This is to certify that the

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Interacting Carbon and Light
Limits to Macrophyte Growth

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Sarah Kate Liehr

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INTERACTING CARBON AND LIGHT LIMITS TO MACROPHYTE GROWTH

By

Sarah Kate Liehr

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ABSTRACT

INTERACTING CARBON AND LIGHT LIMITS
TO MACROPHYTE GROWTH

By

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This study was conducted to develop a laboratory technique for measuring the growth kinetics of macrophytes. Elodea canadensis and Ceratophyllum demersum were grown under a variety of light conditions in microcosms containing defined medium. The pH was measured at regular intervals, and the amount of carbon fixed by the plants was calculated. Although there was a problem with algal interference, it was possible to obtain usable data by this method. The data were used to calculate Monod growth equations describing the interaction of limiting carbon and light levels on the growth of the plants. The plants were found to respond in a manner similar to the response of algae grown under similar conditions. The data indicate that elodea is able to grow at a faster rate than ceratophyllum and that the macrophytes may be able to outcompete the green alga Chorella vulgaris at low CO_2 levels.

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I would especially like to thank Darrell L. King for his guidance throughout this study, and for making my Master's program a valuable educational experience.

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INTRODUCTION

As the population of man on earth increases, increased nutrient loading of our aquatic systems is inevitable. This nutrient loading stimulates plant production, resulting in extensive weed growth, a process often referred to as eutrophication. Although abundant plant growth is usually considered a nuisance, some aquatic plants are more desirable or useful than others in specific situations. Water used for different purposes, such as human consumption, recreation, land irrigation, have different criteria for determining which plants are most desirable. For example, when aquatic plants are used in the treatment of wastewater, it is desirable to have a plant species that can grow in high pH conditions, can easily be harvested, and can be used for some purpose after it is harvested, such as for livestock feed. Therefore, it is desirable to be able to manage aquatic systems for the selection of the dominant plant species. This can be done by comparing growth kinetics of different plants. The purpose of this study was to develop a technique for the measurement of the growth kinetics of two macrophytes, Elodea canadensis and Ceratophyllum demersum, and to determine how their growth is affected by limiting levels of carbon and light.

METHODS

Experimental Procedures

The purpose of this study was to quantify the interactive effect of carbon and light limits on growth responses of Elodea canadensis and Ceratophyllum demersum. Plants were collected from natural populations and then were grown in the laboratory under artificial lights in microcosms containing a defined inorganic nutrient medium.

Microcosms

The experimental microcosms used in this study were similar to the microcosms used by Sievers (1971), Young (1972), and Klemovich (1973). They consisted of one-liter Erlenmeyer flasks with rubber stoppers in which two holes had been drilled. One hole contained a glass tube with an air lock to maintain atmospheric pressure within the microcosm while minimizing recarbonation from the atmosphere. The other hole contained a rubber serum cap for the removal of samples (Figure 1). Samples were taken with a hypodermic syringe through the rubber serum cap so that the medium was not exposed to the atmosphere. The growth medium (Kevern and Ball, 1965) contained all nutrients in excess except carbon, which was limited by the alkalinity of the medium (see Appendix). A running total of the volume removed for sampling was recorded.

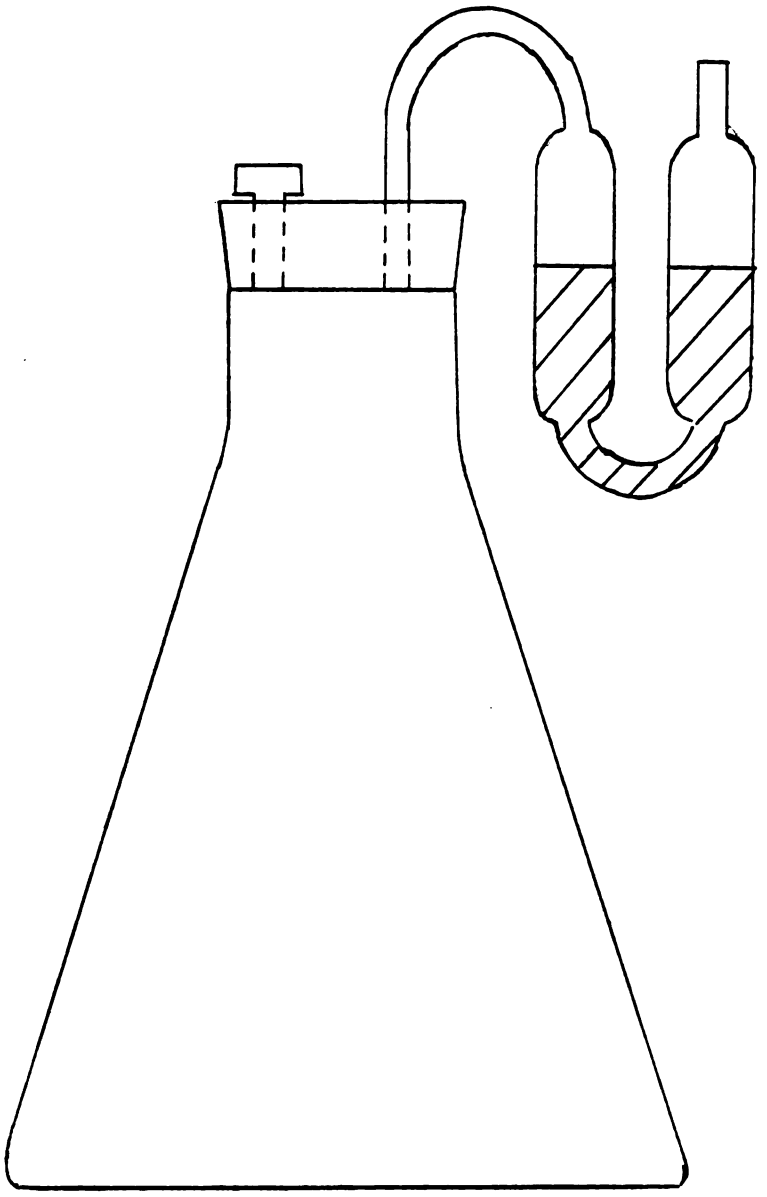


Figure 1. Microcosm with air lock used to study growth rates of C. demersum and E. canadensis.

Light

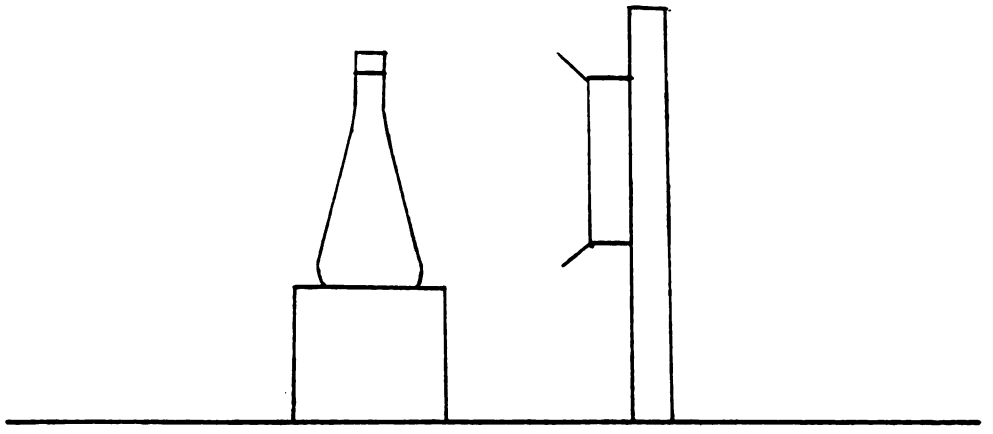
Continuous light was provided by two 40-watt "Gro Lux" fluorescent lights mounted on a wooden frame (Figure 2). A range of light intensities was obtained by covering the lights with various combinations of black cheese cloth and fine mesh, black wire screen. Light intensity and energy units were measured with a Weston footcandle meter Model 756 and a LI-COR Quantum Sensor, Model LI-192S.

Alkalinity, pH

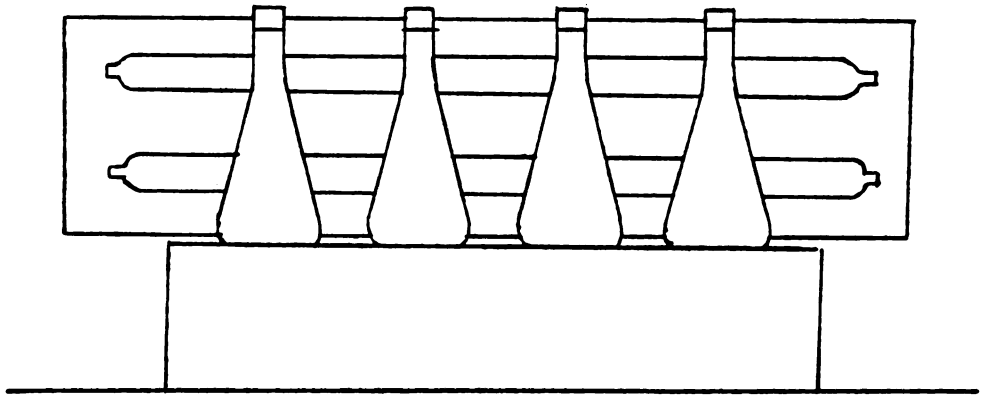
The initial carbonate-bicarbonate alkalinity was measured by the titration method (Standard Methods, 1971). All pH measurements were obtained with a Corning Model 12 research pH meter with a general purpose glass semi-microelectrode. The pH meter was standardized against standard buffer solutions at each sample time, and the standardization was checked between sample measurements.

Sampling

Each sample, collected from the microcosm with a syringe, was injected into a 50 ml beaker which contained nitrogen gas, and was capped with a rubber stopper. The rubber stopper had one hole for injecting the sample and the nitrogen gas into the beaker, and a second hole for the pH electrode. The purpose of this method was to minimize recarbonation from the atmosphere during the time required to record an accurate pH measurement of the sample.



side view



front view

Figure 2. Lighting arrangement used in macrophyte growth study.

Collection of Plants

Elodea canadensis was collected from the second lake of the Water Quality Management Project on the south portion of the Michigan State University campus at East Lansing. *Ceratophyllum demersum* was collected from the concrete ponds in back of the Limnology Laboratory on Kalamazoo Street on the Michigan State University campus. The plants were collected and taken to the laboratory where they were allowed to acclimate to room temperature. After they were sorted and put in beakers of clean medium, the plants were placed under experimental light conditions to allow them to acclimate to light prior to the initiation of the experiment.

Initial Plant Carbon Content

A sample of plants collected for each experiment was weighed, dried at 105° C for 24 hours, and weighed again. These data were used to obtain a wet weight vs. dry weight curve. Initial carbon content of these dried plants was analyzed using a Perkin-Elmer Model 240 Elemental Analyzer. The plants actually used in the experiments were weighed for wet weight, and the carbon mass was extrapolated from the curves.

Data Calculation Procedures

In this study, plant growth was measured as a net uptake of inorganic carbon from the medium. As carbon dioxide is fixed by the plants and carbon is removed from the medium, the CO_2 concentration is controlled by the following equilibrium reactions (King, 1970):

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + HOH \quad (1)$$

$$HCO_3^- + OH^- \rightleftharpoons CO_3^= + HOH \quad (2)$$

These reactions can be added, resulting in

$$2\ HCO_3^- + HOH \rightleftharpoons CO_2 + CO_3^= + 2\ HOH \quad (3)$$

As CO_2 is removed by photosynthesis, the carbonate ion dominates the system. If carbonates do not precipitate from solution, as is the case with media dominated by monovalent cations, CO_2 is further removed by the following reaction:

$$CO_3^= + 2\ HOH \rightleftharpoons CO_2 + HOH + 2\ OH^- \quad (4)$$

As this reaction occurs, carbonate-bicarbonate alkalinity is converted to hydroxyl alkalinity, but the total alkalinity does not change (King, 1970).

Carbon Calculations

Growth, or net uptake of inorganic carbon, was determined by calculating the change in total inorganic carbon in the medium as a function of time. The amount of inorganic carbon in the system at any time can be calculated by the following equation (Sievers, 1971):

$$\Sigma CO_2 = a \frac{\frac{H^2}{K_1} + H + K_2}{H + 2K_2}$$

where:

ΣCO_2 = total inorganic carbon moles/l

a = carbonate-bicarbonate alkalinity, corrected for hydroxyl ion concentration, eq/l

H = hydrogen ion concentration, moles/l

K_1 = first dissociation constant of carbonic acid

K_2 = second dissociation constant of carbonic acid.

To calculate total inorganic carbon by this formula, it is necessary to know the initial carbonate-bicarbonate alkalinity, a; the temperature, to calculate K_1 and K_2; and the pH at given time intervals. As the pH rises with plant carbon fixation, the alkalinity has to be corrected by subtracting the increasing hydroxyl ion concentration. An increase in plant biomass, or carbon fixed (C_{fixed}) can be calculated by measuring pH and calculating the total inorganic carbon (ΣCO_2) at the specified time intervals and applying the following equation (Young, 1972):

$$C_{fixed} = \Delta\Sigma CO_2 = \Sigma CO_2(initial) - \Sigma CO_2(final) \quad (6)$$

The relationship of pH with time and of C_{fixed} with time can be represented by similar curves with the general shape shown in Figure 3.

Growth Rate

Plant biomass can be described at any time t by the equation

$$M_t = M_o e^{\mu t} \quad (7)$$

where:

M_t = biomass at time t

M_o = initial biomass (t=0)

μ = specific growth rate.

The specific growth rate (μ), or the instantaneous rate of change of biomass per unit biomass, does not have to be considered as an intrinsic constant value. In this study, μ was considered to be a variable, and was calculated as follows (Young and King, 1973):

$$\mu_{\Delta t} = \frac{\Delta M/\Delta t}{m} = \frac{(M_{t2} - M_{t1})/(t_2 - t_1)}{(M_{t2} + M_{t1})/2} \quad (8)$$

where:

$\mu_{\Delta t}$ = specific growth rate during time increment, hr^{-1}

M = biomass increment (inorganic carbon fixed), moles C/l

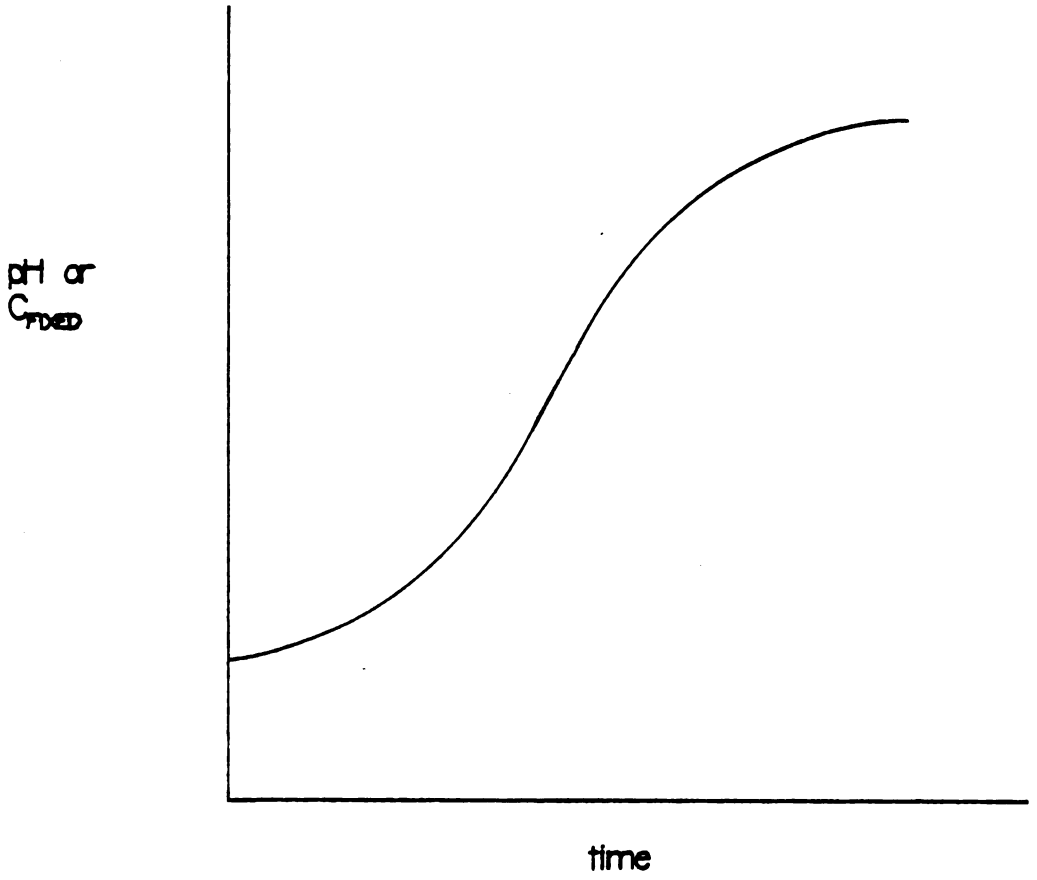


Figure 3. Typical curve of pH or C_{FIXED} with time for microcosm studies.

t_1, t_2 = boundary parameters of time increment, hr^{-1}

$\Delta t = t_2 - t_1$ = time increment, hr^{-1}

m = average standing crop biomass during the increment, moles C/l.

The specific growth rate can be related to some limiting nutrient concentration by Monod's application of the Michaelis-Menten enzyme kinetic equation to whole organisms,

$$\mu = \mu_{max} \frac{S}{K_s + S} \tag{9}$$

where:

μ = growth rate, hr^{-1}

μ_{max} = maximum growth rate, hr^{-1}

S = substrate concentration

K_s = substrate concentration at $\frac{1}{2}\mu_{max}$

The limiting nutrient in this study is carbon. Assuming plants can only use free carbon dioxide (CO_{2f}), the growth rate can be written as a function of CO_{2f}. The CO_{2f} concentration can be calculated by the following equation derived by Harvey (1957) and Park (1969):

$$CO_{2f} = a \frac{H^2}{K_1(H + 2K_2)} \tag{10}$$

where:

$CO_{2f} = H_2CO_3^*$ (aq) including CO_2 (g), moles/l

a = carbonate-bicarbonate alkalinity corrected for hydroxyl ion concentration, eq/l

H = hydrogen ion concentration, moles/l

K_1 = first dissociation constant for carbonic acid

K_2 = second dissociation constant for carbonic acid.

Equation 9 is usually used to describe relationships between specific growth rate and a limiting nutrient in situations where an assumption is made that growth will continue until the substrate has been completely removed. This assumption cannot be made with regard to aquatic plants and CO_{2f} concentration, because there is a certain minimum CO_{2f} concentration required by plants for growth to be sustained (Klemovich, 1973). This threshold concentration (S_q) can be accounted for by modifying Equation 9 as follows:

$$\mu = \mu_{max} \frac{S - S_q}{(K_s - S_q) + (S - S_q)} \qquad (11)$$

where:

S_q = the minimum substrate concentration required to sustain growth.

The constant S_q is obtained from the data as the substrate concentration where μ goes to zero. The other constants, μ_{max} and K_s were calculated using the S/μ vs. S transformation of Equation 9 shown in Equation 12 and in Figure 4.

$$S/\mu = K_s/\mu_{max} + (1/\mu_{max})\, S \qquad (12)$$

This transformation was chosen because it gives more accurate estimates of the constants than the commonly used double-reciprocal transformation (Dowd and Riggs, 1964).

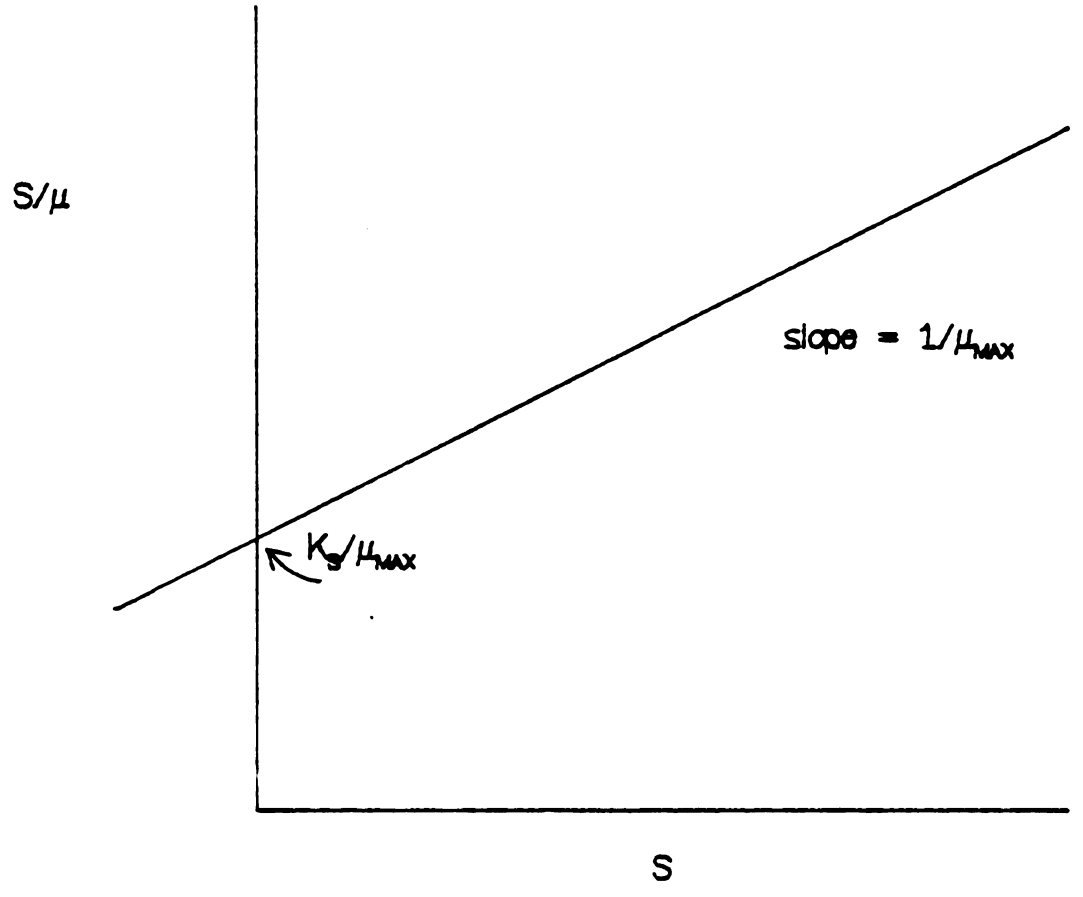


Figure 4. Graphical representation of the S/μ vs. S transformation of the growth rate formula.

RESULTS AND DISCUSSION

This study involved the application of a microcosm method previously used to study algal growth kinetics to the study of macrophyte growth kinetics. Since the method had not been used for this purpose before, preliminary experiments were necessary to determine if the method could be applied to macrophytes. The preliminary experiments showed that the plants did grow within the microcosms, but that diatoms and green and bluegreen algae naturally associated with the macrophytes also fixed carbon from the medium. This algal interference made it impossible to quantify the carbon fixed by the macrophytes alone. Therefore, to be able to use plants from natural populations, it was necessary to develop some techniques to eliminate or at least minimize algal interference.

Scanning electron photomicrographs of elodea indicated that significantly fewer algae were present on the tips than on the older parts of the plants (L. Koivuniemi, personal communication). To minimize the initial algal population introduced into the microcosms, only the 2-4 cm tips of elodea and the 6-10 cm tips of ceratophyllum were used in this study.

Copper sulfate was added to the medium at a concentration of 15 μmoles Cu/l to slow algal growth. This concentration was selected after preliminary studies indicated

that it did slow algal growth while not perceptibly changing the initial growth rate of the plants. This concentration of 15 μmoles Cu/l was not high enough, however, to completely stop algal growth, but it did postpone any noticeable growth of the algae.

Another strategy used in the attempt to minimize the effect of the algae was to start with a large plant biomass. A larger initial biomass results in a faster macrophyte carbon fixation rate. This strategy was used with the hope that the carbon would be removed from the medium by the macrophytes rapidly, yielding a free carbon dioxide concentration which limited further macrophyte activity before the algae had a chance to become established.

None of these techniques were entirely successful at eliminating algal growth. The preliminary studies indicated, however, that data could be obtained if all three methods were used simultaneously. Therefore, the study was conducted using all three methods to minimize algal interference.

The combined technique for elimination of algal interference worked fairly well at the high light intensities, especially for ceratophyllum. Under light intensities of 3875, 2585, and 1290 lux, ceratophyllum removed the carbon fast enough to lower the CO_{2f} concentration to the point where growth stopped before the algae started to grow. Under a light intensity of 690 lux, ceratophyllum did not grow fast enough to allow determination of the point in time

when the macrophyte stopped growing. It was not possible to distinguish this point for elodea under many of the light conditions used, even though elodea generally grew faster than ceratophyllum. Elodea appears to have had larger populations of algae associated with it.

The preliminary experiments also were used to determine the range of light conditions for the study. Plants under light intensities less than 540 lux did not grow well, so only light intensities over 540 lux were used. Alkalinities between 3.4 and 4.0 meq/l were used in all experiments. The bottles were kept at room temperature, and remained at a fairly constant temperature of 25° C. Initial conditions for the experiments are given in Table 1.

The pH measurements were taken at 12 hour intervals until the pH stopped rising, usually between 10 and 16 days. A typical curve of pH as a function of time is shown in Figure 5. A smooth curve was drawn through the data points to eliminate deviations due to incomplete mixing and slight temperature variations. As can be seen in Figure 5, the pH rose sharply, then reached a plateau before starting to rise again. This plateau was interpreted as the point where macrophyte growth stopped. The subsequent rise in pH was due to algal growth.

Representative pH curves for ceratophyllum under four light conditions are shown in Figure 6. The curves indicate that the plants were able to continue growing to higher pH values under higher light intensities. This figure also

TABLE 1. Initial conditions for the ceratophyllum and elodea microcosms.

	Light (lux)	(μ einstein m^{-2} sec^{-1})	Alkalinity (meq/l)	Initial Organic Carbon (mmoles C/l)
Ceratophyllum				
	3875	79.8	3.759	17.457
	3875	79.8	3.759	19.666
	3885	79.8	3.981	17.816
	3875	79.8	3.981	18.752
	3875	79.8	3.835	13.514
	3885	79.8	3.835	13.785
	2585	62.3	3.981	19.656
	2585	62.3	3.981	18.819
	2585	62.3	3.835	14.169
	2585	62.3	3.835	14.216
	1290	26.7	3.981	21.774
	1290	26.7	3.981	22.332
	1290	26.7	3.835	15.145
	1290	26.7	3.835	15.506
	690	17.6	3.981	25.841
	690	17.6	3.981	28.663
	690	17.6	3.835	16.762
	690	17.6	3.835	17.144
Elodea				
	3875	79.8	3.483	17.521
	3875	79.8	3.483	35.310
	3875	79.8	3.474	2.579
	3875	79.8	3.474	4.941
	3875	79.8	3.474	6.782
	3875	79.8	3.759	8.509
	3875	79.8	3.759	12.111
	2585	62.3	3.759	11.191
	2585	62.3	3.759	12.518
	1290	26.7	3.981	13.500
	1290	26.7	3.981	12.072
	690	17.6	3.981	14.588
	690	17.6	3.981	16.615

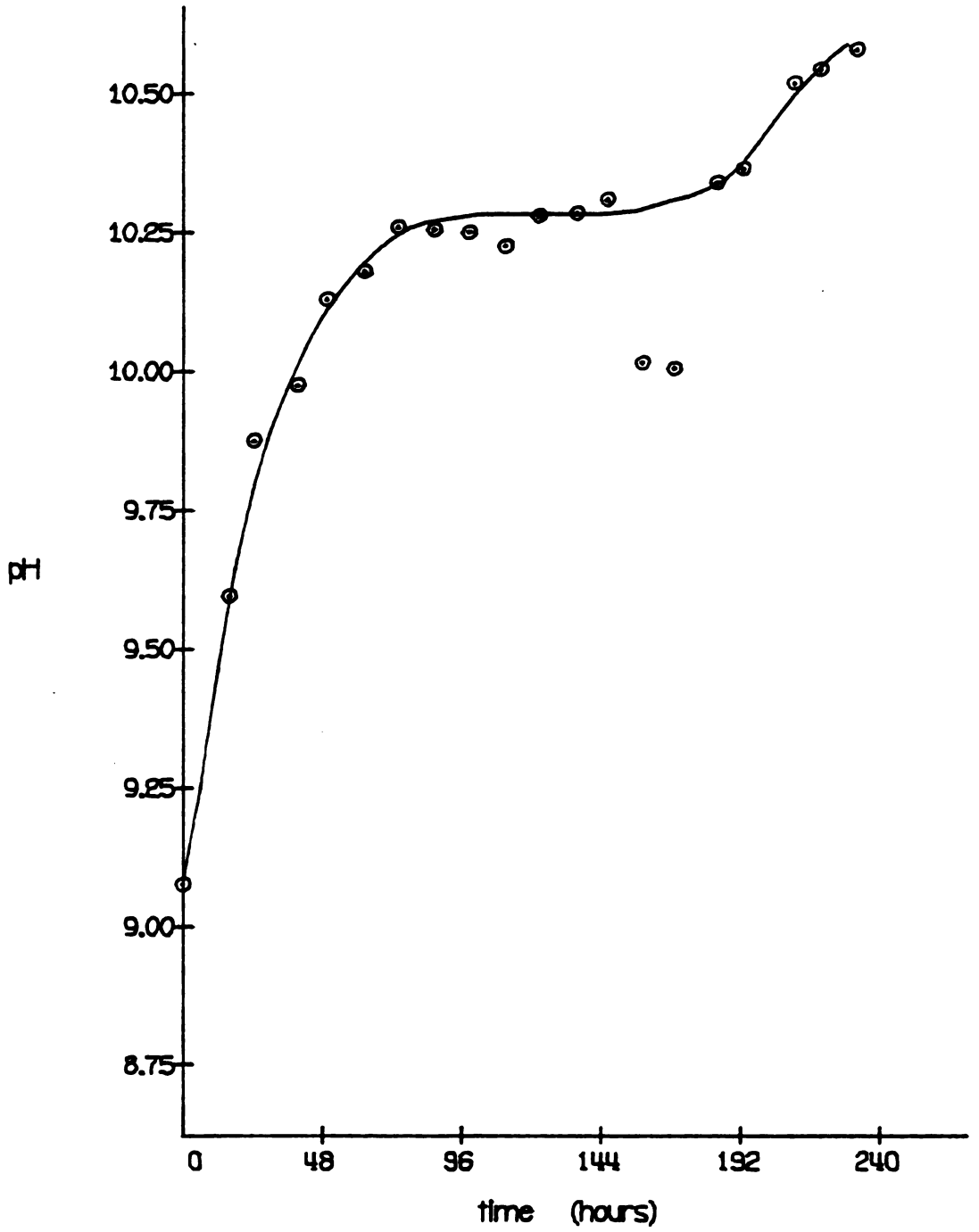


Figure 5. Typical curve of pH as a function of time for a C. demersum microcosm under a light intensity of 3875 lux.

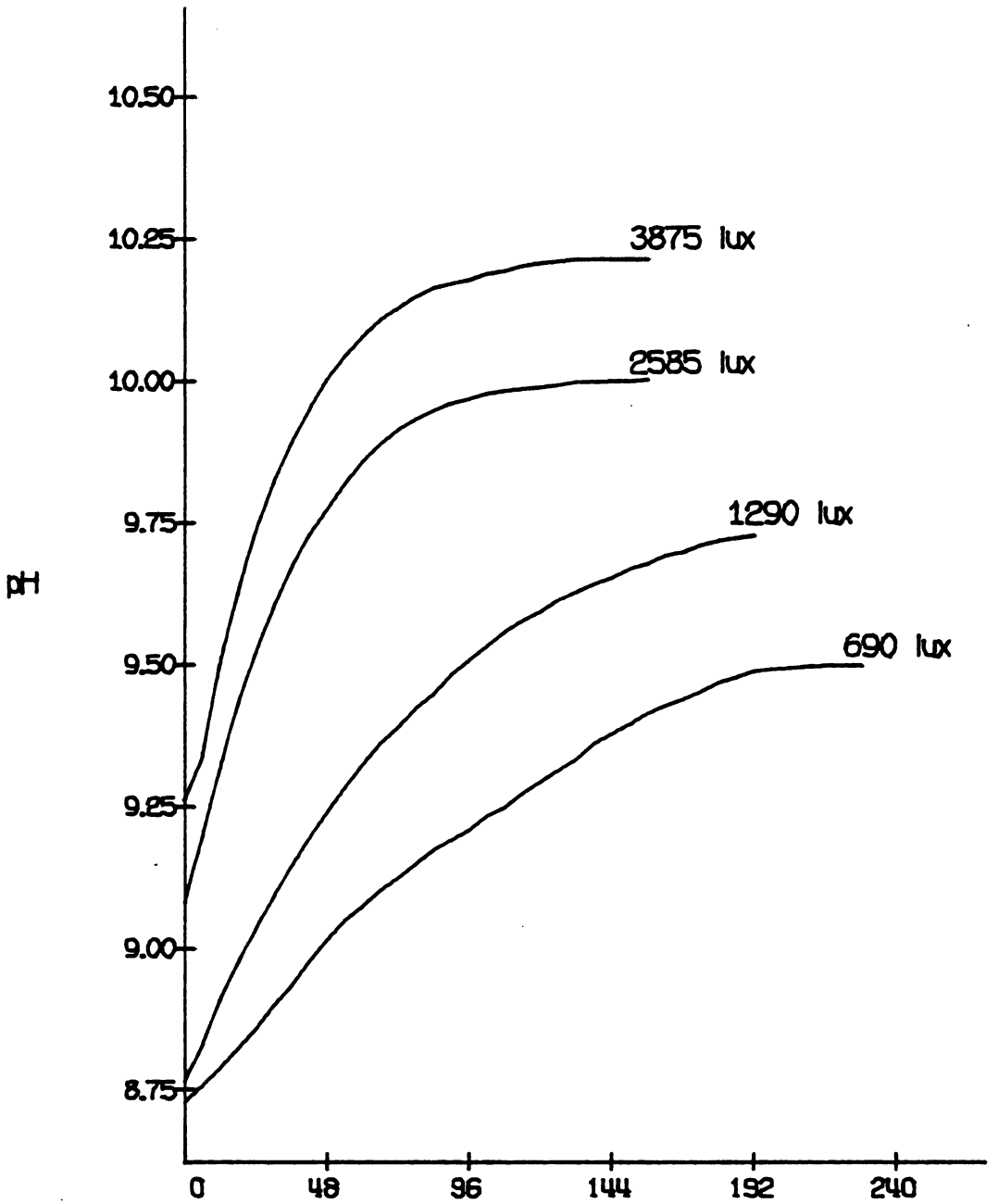


Figure 6. Representatiive curves of pH as a function of time for C. demersum under four light intensities.

shows that higher light intensities result in steeper pH curves, and thus the rate at which the pH rises increases with increasing light intensities.

Similar pH curves are given in Figure 7 for elodea with three light intensities. There was too much algal interference at the lowest light intensity (690 lux) to obtain usable data. These curves demonstrate that elodea responds to various light intensities in a manner similar to ceratophyllum. The two highest light intensities allowed growth to continue to almost the same pH value. The curves illustrate, however, that there was a difference in the rate at which that pH was attained.

The pH data were used to calculate the amount of carbon fixed by Equations 5 and 6. The results are shown in the top half of the graphs in Figures 8 and 9 for ceratophyllum and elodea respectively. Even though the initial biomass is not taken into consideration, the curves demonstrate that as light intensity increases, the amount of carbon that can be fixed by the plants and the rate at which that carbon is fixed also increases.

Concentrations of CO_{2f} were calculated using Equation 10. The lower half of the graphs in Figures 8 and 9 show the mirror-image relationship of CO_{2f} to carbon fixed. These graphs indicate that higher light intensity not only allows the plants to fix more carbon, but also allows the plants to grow to lower CO_{2f} concentrations.

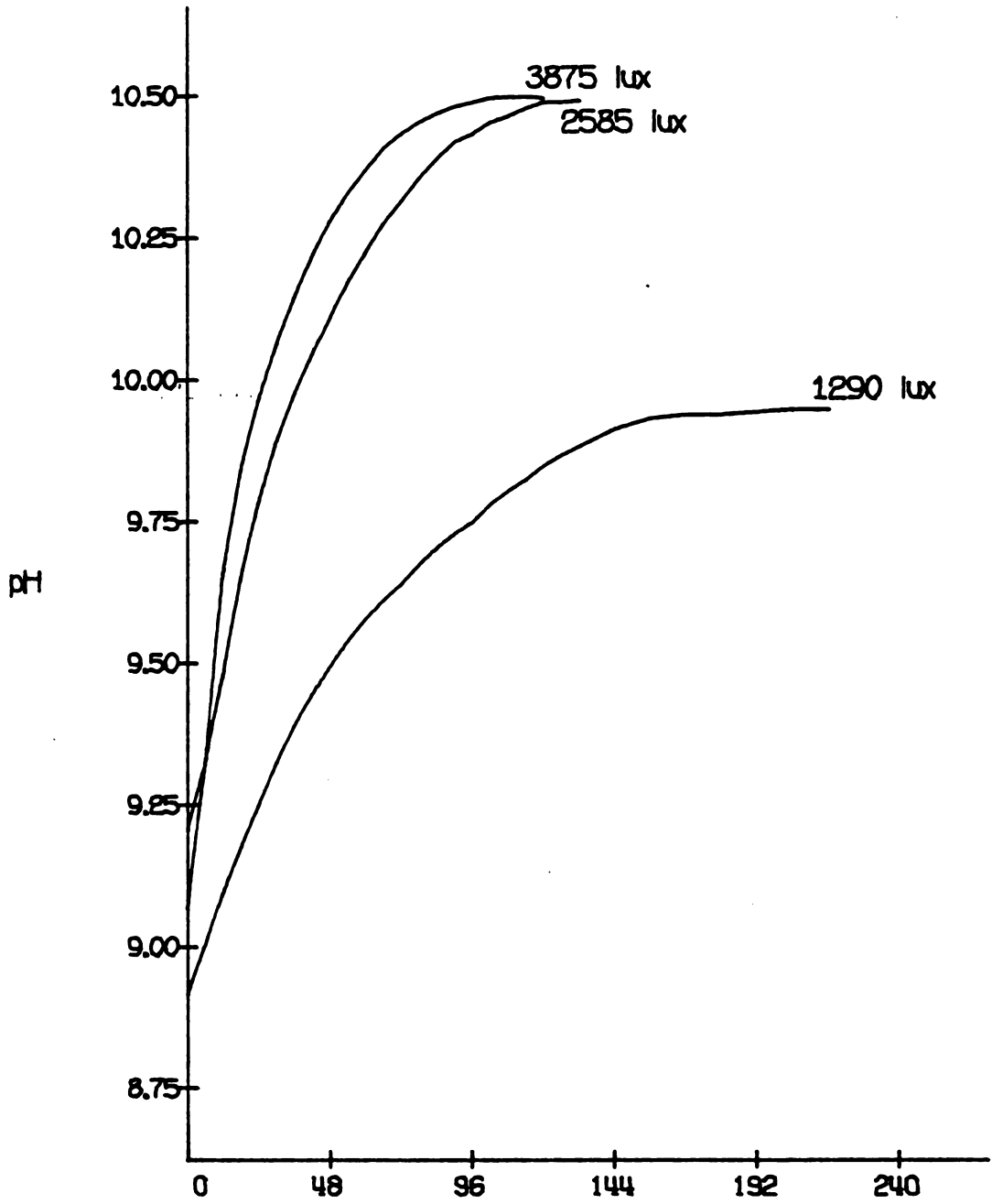


Figure 7. Representative curves of pH as a function of time for E. canadensis under three light intensities.

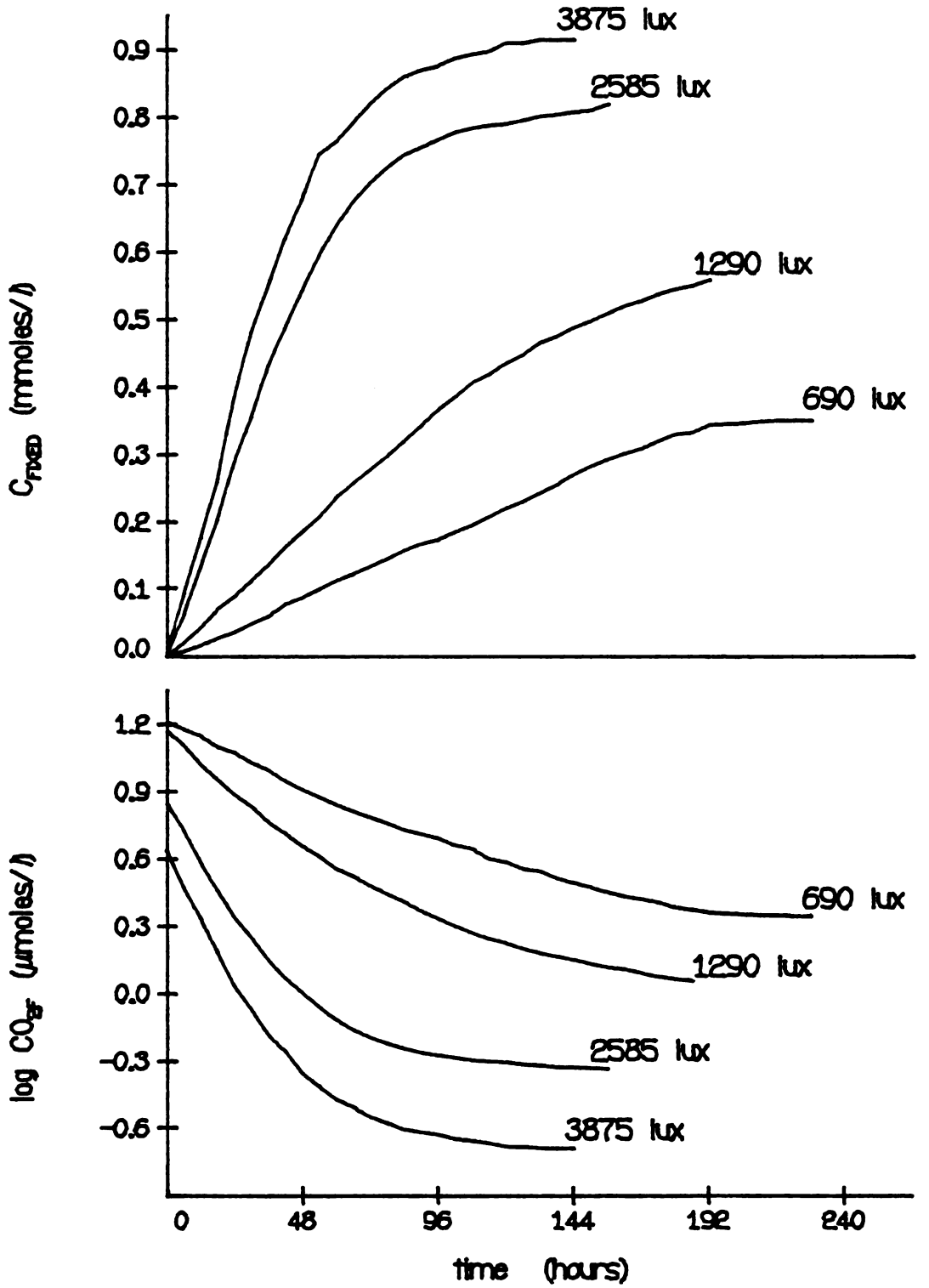


Figure 8. Representative curves of carbon fixed and free CO_2 as functions of time for C. demersum under four light intensities.

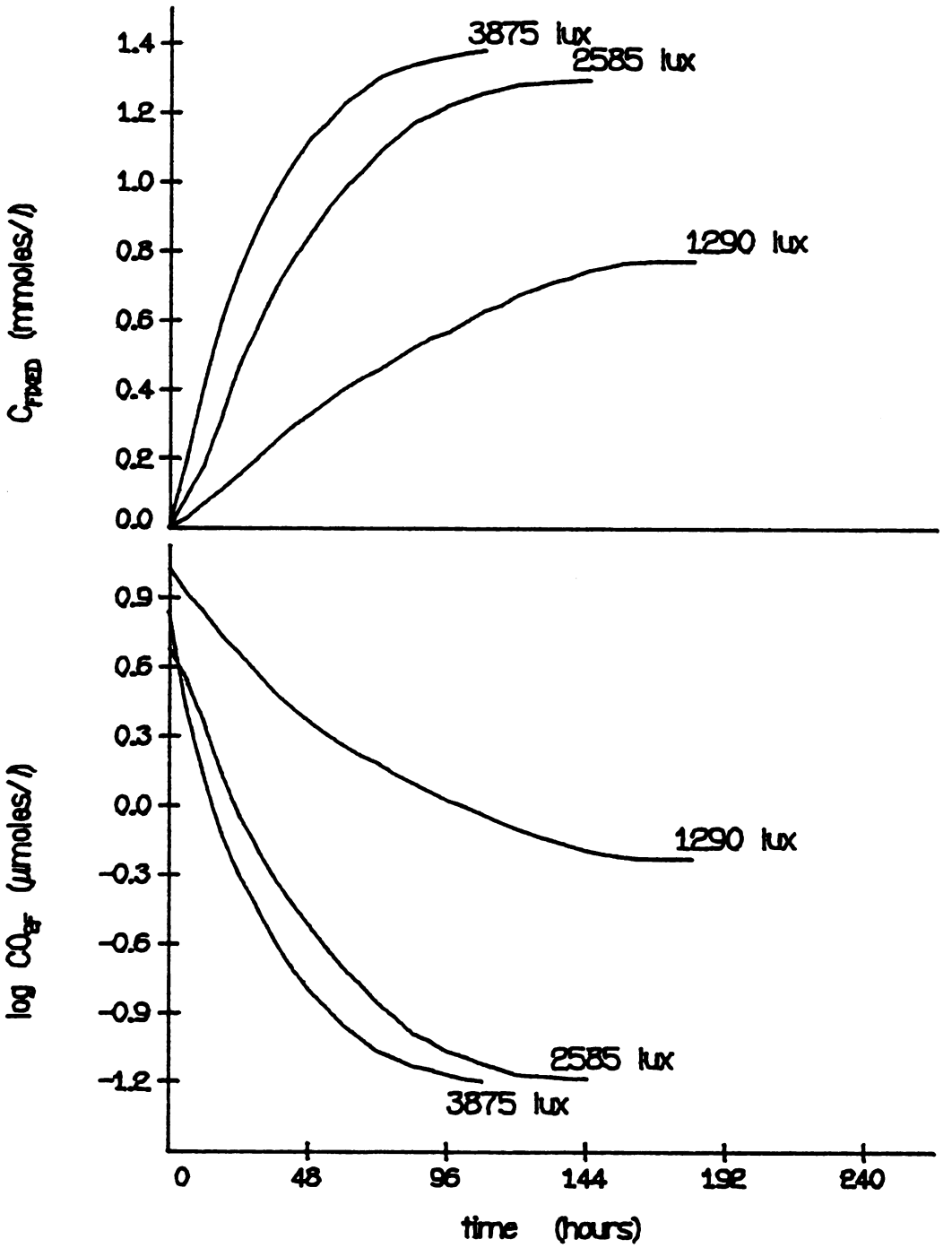


Figure 9. Representative curves of carbon fixed and free CO_2 as functions of time for E. canadensis under three light intensities.

The C_{fixed} curves were next incremented by time to obtain the change in carbon biomass per time increment. Average carbon biomass was calculated by adding the total C_{fixed} to the initial carbon biomass and averaging over the time increment. These data were then used to calculate the specific growth rates by Equation 8.

Growth Rates as a Function of Carbon

There is some question as to what form of carbon aquatic plants, such as ceratophyllum and elodea, use as their actual carbon source. There have been reports in the literature (e.g. Raven, 1968, 1970; Steemann Nielson, 1947, 1960) of aquatic plants and algae directly taking up bicarbonate ions as a carbon source at high pH. These studies, however, do not offer definitive proof that it is the bicarbonate ions and not the equilibrium CO_{2f} that is actually taken up by the plants.

There have also been reports that algae respond to the total inorganic carbon concentration. Goldman, et al (1974), using chemostat studies, concluded that the green algae Scenedesmus quadricauda and Selenastrum capricornutum respond kinetically to the total inorganic carbon concentration in the water, even though only one form of carbon may be assimilated. King and Novak (1974) used Goldman, et al's data to compare K_s values calculated by using total inorganic carbon, bicarbonate ion, and equilibrium CO_2 as the

substrate concentrations. The conclusion of this recalculation was that it is more likely that the algae were responding to CO_{2f} concentration than to total carbon or bicarbonate ion concentrations.

There is also direct evidence from field data that ceratophyllum uses CO_{2f} as its carbon source. Craig (1978) constructed isopleths of percents of μ_{max} with alkalinity vs. pH, using CO_{2f} and HCO_3^- concentrations as the substrates in calculating the percentages of μ_{max}. When HCO_3^- was used as the substrate, the isopleth representing a μ of zero eliminated some of the alkalinity - pH range where ceratophyllum is known to occur. When CO_{2f} was used as the substrate, none of the known range of ceratophyllum was eliminated. Thus, Craig concluded that ceratophyllum responded only to the CO_{2f} concentration.

The examples cited support the theory that aquatic plants use only CO_{2f} as their carbon source. Since there is no conclusive evidence that aquatic plants do respond to other inorganic carbon concentrations, CO_{2f} was used as the carbon substrate in this study.

Threshold CO_{2f}

Since aquatic plants do not continue to grow until the CO_{2f} concentration reaches zero, it is necessary in the calculation of growth rates as a function of CO_{2f} to know how low the CO_{2f} concentration can drop before the plants are no

longer able to fix carbon. This threshold CO_{2f} concentration (CO_{2q}) was obtained from the data as the CO_{2f} concentration that resulted in a μ of zero. Due to algal interference, however, it was not possible to define the exact threshold concentration in all microcosms. The ceratophyllum microcosms were relatively free of algae except at the lowest light intensity (690 lux). The elodea microcosms, however, had significant algal interference in the low CO_{2f} ranges at all light intensities. In the microcosms where algal interference occurred at low CO_{2f} concentrations, it was impossible to get reliable estimates of the CO_{2q}.

Kinetic Equations of μ as a Function of CO_{2f}

To describe μ as a function of CO_{2f}, it is necessary to know μ_{max} and K_s as well as the CO_{2q}, as described in Equation 11. The constants μ_{max} and K_s were calculated in Equation 12. Table 2 is a table of these kinetic constants as the average of all experiments at a given light intensity. It is obvious from the 95% confidence intervals that algal interference was particularly significant for elodea and for both plants at the lower light intensities.

The values listed in Table 2 were used as the values of the parameters in Equation 11, resulting in equations for the growth rate of ceratophyllum and elodea. Figure 10 is a graph of the growth rate equations for ceratophyllum for

TABLE 2. Growth kinetic constants (average) with 95% confidence intervals.

Light (lux)	μ_{max} (hr^{-1})	K_s (μmoles CO_{2f}/l)	CO_{2q} (μmoles CO_{2f}/l)
Ceratophyllum			
3875	.001012 ± .000256	.50999 ± .08623	.26451 ± .08276
2585	.000773 ± .000094	.79134 ± .19636	.47757 ± .13547
1290	.000267 ± .000124	2.0497 ± 1.8799	1.6999 ± 1.7350
690	.000196 ---- *	5.2919 ---- *	---- * ---- *
Elodea			
3875	.003538 ± .008843	.35229 ± .23504	---- *
2585	.002218 ---- *	.26353 ---- *	---- *
1290	.000543 ---- *	.83101 ---- *	---- *

* - not available

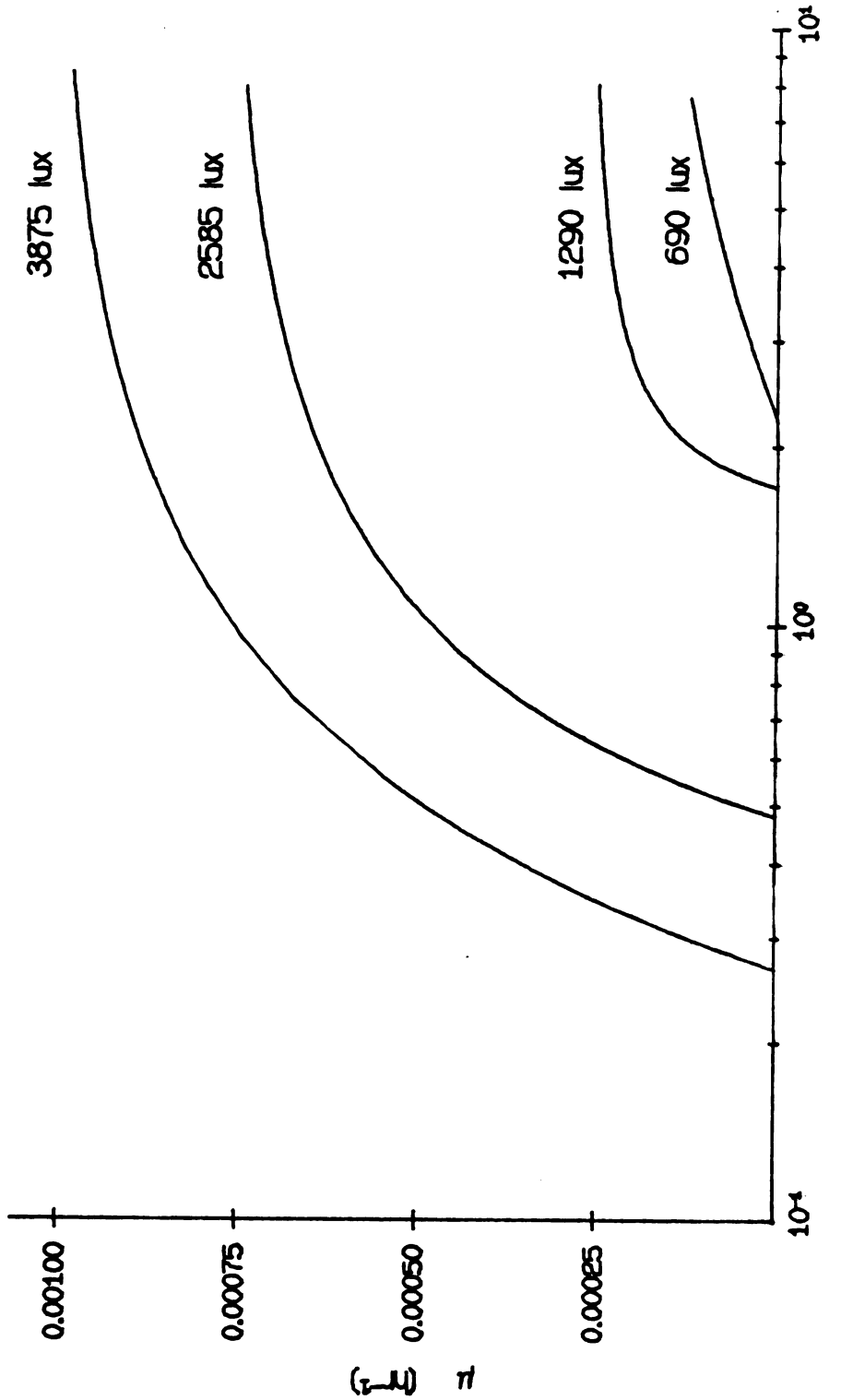


Figure 10. Growth equations for C. demersum under four light intensities.

four light intensities. These curves illustrate the dramatic effect of light intensity both on the rate and the extent, or minimum CO_{2f}, to which the plants will grow. The poor estimate of the CO_{2q} from the 690 lux data is responsible for flattening the growth rate curve for that light intensity. It is still possible, however, to compare the maximum growth rate at this light intensity with the maximum growth rates at the other light intensities in this figure. There is clearly a relationship between light and μ_{max} such that μ_{max} decreases as the light intensity decreases. It can also be seen in the top three curves that the CO_{2q} increases with decreasing light intensity. In other words, the plants require higher CO_{2f} concentration to sustain growth as the available light decreases.

The growth rate curves for elodea are shown in Figure 11. Again, poor estimates of the CO_{2q} were responsible for flattening the lower ends of the growth curves. The μ_{max} values show the same trend as ceratophyllum of decreasing with decreasing light. This figure indicates that there is a possibility that CO_{2q} increases with decreasing light for elodea as it does for ceratophyllum, but the data here are not adequate for drawing a definitive conclusion.

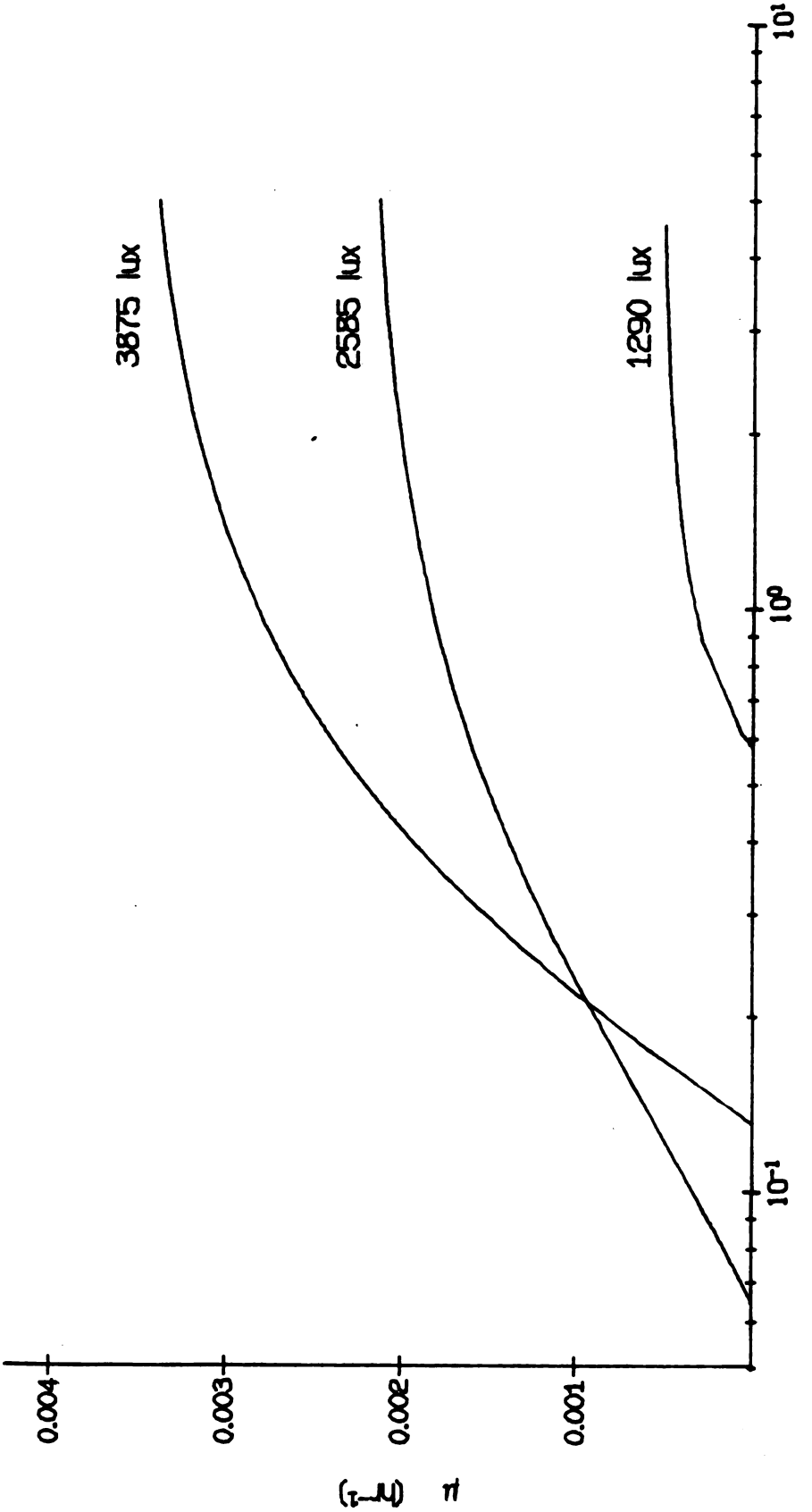


Figure 11. Growth equations for E. canadensis under three light intensities.

Kinetic Equations of μ as a Function of CO_{2f} and Light

The above discussion clearly shows that the parameters of the growth equation are related to the available light intensity. These relationships can be expressed as mathematical equations. King and King (1974) found the relationship of μ_{max} with light for some green algae to be linear, or:

$$\mu_{max} = a + bL$$

where: L = light intensity

If macrophytes respond in a similar manner, then they should also show a linear relationship. This theory was tested for μ_{max} vs. light by using linear regression to calculate a and b as shown in Figure 12, resulting in the following equations:

$$\mu_{max}(\text{ceratophyllum}) = -3.43 \times 10^{-5} + 2.78 \times 10^{-7} L \quad hr^{-1} \quad r=.897 \qquad (14)$$

$$\mu_{max}(\text{elodea}) = -8.74 \times 10^{-4} + 1.15 \times 10^{-6} L \quad hr^{-1} \quad r=.927 \qquad (15)$$

where: L = light intensity in lux

The correlation coefficients of .897 and .927 indicate that the equations gave a fairly good fit to the data.

The parameter K_s and CO_{2q} for ceratophyllum are also functions of light and can be expressed as a linear relationship on a log-log scale as shown in Figures 13 and 14.

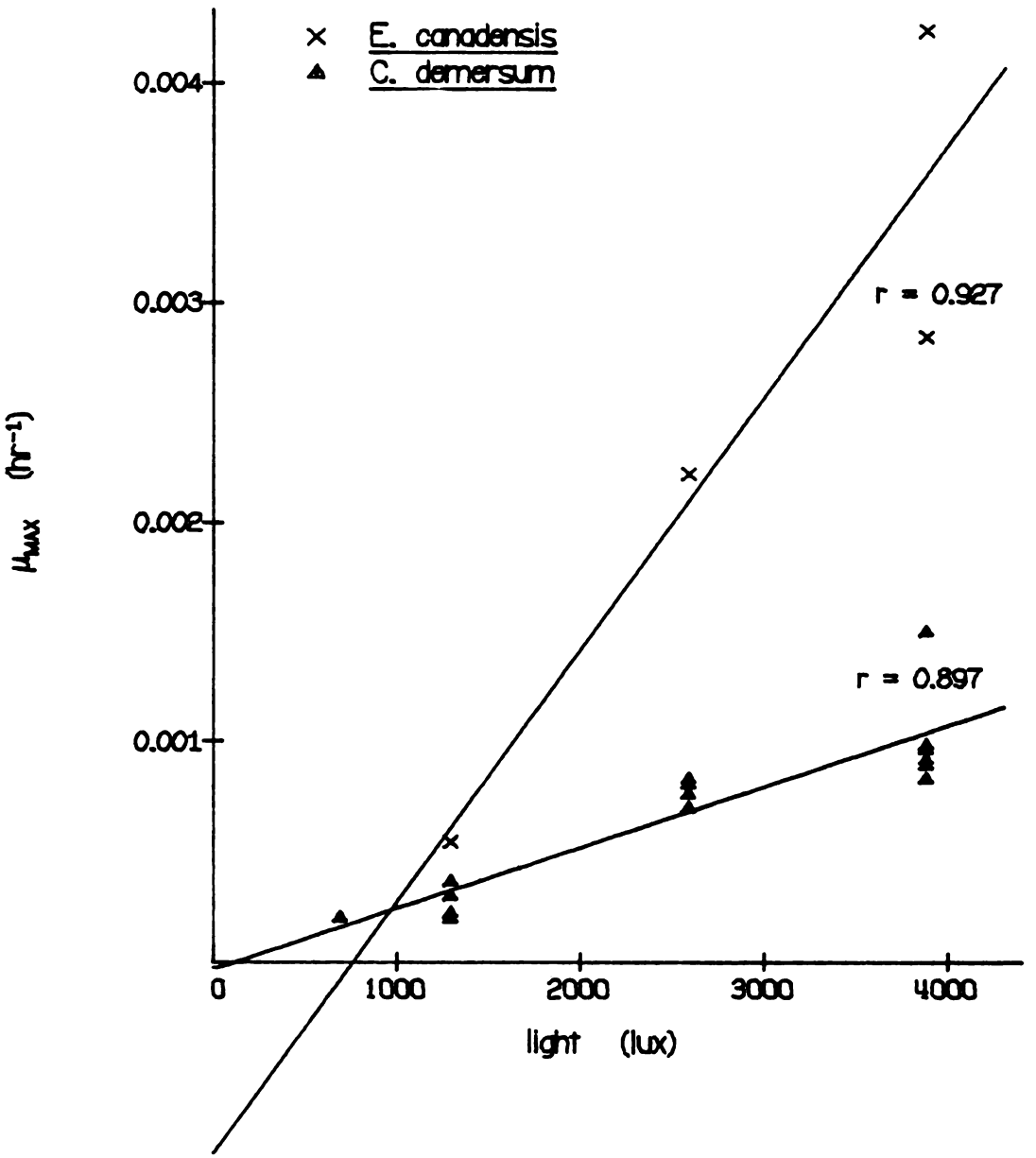


Figure 12. Linear relationship of μ_{MAX} with light for C. demersum and E. canadensis.

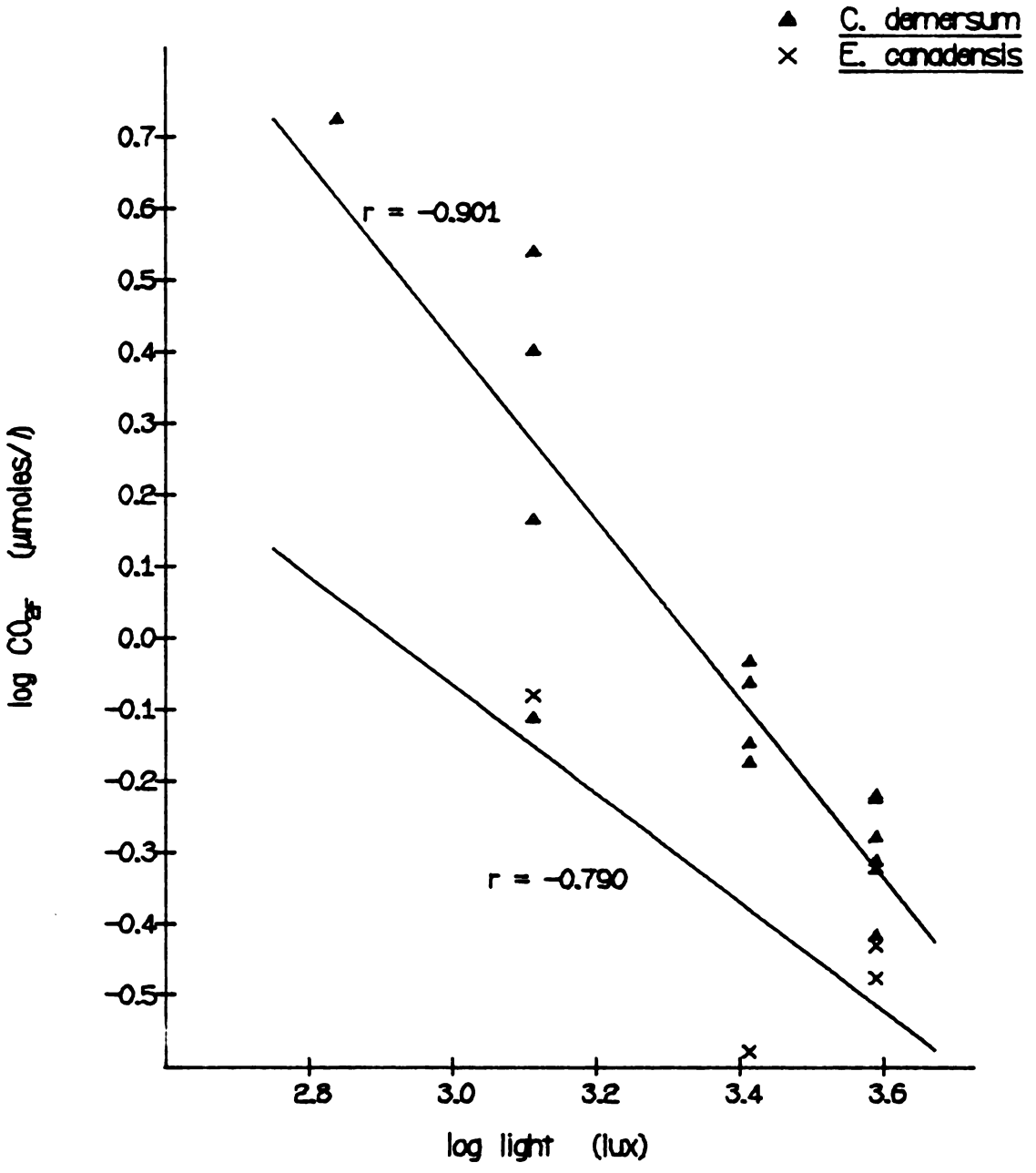


Figure 13. Linear relationship of log K_s with log light for C. demersum and E. canadensis.

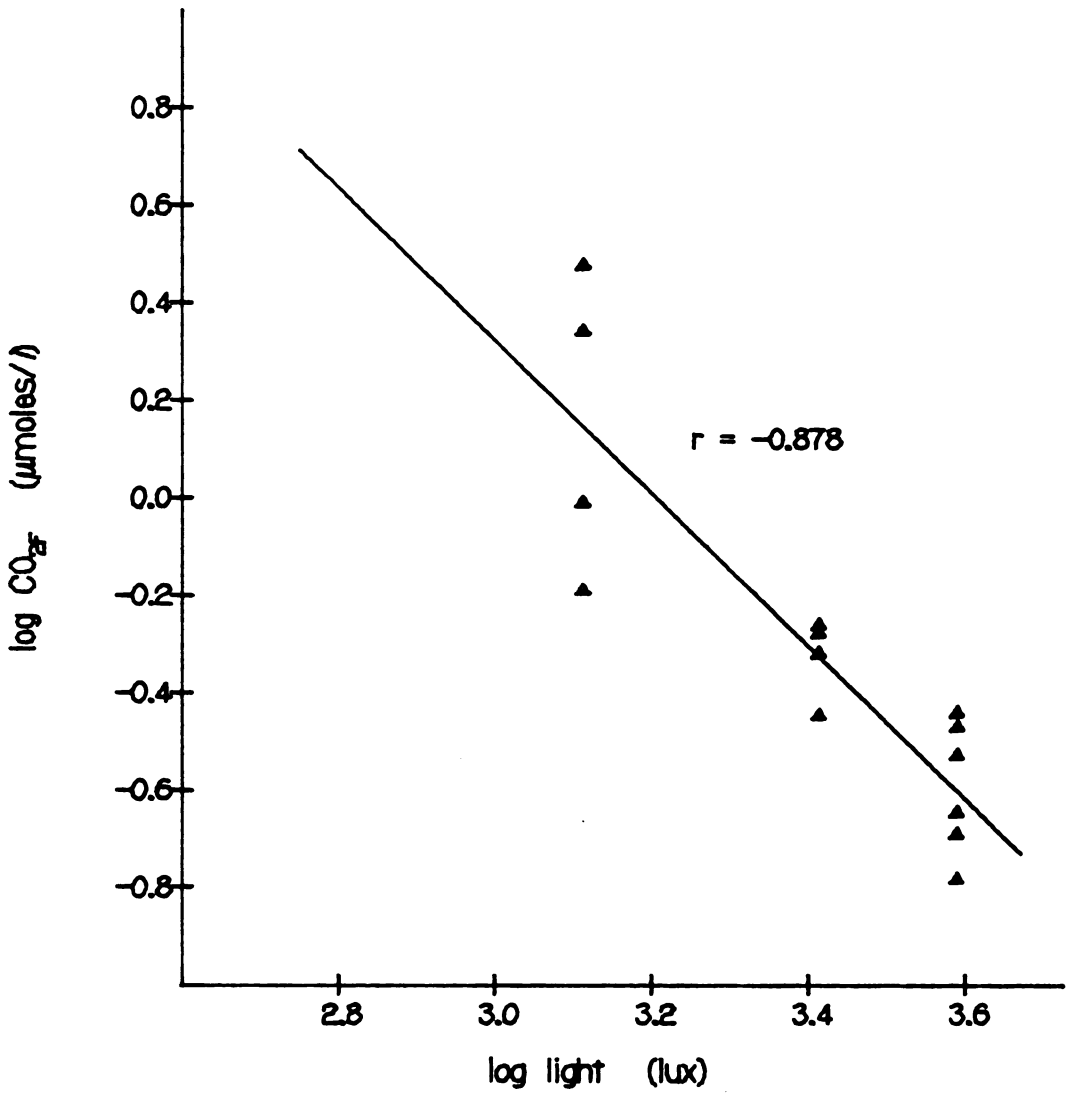


Figure 14. Linear relationship of log CO_{2Q} with log light for C. demersum.

Again using linear regression, the following equations were obtained:

$$K_s(\text{ceratophyllum}) = 1.45 \times 10^4 \, L^{-1.25} \underset{r=-.901}{\mu\text{moles } CO_{2f}/l} \quad (16)$$

$$K_s(\text{elodea}) = 1.63 \times 10^2 \, L^{-.76} \underset{r=-.790}{\mu\text{moles } CO_{2f}/l} \quad (17)$$

$$CO_{2q}(\text{ceratophyllum}) = 1.07 \times 10^5 \, L^{-1.57} \underset{r=-.878}{\mu\text{moles } CO_{2f}/l} \quad (18)$$

where: L = light intensity in lux

These equations can be seen graphed on a straight scale in Figure 15. The relatively low correlation coefficient for K_s(elodea) again indicates difficulty with algal interference.

Equations 14, 16, and 18 can now be substituted for the parameters in Equation 11 to obtain an expression of the growth rate of ceratophyllum as a function of light for the light conditions of this study. The resulting equation is:

$$\mu = f_1(L) \; \frac{S - f_3(L)}{(f_2(L) - f_3(L)) + (S - f_3(L))} \; hr^{-1} \quad (19)$$

where: $f_1(L) = \mu_{max}$(ceratophyllum) Equation 14

$f_2(L) = K_s$(ceratophyllum) Equation 16

$f_3(L) = CO_{2q}$ (ceratophyllum) Equation 18

$S = CO_{2f}$ in μmoles/l

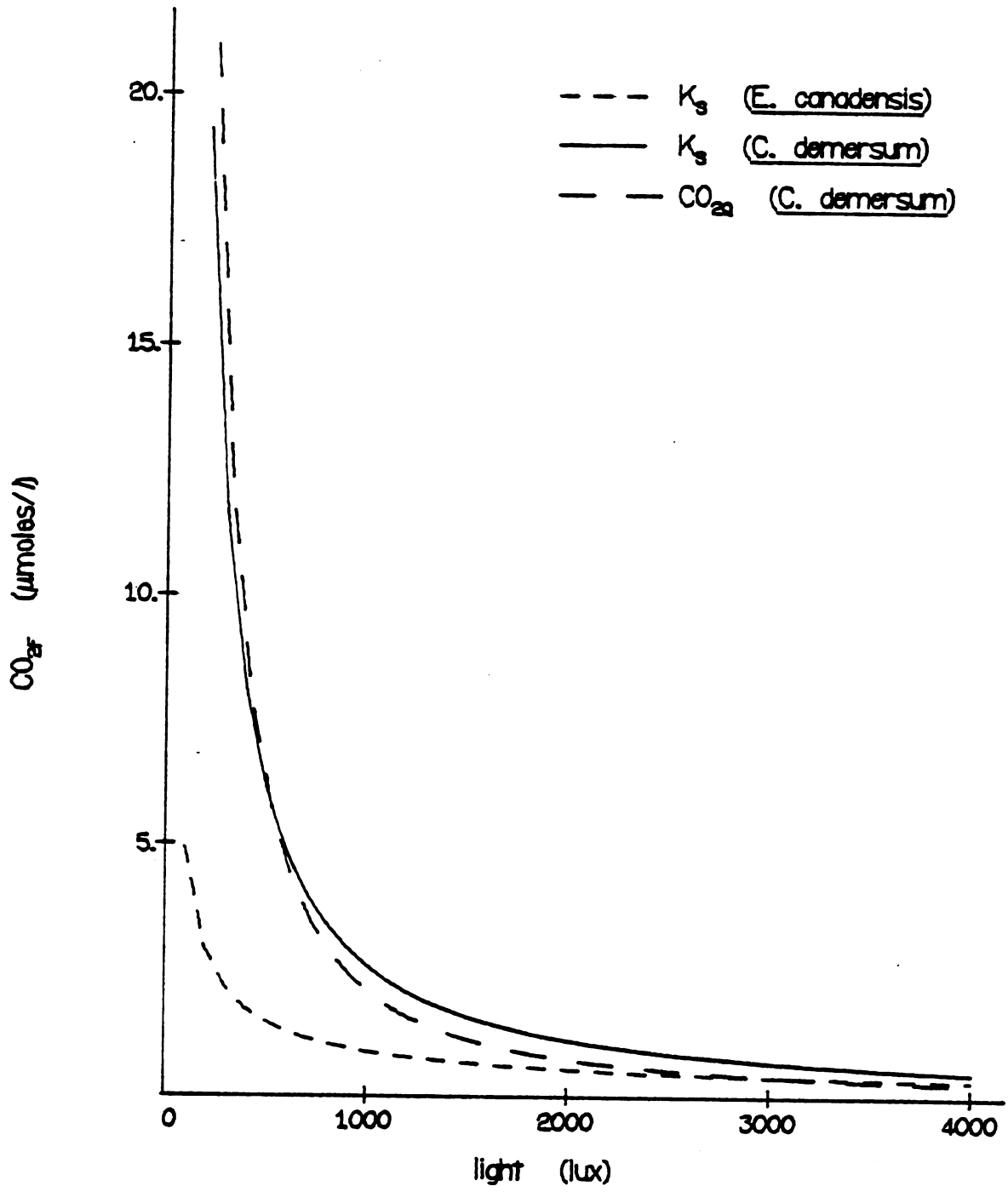


Figure 15. Relationship of K_s and CO_{2g} with light.

It is not possible to write such an equation for elodea since no CO_{2q} data are available.

Utilization of Kinetic Growth Analyses

Analyses of kinetic growth rates, such as that attempted in this study, allows us to compare the relative ability of different species of organisms to compete with each other. Organisms in nature must compete for resources which are limited, and a prime means of competition is their growth rate. The set of conditions that physiologically allows an organism to grow is often referred to as that organism's niche. The niche that an organism occupies is defined, or bounded, by the threshold concentrations of limiting resources. As seen in this study, these threshold concentrations are not determined by single limiting factors, but by the interaction of limiting factors. The growth rate is not constant under all conditions where the organism is physiologically able to exist, but approaches zero as the substrate concentration approaches the boundaries of the organism's niche. This can be seen graphically by the typical curves shown in Figure 16 of biomass and substrate concentration with time, obtained from microcosm studies. The top curve, of biomass with time, is a typical logistic growth curve. Initially, the biomass increases exponentially, but as the substrate concentration decreases, so does the rate at which the biomass increases. Finally at time t, when the substrate concentration has been reduced to

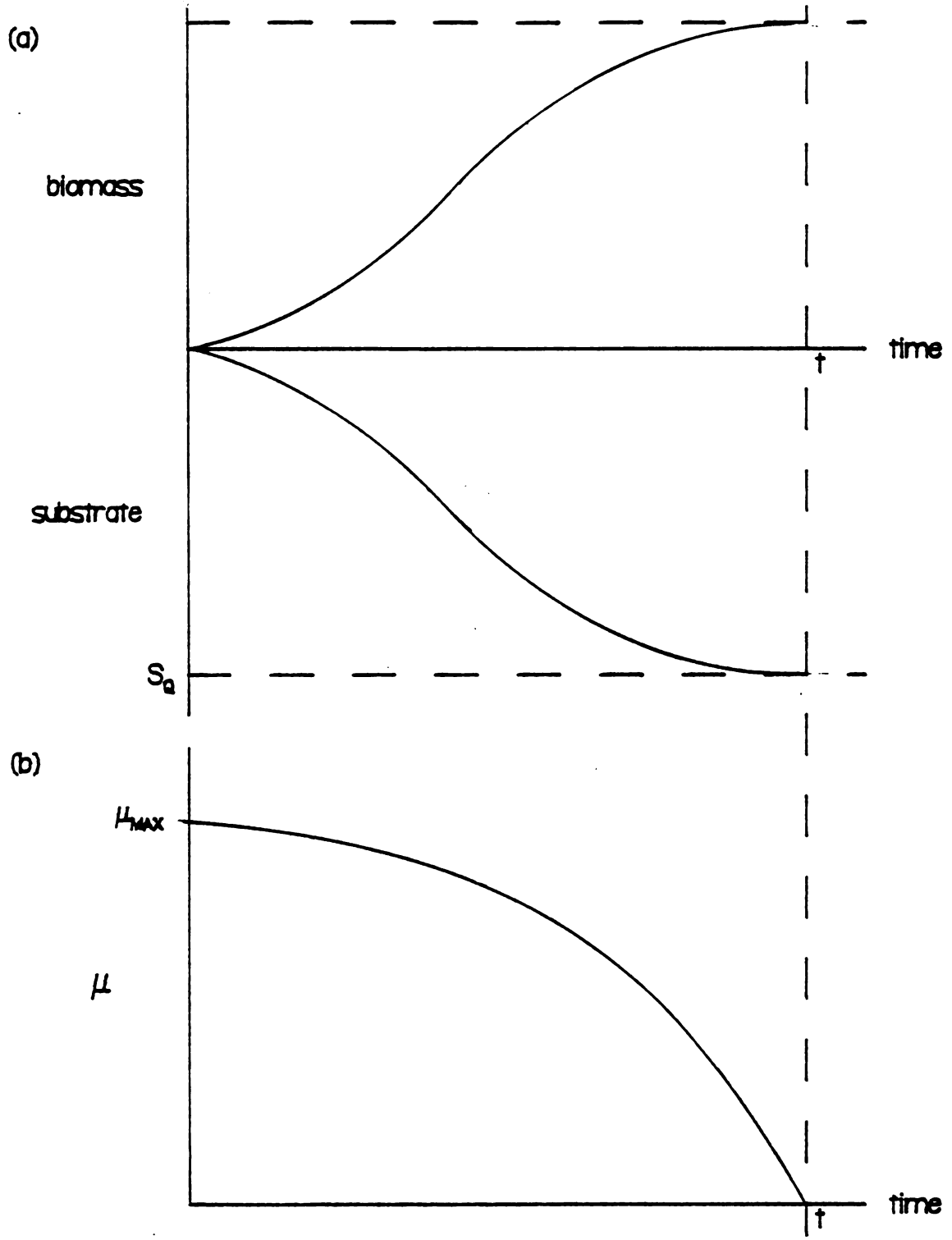


Figure 16. Typical curves of biomass and substrate concentration with time (a), and specific growth rate with time (b) from microcosm studies.

the threshold concentration, growth stops and the biomass no longer increases. The bottom curve of Figure 16 shows graphically this effect of the substrate concentration on the specific growth rate.

When growth stops, the population has reached its maximum biomass. This limit of the logistic growth curve is commonly described as a carrying capacity. The carrying capacity approach to the logistic growth curve is purely empirical and offers no explanation of the environmental factors that impose this limit. For that reason, it is of very limited use in designing management strategies. The kinetic approach, however, directly relates the growth rate of the organism to the limited substrate concentrations, and therefore offers management application.

The niche of an organism only defines the conditions under which the organism has the physiological ability to grow. The ability to grow under certain conditions, however, is not sufficient to determine how competitive that organism will be when challenged by others. The ability to compete depends on the rate at which an organism grows relative to the rate of growth of competing organisms. By using this kinetic approach, it is possible to compare growth rates under defined conditions, and thus compare the relative competitive abilities of the organisms. This comparison can be made graphically by drawing the growth rate curves on the same graph, as in Figure 17. Figure 17 is a theoretical graph of the growth rates of two organisms, A

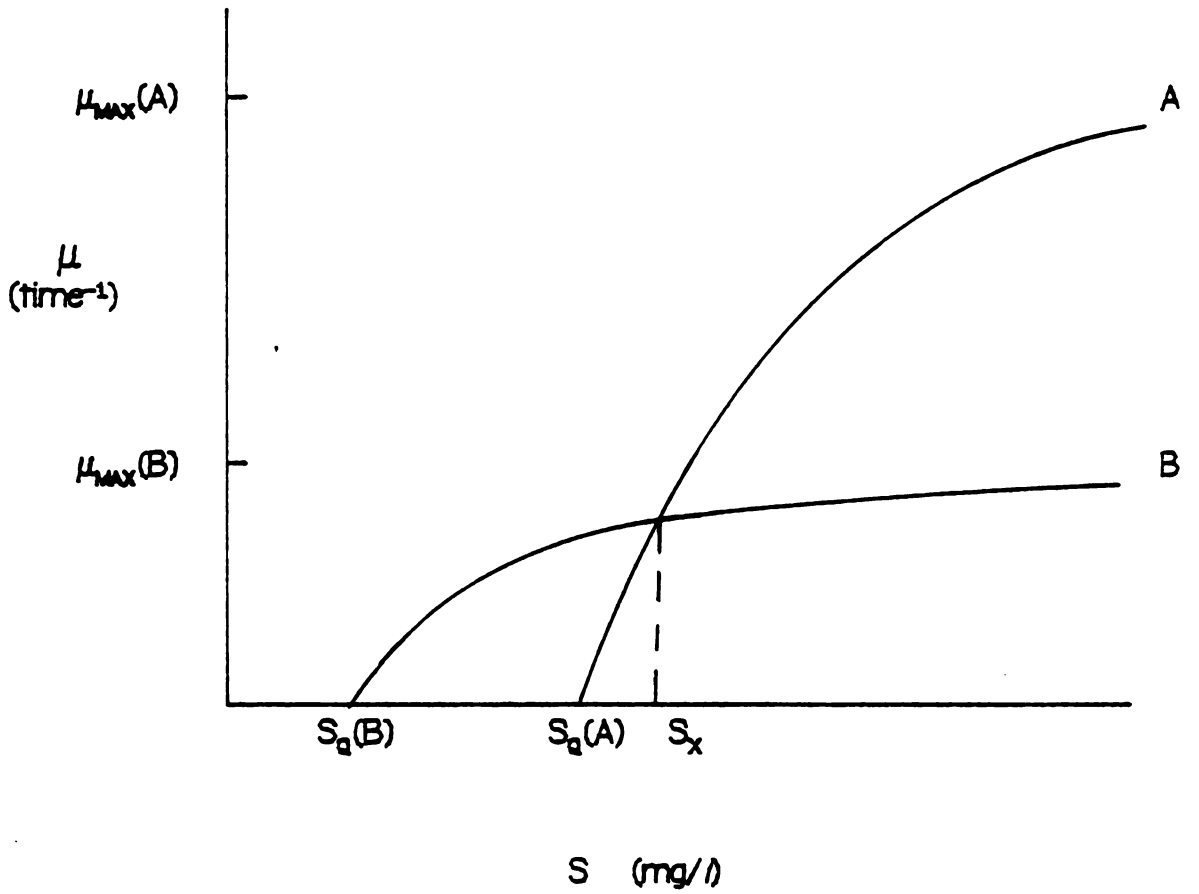


Figure 17. Theoretical graph of the specific growth rates of two organisms competing for substrate S.

and B, competing for substrate S. As seen in this figure, when the substrate concentration is greater than S_x, organism A has a larger growth rate, and thus would be dominant over organism B. When the substrate concentration is less than S_x, organism B has the competitive advantage and would be expected to dominate. When the substrate concentration equals S_x, neither organism has a competitive advantage over the other. This theoretical example illustrates how easily the relative competitive abilities of organisms can be compared by this method.

Comparison of the Growth Rates of Ceratophyllum and Elodea

The conditions of this study might represent a nutrient enriched, hyper-eutrophic situation in which carbon and light are the limiting factors to the growth of plants. In such a situation, plants compete with their μ values within the boundaries defined by their CO_{2q}. The growth rates of ceratophyllum and elodea are compared in Figure 18 for 3875, 2585, and 1290 lux. The lower ends of the elodea curves are dashed to indicate best estimates of CO_{2q} values. The significance of these curves is that they indicate that elodea is a better competitor for carbon, i.e., has a higher growth rate, at all light levels used in this study. This relationship is dramatically illustrated by the three-dimensional graphs in Figure 19. This implies that elodea should be able to out-compete ceratophyllum in carbon limited situations, at least when the light conditions are within the

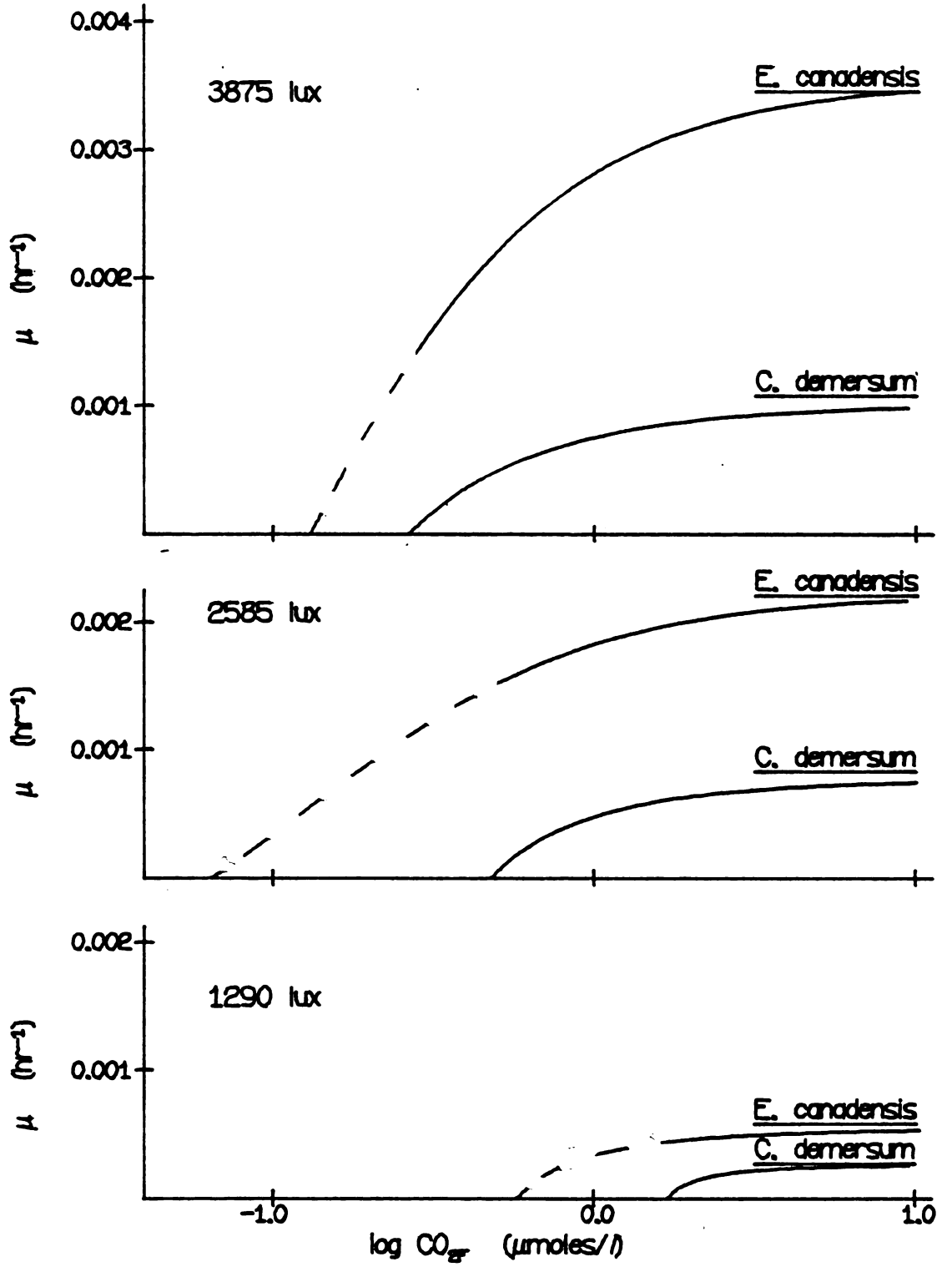


Figure 18. Specific growth rates of C. demersum and E. canadensis under three light intensities.

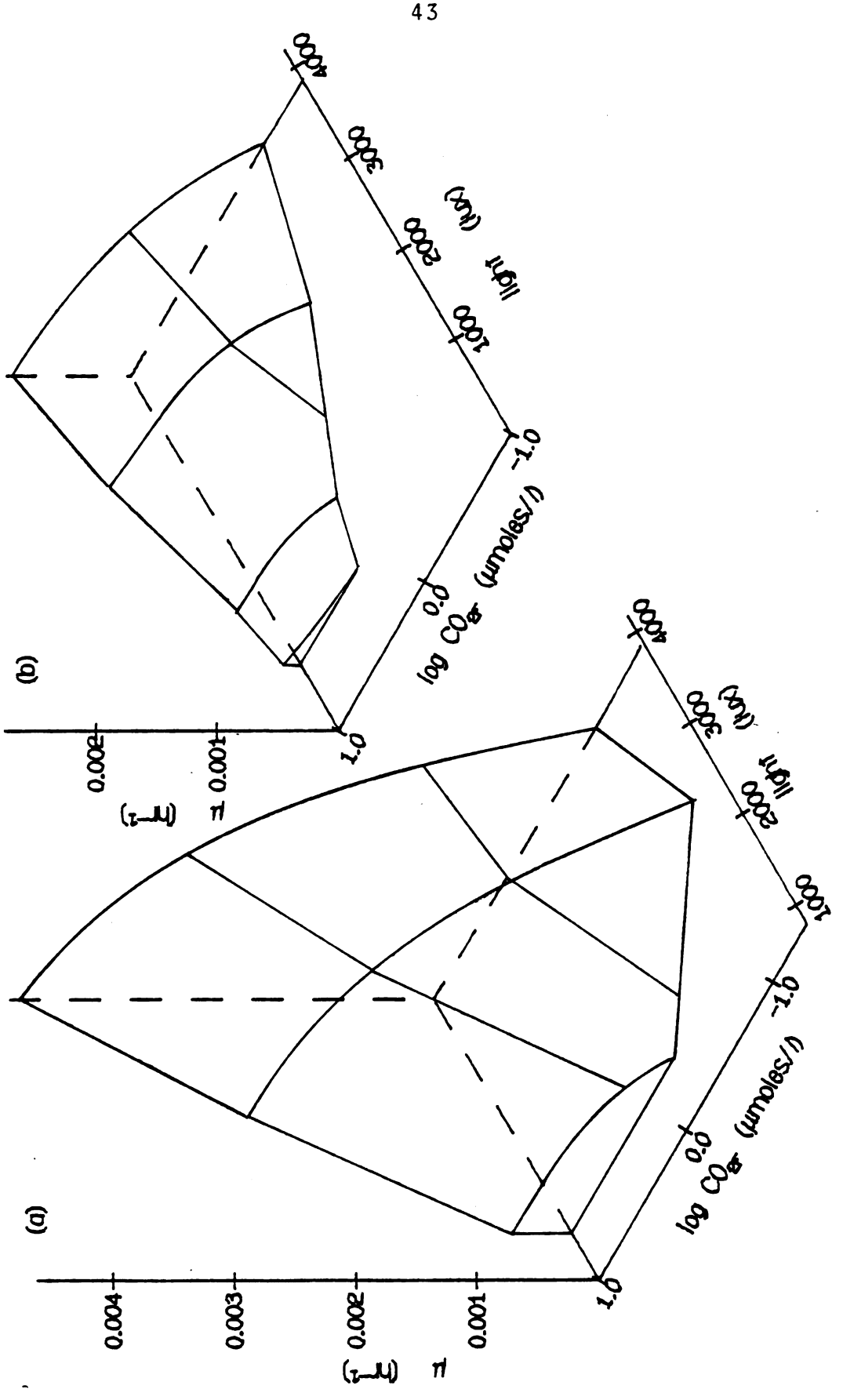


Figure 19. Three dimensional graphs of the specific growth rates of E. canadensis (a) and C. demersum (b).

range used in this study. However, ceratophyllum does exist in some nutrient enriched, highly eutrophic situations where it is not excluded by the growth of elodea. It is possible that elodea has never been introduced into these lakes, but if it has been introduced, the existence of ceratophyllum indicates that the limit to plant growth in these situations is more complex than a relatively simple carbon-light interaction. Data are not now available to determine growth rates of macrophytes relative to phosphorous, nitrogen, or other nutrients and their interaction with limiting carbon and light.

Comparison of the Growth Rates of Macrophytes with Algae

This type of kinetic analysis also can be used to describe competition between macrophytes and algae. In a study done by Hill (1977), microcosm techniques similar to the ones used in this study were used to obtain kinetic data for the growth of the green alga Chlorella vulgaris. When used for algae, the type of microcosm used here provides data on the physiological ability of the algae to fix carbon, since algae that sink to the bottom of the microcosm continue photosynthesis. This is not very realistic when compared to a lake, in which algae that sink are often removed from the photic zone. Therefore, in addition to the "light" microcosms used here, Hill used bottles in which the lower half of the microcosm was shaded. This technique provides a situation in which algae that sink to the bottom

of the microcosm are not able to continue photosynthesis. In this way he was able to get data for the physiological growth abilities of the algae from the "light" microcosms and data on the effective growth rate from the shaded microcosms, which take into consideration the sink characteristics of algae.

Data from Hill's "light" microcosms were used to calculate equations for μ_{max} for chlorella as a function of light (D. L. King, personal communication). Figure 20 shows the linear relationship of μ_{max} for chlorella with light. The μ_{max} light functions for ceratophyllum and elodea are included on this figure. The lines indicate that chlorella has a much higher maximum growth rate than either elodea or ceratophyllum. The line for chlorella, however, represents its physiological ability to fix carbon and does not consider the fact that algae sink.

Hill's data also indicate that chlorella is physiologically able to grow at much lower CO_{2f} concentrations than ceratophyllum. Again, this is not a true indication of chlorella's actual ability to compete. Figure 21 shows recalculations of Hill's data (D. L. King, personal communication) for the physiological CO_{2q} and the CO_{2q} from the shaded microcosms compared to the CO_{2q} for ceratophyllum obtained in this study. The curves indicate that if the algae did not sink, they would be able to grow to much lower CO_{2f} concentrations than ceratophyllum. However, when sink properties of algae are taken into consideration,

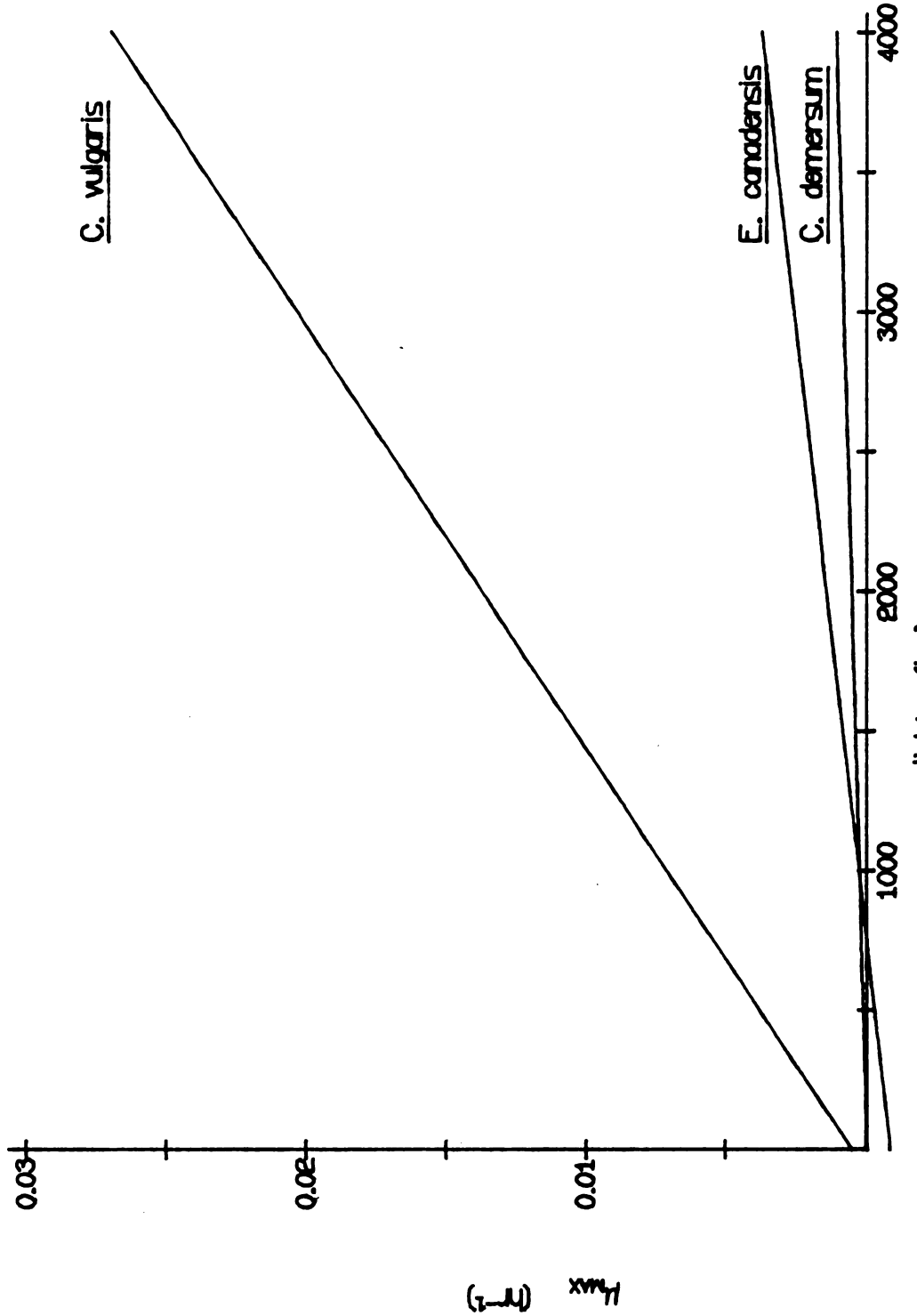


Figure 20. Comparison of the light relationships of μ_{max} for C. vulgaris, C. demersum and E. canadensis.

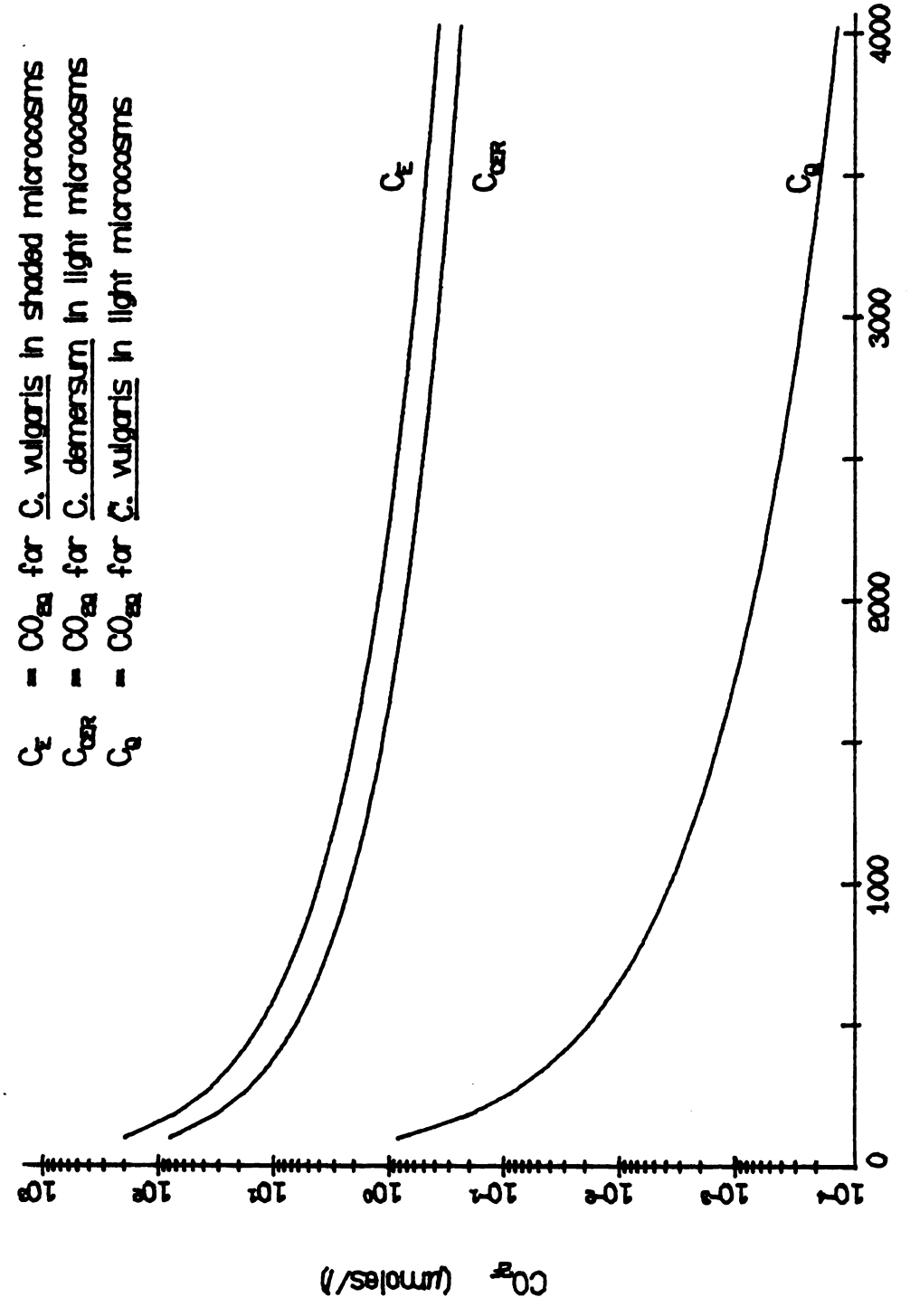


Figure 21. Comparison of the light relationships of CO_{aq} for C. vulgaris and C. demersum.

ceratophyllum can grow to lower CO_{2f} concentrations than chlorella for some light intensities.

A growth rate equation also was calculated from Hill's data for both the physiological growth rate and the "effective" growth rate of chlorella under a light intensity of 3875 lux. Figure 22 shows these curves compared to the growth rate curve for ceratophyllum under 3875 lux. Again, the physiological growth rate of chlorella far exceeds that of ceratophyllum but when sink characteristics of the chlorella are taken into consideration, ceratophyllum has a higher competitive ability under some conditions. Chlorella has a relatively high specific growth rate, but it tends to sink out of the photic zone, especially when subjected to stress conditions (Hill, 1977). Even though ceratophyllum has a relatively low specific growth rate, its ability to maintain its biomass in the photic zone gives it a competitive advantage over chlorella.

Figure 22 can be used to illustrate how growth kinetics can be used as a management tool. For example, if chlorella and ceratophyllum were the only competing plant species in a waste stabilization lagoon where carbon and light were the limiting factors, the dominant species could be chosen by adjusting the detention time of the water flowing through the lagoon. Longer detention times allow the plants to fix more carbon and thus create lower CO_{2f} concentrations. From Figure 22, chlorella and ceratophyllum are equally competitive when the CO_{2f} concentration is approximately

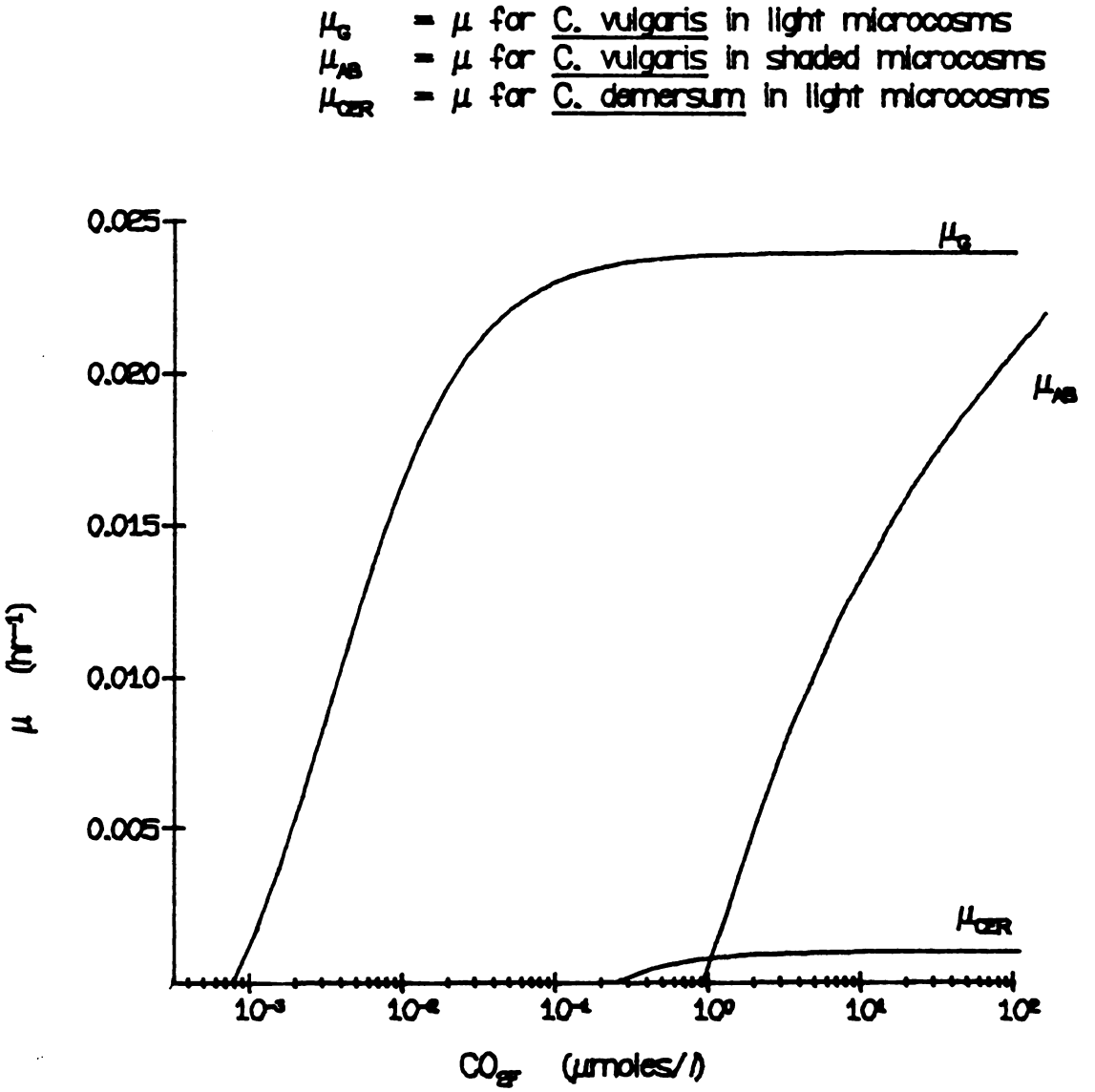


Figure 22. Comparison of the specific growth rates of C. demersum and C. vulgaris.

1 μmole/l. If the detention time is adjusted so that plant photosynthesis could lower the CO_{2f} concentration to a value greater than 1 μmole/l, chlorella would dominate. If the detention time was increased so that the plants could lower the CO_{2f} concentration to less than 1 μmole/l, then ceratophyllum would dominate.

This type of simple model should be used with caution, since it does not take all factors into consideration, such as shading effects from the plant species that becomes dominant first. Models of this sort also cannot always be applied directly to the field, as indicated by field data from Craig's study (1978) of ceratophyllum in an enriched system. Craig reported a μ_{max} of .067 day^{-1}, or approximately .0048 $hour^{-1}$, which is considerably higher than the μ_{max} of .0010 $hour^{-1}$ found in this study at 3875 lux. This could easily be a result of higher light intensity in the field situation. He also reported a CO_{2q} of 1.3 moles CO_{2f}/l, which is much higher than the value obtained in this study of .265 μmoles CO_{2f}/l at 3875 lux. Craig's pH data were not collected directly in the plant mat, and thus the CO_{2q} value represents an average for the water column. This average would be expected to be higher than the CO_{2f} concentration present in the plant mat. It is obvious that more data are necessary to determine if laboratory studies of this kind can be applied directly to field situations.

This study was an attempt to develop a technique for designing a small model of plant growth that could have management application. At the same time, it points to some of the downfalls of the large universal eutrophication models currently being attempted. These models often are composed of unjustified simplifications, such as constant algal sink rates, no threshold values for limiting nutrients, single limiting factors, empirically derived rate constants and simple ballpark estimates (e.g. Bierman, et al, 1973; Bloomfield, et al, 1973). It is obvious from this study and studies done with algae (e.g. Hill, 1977; King and King, 1974) that plant growth cannot be accurately represented in such a simplified manner but that environmental factors interact to affect growth characteristics of plants. This study points out that μ_{max} and CO_{2q} are not constants, but vary as a function of light. It is very likely that these parameters also vary as a function of other limiting nutrients, creating a very complex set of interactions in nature. Until we learn more about these interactions, attempts at constructing universal management models are futile.

CONCLUSIONS

1) This microcosm method of studying growth kinetics can be used with macrophytes, provided the plants used are free of algae.

2) Ceratophyllum demersum and Elodea canadensis follow Monod growth kinetics in relation to carbon if all other growth requirements are met.

3) Carbon and light availability interact to control the growth kinetics of Ceratophyllum demersum and Elodea canadensis in the same manner previously observed for the green alga Chlorella vulgaris.

4) Elodea canadensis is a better competitor for carbon than Ceratophyllum demersum at all light intensities used in this study.

5) There are insufficient data at this time to determine whether or not laboratory data collected in this manner are directly applicable to field situations.

APPENDIX

Composition of Inorganic Nutrient Medium

Nutrient	Concentration
$NaHCO_3$	varies
KNO_3	114.0 mg/liter
$CaCl_2$	43.3 mg/liter
$FeCl_3$	4.0 mg/liter
$MgSO_4 \cdot 7H_2O$	40.0 mg/liter
EDTA	2.0 mg/liter
K_2HPO_4	8.0 mg/liter
Microelement Solution	1.0 ml/liter

Composition of Microelement Solution

Nutrient	Concentration
H_3BO_3	2.86 g/liter
$MnCl_2 \cdot 4H_2O$	1.81 g/liter
$ZnSO_4 \cdot 7H_2O$	0.22 g/liter
$(NH_4)_6Mo_7O_{24}$	0.18 g/liter
$CuSO_4$	0.05 g/liter
$Co(NO_3)_2 \cdot 6H_2O$	0.49 g/liter

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