N and P concentrations in *E. crassipes*, derived from the Y_c values for these nutrients, in Droop's simplified hyperbolic equation, it was shown that specific growth rates of marginal plants in the field were fairly accurately predicted from the growth rate limiting nutrient (N or P) concentrations in the plants. The largest differences between the predicted and measured specific growth rates occurred during midsummer (November to February) and midwinter (June and July), when the air temperatures and diffuse radiant fluxes in the field were at their highest and lowest levels respectively. It was suggested that the minimum N and P concentrations in *E. crassipes* were dependent on the temperature and radiant flux density. This may partly explain the poor correlation between the predicted and measured specific growth rates during the midsummer and midwinter months.

In general specific growth rates of marginal plants in the field were less accurately predicted from the growth rate limiting nutrient (N or P) concentrations in the plants than from the growth rate limiting nutrient (total N or total P) concentrations in the water.

It was concluded from this study that apart from the yield coefficient, kinetic coefficients were not consistently determined for *E. crassipes* growing under N or P growth rate limitation in culture using a batch culture approach. Some suggestions are made as to the manner in which the batch culture method used for determining kinetic coefficients for *E. crassipes*, growing under specific nutrient growth rate limitation in

culture, in this study could be improved. In addition, some possible further refinements to the model are outlined, particularly with regard to using the model for predicting growth rates for *E. crassipes* growing in densely crowded field populations. The application of the refined model for predicting yields, growth rates and amounts and frequencies of harvest for *E. crassipes* growing in loosely crowded field populations in a hypothetical culturally eutrophied water body is illustrated.

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APPENDIX I

PILOT STUDIES

I THE INFLUENCE OF THE MACRONUTRIENT CONCENTRATIONS IN THE CULTURE MEDIUM ON THE SPECIFIC GROWTH RATE OF *E. CRASSIPES*.

1 Introduction

In order to study the kinetics of N or P growth rate limitation of *E. crassipes* in culture, it was required that either N or P be the only growth rate limiting nutrients. This occurs only when all other nutrients supplied to culture are in excess of requirements. However, as the growth rate of *E. crassipes* may be adversely affected if the levels are too high, it is desirable to determine at least for certain macronutrients such as N, K, Ca and Mg the concentrations, and relative proportions in the case of K, Ca and Mg, required to permit growth of *E. crassipes* to proceed at a maximum rate. Mitchell (1970) observed that the growth of *Salvinia auriculata* Auct. (*S. molesta* Mitch) was significantly influenced by different relative proportions of K, Ca and Mg supplied to the culture medium.

IA THE INFLUENCE OF THE N CONCENTRATION IN THE CULTURE MEDIUM ON THE SPECIFIC GROWTH RATE OF *E. CRASSIPES*

2 Methods

Sixty *E. crassipes* plants collected from a population growing in a maturation pond were rinsed through 3 changes of deionised-distilled water, shaken to dislodge adhering water, and their fresh masses recorded on an electronic top loading balance. One plant was placed in each culture vessel containing 5 litres of culture solution deficient in N. Ten different levels of N were added to the cultures to obtain 10

treatments (6 replicates per treatment) in which the N concentrations ranged from 3,61 to 12,64 x 10³ ug N l⁻¹ (16 to 56 x 10³ ug NO₃ l⁻¹). A randomized complete block design was adopted (Rayner, 1967). Culture solutions were changed weekly and adjusted to pH 7,0 weekly, using 5% H₂SO₄ and 10% NaOH, as maximum growth of *E. crassipes* occurs at this pH in culture (Chadwick and Obeid, 1966). Evaporation loss from the cultures was replaced daily with deionised-distilled water. Plants were grown in a greenhouse for 4 weeks after which they were harvested from culture, allowed to drain for 2 minutes, shaken to dislodge adhering water, and reweighed.

Specific growth rates, over the 4 week growth period, were determined for each plant grown at each level of N supplied using the general growth equation (Malek and Fencel, 1966). Specific growth rate data was subjected to an analysis of variance (Rayner, 1967).

3 Results

The average specific growth rates (means of 6 replicates) of *E. crassipes*, over the 4 week growth period, increased significantly ($P = 0,05$) with an increase in the level of N supplied in culture (Table I). The highest specific growth rate was attained by plants where N was supplied at a concentration of 9,03 x 10³ ug N l⁻¹ (40 x 10³ ug NO₃ l⁻¹).

4 Discussion

The N concentration of 9,03 x 10³ ug N l⁻¹ (40 x 10³ ug NO₃ l⁻¹) at which *E. crassipes* attained a maximum growth rate in culture compares favourably with the nitrate concentration of 42 x 10³ ug NO₃ l⁻¹ below which Gosset and Norris (1971) suggested that N as nitrate was limiting to the growth rate of *E. crassipes* in culture.

5 Conclusions

It is concluded that, provided culture solutions are changed weekly, maximum growth rate of *E. crassipes* will occur in cultures where N is supplied at a concentration of $9,03 \times 10^3$ ug N l⁻¹ (40×10^3 ug NO₃ l⁻¹). Consequently, in the kinetic studies where *E. crassipes* needs to be grown in cultures that are not changed, it will be necessary to supplement the cultures with additional N at a concentration of $9,03 \times 10^3$ ug N l⁻¹, at least at weekly intervals, to ensure that the growth rates of plants are maintained at maximum levels.

IB THE INFLUENCE OF THE K, Ca AND Mg CONCENTRATIONS AND THEIR RELATIVE PROPORTIONS IN THE CULTURE MEDIUM ON THE SPECIFIC GROWTH RATE OF *E. CRASSIPES*

2 Methods

K, Ca and Mg stock solutions were prepared according to the method of Hamner, Lyon and Hamner (1942) (Table II). Apart from the sulphate ion, all other ions were supplied to the stock solutions at concentrations identical to those supplied in the culture solution used for growing *E. crassipes* in all other investigations (Table 2.6). Nutrient triangle combinations were derived by combining the stock solutions in different relative proportions (Table III) to give 28 cation combination treatments. The concentrations and relative proportions of K, Ca and Mg varied in the different cation combination treatments whereas those of the anions and the total ionic concentration in each remained constant.

One hundred and sixty eight *E. crassipes* plants were collected from a population growing in a maturation pond (see Pilot Study IA). Two plants were placed in each culture vessel containing 5 litres of a cation combination solution. There were 28 cation combination treatments (Table III) with 6 replicates per treatment. A randomized complete block design was adopted (Rayner, 1967). Cation combination solutions were changed

weekly and adjusted to pH 7,0 weekly. Evaporation loss from the cultures was replaced daily with deionised-distilled water. Plants were grown in a greenhouse for 4 weeks after which they were harvested from culture, allowed to drain for 2 minutes, shaken to dislodge adhering water, and reweighed.

Specific growth rates, over the 4 week growth period, were determined for each plant grown at each cation combination treatment using the general growth equation (Malek and Fencl, 1966). Specific growth rate data was subjected to an analysis of variance (Rayner, 1967).

3 Results

The average specific growth rates (means of 6 replicates) of *E. crassipes*, over the 4 week growth period, were only significantly reduced ($P = 0,05$) in those cultures deficient in Ca and/or K (Table IV). They were not significantly influenced ($P = 0,05$) by the different relative proportions of K, Ca and Mg supplied in the various cultures. In general, however, specific growth rates of *E. crassipes* appeared somewhat higher in those cultures where K, Ca and Mg were supplied in more or less equal relative proportions.

4 Discussion

As the specific growth rates of *E. crassipes* were only significantly reduced in those cultures deficient in K and Ca, it would appear that low concentrations of K, Ca and Mg, ca $4,4 \times 10^3 \text{ ug l}^{-1}$, are required in culture to produce a maximum growth rate in *E. crassipes*. These cations concentrations, however, are considerably lower than those required to produce maximum growth in other aquatic plants. For example, Anderson (1958) reported that maximum growth of *Chara zeylanica* occurs at concentrations of $40 \text{ to } 80 \times 10^3 \text{ ug K l}^{-1}$, $50 \times 10^3 \text{ ug Ca l}^{-1}$ and $40 \times 10^3 \text{ ug Mg l}^{-1}$ in culture. Comparable results were obtained for K and Ca by Starling et al. (1974) for *Nitella hookerii* although this species shows maximum growth at much

lower Mg concentrations (ca 6×10^3 ug Mg l⁻¹) in culture. As *E. crassipes* plants for this experiment were collected from a population growing in secondary treated waste-water effluent in a maturation pond, it is possible that luxury quantities of K, Ca and Mg stored in the plants may have influenced their subsequent growth rates in the various cation combination treatments. This may explain why the specific growth rates of *E. crassipes* were only significantly reduced in those cultures deficient in Ca and/or K.

The observation that the specific growth rates of *E. crassipes* appeared somewhat higher in those cultures where K, Ca and Mg were supplied in more or less equal relative proportions compares with the data of Mitchell (1970) which suggests that growth of *Salvinia auriculata* is also significantly higher in those cultures where these cations are supplied in more or less equal relative proportions.

5 Conclusions

It is concluded that, provided that *E. crassipes* is collected for culture from populations growing in secondary treated waste-water effluent and cultures are changed weekly, this plant will grow at a maximum rate in cultures where K, Ca and Mg are supplied at concentrations of, or in excess of, $4,4 \times 10^3$ ug l⁻¹ and in more or less equal relative proportions. Consequently, in the kinetic studies where *E. crassipes* needs to be grown for longer periods than 4 weeks in culture and in cultures that are not changed, it will be necessary to supply these cations to the cultures at much higher concentrations than $4,4 \times 10^3$ ug l⁻¹ to ensure that the growth rates of plants are maintained at maximum levels.

II THE INFLUENCE OF AERATION OF THE CULTURE MEDIUM ON THE SPECIFIC GROWTH RATE OF *E. CRASSIPES*.

1 Introduction

Plant species differ in their ability to adapt to anaerobic conditions in the rhizosphere (Crawford, 1966) and aeration of the culture medium may, therefore, be an important factor influencing the growth rate of *E. crassipes*. The literature shows that *E. crassipes* has been grown in aerated (Chadwick and Obeid, 1966), but more frequently in unaerated cultures (Bock, 1969; Haller and Sutton, 1973; Pieterse et al. 1976; Tag El Seed, 1978). In order to provide conditions conducive for the maximum growth rate of *E. crassipes* in the kinetic investigations, this experiment was designed to determine the effect of aeration of the culture medium on the growth rate of this plant.

2 Methods

Sixty *E. crassipes* plants were collected from a population growing in partially anoxic water (dissolved oxygen concentration ca 1 mg l^{-1}) in a maturation pond (see Pilot Study IA). One plant was placed in each culture vessel containing 5 litres of culture solution. There were 2 treatments (30 replicates per treatment) in which culture solutions were aerated for 10 min. hr^{-1} by a steady stream of air bubbles in one treatment and not aerated in the other treatment. A randomized complete block design was adopted (Rayner, 1967). Culture solutions were changed weekly and adjusted to pH 7.0 weekly. Evaporation loss from the cultures was replaced daily with deionised-distilled water. Plants were grown in a greenhouse for 3 weeks after which they were harvested from culture, allowed to drain for 2 minutes, shaken to dislodge adhering water, and reweighed.

Specific growth rates, over the 3 week growth period, were determined for each plant grown at each treatment using the general growth equation (Malek and Fencl, 1966). Specific growth rate data was subjected to an analysis of variance (Rayner, 1967).

3 Results

The average specific growth rate (mean of 30 replicates) of *E. crassipes*, over the 3 week growth period, was significantly higher ($P = 0,001$) in unaerated than in aerated cultures (Table V).

4 Discussion

Plants for culture were collected from a population growing in partially anoxic water. Consequently, it is possible that their adaptation to the previous environment may have adversely affected their subsequent growth rate in aerated cultures. Alternatively, it is suggested that the higher growth rate of *E. crassipes* in unaerated cultures may have been the result of an increase in the rate of uptake of nitrate-nitrogen by plants in response to the partially anoxic conditions in culture, the nitrate ion providing the roots with an alternative electron acceptor to oxygen. This phenomenon is known from observations on the respiratory activity of roots in the presence of nitrate. Addition of this ion to the medium surrounding roots gives a marked increase in the respiratory quotient (Burstrom, 1945; Ruhland and Ulrich, 1972). The importance of this electron sink when the oxygen supply is reduced has been demonstrated by Arnon (1937) and Malavolta (1954) who found in barley and rice respectively that these species withstood flooding better if the N supply was as nitrate than as ammonium. A marked increase in the nitrate reductase activity in the roots and leaves of flood tolerant species, when flooded, has been reported by Garcia-Novo and Crawford (1973). Willis and Yemm (1955) found that a rise in the respiratory quotient value in the presence of nitrate is due to the incorporation of reduced N into amino acids and is associated with an increase in carbohydrate utilization. Garcia-Novo and Crawford (1973) found that flood tolerant species had a greater ability to synthesize amino acids under conditions of anoxia than species intolerant of flooding. They suggested that this increase in turnover of amino acids provides a means of

disposal of hydrogen atoms, possibly through the animation of pyruvate, with the various products of glycolysis including amino acids being translocated to the aerial portions of the plant allowing the oxygen debt of the poorly aerated roots to be transferred to the well aerated shoots. As low oxygen tensions have frequently been recorded beneath densely crowded natural populations of *E. crassipes* (Lynch et al., 1947; Tabita and Woods, 1962; Ultsch, 1973), it would appear that such an adaptive mechanism to conditions of partial anoxia may exist in this plant.

5 Conclusions

It is concluded that the higher growth rate of *E. crassipes* in anaerated cultures may be due to an increase in the rate of uptake of nitrate-nitrogen by plants in response to the partially anoxic conditions, but this may also be dependent on a preconditioning of plants to a partially anoxic environment. Consequently, to provide conditions conducive for maximum growth rate and rate of N uptake by *E. crassipes* in the kinetic investigations, particularly in those conducted under N growth rate limitation, it is suggested the plants should be grown in anaerated cultures and collected for culture from populations growing in partially anoxic waters.

III THE RELATIONSHIP BETWEEN THE NUTRIENT CONCENTRATIONS IN THE WATER ENVIRONMENT AND THE NUTRIENT DISTRIBUTION PATTERN IN *E. CRASSIPES*.

1 Introduction

In order to determine whether specific growth rates of *E. crassipes* in the field could be predicted from the growth rate limiting nutrient concentrations in the plants, it was necessary to establish whether chemical analyses should be performed on whole plants or the various plant parts and separately on plants of the marginal and central growth form. Previous attempts to correlate the chemical composition of

E. crassipes with the water environment have yielded conflicting results. Boyd and Vickers (1971) analyzing whole plants, collected from 17 sites in the field, found no significant correlation between the nutrient concentrations in the plant tissues and those in the water environment. Gosset and Norris (1971), on the other hand, by separately analyzing the roots, petioles and pseudolaminae of cultured plants, demonstrated a positive correlation between the N and P concentrations in the plant tissues and those in the culture medium. The differences in P/R ratios between large water hyacinths (central plants) and dwarf water hyacinths (marginal plants) reported by Mitsch (1977) suggests that there may be differences in their chemical composition. As both marginal and central plants occur in the field, this may be one factor contributing to the poor correlation reported by Boyd and Vickers (1971) between the chemical composition of *E. crassipes* and the water in field samples. The good correlation reported by Gosset and Norris (1971) may be attributable to the fact that only the marginal growth form is present where plants grow individually or in loosely crowded populations (Ra0, 1920; Bruhl and Gupta, 1927; La Garde, 1930) as in culture.

2 Methods

E. crassipes plants of the marginal and central growth form were collected during summer (September) from 3 sites along the Natal coastal plain, viz the Enseleni River ca 20 km from the sea, the Umlaas River ca 5 km from the sea and the Isipingo Canal ca 2,5 km from the sea (Figure I). Batches of marginal and central plants were collected at random from loosely crowded and densely crowded populations respectively at each site. Three batches of each growth form were collected at each site. Each batch of marginal and central plants was picked free of debris, separated into roots, petioles and pseudolaminae, washed thoroughly in tap water to remove all extraneous particles, and rinsed through 3 changes of deionised-distilled water. The plant tissues were dried in a forced draft oven at 60°C. The dry 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.

Nitrogen and P concentrations were determined in each batch of dry, ground, plant tissues according to methods published in the Association of Official Agricultural Chemists (1975). Na, K, Ca and Mg concentrations were analyzed in each batch of dry ground, plant tissues with the aid of an atomic absorption spectrophotometer (Isaac and Kerber, 1971).

Water samples collected from the immediate vicinity of the marginal and central plant populations at each site were chemically analyzed. Kjeldahl nitrogen ($\text{NH}_4\text{-N}$), nitrate-nitrogen ($\text{NO}_3\text{-N}$) and soluble reactive phosphorus (SRP) concentrations (Twinch and Breen, 1980) were analyzed in the water samples using methods published in the Environmental Protection Agency (1974) and Standard Methods (1975). Na, K, Ca and Mg concentrations were determined in the water samples using an atomic absorption spectrophotometer (Isaac and Kerber, 1971).

Chemical analysis data was subjected to an analysis of variance (Rayner, 1967). Tests for significant differences between sites, plant parts, growth form (marginal and central plants) were made for each nutrient analyzed in the plant tissues.

3 Results and Discussion

(a) Water analysis

The nutrient concentrations (means of 3 replicates) analyzed in the water from the vicinity of the marginal and central plant populations at each site are given in Table VI. The cationic dominance order of the 3 sites was $\text{Na} > \text{Ca} > \text{Mg} > \text{K}$. This order is characteristic of water influenced principally by geochemical enrichment (Gorham, 1955) and, therefore, the proximity of the sea, particularly at the Isipingo Canal site, appeared to have little influence on the chemical composition

of the water. There was, however, a general increase in the nutrient status of the water in the sequence Enseleni, Umlaas, Isipingo except for K, SRP and N (both $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) where the order was reversed for the Enseleni and Umlaas Rivers. A possible explanation for this reversal may be in the influence of nutrient enrichment by runoff from the sugar cane fields in the Enseleni River catchment. This suggestion is supported by the higher $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, SRP and K concentrations and lower Na, Ca and Mg concentrations in the Enseleni River water.

Na, K, Ca and Mg concentrations showed no consistent differences in the water taken from the vicinity of the marginal and central plant populations at the 3 sites (Table VI). $\text{NH}_4\text{-N}$ and SRP concentrations, however, were higher in the water taken from the vicinity of the central plant populations and $\text{NO}_3\text{-N}$ concentrations were higher in the water taken from the vicinity of the marginal plants populations at all 3 sites. Several researchers (Lynch *et al.*, 1947; Tabita and Woods, 1962; Ultsch, 1973) have reported reduced oxygen and increased carbon dioxide tensions in the water from the vicinity of densely crowded populations of *E. crassipes*. This may explain the higher $\text{NH}_4\text{-N}$ and SRP concentrations, and lower $\text{NO}_3\text{-N}$ concentrations, recorded in the water taken from the vicinity of the central plant populations. With respect to N, the higher oxygen tensions in the water from the vicinity of loosely crowded marginal plant populations would favour the oxidation of $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$, particularly as the rate of nitrification is influenced by the redox status of the water (Keeny, 1973). Under densely crowded central plant populations, where oxygen levels are reduced, the rate of nitrification would be lower and $\text{NH}_4\text{-N}$ would accumulate. With respect to P, the greater release of P from sediments under anoxic conditions is well documented (Mortimer, 1941). Vollenweider (1972) has shown that oxygen depletion is accompanied by a breakdown in the ability of sediments to fix P resulting in the sediments acting as a source rather than a sink for P. In addition, slower water movement beneath densely crowded central plant populations would permit organic material to accumulate which would yield P on decomposition (Jewell, 1971).

(b) Plant analysis

(i) Whole plants

Despite the differences in the cation concentrations of the water between sites, plant tissues from all sites were consistent in having concentrations of $K > Ca > Mg > Na$ (Table VII). Their relative contributions, however, were influenced by site so that the ratios between ions in the plant tissues varied considerably. For example, $K:Na$ ranged between 9,2 and 15,1. Na, Mg and P concentrations in whole plants showed significant differences ($P = 0,01$) between sites (Table VIII) with the Isipingo Canal populations being most different (Table VII). The relatively higher Na and SRP concentrations recorded in the water at the Isipingo Canal site were reflected in the plant tissue analysis whereas Mg was not. The smaller differences in the Na, SRP and Mg concentrations recorded in the water at the Umlaas and Enseleni Rivers were, however, not reflected in the plant tissue analysis. It appears, therefore, that in some instances only, can increased concentrations of nutrients in the water, particularly if these are fairly large, be detected by whole plant analysis as reported by Dymond (1948). The chemical composition of plants may, however, also reflect the earlier rather than the present chemical status of the water.

(ii) Plant parts

All nutrients analyzed showed significant differences ($P = 0,01$) between the various plant parts when these were compared irrespective of site or growth form (Table VIII). The lowest concentrations of all nutrients analyzed were consistently found in the roots. The cations (Na, K, Ca and Mg) were maximal in the petioles and the anions (N and P) were maximal in the pseudolaminae (Table IX). As the pseudolaminae of *E. crassipes* are

modified petioles (Arber, 1920; Bock, 1966), it would appear that luxury quantities of nutrients are stored in the petioles of *E. crassipes*. Luxury quantities of nutrients have also been reported to be stored in the petioles of other higher plants. For example, Ulrich (1955) found that luxury quantities of N are stored in the petioles of sugar beet (*Beta vulgaris*).

Apart from Na, the nutrient concentrations in the various plant parts of *E. crassipes* were not significantly influenced ($P = 0,05$) by site (Table VIII). As the nutrient concentrations in whole plants were in some instances significantly influenced by site, it is suggested that more reliable correlations may be obtained between the nutrient concentrations in the plant tissues and those in the water environment by analyzing whole plants rather than the various plant parts.

(iii) Marginal and central plants

Apart from Ca, all nutrients analyzed differed significantly (at or less than $P = 0,05$) in the tissues of marginal and central plants (Table VIII). These differences were reflected in the roots, petioles and pseudolaminae (Table XI). With the exception of Ca and P, they showed significant differences (at or less than $P = 0,05$) in the various plant parts between the two growth forms (Table VIII). Na, Mg and N concentrations were higher in the tissues of marginal plants whereas K and P concentrations were higher in the tissues of central plants (Table X). With respect to Na, K and Mg, it does not seem reasonable to explain the differences in the concentrations of these nutrients between marginal and central plants in terms of the nutrient concentrations in the water as the latter were fairly similar in the vicinity of the marginal and central plant populations at each site (Table VI). One is, therefore, left to postulate that these differences reflect some aspect of

the physiology of plants growing under different degrees of crowding. With respect to N and P, however, the higher P concentrations determined in the tissues of central plants may be related to the higher SRP concentrations recorded in the water from the vicinity of the central plant populations (Table VI), whereas the higher N concentrations determined in the tissues of marginal plants may be related to the higher $\text{NO}_3\text{-N}$ concentrations recorded in the water from the vicinity of the marginal plant populations. The latter suggestion, however, is based on the assumption that $\text{NH}_4\text{-N}$ does not serve as a N source for aquatic plants as suggested by Sculthorpe (1967). With the exception of Na, the concentrations of the various nutrients in the 2 growth forms were not significantly influenced ($P = 0,05$) by site (Table VIII). This suggests that growth form has a greater influence on the nutrient concentrations in *E. crassipes* and their distribution within the plant than site. The influence of site on the Na concentrations in plants of different growth form, however, is not clear. It is possible that interaction between plants in densely crowded populations may influence their physiology of Na uptake in some undetermined manner.

4 Conclusions

It is concluded that the nutrient concentrations in whole *E. crassipes* plants may in some instances be influenced by site, whereas those in the various plant parts are not influenced by site. This suggests that more reliable correlations may be obtained between the nutrient concentrations in the plant tissues and those in the water environment by analyzing whole plants rather than the various plant parts. Consequently, specific growth rates of *E. crassipes* in the field should also be more accurately predicted from the growth rate limiting nutrient concentrations as analyzed in whole plants rather than as analyzed in the various plant parts. Most important, however, is the observation that the nutrient

concentrations in *E. crassipes* are significantly influenced by the growth form of plants. Consequently, in order to accurately predict specific growth rates of *E. crassipes*, growing in loosely and densely crowded field populations respectively, from the growth rate limiting nutrient concentrations in the plant tissues, it will be necessary to analyze these separately in marginal and central plants.