INTERACTIONS BETWEEN LIGHT AND CARBON DIOXIDE AVAILABILITIES AS A CONTROL OF ALGAL SPECIES SUCCESSION

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INTRODUCTION

In attempting to gain knowledge of or insight into the requirements of algal production and the rate of algal production investigators turned to both field studies of natural water and laboratory studies. From these studies there has been an extensive effort to derive mathematical models to predict the rate and degree which aquatic production will be increased upon the addition of various nutrients.

This study was conducted using methods similar to those used by Klemovich (1). The purpose of this study was to determine the effect of various light intensities on the relative ability of different algae to extract carbon from the carbonate-bicarbonate alkalinity, when only the carbon available was that from the carbonate-bicarbonate alkalinity.

To accomplish this aim, two separate investigations were conducted. The first investigation allowed evaluation of differences in the ability of algae to extract carbon from a single concentration of carbonate-bicarbonate alkalinity under different light intensities. The second investigation allowed evaluation of the ability of algae to extract carbon from different carbonate-bicarbonate alkalinities at a single constant light intensity.

The primary objective of this study was to determine the degree of interaction between simultaneous limiting carbon dioxide concentration and light intensities on the rate and extent of algal production.

EXPERIMENTAL PLAN AND ANALYTICAL TECHNIQUES

Experimental Plan

The basic objective of this laboratory study was to examine the rate and extent of photosynthetic carbon fixation by algae simultaneously limited by light intensity and alkalinity concentration. Since two primary variables were of concern, two separate studies were necessary, one for light intensity and one for alkalinity.

Microcosms and Apparatus

The microcosms used throughout this study were similar to those used by Sievers (2), Young (3), and Klemovich (1) in conducting their algal growth studies. The microcosms were one-liter Erlenmeyer flasks sealed with number 11 rubber stoppers as illustrated in Figure 1.

A 1.5 cm diameter hole was drilled in each rubber stopper for the placement of a rubber serum cap. This serum cap allowed samples to be withdrawn with a hypodermic syringe without exposing flask contents to the atmosphere. To allow for the escape of photosynthetically produced oxygen and to allow for atmospheric pressure to be maintained in the microcosm each rubber stopper was fitted with a 2 mm diameter piece of glass tubing. This vent also eliminated recarbonation from atmospheric carbon dioxide.

Both experiments conducted in this study were similar to that of Klemovich (1) in his second experiment. Light limitation

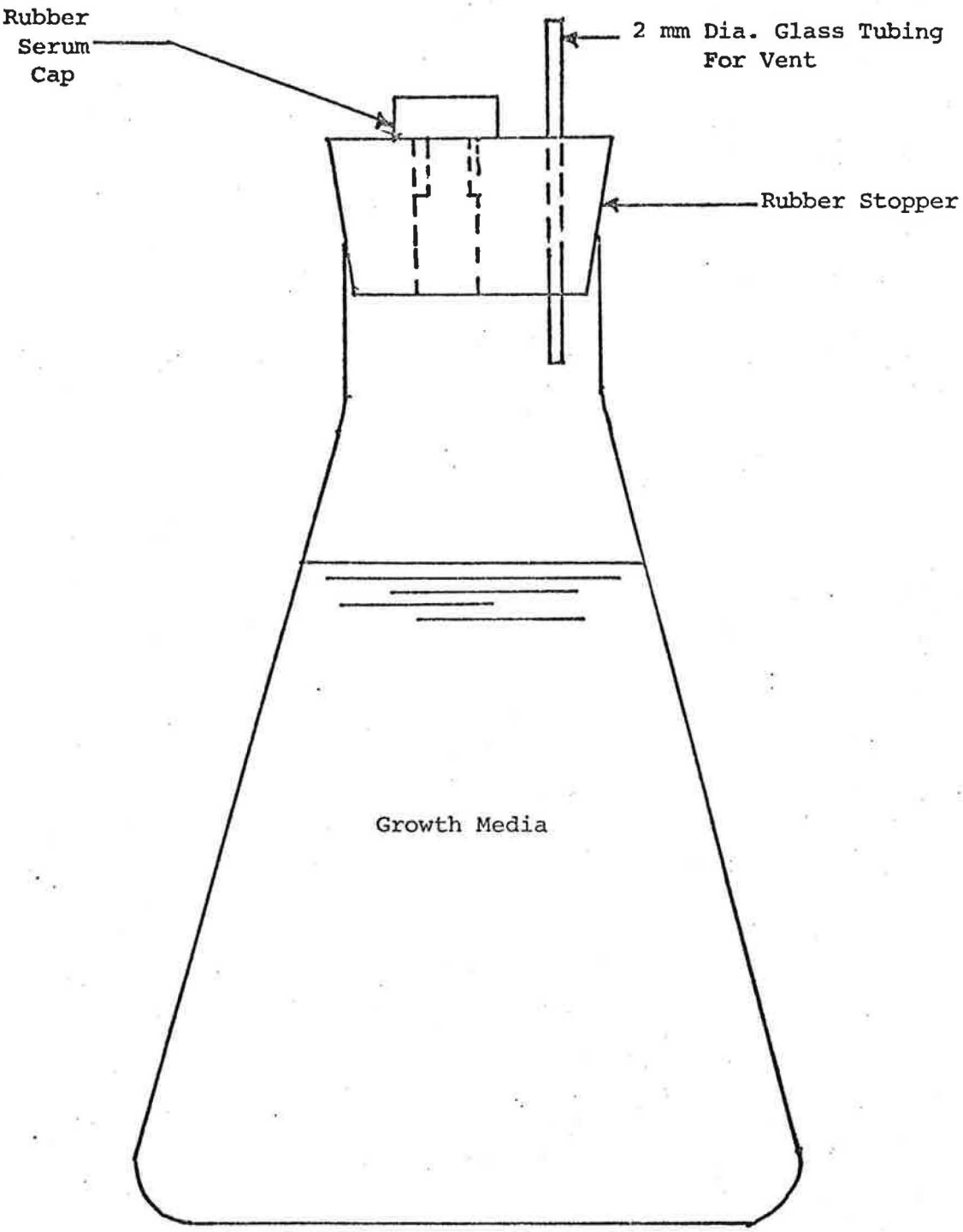

Figure 1

One Liter Erlenmeyer Flask Used
As Microcosms For Study

was accomplished by four light boxes which were constructed specifically to transmit varying intensities of light as shown in Figure 2. The units consisted of wooden frames covered by varying layers of black nylon and window screen painted flat black. The unit shown in Figure 3 allowed the transmission of 42 footcandles of incident light to the microcosm.

Table 1 is a list of the coverings used for all light boxes and the resulting incident light transmission to the microcosms. A variation in incident light intensity from 130 footcandles to 5 footcandles was achieved with this apparatus. The light source consisted of four forty watt Gro-Lux fluorescent light bulbs positioned 16 inches above the table and two twenty watt Gro-Lux fluorescent light bulbs positioned 13 inches above the table. The table was covered with black polyethylene to prevent reflection.

For both experiments, 24 microcosms were prepared, six of which were seeded with Scenedesmus acutiformis, six with Phormidium olivacea, six with Pediastrum biradiatum, and six with Anabaena variabilis. One microcosm of each species was placed under each light intensity.

The intensity of illumination provided by the light sources was measured with a Model 614 Weston footcandle meter.

Cultures

Five genera of algae were obtained from the Indiana University Department of Botany. The five genera included Pediastrum biradiatum, Scenedesmus acutiformis, Anabaena variabilis, Chlamydomonas

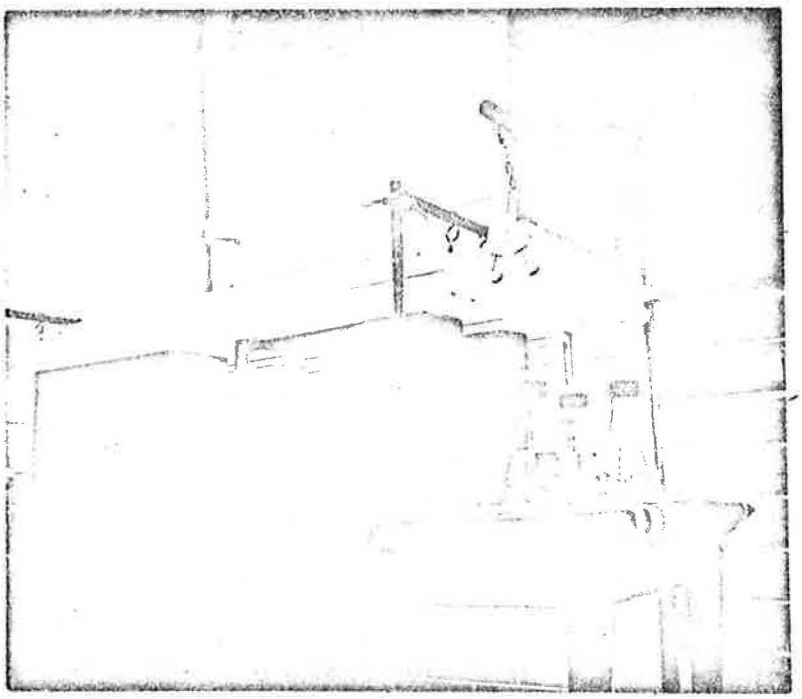

Figure 2
Laboratory Set-Up of Four Light
Boxes Used in This Study

Figure 3
Shown Is The Typical Construction Used For the Cage Units.
The Cage Unit Pictured is Covered By One Layer
Of Window Screen Painted Flat Black.

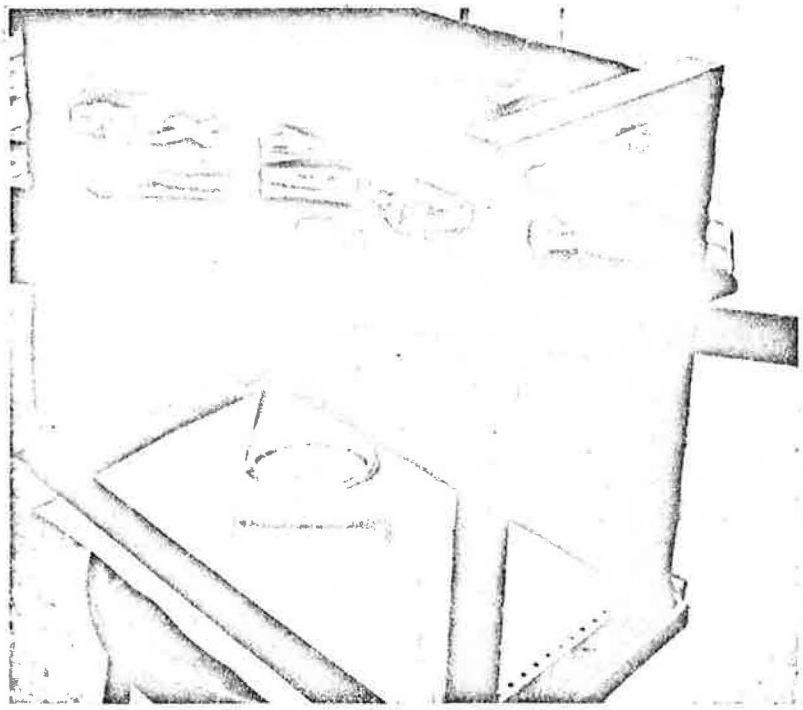

Table 1

Materials Used to Cover the Light Box Frames to Vary Incident Light Transmission Listed With the Resulting Light Intensity Levels Inside the Light Boxes

Coverings	Light Intensity Transmitted (footcandles)
No Light Box	130
No Light Box	74
1 Layer Window Screen	42
1 Layer Window Screen + 1 Layer Black Nylon	20
2 Layers of Black Nylon	10
3 Layers of Black Nylon	5

rotula, and Phormidium olivacea. Pediastrum, Scenedesmus, and Chlamydomonas are green algae while Anabaena and Phormidium are blue-green algae.

These cultures were maintained on their original agar slant and aseptically transferred to the seed cultures. The liquid media and flasks used to grow the seed cultures were autoclaved at 250°F, 15 psi, for twenty minutes prior to seeding.

The seed culture employed the same growth media as used in the microcosm except the initial phosphorus concentration was 20 µg P/liter. The algae in the culture were considered to be phosphorus starved when the pH of the seed culture growth medium reached its highest or peak pH value.

In this study Pediastrum, Anabaena, Scenedesmus, and Phormidium were the only algae used. A seed culture of Chlamydomonas could not be established in the media used. Forester (4) found that a community of Chlamydomonas was limited at free carbon dioxide concentrations less than 25 micromoles per liter.

Growth Medium and Culture Methods

In this study a growth medium described by Kevern and Ball (5) and modified by Sievers (2) was used in all microcosm flasks. The growth medium composition can be found in Appendix A. All nutrients except carbon were in excess and carbonate-bicarbonate alkalinity could be maintained at a desired concentration. The medium, and culture flasks were autoclaved and then aerated for

24 hours prior to seeding to allow atmospheric saturation of carbon dioxide to be achieved.

After the algae in the seed culture reached maximum standing crop they were centrifuged and the excess growth medium decanted. A Beckman Model IR-315 infrared carbonaceous analyzer was used to measure the total organic carbon content of the seed culture. This procedure allowed for the addition of known amounts of biomass to be used as seed in each microcosm.

Sampling

To insure that a uniform sample was obtained for all determinations the microcosms were well shaken prior to extraction of a sample. Anabaena and Phormidium grew as periphyton and shaking the microcosms caused the algae to form balls. Thus, microcosms containing Anabaena and Phormidium were gently swirled to prevent the forming of balls.

All samples were extracted with a 20 ml syringe inserted through the rubber serum cap. Both the alkalinity and pH readings required the extraction of 20 ml samples. A separate syringe and needle was used for each algal species to avoid cross-contamination.

Measured Parameters

Alkalinity. Determinations of the carbonate-bicarbonate alkalinity concentrations were made immediately after seeding the microcosm flasks. The samples were titrated according to the method

listed in Standard Methods (6). A concentration of 0.02 N sulfuric acid was used as the titrant standard.

pH. All pH determinations were obtained with a Corning Model 12 research pH meter with a general purpose glass semi-microelectrode. The pH meter was calibrated periodically against a group of Fisher Brand Standard Buffer solutions.

The 20 ml samples extracted for pH determinations were injected into a sealed 50 ml beaker which contained nitrogen gas. A No. 9-1/2 rubber stopper sealed the 50 ml beaker containing the nitrogen gas. The rubber stopper had two holes drilled into the top. One hole allowed for the sample and nitrogen gas to be injected and the other hole was for the insertion of the pH electrode. This method minimized recarbonation of the sample with CO_2 from the atmosphere. Measurements were obtained once a day for each microcosm under high light intensity and every three days for each microcosm under low light intensity.

Carbon Calculations

All inorganic carbon values were calculated in the same manner used by Klemovich (1). Klemovich used the following equation derived and presented by Sievers (2), based on steady state concentrations of inorganic carbon in water.

$$\Sigma CO_2 = a \frac{\frac{H^2}{K_1} + H + K_2}{H + 2K_2} \qquad (1)$$

where:

ΣCO_2 = total inorganic carbon, mole/liter

a = carbonate-bicarbonate alkalinity in eq/liter corrected for hydroxyl ion concentration

H = hydrogen ion concentration, mole/liter

K_1 = first dissociation constant of carbonic acid

K_2 = second dissociation constant of carbonic acid

Theoretically it is possible to use Equation 1 and time incremented pH to calculate the increase in algal biomass or carbon fixed. Equation 2 is how Young (3) stated this relationship.

$$C_{fixed} = \Delta\Sigma CO_2 = \Sigma CO_{2_{initial}} - \Sigma CO_{2_{final}} \qquad (2)$$

Young obtained a coefficient of correlation of 0.986 between the total organic carbon as measured on the Beckman Model 315 infrared carbonaceous analyzer and carbon fixed values calculated using Equations 1 and 2. Based on this correlation coefficient, all values of carbon fixation calculated for this study were obtained from Equations 1 and 2 through the use of the computer program derived by Young (3).

A third equation used in this study for calculating the free carbon dioxide concentration (CO_{2_f}) present in water at equilibrium was derived by Harvey (7) and Park (8).

$$CO_{2_f} = a \frac{H^2}{K_1(H + 2K_2)} \quad (3)$$

where:

CO_{2_f} = moles/liter of H_2CO_3(aq) including free CO_2(g)

a = carbonate-bicarbonate alkalinity in eq/liter corrected for hydroxyl ion concentration

H = hydrogen ion concentration, moles/liter

K_1 = first dissociation constant of carbonic acid

K_2 = second dissociation ion constant of carbonic acid

Free carbon dioxide values were calculated through the use of Young's (3) computer program.

RESULTS AND DISCUSSION

Laboratory microcosms offer a means of controlling variables in an otherwise continuously changing natural ecosystem and allow detailed studies of the effects of various ecosystem variables. However, there are limits imposed when analyzing the results from such studies. In this study, the algae were maintained continuously within the photic zone under constant light and could continue photosynthesis even after they had settled to the flask bottom; a characteristic not found in lakes.

First Investigation

Scenedesmus, Anabaena, Phormidium and Pediastrum were used in this investigation aimed at evaluating their ability to extract carbon, as carbon dioxide, from the carbonate-bicarbonate alkalinity system under different constant light intensities. As was noted previously, the light intensity varied from 130 footcandles to 5 footcandles for this investigation and the temperature throughout the study did not vary more than ± 1°C from 26°C. Attempts were made to attain constant alkalinity concentration of 2 meq/liter in all flasks, but this varied slightly for each microcosm. The initial alkalinity concentrations are given in Appendix B.

When light is applied to a system containing algae and the nutrients essential for algal growth the response in the system is an increase in pH caused by algal extraction of free CO_2 from the

alkalinity system. As the algae continue photosynthesis the pH will continue to rise until the rate of free CO_2 extraction from the carbonate-bicarbonate alkalinity equals the rate of respiration by the algae.

Figure 4 shows the increase in pH with elapsed time for Anabaena, with results typical of those obtained by previous investigators (1),(2),(3) who used the type of microcosm used in this study. These curves show that the microcosms with the highest light intensities are able to attain significantly greater pH values than those at lower light intensities. This figure also shows that with an increase of light intensity the rate of change of pH to maximum pH is significantly greater. Figure 5 shows that Phormidium is able to attain the greatest maximum pH value when compared to Anabaena, Scenedesmus, and Pediastrum under a constant light intensity of 74 footcandles.

The pH values from Figure 4 were incremented with time and substituted into Equation 1 to obtain total inorganic carbon values (ΣCO_2). These values were then substituted into Equation 2 to obtain estimates of carbon fixed as biomass by the algae.

Figure 6 is a plot of total carbon fixed with respect to elapsed time for Anabaena. By examining the curves, one sees an increase in the amount of total carbon fixed by the algae with increasing light intensity. These responses are typical of those found by Klemovich (1). It can also be seen that there is a sig-

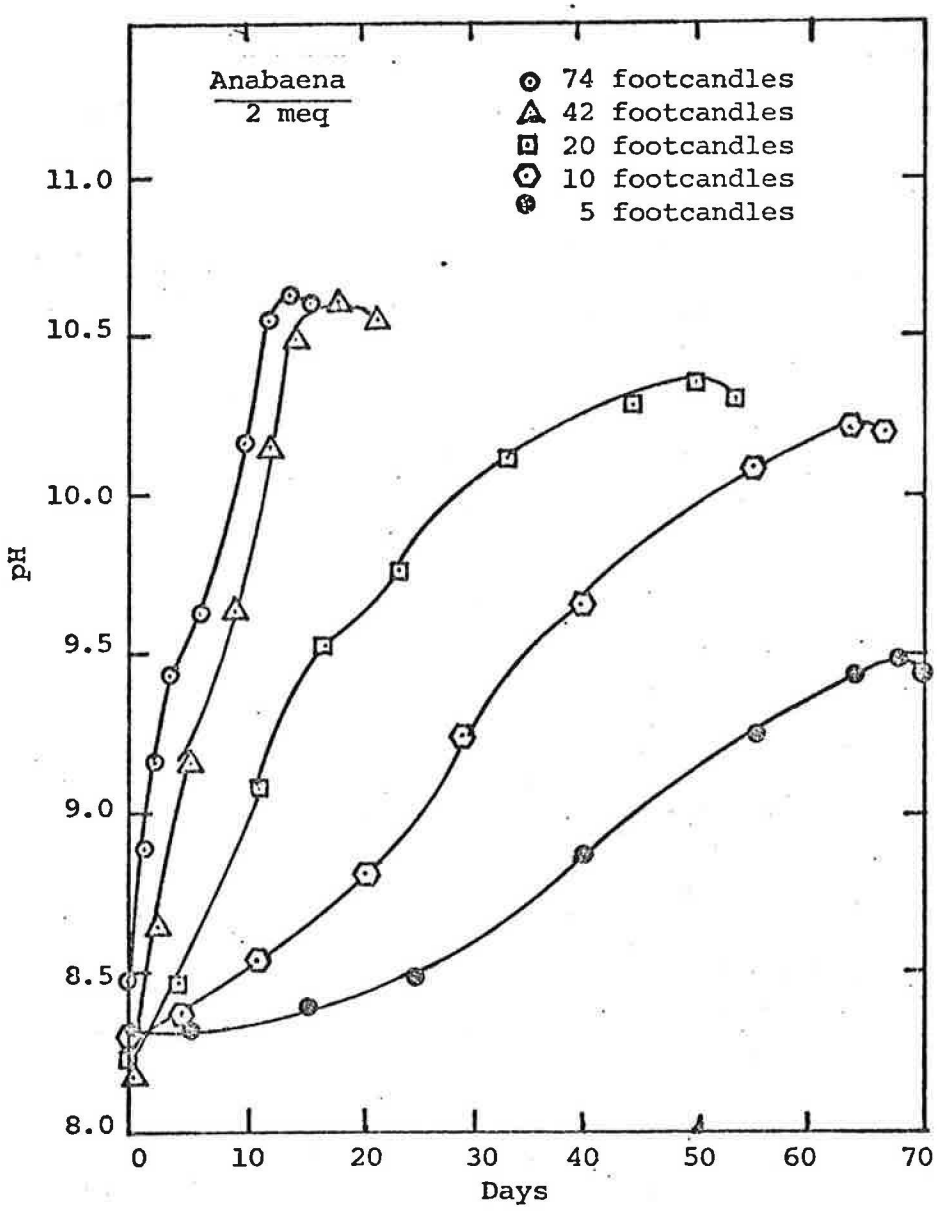

Figure 4

Time pH Response Under Various Light Intensities For Anabaena variabilis Recorded During First Investigation

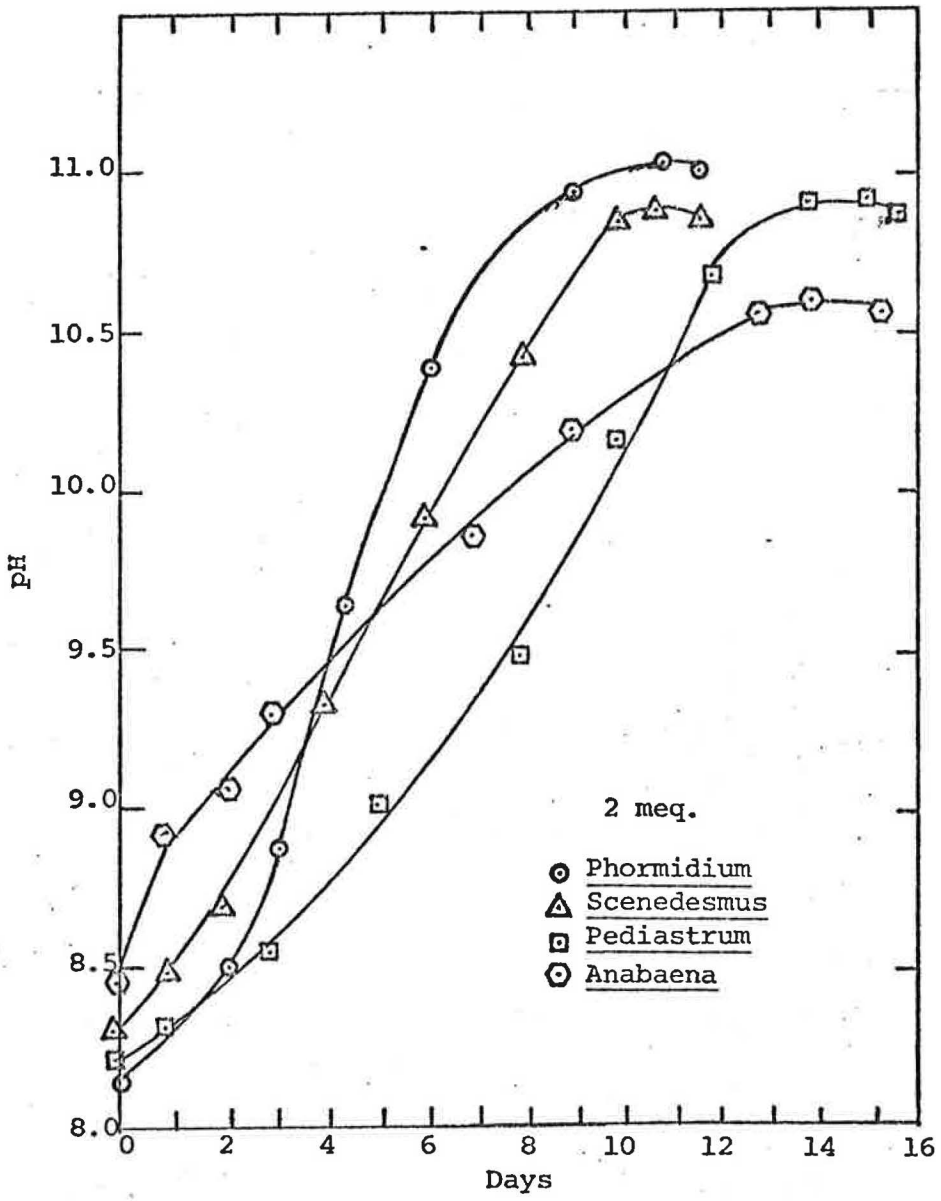

Figure 5

Time pH Response Under a Constant Light For Anabaena variabilis, Scenedesmus acutiformis, Pediastrum biradiatum, and Phormidium olivacea During First Investigation

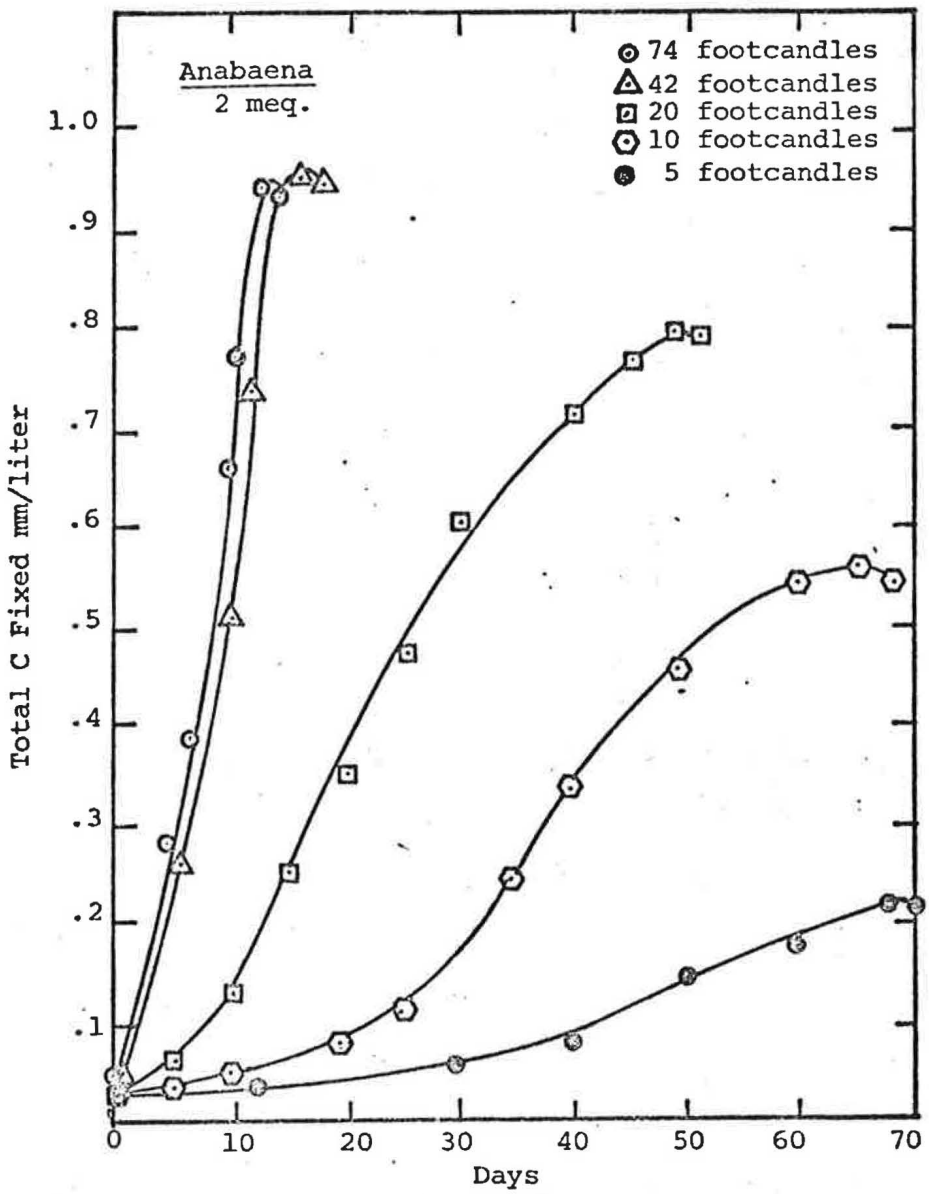

Figure 6
Time Related Accrual of Carbon Fixed by Anabaena variabilis under Various Light Intensities During First Investigation

nificantly greater rate of total carbon fixation with an increasing light intensity.

A similar plot is shown in Figure 7 for Pediastrum, Anabaena, Scenedesmus, and Phormidium under a constant light intensity of 74 footcandles. Pediastrum is shown to have fixed the greatest amount of total carbon from the carbonate-bicarbonate alkalinity. This was due to Pediastrum having a greater initial alkalinity concentration than Phormidium.

Figure 8 shows the time related decrease in the equilibrium free carbon dioxide concentrations, which is designated as CO_{2_f}, for Anabaena cultures. These values in Figure 8 were calculated with Equation 3 and the experimental pH values from Figure 4. This log-scale plot of the CO_{2_q} concentration in μmoles CO_2/liter shows how the equilibrium CO_{2_f} concentration decreases with time as the algae continue to extract and fix carbon from the carbonate-bicarbonate alkalinity of the growth medium.

The CO_{2_f} curves attained by Anabaena showed the same response noted by Klemovich (1) for his algae. That is the minimum CO_{2_f} concentration attained is successively lowered by increasing the light intensity. This response can be shown in the following equation:

$$P_N = P_G - R \qquad (4)$$

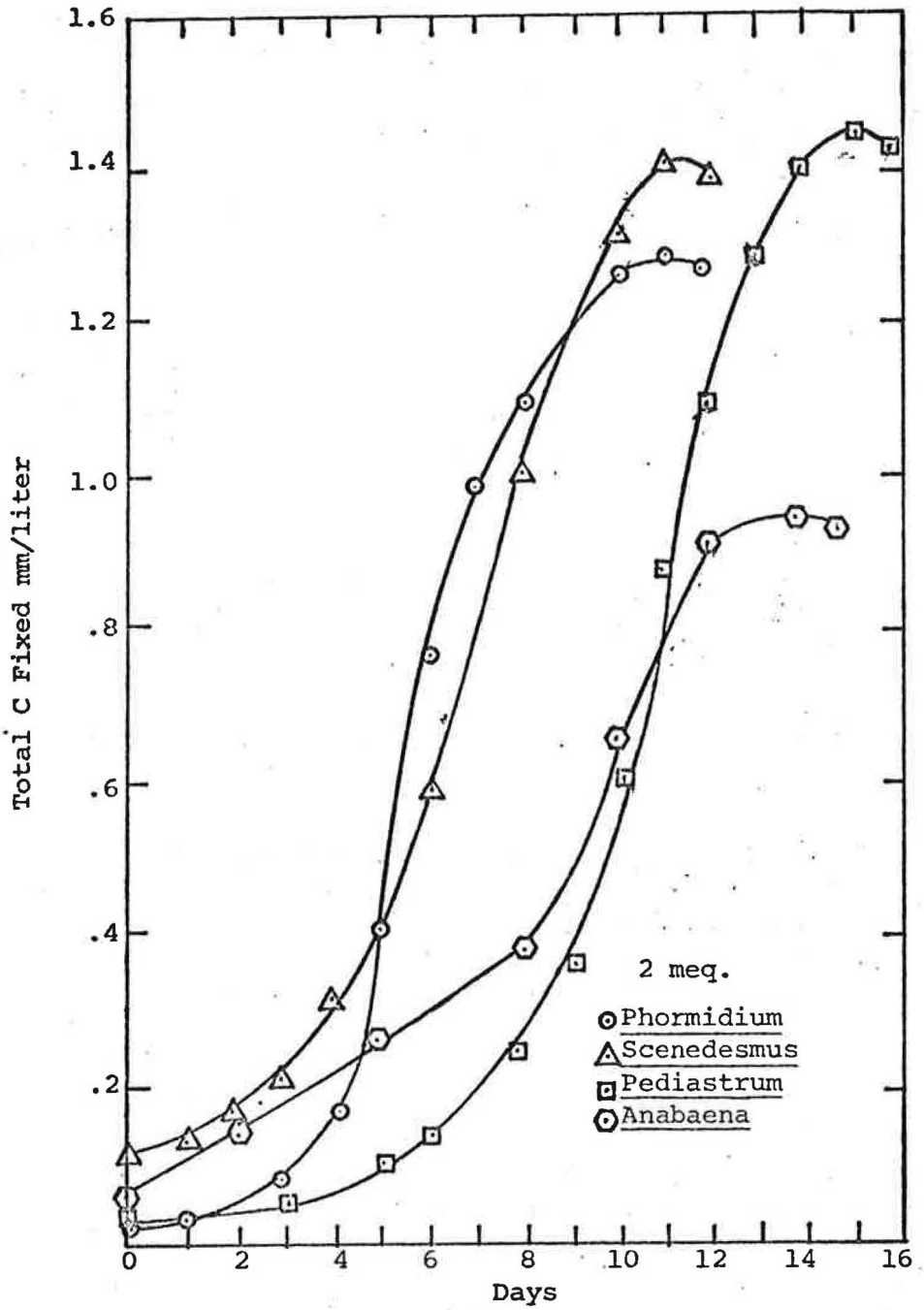

Figure 7

Time Related Accrual of Carbon Fixed Under Constant Light for Anabaena variabilis, Phormidium olivacea, Pediastrum biradiatum, and Scenedesmus acutiformis During First Investigation

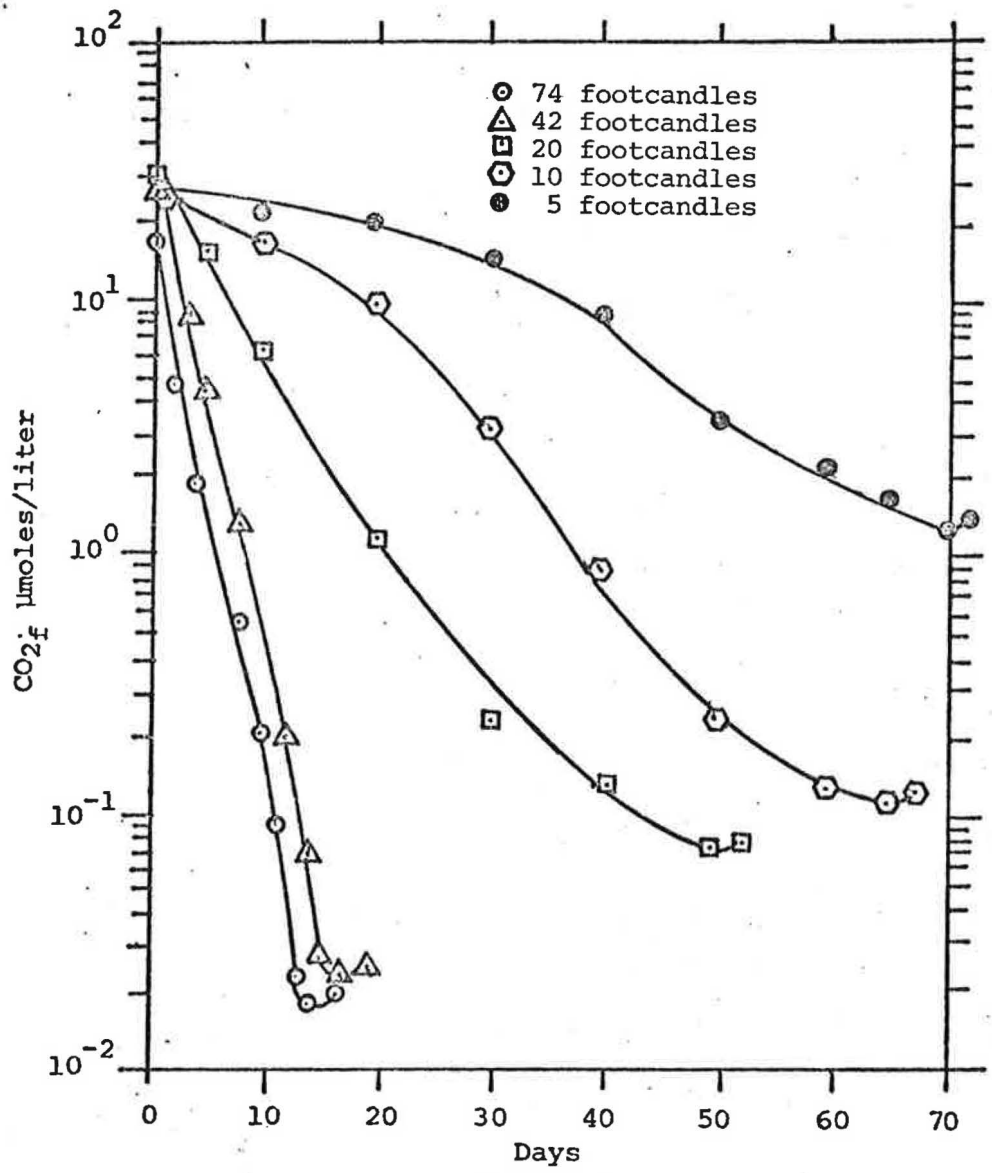

Figure 8

Time Related CO_{2f} Concentration Under Various Light Intensities for Anabaena variabilis During First Investigation

where:

P_N = net production; mg C/liter

P_G = gross production; mg C/liter

R = respiration of algae; mg C/liter

As noted previously, algae use the CO_{2_f} from the carbonate-bicarbonate alkalinity to produce oxygen and more algae. Equation 4 suggests that as long as gross production is greater than the respiration of algae there will be a net production of biomass. The rates at which photosynthesis takes place are given in Equations 5-7.

$$P_N = P_{N_\mu} B \tag{5}$$

$$P_G = P_{G_\mu} B \tag{6}$$

$$R = R_\mu B \tag{7}$$

where:

B = biomass; mg C/liter

P_{N_μ} = rate of net carbon production; (hr^{-1})

P_{G_μ} = rate of gross carbon production (hr^{-1})

R_μ = respiration rate of algae (hr^{-1})

Substituting Equations 5-7 into Equation 4 will give the rate of net production for algae, as shown in Equation 8.

$$B\,(P_{N_\mu}) = B\,((P_{G_\mu}) - (R_\mu)) \tag{8}$$

As shown in Figure 8 the algae were able to continue to extract carbon from the carbonate-bicarbonate alkalinity and lower the CO_{2_f} concentration until a minimum CO_{2_f} value was reached at which net photosynthetic activity ceased under that light intensity. This minimum CO_{2_f} concentration is designated as CO_{2_q}. At this CO_{2_q} concentration the rate of gross production (P_{G_μ}) equals the rate of respiration (R_μ) and there is no increase in net production. The lowering of the CO_{2_q} value by increasing light intensity (Figure 8) allowed more carbon to be fixed as net production before P_{G_μ} equaled R_μ. The curves for the two high light intensities (Figure 8) indicate a difference between the CO_{2_q} values of only 0.003 µmoles/liter, suggesting that saturating light intensity had been approached for Anabaena.

The CO_{2_f} concentration with respect to elapsed time is compared for the four algal species in Figure 9. Phormidium is seen to possess the ability to continue to extract carbon from the carbonate-bicarbonate alkalinity to a lower CO_{2_f} concentration than Pediastrum, Scenedesmus, or Anabaena at a light intensity of 74 footcandles. It can be seen in Figure 9, that Anabaena was unable to extract carbon much below a CO_{2_f} concentration of 0.018 µmoles CO_{2_f}/liter, under conditions which allowed Phormidium, Pediastrum, and Scenedesmus to extract carbon to CO_{2_f} concentrations of 0.00225 µmoles/liter, 0.0051 µmoles/liter, and 0.0062 µmoles/liter, respectively. Thus, on the basis of CO_{2_f}, Anabaena is unable to compete for carbon with these other algae at a low CO_{2_f} concentra-

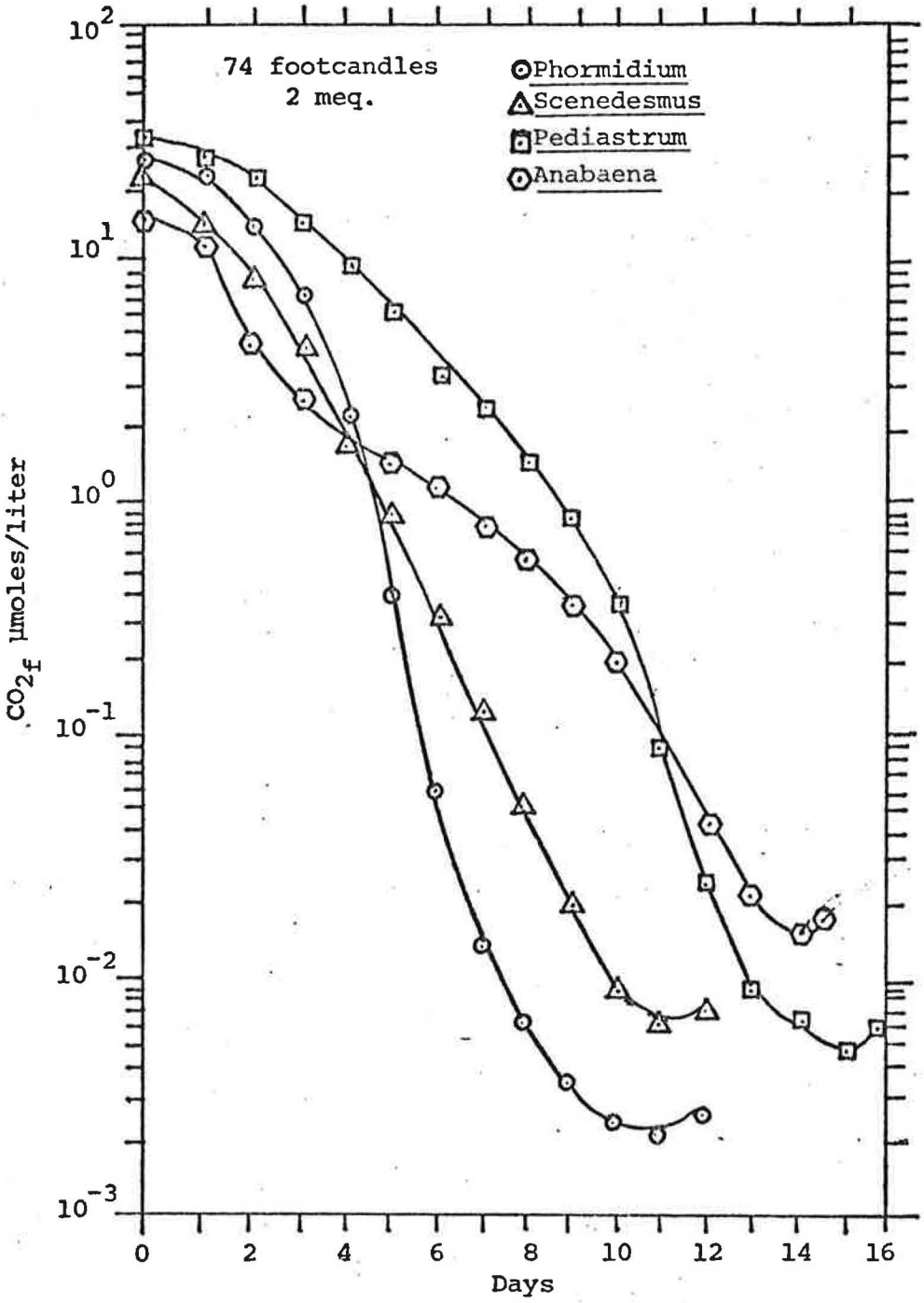

Figure 9

Time Related CO_{2f} Concentration Under Constant Light for Anabaena variabilis, Scenedesmus acutiformis, Phormidium olivacea, and Pediastrum biradiatum During First Investigation

tion. On this same competitive basis Phormidium has the advantage for carbon over both Pediastrum and Scenedesmus.

Rate of Growth

The calculated specific growth rates for the four algal genera used in this investigation with respect to CO_{2_f} concentration are shown in Figures 10-13. The specific growth rates are defined as follows:

$$\mu = dm/dt/M \qquad (9)$$

where:

μ = specific growth rate = hr^{-1}

M = average biomass; mg C/liter

t = time; hours

From Equation 9 it is evident that the specific growth rate is the instantaneous time rate of change of biomass per unit biomass. Specific growth rates (μ) were obtained by incrementing the carbon fixed curves with time (Figure 7). The increase in carbon fixed over a time increment was then divided by the associated average biomass to obtain μ values. In Figures 10-13 specific growth rates are plotted against average CO_{2_f} concentrations, during the time increment for which the associated μ was calculated. This is more fully discussed by Young (3).

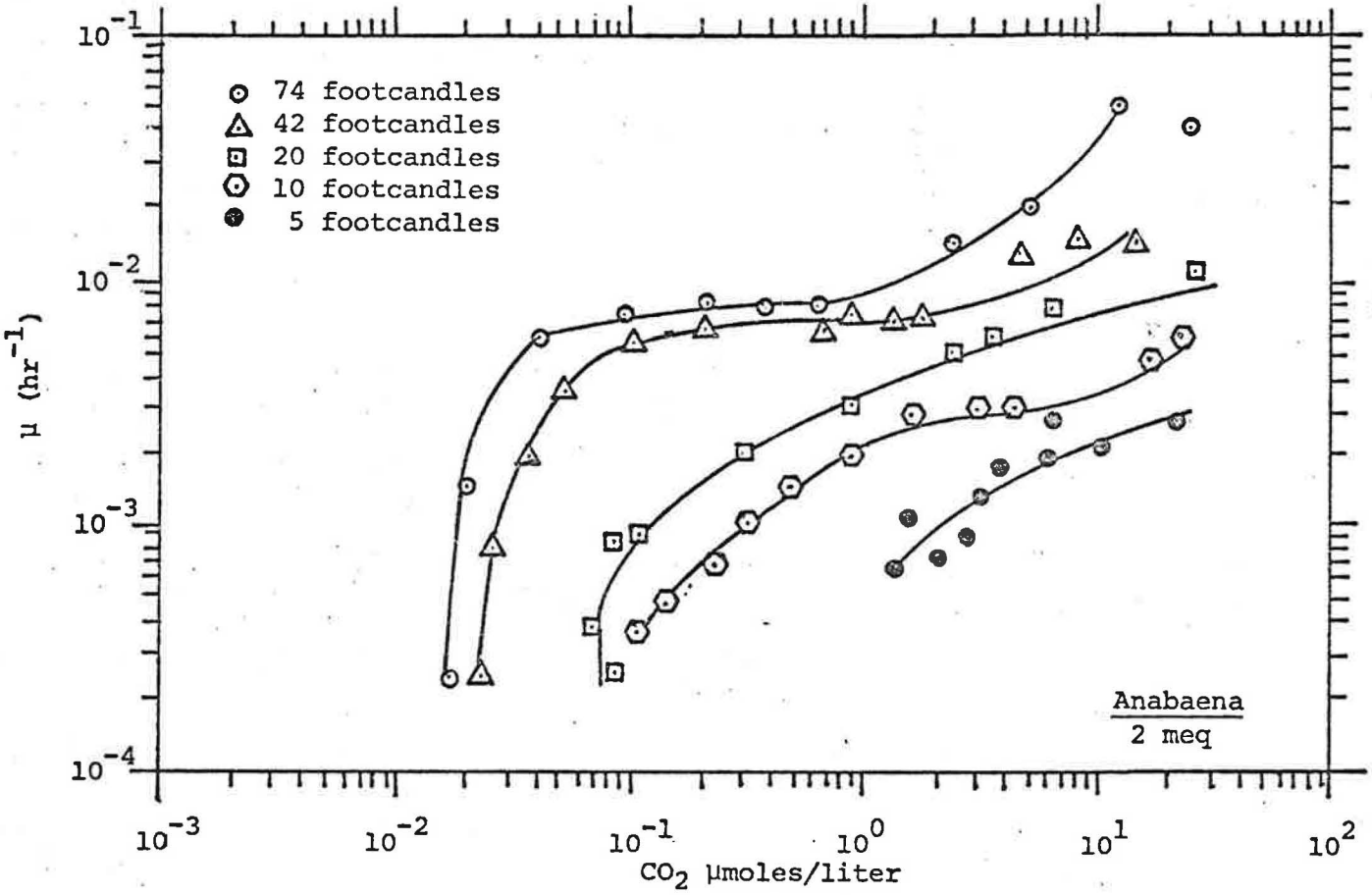

Figure 10

Variation of Specific Growth Rate With CO_2 Concentration for *Anabaena variabilis* Under Various Light Intensities During First Investigation

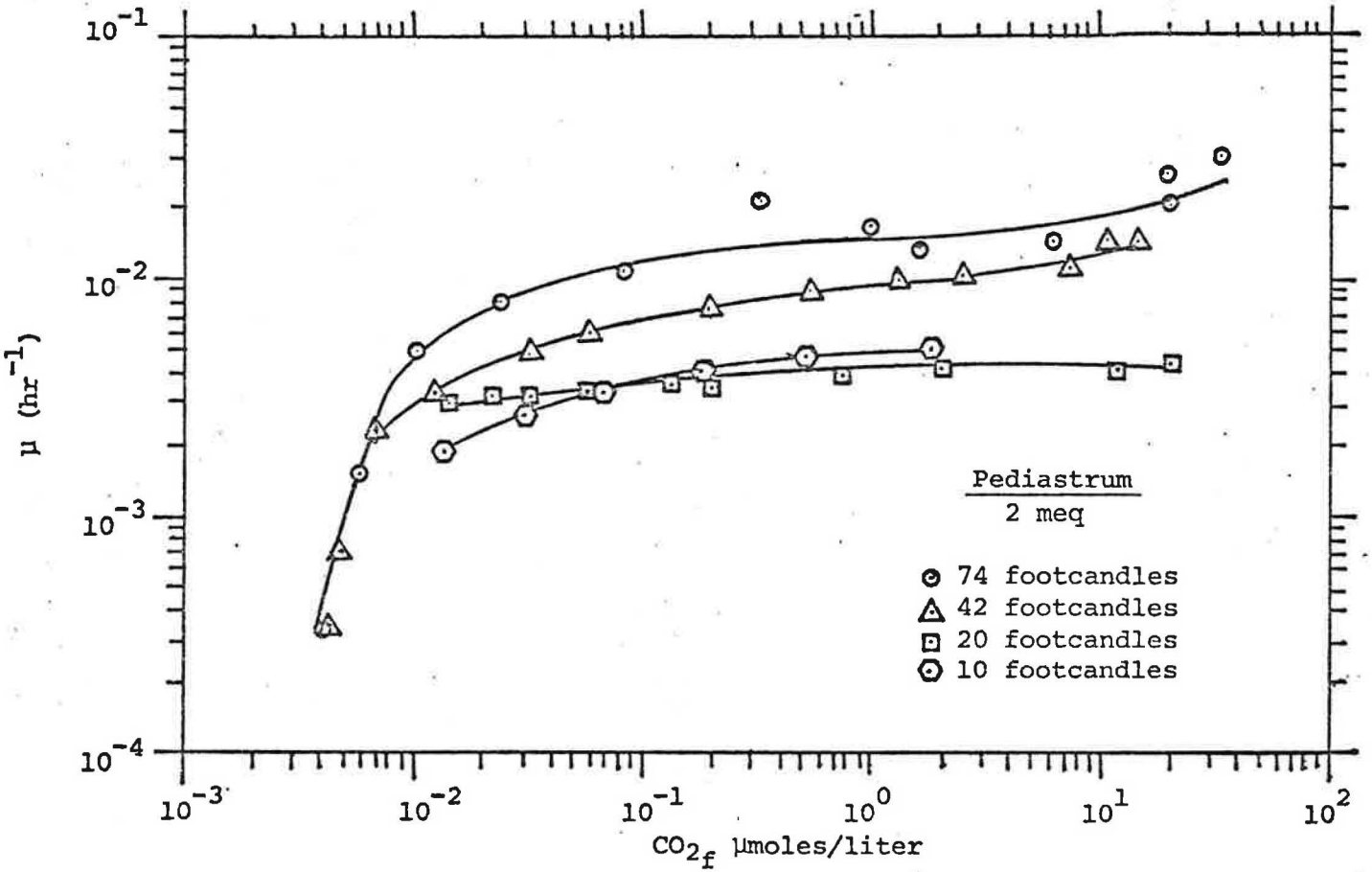

Figure 11

Variation of Specific Growth Rate With CO_{2_f} Concentration for Pediastrum biradiatum Under Various Light Intensities During First Investigation

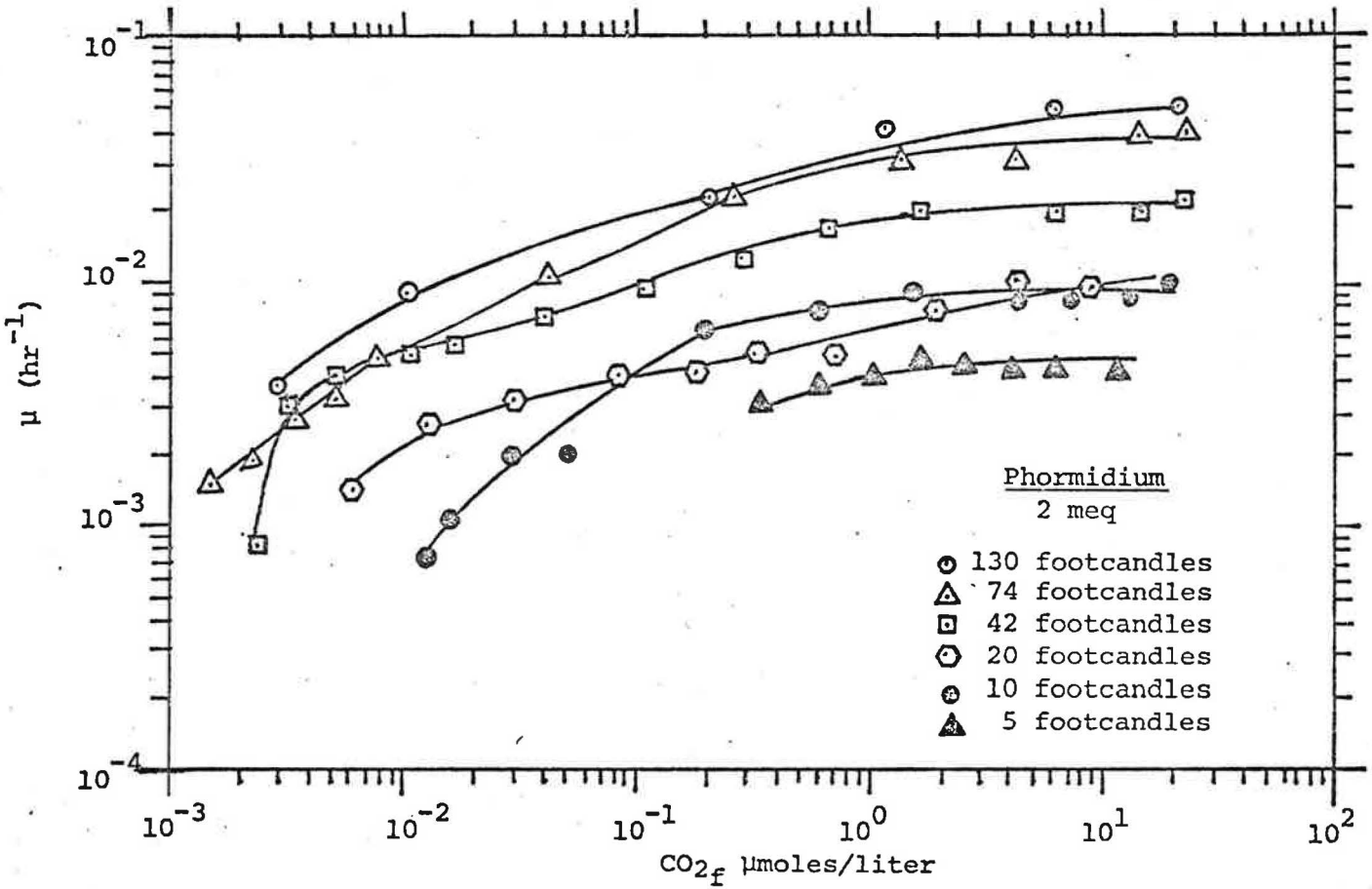

Figure 12

Variation of Specific Growth Rate With CO_{2_f} Concentration for Phormidium olivacea Under Various Light Intensities During First Investigation

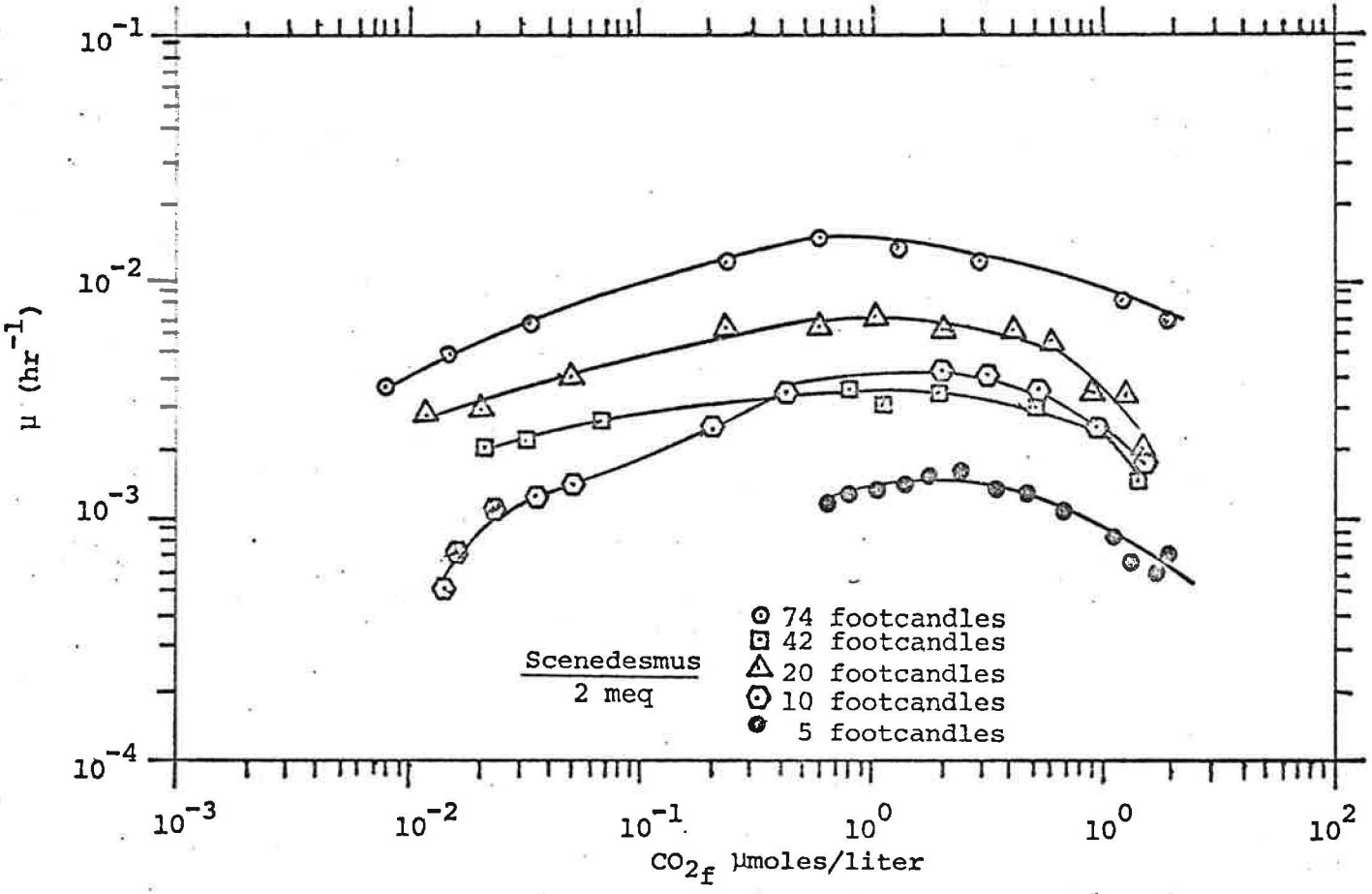

Figure 13

Variation of Specific Growth Rate With CO_{2_f} Concentration for Scenedesmus acutiformis Under Various Light Intensities During First Investigation

It should be noted that Figures 10-13 are log-log plots and the curves represent the respective growth rate under each variation of light intensity. It is evident from these plots that specific growth rates for a constant light intensity is a function of the CO_{2_f} concentration. That is, as the CO_{2_f} concentration decreases, specific growth rate decreases, as shown for Pediastrum, Anabaena, Phormidium, and Scenedesmus in Figures 10-13. It is also evident that for all CO_{2_f} concentrations, specific growth rate decreases as light intensity decreases. This was noted by Klemovich (1) for Chlorella and Nostoc.

In Figure 13, Scenedesmus is shown to have low specific growth rates during the early stages of the runs for all light intensities studied. This may be due to difficulties in seeding or perhaps Scenedesmus functions better at higher pH values.

Specific growth rates with respect to CO_{2_f} concentration for the four algal genera under a constant light intensity of 74 footcandles is shown in Figure 14. It is apparent that differences exist in specific growth rates between algae for all CO_{2_f} concentrations. Phormidium is seen to exhibit the highest growth rate for all CO_{2_f} concentrations, followed by Pediastrum, Scenedesmus and then Anabaena. Thus, Phormidium is seen to have a competitive advantage for carbon over Pediastrum, Scenedesmus and Anabaena, not only on a CO_{2_q} basis but also on a rate basis.

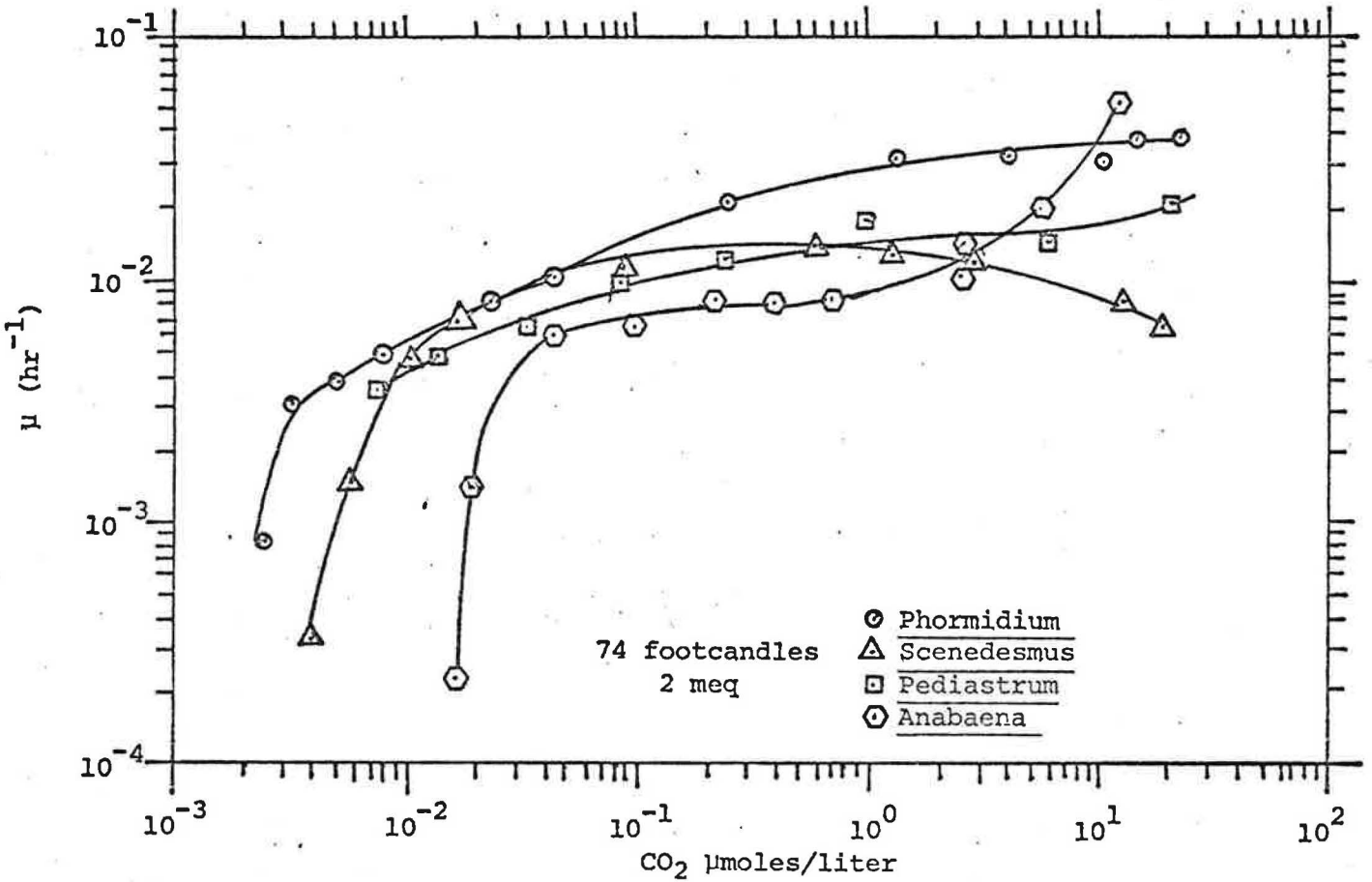

Figure 14

Variation of Specific Growth Rate With CO_2, Concentration Under a Constant Light Intensity of 74 footcandles for Anabaena variabilis, Phormidium olivacea, Pediastrum biradiatum and Scenedesmus acutiformis During First Investigation

Second Investigation

In the first investigation the effects of varying light intensity upon the ability of algae to extract carbon from a single carbonate-bicarbonate alkalinity was determined. In the second investigation consideration was given to the relative ability of Pediastrum and Scenedesmus to extract carbon from different carbonate-bicarbonate alkalinities under a constant light intensity. The objective of this investigation was to determine if CO_{2_q} for Scenedesmus and Pediastrum was independent of alkalinity at a fixed light intensity.

The light intensities used for this experiment were 130 footcandles and 42 footcandles for Pediastrum and 130 footcandles for Scenedesmus. Alkalinities used were 1, 2, and 3 meq/liter under each light intensity. The temperature throughout this experiment did not vary more than $\pm$1°C from 26°C.

Figure 15 shows that by increasing the alkalinity, under a constant light intensity, Pediastrum is able to attain a greater maximum pH. This relationship also was noticed for Pediastrum under a light intensity of 42 footcandles. Due to the limited buffer capacity associated with the one meq/liter alkalinity, the rate of change of pH to the maximum pH, was greater than for the higher carbonate-bicarbonate alkalinity systems.

King (9) has shown that as the alkalinity increases there is an increase in the total inorganic carbon, ΣCO_2. As shown in Figure 16, the amount of carbon fixed as biomass by Pediastrum

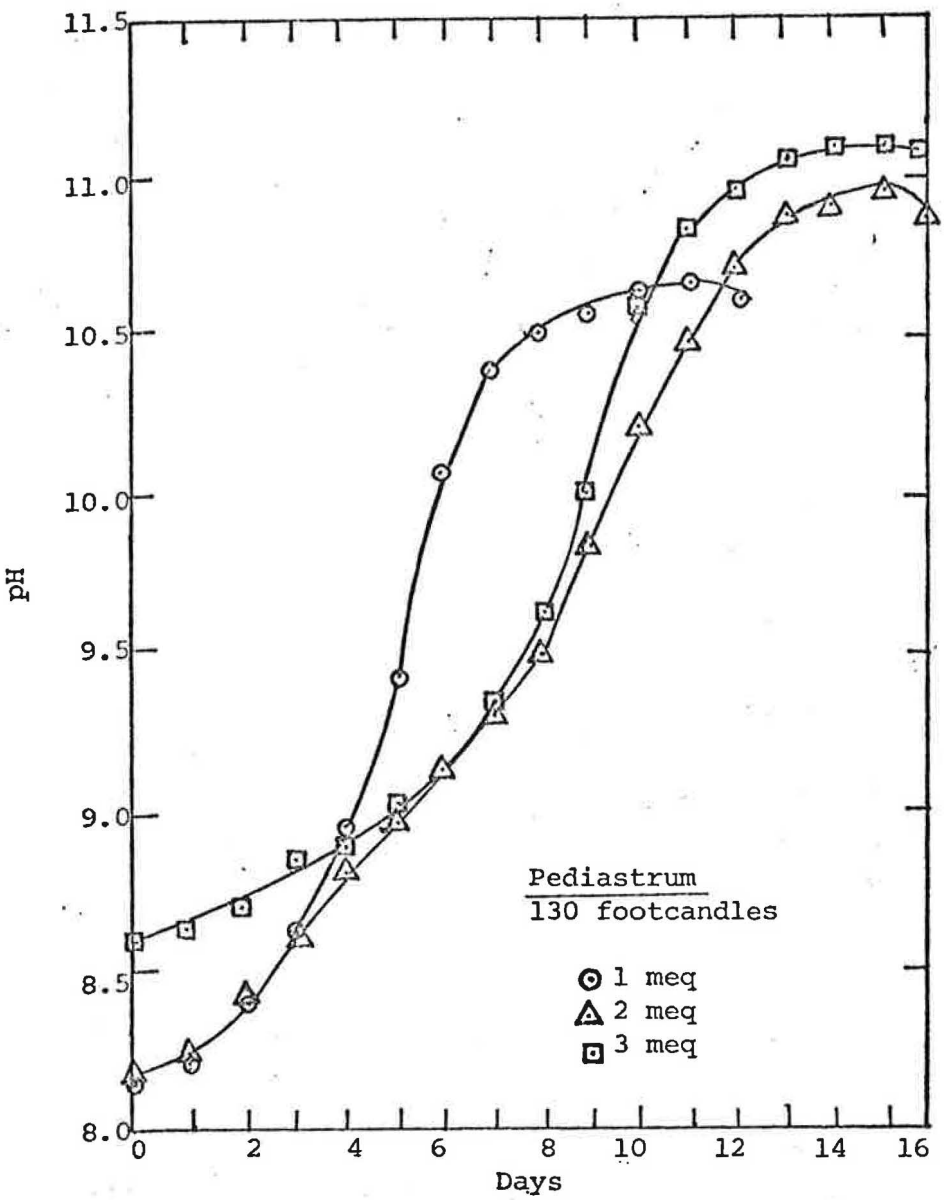

Figure 15

Time pH Response For Pediastrum biradiatum for Various Alkalinity Concentrations at a Constant Light During Second Investigation

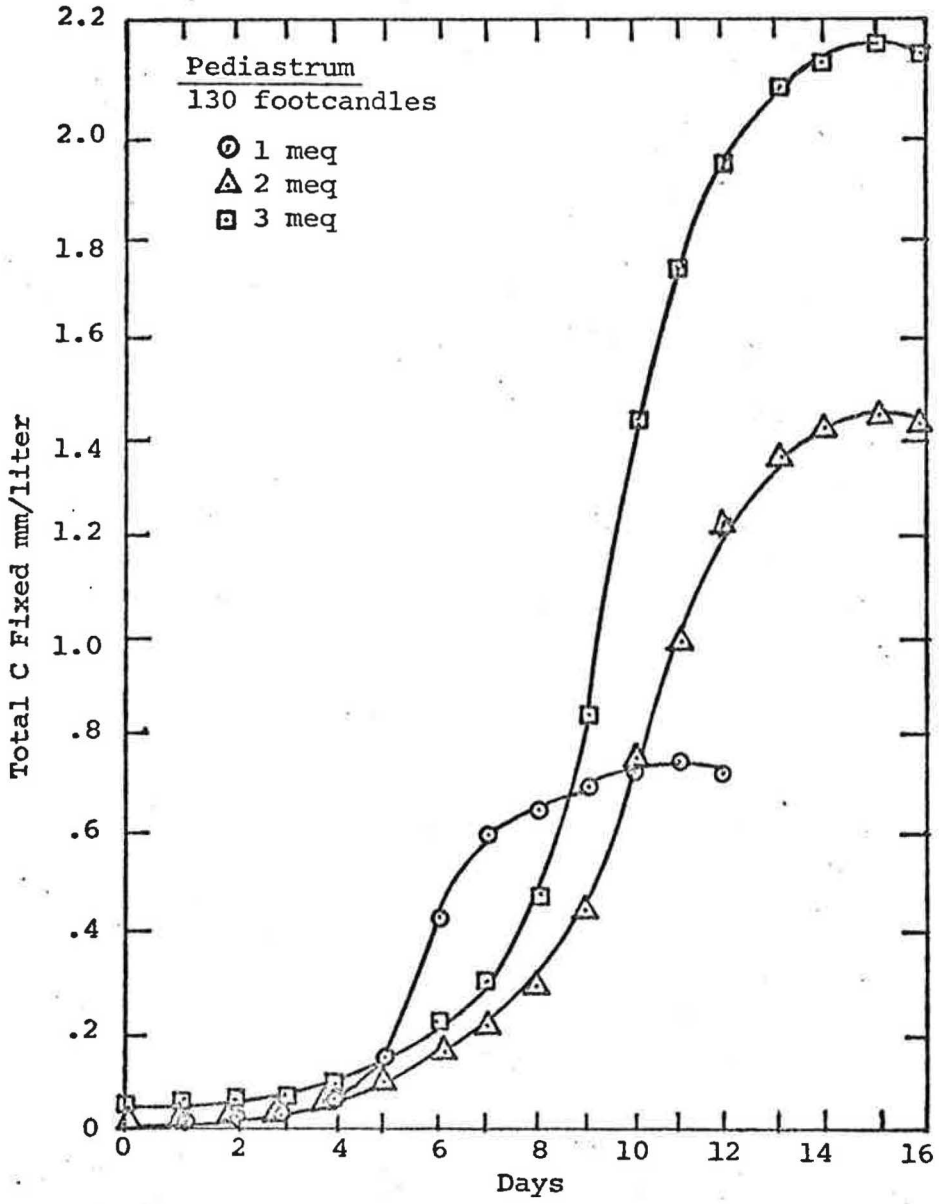

Figure 16

Time Related Accrual of Carbon Fixed by Pediastrum biradiatum for Various Alkalinity Concentrations at a Constant Light Intensity During Second Investigation

increased with the increased available carbon supplied by increased carbonate-bicarbonate alkalinity.

The amount of carbon fixed at a light intensity of 130 footcandles gave a ratio of 1:2:3 for the respective 1:2:3 meq/liter alkalinities. However under a light intensity of 42 footcandles this ratio fell to 1:1.4:2.2 for the alkalinities 1, 2, and 3 meq/liters, respectively.

Figure 17 shows the time related decrease in the equilibrium CO_{2_f} concentration for Pediastrum under a constant light intensity. It is evident from these curves, that Pediastrum is able to continue photosynthesis to a lower equilibrium CO_{2_f} concentration when the alkalinity concentration is increased. This suggests that under a light intensity of 130 footcandles, carbon fixation is dependent at least in part on the alkalinity concentration. However, when Pediastrum was grown under a light intensity of 42 footcandles the CO_{2_f} concentration was approximately equal for each microcosm regardless of the alkalinity. This suggests that for this intensity of light carbon fixation is dependent on the concentration of the CO_{2_f} present in the water and is independent of alkalinity.

The specific growth rate of Pediastrum at various CO_{2_f} concentrations under a constant light-intensity of 130 footcandles are shown in Figure 18. These growth rates were calculated using the carbon values from Figure 16 and the method presented in the discussion of the first experiment. The associated CO_{2_f} concentrations were obtained from Figure 17. Figure 18, a log-log plot,

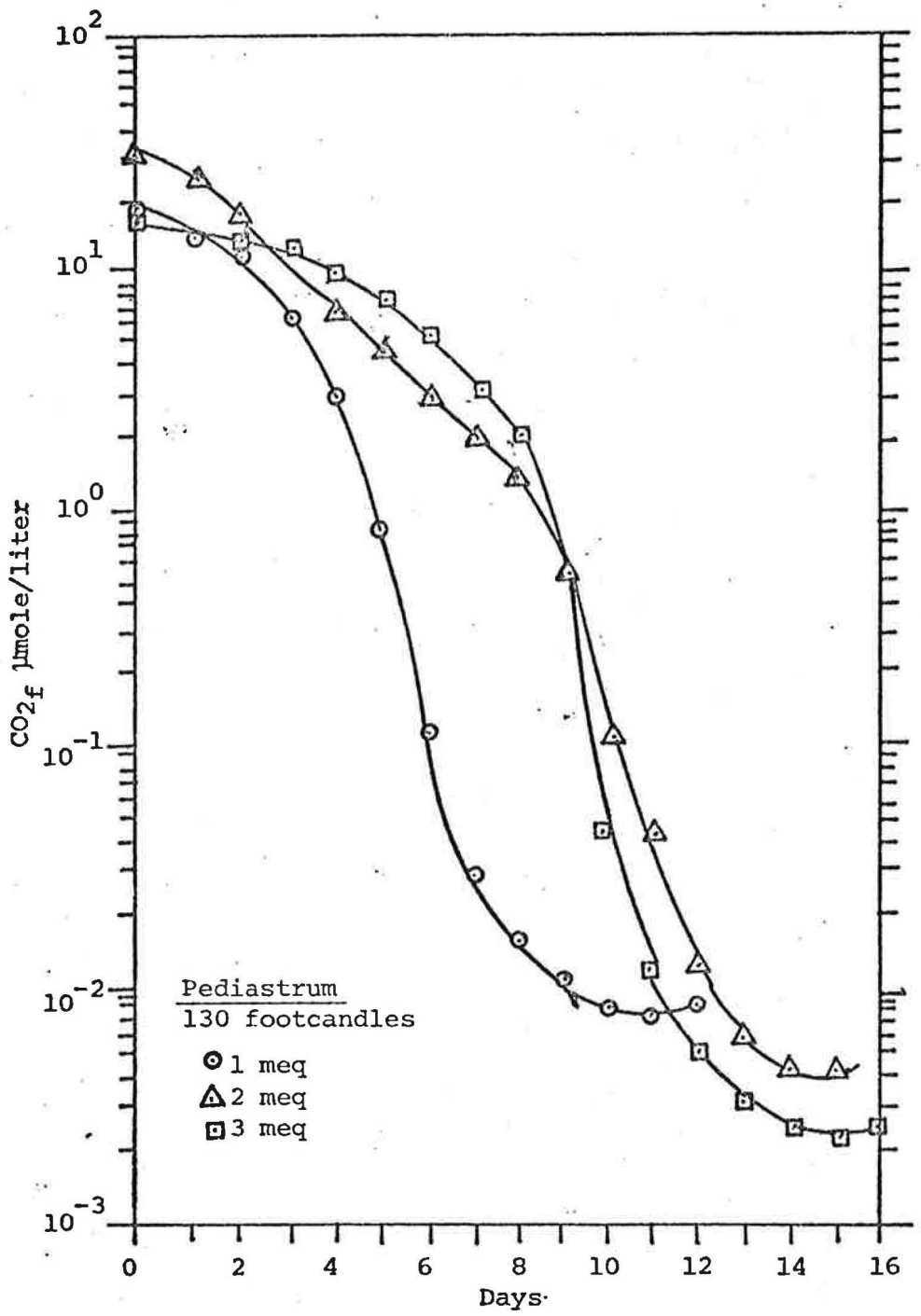

Figure 17

Time Related CO_{2f} Concentration Under Various Alkalinity Concentrations for Pediastrum biradiatum at a Constant Light Intensity During Second Investigation

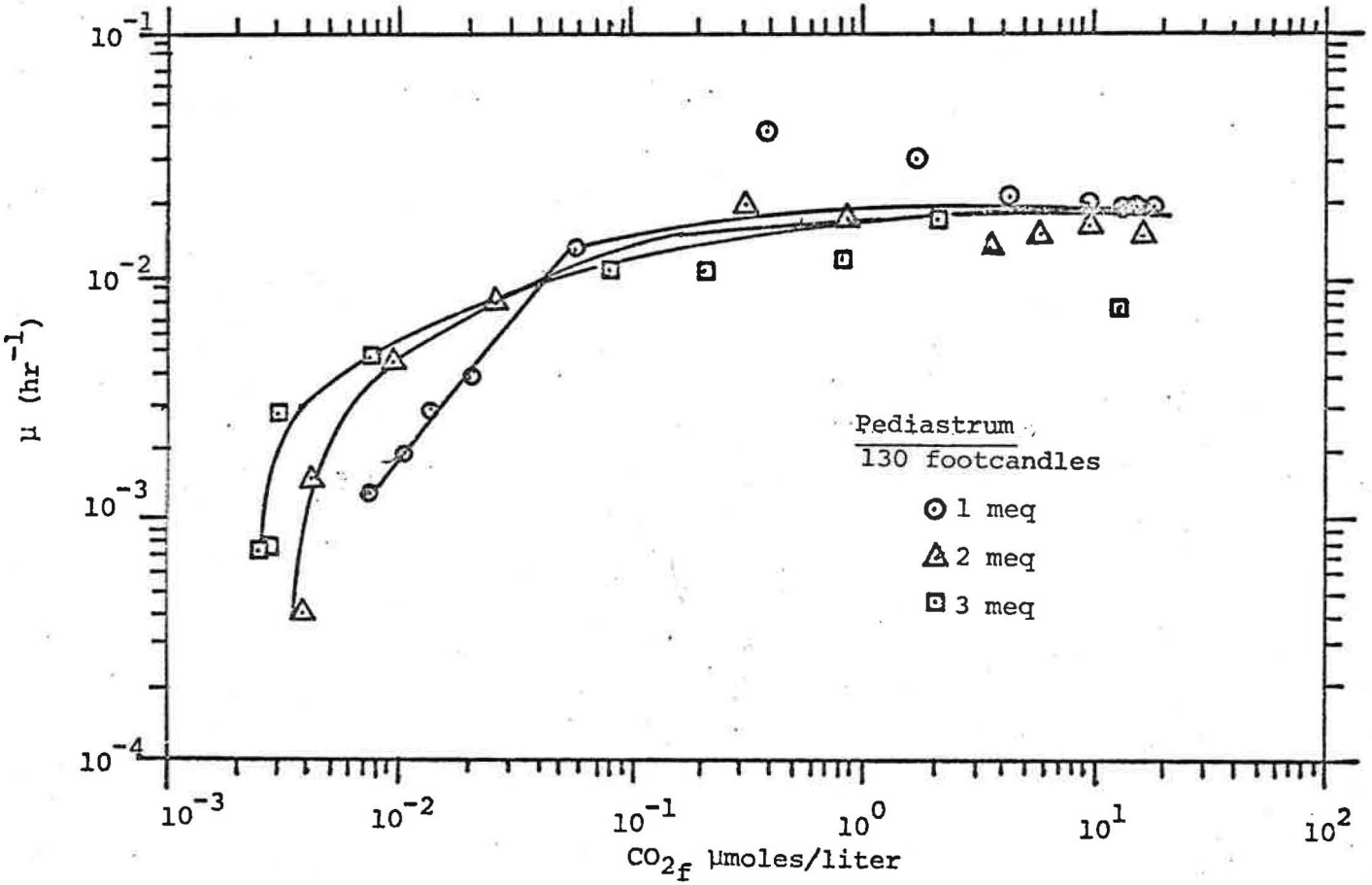

Figure 18

Variation of Specific Growth Rate with CO_{2_f} Concentration for Pediastrum biradiatum Under Various Alkalinity Concentrations at a Constant Light Intensity of 130 Footcandles During the Second Investigation

serves to illustrate that under a constant light intensity increased alkalinity will increase the specific growth rate at lower CO_{2_f} concentrations. As previously noted, there is a greater amount of carbon in a higher alkalinity concentration, therefore more biomass would have accumulated by the time a given CO_{2_f} concentration was reached. This increase in biomass would result in more surface area of algae and perhaps there would be a greater CO_{2_f} driving force in the higher alkalinity. As shown in Figure 18 at high concentration there was considerable scatter.

For Pediastrum under a light intensity of 42 footcandles this similarity did not hold true. As shown in Figure 19, for Pediastrum at 1, 2, and 3 meq/liter alkalinity the specific growth rate curves crossed over each other at various CO_{2_f} concentrations and no generalized conclusion could be drawn. However, comparison of Figure 18 and Figure 19, again indicates the role of light intensities plays in determining CO_{2_q}.

Scenedesmus also was subjected to varying alkalinity concentrations of 1, 2, and 3 meq/liter under a constant light intensity of 130 footcandles.

Figure 20 shows this increase in pH with elapsed time for Scenedesmus at varied alkalinity concentrations. The measurable difference between the maximum pH values at varied concentrations was 0.04 pH units. This very slight difference in pH can be attributed to the error in calibrating the pH meter, especially at these high pH values. From this it is assumed that Scenedesmus,

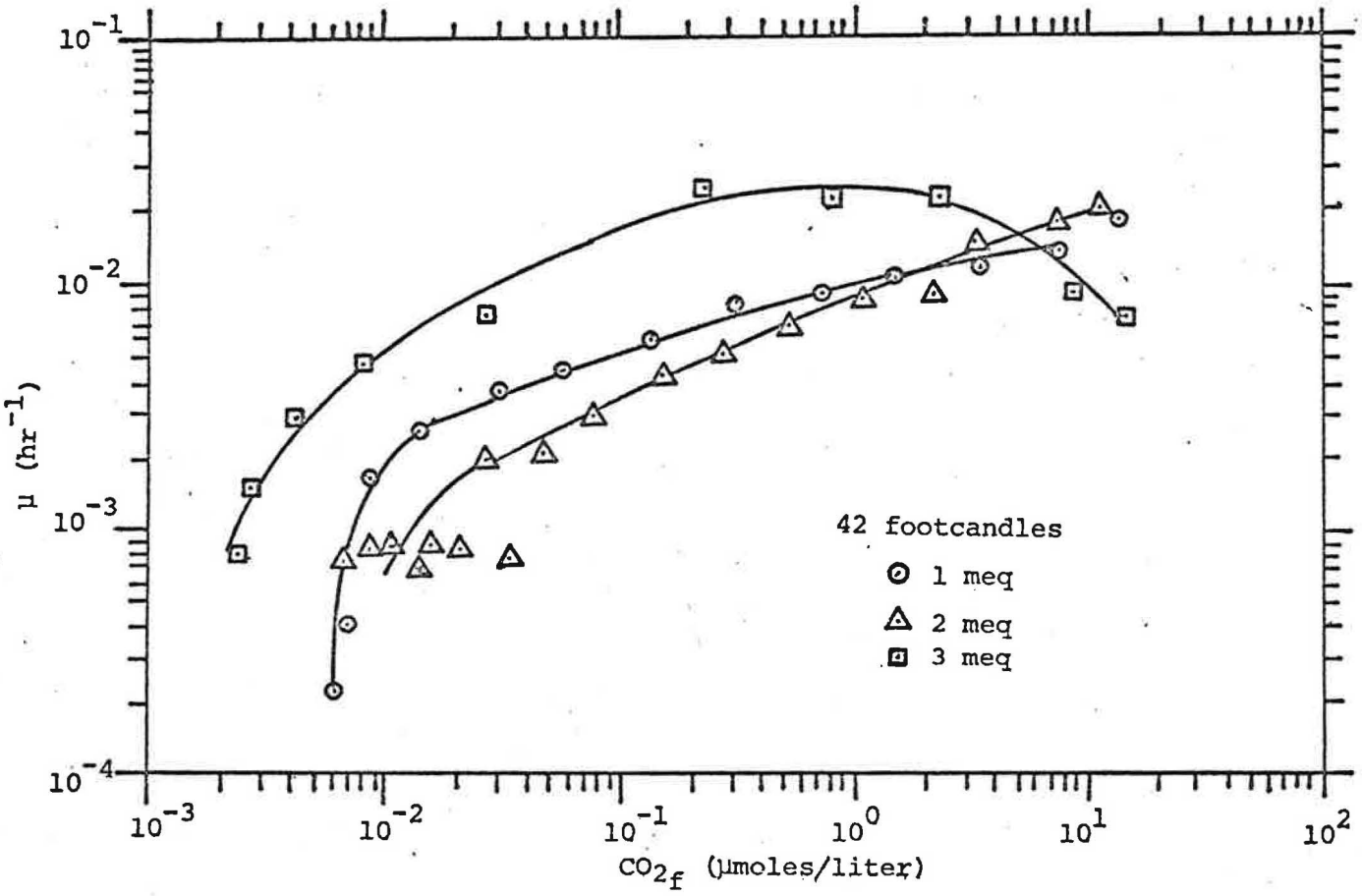

Figure 19

Variation of Specific Growth Rate With CO_{2_f} Concentration For Pediastrum biradiatum Under Various Alkalinity Concentrations at a Constant Light Intensity of 42 Footcandles During Second Investigation

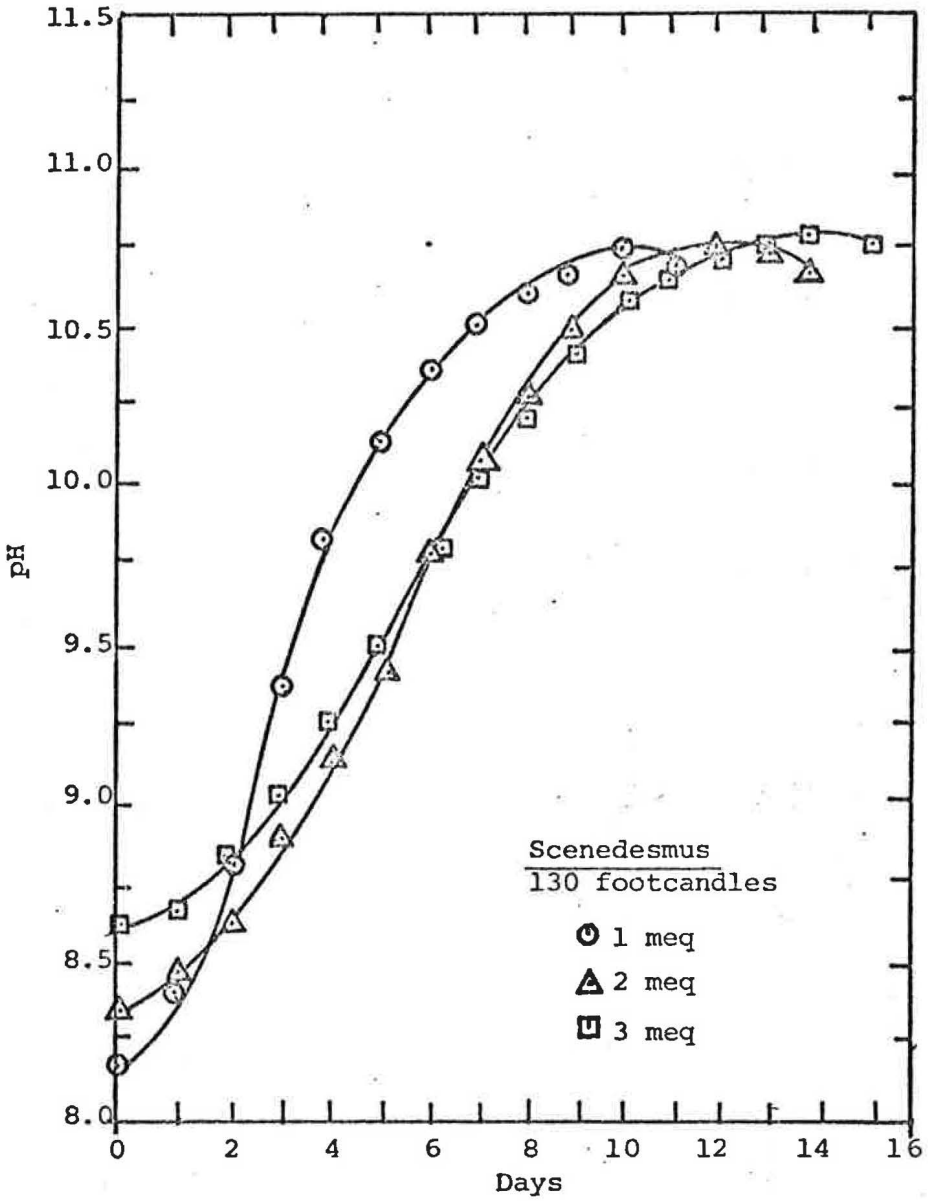

Figure 20

Time pH Response for Scenedesmus acutiformis for Various Alkalinity Concentrations at a Constant Light Intensity of 130 Footcandles During the Second Investigation

under a light intensity of 130 footcandles, is not able to increase its maximum pH value with an increase in carbonate-bicarbonate alkalinity. The maximum pH attained for Scenedesmus was 10.80 which was what Klemovich (1) found in his work with Scenedesmus. The ability of Scenedesmus to fix carbon from the carbonate-bicarbonate alkalinity is shown in Figure 21. This figure shows that Scenedesmus is able to fix the greater available carbon associated with an increased alkalinity. But the ratio of total carbon fixed for alkalinities of 1, 2, and 3 meq/liter was only 1:1.47:1.95. This low efficiency of total carbon fixed, as biomass, is associated with the inability of Scenedesmus to continue to extract carbon from the carbonate-bicarbonate alkalinity above a maximum pH of 10.80.

Figure 22 shows the time related decrease in the equilibrium free carbon dioxide concentration for Scenedesmus under varied alkalinity concentrations. The microcosm with 1 meq/liter alkalinity reached the minimum CO_{2_f} concentration (CO_{2_q}) as would be expected if Scenedesmus was pH limited. Since there is less CO_{2_f} at a given pH and a lower alkalinity and since all microcosms reached the same maximum pH value, it would be expected that the higher alkalinity would have a greater CO_{2_f} concentration remaining when growth stopped as is shown in Figure 22.

Thus, Scenedesmus could not continue to fix carbon beyond a pH of 10.80. The limitation of carbon fixation by Scenedesmus was not associated with either a CO_{2_q} concentration or an alkalinity

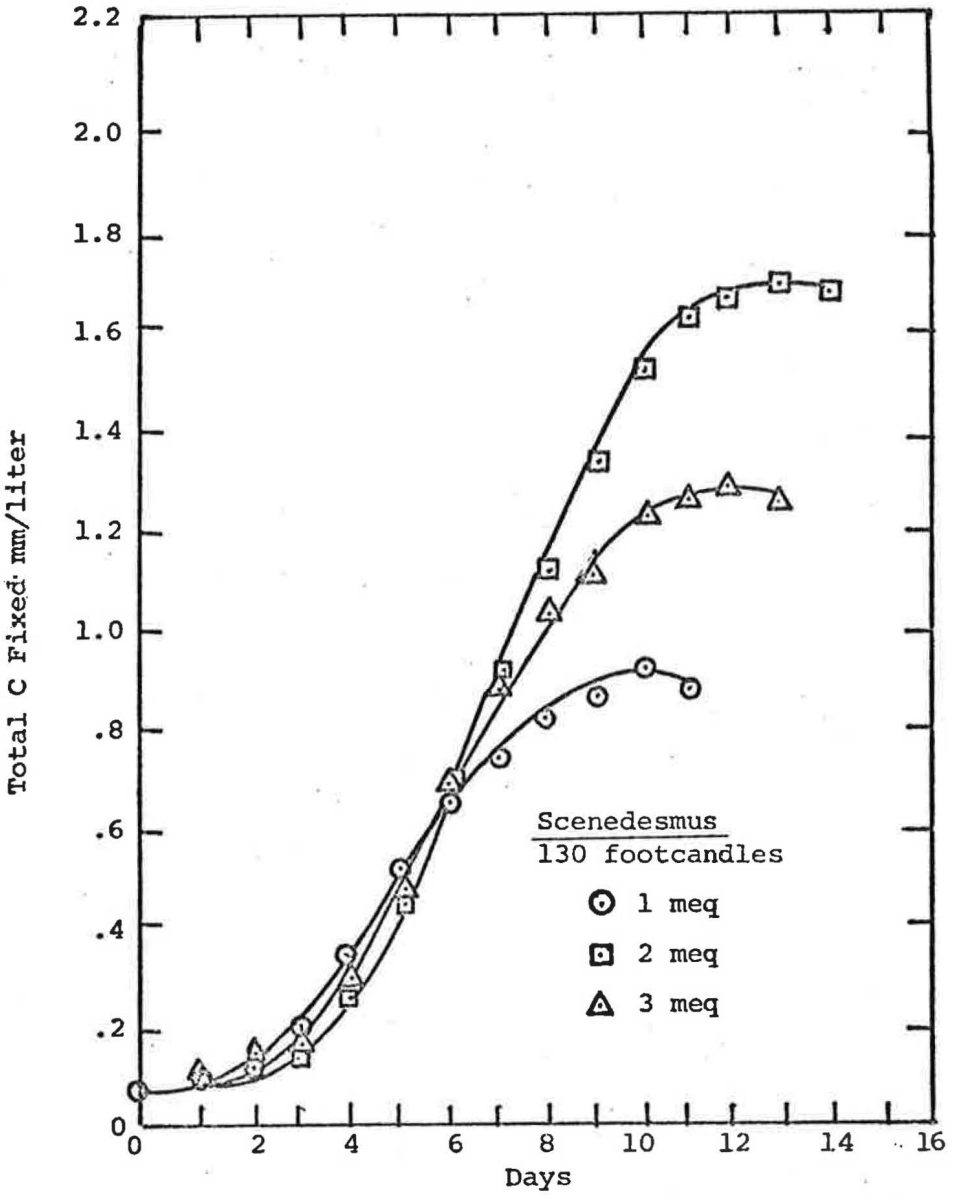

Figure 21

Time Related Accrual of Carbon Fixed by Scenedesmus acutiformis for Various Alkalinity Concentrations at a Constant Light Intensity During the Second Investigation

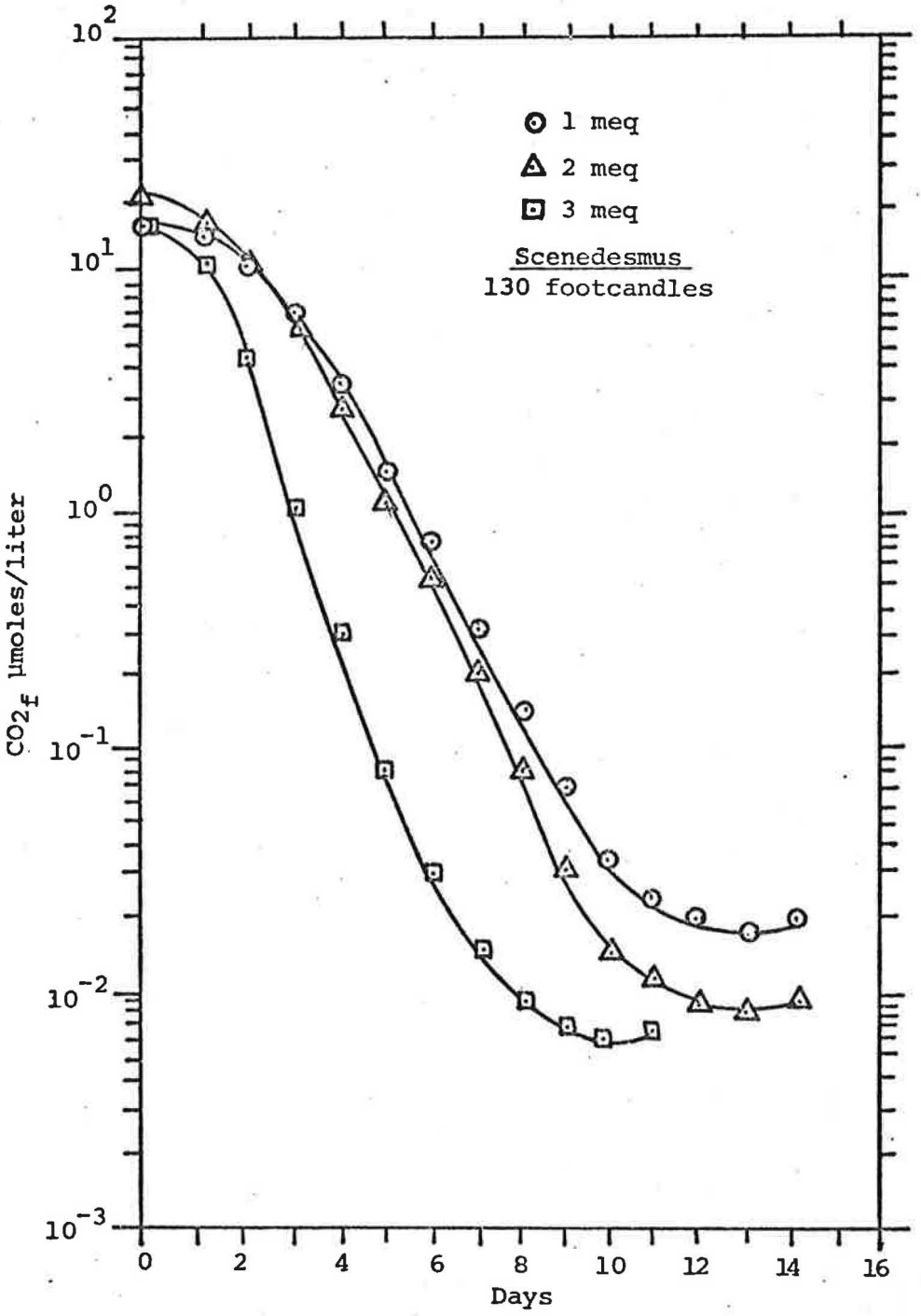

Figure 22

Time Related CO_{2f} Concentrations Under Various Alkalinity Concentrations for Scenedesmus acutiformis at a Constant Light Intensity During the Second Investigation

concentration, but rather the extent of carbon fixation was limited by a pH maximum which limited the ability of the algae to continue to fix carbon from the carbonate-bicarbonate alkalinity, under a constant light intensity of 130 footcandles.

Light-Carbon Interactions

The results from this study have shown that there is a relationship between the relative ability of different algae to extract carbon from the carbonate-bicarbonate alkalinity and the intensity of light algae receive. Klemovich (1) defined this relationship for Chlorella and Nostoc and this study defined this relationship for Scenedesmus, Phormidium, Anabaena and Pediastrum as the extent of carbon fixation from a set alkalinity and as the rate of carbon fixation, both of which are related to the light intensity algae receive. As previously shown the extent of carbon fixation for these algae is directly related to their relative ability to continue photosynthetic activity to a light intensity limited CO_{2_f} concentration (CO_{2_q}). It was also shown that the utilization rate of carbon, or specific growth rate is dependent upon the CO_{2_f} concentration present and will decrease significantly as the light intensity determined CO_{2_q} is approached. Thus, this difference in the ability of algae to extract CO_{2_f} to a limiting CO_{2_f} concentration (CO_{2_q}) as a function of light intensity appears to be of some importance in determining the relative competitiveness of different algae and would appear to play a role in algal succession.

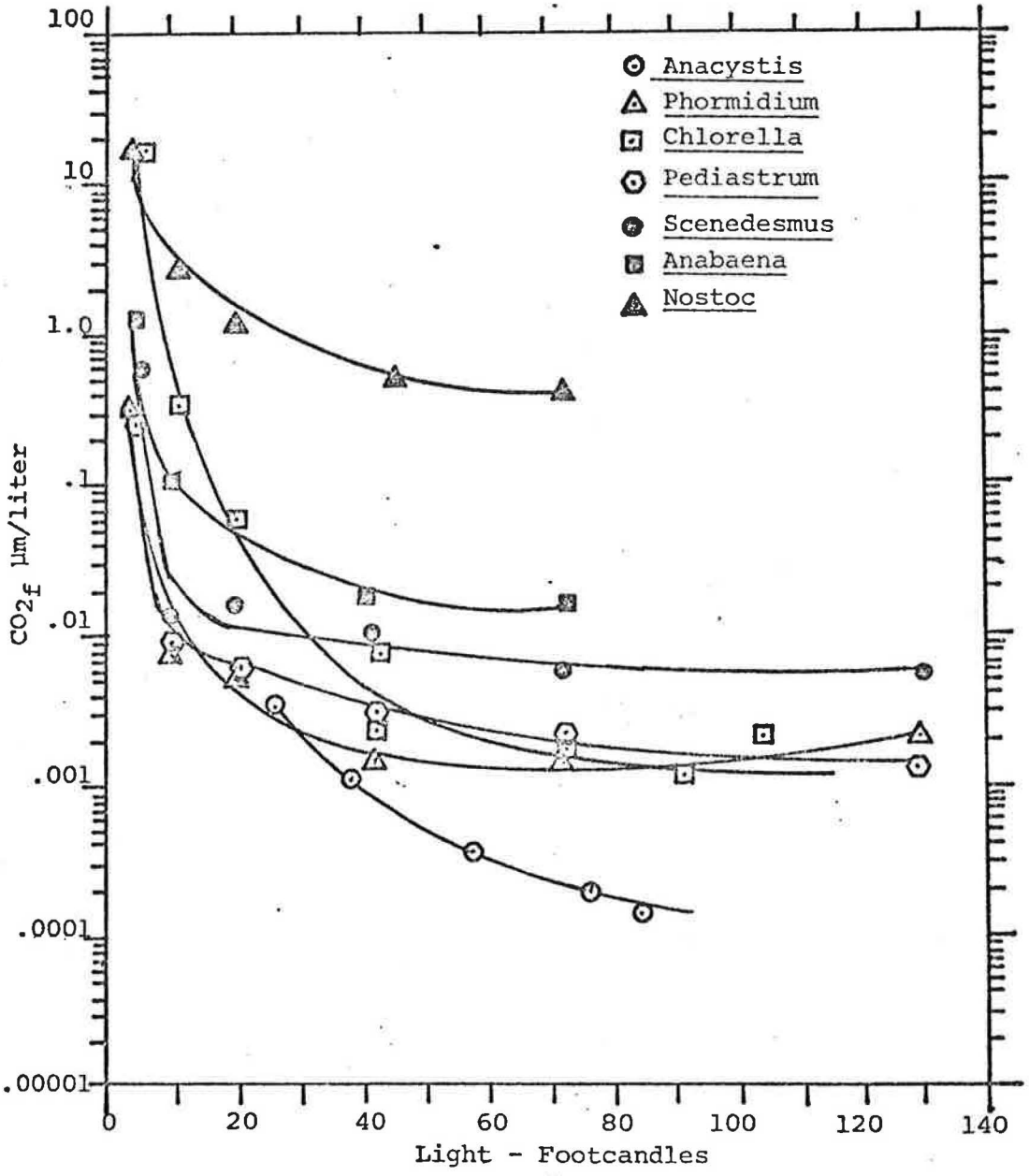

Figure 23

The Limiting CO_{2f} Concentrations (CO_{2_g}) for a Variance of Light. Five Footcandles to 130 Footcandles

For example, Scenedesmus will have a competitive advantage for carbon in the carbonate-bicarbonate alkalinity system until a light intensity of 30 footcandles is reached. At this light intensity there is a trade-off of relative competitiveness for carbon and Chlorella will dominate over Scenedesmus for light intensities greater than 30 footcandles.

If Scenedesmus, Pediastrum and Phormidium algal cells were in the same microcosm, Pediastrum would be the dominant species for light intensities under 20 footcandles. At light intensities greater than 20 footcandles Phormidium will have the competitive advantage for carbon until the light reaches an intensity of 70 footcandles where Chlorella will then dominate for microcosms that are only carbon limited.

Also Figure 23 shows for a single constant light intensity, which algae would be able to continue photosynthesis if only carbon were the limiting nutrient. For a light intensity of 60 footcandles, Nostoc would be able to reach a CO_{2_f} concentration of only 0.2 µmoles /liter until photosynthesis ceased. But Anabaena and Anacystis would be able to continue to extract carbon from the carbonate-bicarbonate alkalinity to CO_{2_f} concentrations of 0.019 µmoles/liter and 0.003 µmoles/liter, respectively, before photosynthesis ceased.

King (10) has stated that blue-green algae are able to continue photosynthesis to a lower CO_{2_f} concentration than that tolerated by other algae. Inspection of Figure 23 indicates this to be the case of Anacystis and Phormidium in relation to the green algae but

not for Anabaena and Nostoc. Since there are many different types of blue-green algae it should not be expected that they all will respond in a similar manner to a given set of conditions. Anabaena, Anacystis, and Phormidium are a common problem blue-green alga, but Nostoc usually is not considered in this category.

The curves in Figure 23 for Nostoc, Anabaena, Scenedesmus, Phormidium, Pediastrum and Chlorella are leveling off as the light intensity increases. It would appear the saturation of light is being reached, because the minimum CO_{2_f} concentration is not attaining a significantly lower CO_{2_q} concentration with an increase in light intensity for these algae. While for Scenedesmus this can be shown to be a pH limit relationship.

Figure 23 has shown the light determine CO_{2_q} to be specific for each algal type over the range of light intensities studied. Also as shown in Figure 24, light does limit the extent of carbon fixation from the carbonate-bicarbonate alkalinity for those algae studied. Figure 24 represents the quantity of carbon which would be fixed from an alkalinity of 2 meq/liter by pure cultures of those algae included in Figure 23 from water at atmospheric CO_{2_f} saturation to the light intensity determined CO_{2_q}. Initial concentration was assumed to be atmospheric saturation, or 16 µmoles CO_{2_f}/liter, at a temperature of 25°C. Figure 24 includes the data from Young's (3) Anacystis and the data from Klemovich's (1) Chlorella and Nostoc. All carbon availability calculations were then made through the use of the Fortran computer program derived by Young (3). The values pre-

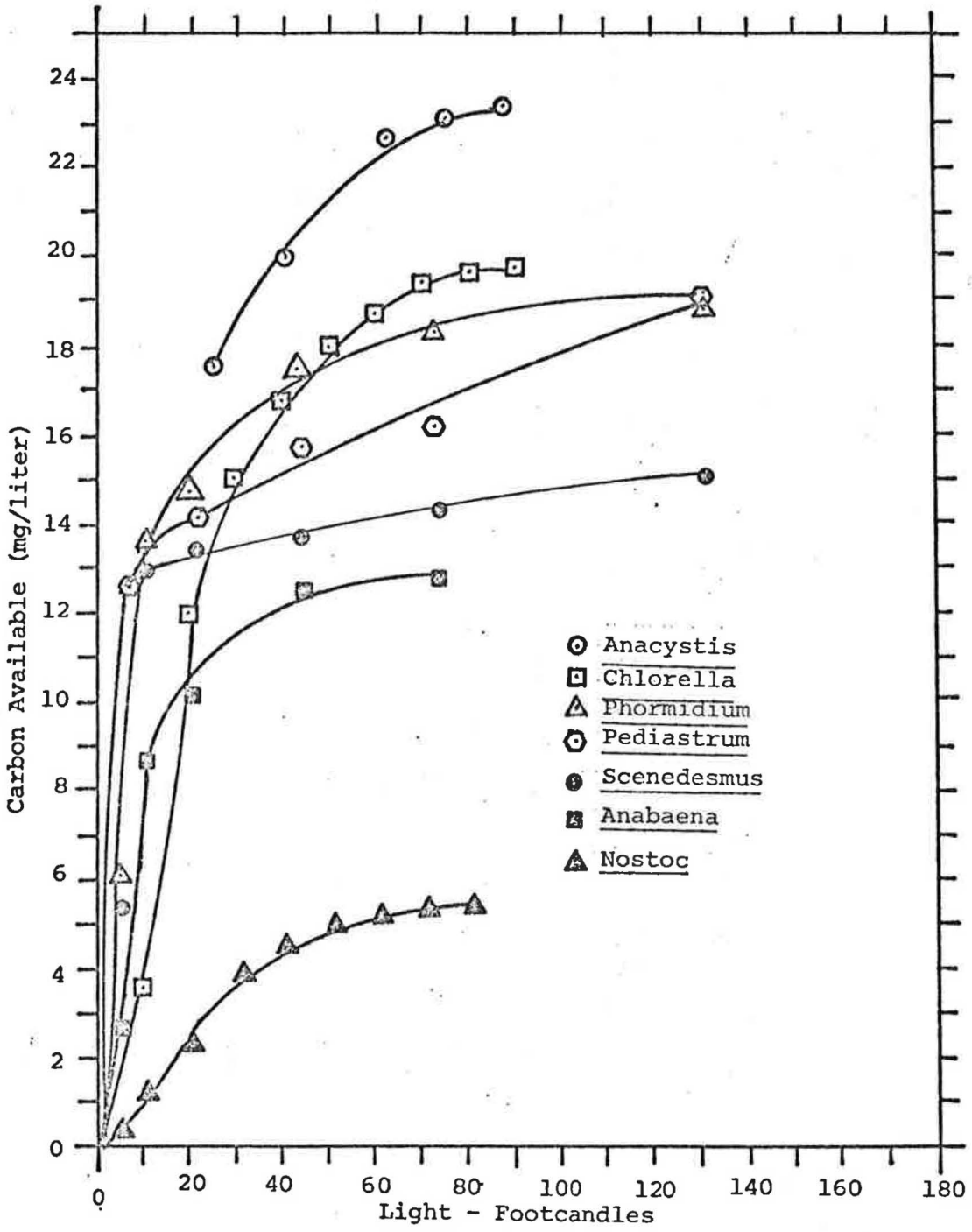

Figure 24

Carbon Available to Seven Separate Genera of Algae From an Alkalinity of 2 meq/liter and Assuming Initial Atmospheric CO_2 Equilibrium at the Indicated Light Intensities

sented in Figure 24 represent the maximum carbon fixation by the individual algae for the light intensities shown assuming that carbon and light were limiting. As Klemovich (1) stated in his study "strict application of these relationships are limited because of the artificial light source used and other constraints of the laboratory system. But the differences between genera are of sufficient magnitude that similar relationships must exist in a natural aquatic system."

Multiplicative Effects

In the preceding sections emphasis was given to relating specific growth rate to either carbon or light limits. The objective of this section is to consider the effect of simultaneous light and carbon limits on the specific growth rates of the four genera of algae studied. The most widely used method of relating specific growth rate to limiting concentration of required nutrients is the Monod Equation (11) which takes the following form.

$$\mu = V_{max} \frac{S}{K_s + S} \qquad (10)$$

where:

μ = growth rate; hr^{-1}

V_{max} = maximum growth rate; hr^{-1} a constant

S = substrate concentration, mass per unit volume

K_s = substrate concentration at 1/2 μ_{max}; mass per unit volume

Figure 25 is a plot of the specific growth rate, (μ_c), for Pediastrum, as a function of CO_{2_f} concentration, at a light intensity of 130 footcandles.

The other curve in Figure 25 was calculated using Equation 10 and the constants V_{max} and K_{s_c}, found from a Lineweaver-Burk plot using the experimental data for Pediastrum at 130 footcandles. Young (3) has shown, with his computer program, that there are significant differences between the specific growth rates calculated from the experimental data and those calculated from Equation 10 as the CO_{2_f} concentration becomes limiting. As can be seen in Figure 25, there are also differences between the specific growth rates calculated from experimental data and those based on the assumption that the data fits the Monod Equation, for Pediastrum. In effect then, specific growth rate as a function of CO_{2_f} appears to fit the Monod Equation only at high concentrations of CO_{2_f}.

Figure 26 is a hypothetical "slice" across the growth rate curves of Figure 11, at external CO_{2_f} concentrations of 10 µmoles CO_2/liter. This figure is a plot of the specific growth rate, (μ_L), for Pediastrum as a function of light intensity at CO_{2_f} concentrations of 10 µmoles/liter.

The other curve in Figure 26 was calculated with Equation 10 and the constants V_{max} and K_{s_L}, found from a Lineweaver-Burk plot using experimental data from the light study.

The apparent effects of increasing separately light intensity and CO_{2_f} concentration upon the extent and rate of algal growth were shown in the previous section.

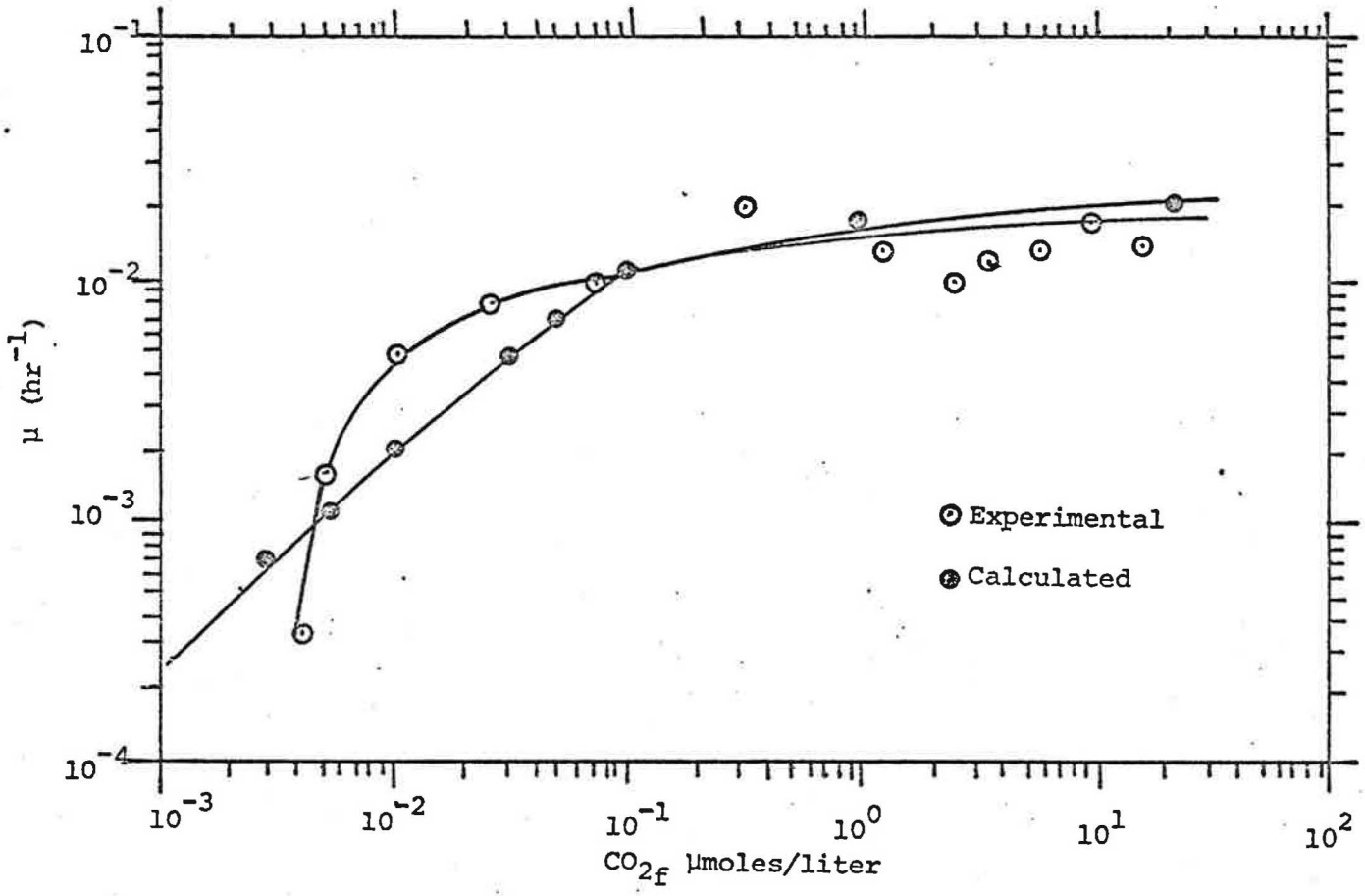

Figure 25

Variation of Specific Growth Rate With CO_{2_f} Concentration and Plot of Monod Equation Generated from Experimental Data for Pediastrum biradiatum Under a Constant Light Intensity of 130 Footcandles; Constants V_{max} and K_{s_c}, Obtained From Regression Analysis of Experimental Data

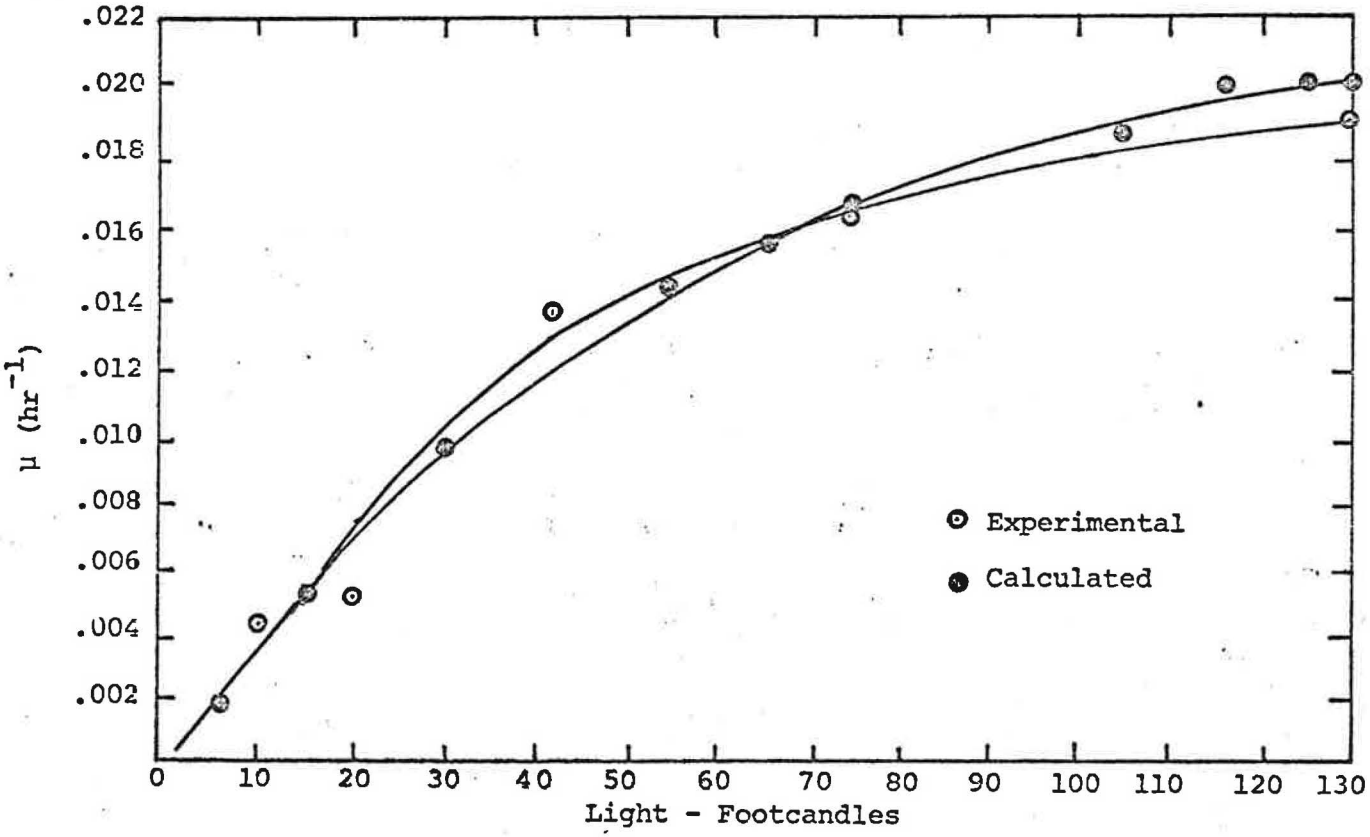

Figure 26

Variation of Specific Growth Rates with Light Intensities for Pediastrum biradiatum, Evaluated at CO_{2_f} Concentration of 10 μmoles CO_{2_f}/liter; From Regression Analysis of Experimental Data

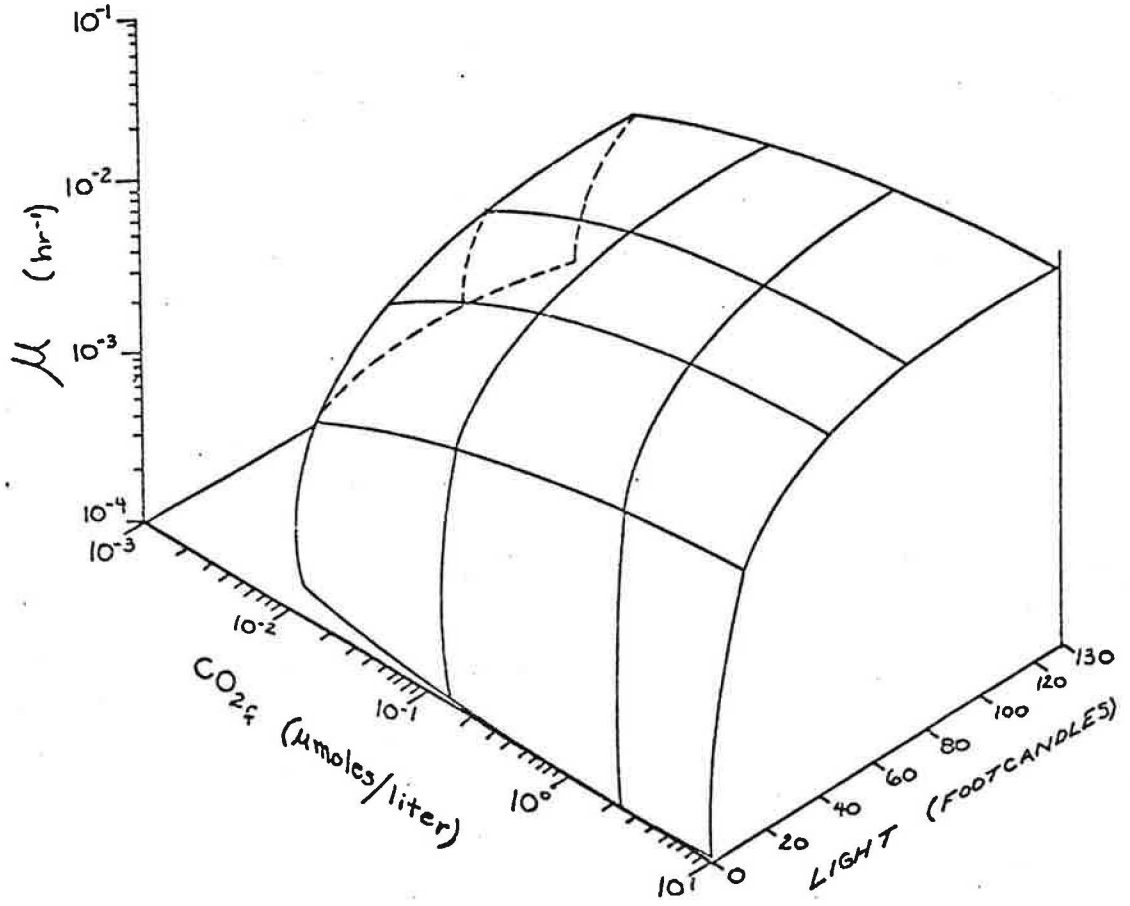

Figure 27

Variation of Specific Growth Rate With CO_{2f} Concentration and Light Intensity for Pediastrum biradiatum

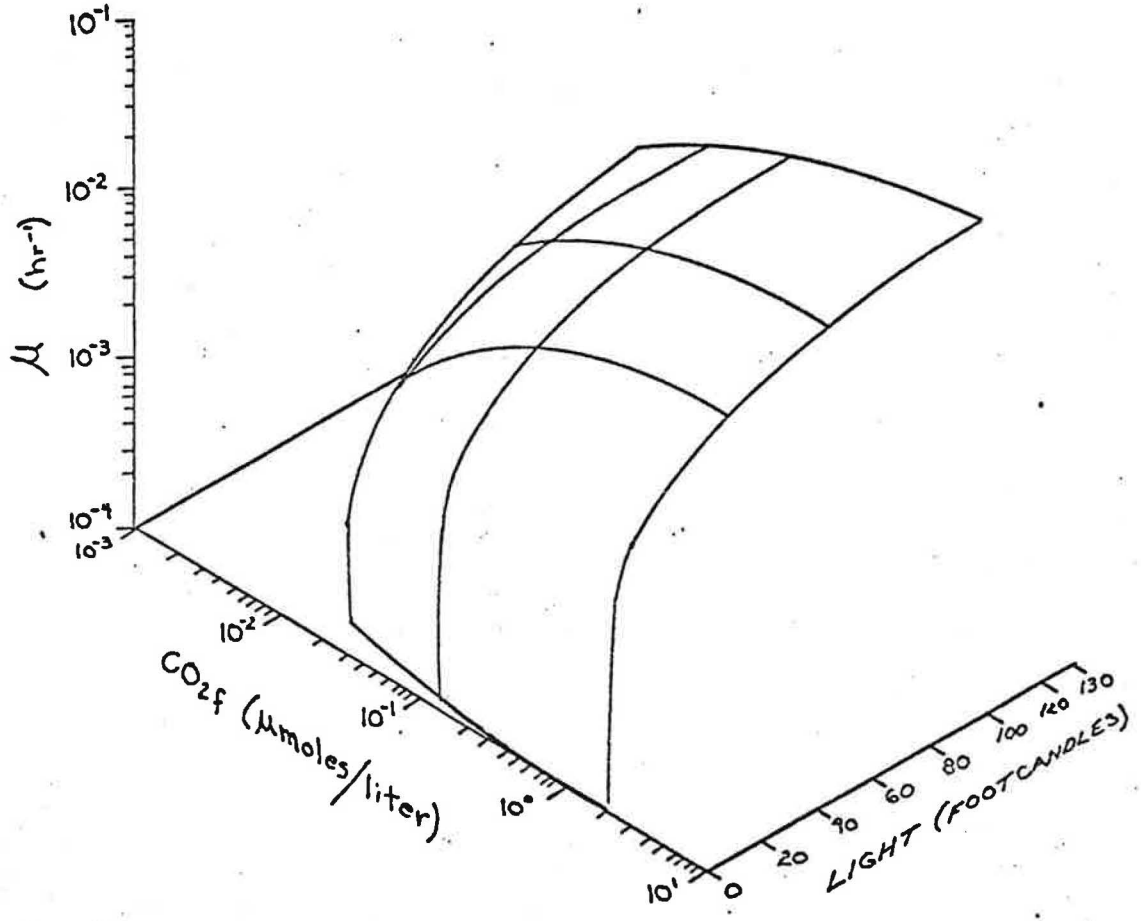

Figure 28

Variation of Specific Growth Rate With CO_{2_f} Concentration and Light Intensity for Scenedesmus acutiformis

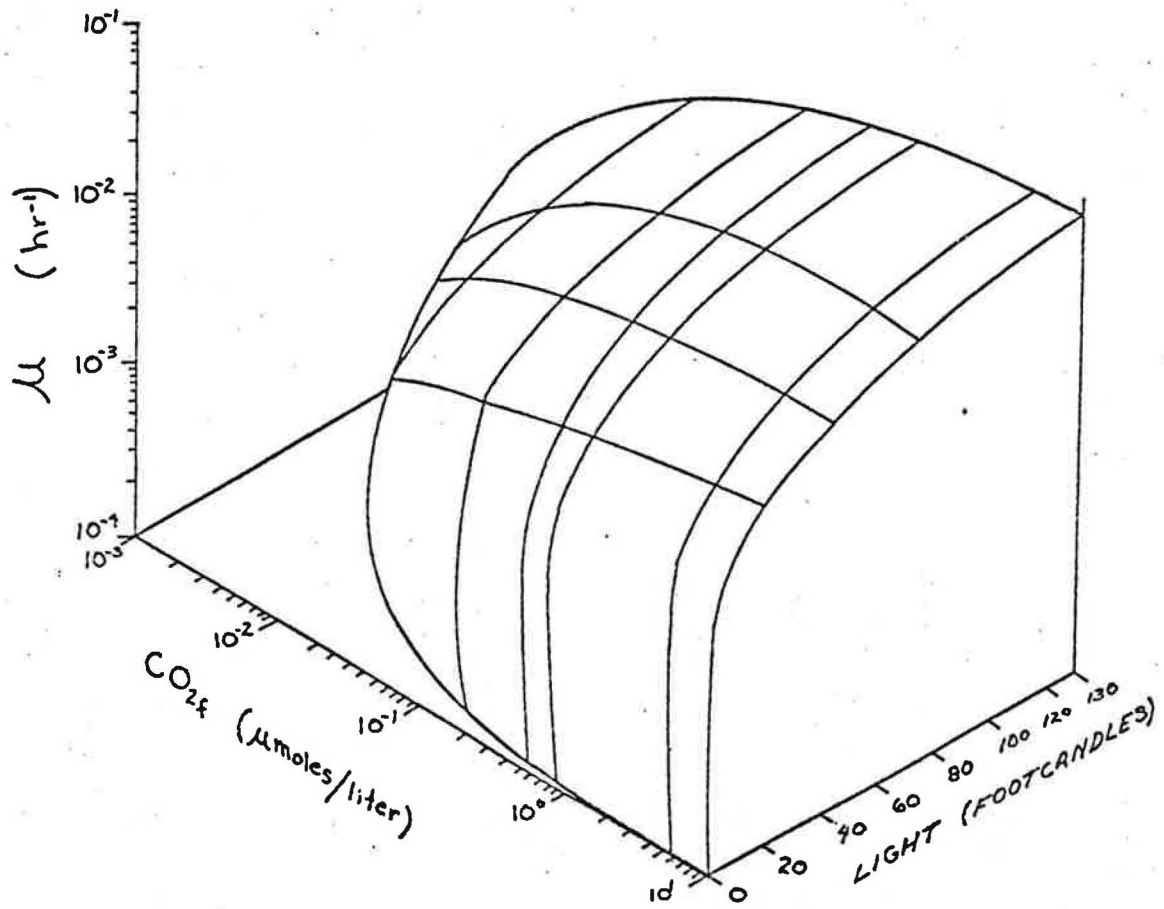

Figure 29

Variation of Specific Growth Rate With CO_{2_f} Concentration and Light Intensity for Phormidium olivacea

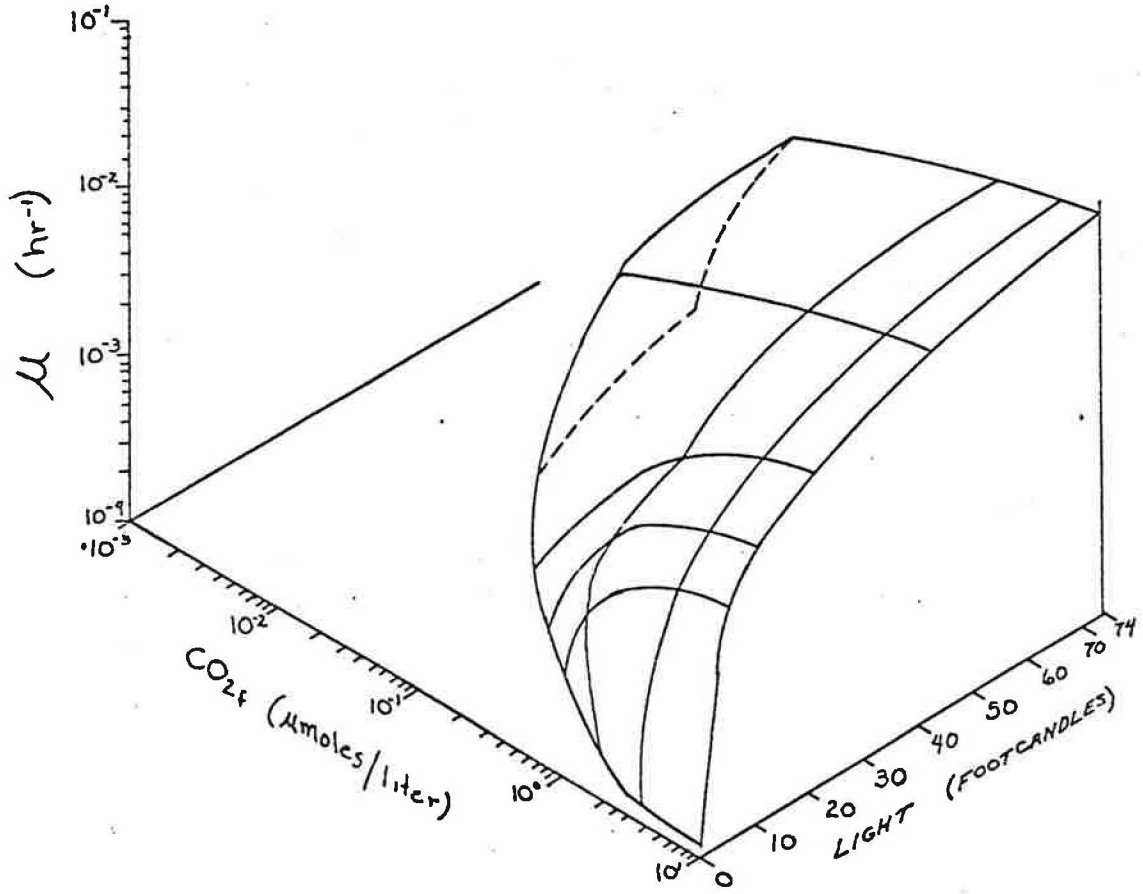

Figure 30

Variation of Specific Growth Rate With CO_{2f} Concentration and Light Intensity for Anabaena variabilis

Since Scenedesmus was limited by pH at each alkalinity at a light intensity of 130 footcandles in the second investigation, the curve in Figure 28 would not differ at high light intensities for Scenedesmus.

If the interacting limits imposed by CO_{2_f} and light intensities are truly multiplicative, as indicated by Figures 27-30, the fractional remainder of μ_c at high light multiplied by the fractional remainder of μ_L at high CO_{2_f} times the maximum μ observed ($\mu_{max_{obs}}$) should yield a calculated μ (μ_{calc}) equal to the experimental μ (μ_{exp}) as follows.

$$\mu_{calc} = \frac{\mu_c}{\mu_{max_{obs}}} \frac{\mu_L}{\mu_{max_{obs}}} \mu_{max_{obs}} \tag{11}$$

$$\mu_{calc} = \mu_{max_{obs}} \frac{\mu_c \mu_L}{(\mu_{max_{obs}})^2} \tag{12}$$

$$\mu_{calc} = \frac{\mu_c \mu_L}{\mu_{max_{obs}}} \tag{13}$$

In the above equations, the specific growth rates as a function of illumination (μ_L) were those growth rates taken from Figure 26 under light intensities of 130, 74, 42, 20, and 10 footcandles. The maximum observable specific growth rate ($\mu_{max_{obs}}$) was at 130 footcandles and high CO_{2_f} and was equal to 0.019 hr^{-1} for Pediastrum at 2 meq/liter alkalinity.

The specific growth rate as a function of CO_{2_f} concentration (μ_c) were taken from Figure 25. Values for μ_{calc}, for Pediastrum, from Equation 13 are compared with μ_{exp} values in Table 2 for light intensities shown in the top row and CO_{2_f} concentrations shown in the first column of Table 2. Similar comparisons are given in Tables 3-5 for the other algae. These calculated specific growth rates (μ_{calc}) were then correlated with the growth rates found experimental (μ_{exp}). The resulting coefficients of correlation for Pediastrum, Scenedesmus, Anabaena, and Phormidium were 0.96, 0.98, 0.99, and 0.99, respectively.

These high correlation coefficients indicate that the specific growth rate can be accurately calculated by the use of Equation 13, even though carbon limits cannot be related directly to the Monod Equation. Although these correlation coefficients are excellent Equation 13 does have limits. To be able to accurately predict the growth rates for a system the $\mu_{max_{obs}}$ must first be known for that system and specific growth rates as a function of CO_{2_f} at high light intensity (μ_c) and specific growth rates as a function of light at high CO_{2_f} concentration (μ_L) must also be known.

As previously noted as the light intensity increased so did the experimental specific growth rate. The specific growth rate would continue to increase, assuming all other nutrients were in excess, until the saturation of light for growth was reached, yielding a maximum specific growth rate (V_{max}). Meyers (13) has stated that

Table 2

Data Illustrating the Experimental Specific Growth Rate and the Calculated Specific Growth Rate With Various CO_{2_f} Concentrations and Various Light Intensities for Pediastrum biradiatum

CO_{2_f} µmoles/liter	Light Intensity (footcandles)									
	130	74		42		20		10	
		exp	calc	exp	calc	exp	calc	exp	calc
10.0	.019	.0165		.0138		.0052		.0044	
1.12	.0152	.0141	.0132	.0101	.0111	.0041	.0041	.0052	.0035
0.70	.01425	.0139	.0124	.0097	.0104	.0038	.0038	.0051	.0033
0.28	.01267	.0127	.0111	.009	.0093	.0035	.0034	.0046	.0029
0.095	.0095	.0110	.0083	.0072	.0069	.0034	.0026	.0036	.0022
0.009	.00475	.0048	.0041	.0026	.0035	-	.0013	-	.0011

Table 3

Data Illustrating the Experimental Specific Growth Rate and the Calculated Specific Growth Rate With Various CO_{2_f} Concentrations and Various Light Intensities for Scenedesmus acutiformis

CO_{2_f} µmoles/liter	Light Intensity (footcandles)									
	130	74		42		20		10	
		exp	calc	exp	calc	exp	calc	exp	calc
2	.0195	.015		.0076		.0045		.0046	
.46	.0156	.013	.012	.0064	.0061	.0037	.0036	.0028	-
.35	.0146	.0124	.0113	.006	.0057	.0036	.0034	.0025	-
.23	.01305	.0113	.0101	.0056	.0051	.0034	.003	.0022	-
.095	.00975	.0088	.0098	.0043	.0038	.0028	.0022	.0018	-
.025	.00488	.0051	.0038	.0032	.0019	.0021	.0011	.0010	-

Table 4

Data Illustrating the Experimental Specific Growth Rate and the Calculated Specific Growth Rate With Various CO_{2_f} Concentrations and Various Light Intensities for Phormidium olivacea

CO_{2_f} µmoles/liter	Light Intensity (footcandles)											
	130	74		42		20		10		5	
		exp	calc	exp	calc	exp	calc	exp	calc	exp	calc
10	.052	.035		.020		.011		.0098		.005	
5	.046	.034	.028	.020	.0158	.0105	.0087	.0096	.0079	.0043	.0042
0.8	.039	.031	.026	.018	.0148	.0068	.0082	.0088	.0074	.0041	.0039
0.48	.0348	.028	.023	.016	.0132	.0058	.0073	.0080	.0066	.0035	.0035
0.18	.026	.021	.017	.012	.0099	.0048	.0055	.0064	.0049	-	-
0.029	.013	.0093	.0087	.007	.0049	.0032	.0028	.0021	.0025	-	-

Table 5

Data Illustrating the Experimental Specific Growth Rate and the Calculated Specific Growth Rate With Various CO_{2_f} Concentrations and Various Light Intensities for Anabaena variabilis

CO_{2_f} µmoles/liter	Light Intensity (footcandles)									
	74	42		20		10		5	
		exp	calc	exp	calc	exp	calc	exp	calc
10	.025	.014		.0076		.0045		.0025	
5.2	.020	.0127	.0117	.0064	.0060	.0035	.0036	.0020	.0020
3.5	.0187	.0119	.0105	.0055	.0056	.0032	.0034	.0016	.019
2.8	.0167	.0113	.0094	.0050	.0050	.0028	.0030	.0014	.017
1.2	.0125	.0098	.0070	.0035	.0035	.0022	.0023	-	-
.058	.00625	.0039	-	-	-	-	-	-	-

due to physiological characteristics unique to each algal type there is a different V_{max} for each algae.

In this study the highest light intensity used was 130 foot-candles which was less than the saturation value for growth. This yielded the $\mu_{max_{obs}}$ of 0.019 hr^{-1} used in Equation 13 which is less than the calculated V_{max} of 0.031 hr^{-1}. If the light intensity or any variable was increased until the maximum specific growth rate (V_{max}) was reached then the $\mu_{max_{obs}}$ would be equal to V_{max} for that algae. This new value could then be substituted into Equation 13 to give the following equation:

$$\mu = \frac{\mu_c \mu_L}{V_{max}} \qquad (14)$$

If μ_c was assumed to follow the Monod Equation, then the Monod Equation for μ_c and μ_L could be substituted into Equation 14. This yields the following equation.

$$\mu_m = V_{max} \frac{c}{K_c + c} \frac{L}{K_L + L} \qquad (15)$$

where:

μ_m = Monod specific growth rate; hr^{-1}

V_{max} = maximum specific growth rate at saturation growth; hr^{-1}

c = CO_{2_f} concentration; mg CO_{2_f}/liter

K_c = CO_{2_f} concentration at 1/2 V_{max}; mg CO_{2_f}/liter

L = light concentration; footcandles

K_L = light concentration at 1/2 V_{max}; footcandles

Values of μ_m calculated from Equation 15 are compared with μ_{exp} values in Table 6. The V_{max}, K_L, and K_c values were those obtained from the Lineweaver-Burk plots for CO_{2_f} concentration and light intensity. The CO_{2_f} concentration and light intensity values used in Table 6 were taken from Table 2.

Comparison of predictive accuracy of Equation 15 can be seen in Table 6 as the rate of μ_m/μ_{exp}. Ratios of μ_m/μ_{exp} of one would indicate perfect agreement.

The V_{max} values for Figure 25 and Figure 26 were 0.02 hr^{-1} and 0.031 hr^{-1} respectively. In all above calculations involving V_{max}, the 0.031 hr^{-1} as a function of light intensities were used, because the light data fit the Monod Equation better than did the carbon data. This can be seen in Figure 31, the V_{max} for illumination gave a μ_m/μ_{exp} ratio closer to a value of one than did the V_{max} for CO_{2_f} concentration at high light and high CO_{2_f}.

Figure 32 shows the correlation between μ_m and μ_{exp} when plotted in the same figure. The line in Figure 32 represents the value of one, which would mean perfect agreement between the specific growth rates calculated from Equation 13 and Equation 15.

Most of the points in Figure 32 are either above or below the line. The correlation between μ_m and μ_{exp} is not perfect, with much of the error being related to the fact that μ_c did not fit the Monod Equation particularly well.

Table 6

Data Illustrating the Monod Specific Growth Rate, Experimental Specific Growth Rate and the Ratio μ_m/μ_{exp} for Pediastrum biradiatum

$$\mu_m = V_{max} \left(\frac{L}{K_L + L}\right) \left(\frac{C}{K_C + C}\right)$$

Where

$$V_{max} = 0.031,\ K_L = 62.5,\ K_C = 0.083$$

L (footcandles)	C (mg CO_{2_f}/liter)	μ_m hr^{-1}	μ_{exp} hr^{-1}	$\frac{\mu_m}{\mu_{exp}}$ hr^{-1}
130	10.0	.0208	.019	1.092
130	1.12	.0195	.0152	1.28
130	0.70	.0187	.01425	1.31
130	0.28	.0161	.01267	1.127
130	0.095	.0112	.0095	1.176
130	0.009	.0020	.00475	0.431
74	10.0	.0166	.0165	1.009
74	1.12	.0156	.0141	1.109
74	0.70	.0150	.0139	1.08
74	0.28	.0129	.0127	1.02
74	0.095	.0089	.0110	0.815
74	0.009	.0015	.0048	0.315
42	10.0	.0123	.0138	.895
42	1.12	.0116	.0101	1.148
42	0.70	.0114	.0097	1.148
42	0.28	.0096	.009	1.06
42	0.095	.0066	.0072	0.923
42	0.009	.00112	.0026	0.431
20	10.0	.0074	.0052	1.43
20	1.12	.0069	.0041	1.70
20	0.70	.0067	.0038	1.762
20	0.28	.0057	.0035	1.625
20	0.095	.0040	.0026	1.539
20	0.009	.00067	.0013	0.519
10	10.0	.0042	.0044	0.957
10	1.12	.00395	.0035	1.1297
10	0.70	.00379	.0033	1.151
10	0.28	.0033	.0029	1.129
10	0.095	.0023	.0022	1.045
10	0.009	.00038	.0011	0.3474

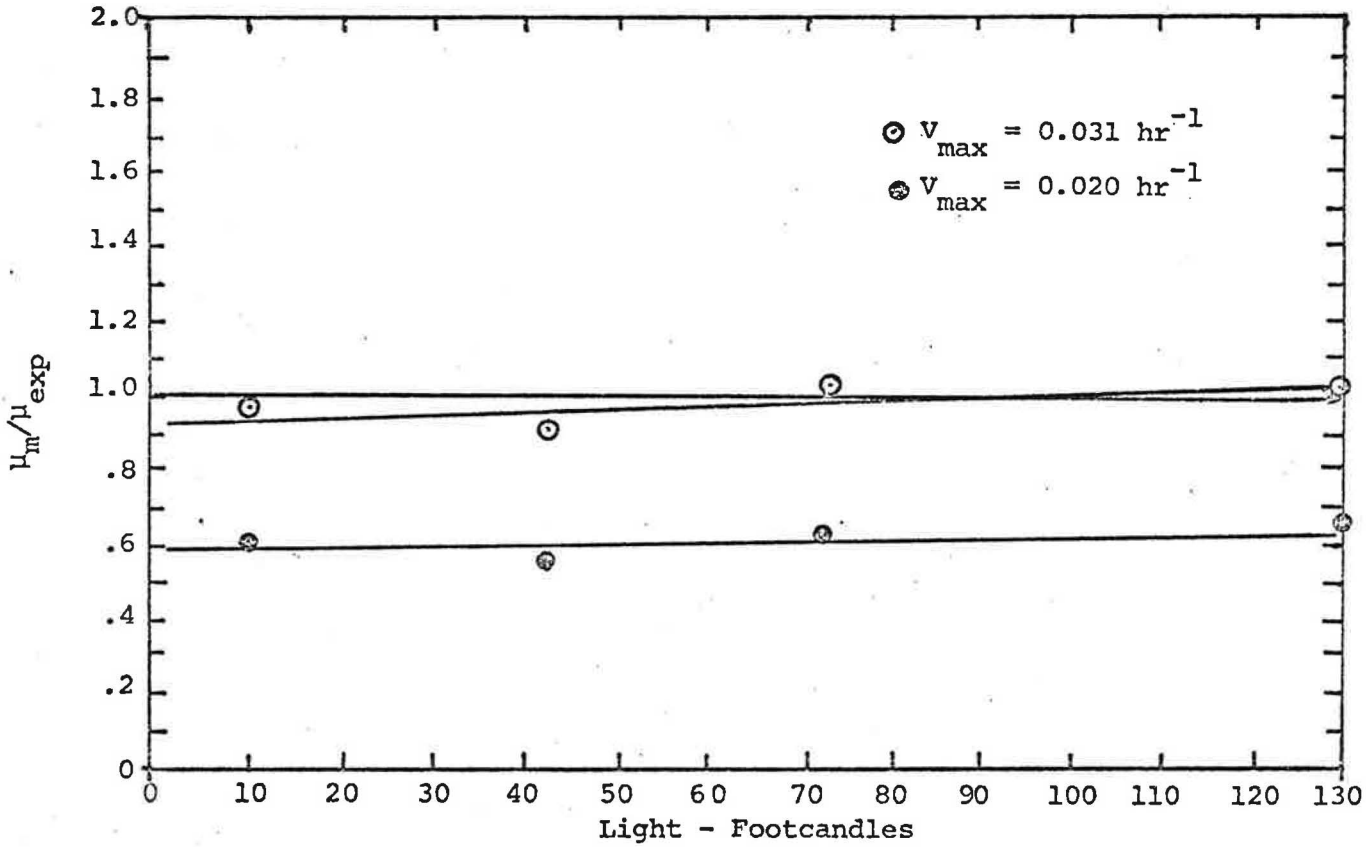

Figure 31

Variation in the Ratio of μ_m/μ_{exp} With Light Intensity for Pediastrum biradiatum Under Various V_{max} Values at a Constant CO_{2_f} Concentration

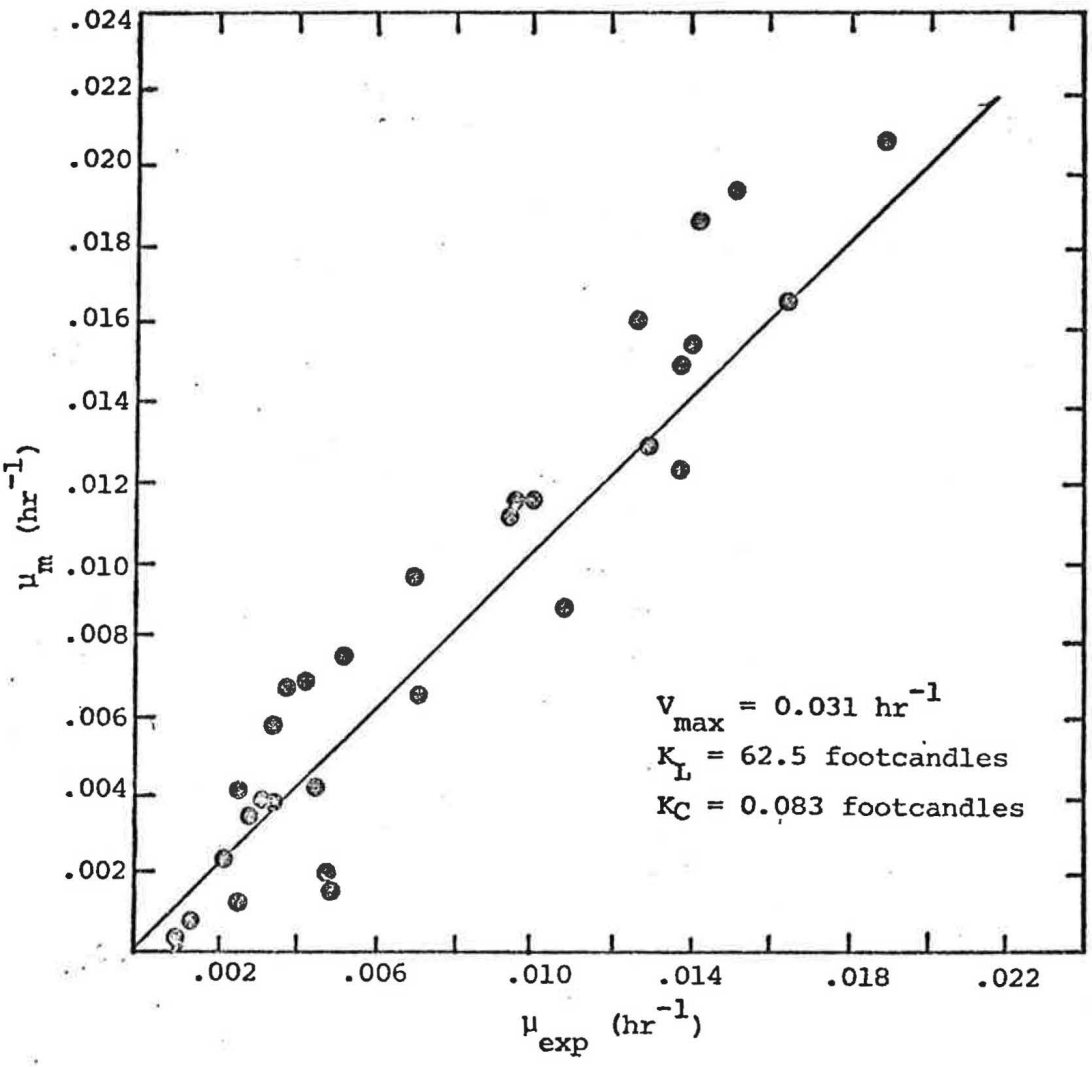

Figure 32

Variation in the Unity Line With μ_m and μ_{exp} for Pediastrum biradiatum Under Various Light Intensities and Various CO_{2_f} Concentration

Only nonconservative parameters can be multiplied in Equation 15. Carbon and light are only utilized as they become available to the algae in the system. This is because algae do not store either carbon or light in the algal cell. Phosphorus and nitrogen could not be used in Equation 15. Sievers' (2) data for C:P and Garcia's (14) data for C:N show that algae are able to store nitrogen and phosphorus in the cell. Because of differences in uptake and growth kinetics for these two conserved nutrients they cannot be added to Equation 15 as simple Monod multiples.

In this study the algae were maintained under a constant light intensity and thus were maintained in the photic zone continuously. However, in a natural ecosystem, except for perhaps the littoral zone, an alga's ability to remain functional and to continue to fix carbon will depend upon its ability to maintain a planktonic nature in the photic zone. As Haase (12) has shown for Chlorella, the ability of algae to extract and fix carbon from an inorganic growth medium is directly governed by the alga's ability to maintain its planktonic nature in the photic zone. The specific growth rates determined in this study represents the maximum ability of growth, but in a natural ecosystem the continuous rain of algae out of the photic zone would effectively decrease the standing crop of biomass which would lead to a decreased population growth rate. This settling property will play a most important role in determining the rate and degree of production of algae in natural aquatic ecosystems.

CONCLUSIONS

1. The free carbon dioxide concentration at which Pediastrum biradiatum, Phormidium olivacea, Anabaena variabilis, and Scenedesmus acutiformis become carbon limited varies markedly with both intensity of light available and the type of algae present.

2. Pediastrum biradiatum was able to fix carbon from the carbonate-bicarbonate alkalinity to a lower free CO_2 value with increasing alkalinity at a light intensity of 130 footcandles but not at a light intensity of 42 footcandles.

3. Scenedesmus acutiformis can fix carbon from the carbonate-bicarbonate alkalinity only to a maximum pH of 10.80.

4. The relations of the specific growth rate of algae to light intensity follows the Monod Equation reasonably well.

5. The relations of the specific growth rate of algae to free CO_2 concentration follows the Monod Equation only at high concentrations of free CO_2.

6. The interactions imposed on the specific growth rate of algae (μ) by simultaneous limitation of carbon availability and light intensity is multiplicative and can be approximated by the equation

$$\mu = V_{max} \frac{c}{K_c + c} \frac{L}{K_L + L}$$

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APPENDICES

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APPENDIX A

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 ml/liter

Composition of Microelement Solution

Nutrient	Concentration
H_3CO_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
$Ca(NO_3)_2 \cdot 6H_2O$	0.49 g/liter

The three dimensional curves in Figures 27-30 show the dramatic differences in the specific growth rate when the light intensity and the CO_{2_f} concentration are both limiting simultaneously. These figures are based on the data presented in the previous section and show the limits imposed on specific growth rates by interaction of CO_{2_f} availability and light intensity on the specific growth rate of Pediastrum, Scenedesmus, Anabaena, and Phormidium.

These figures graphically illustrate a multiplicative relationship between carbon availability, specific growth rate and light intensity. A decrease in light intensity with a decrease in CO_{2_f} concentration appears to yield a multiplicative decrease in specific growth rate. Haase (12) has shown there is also a multiplicative relationship between carbon fixation, carbon extraction and phosphorus availability for Chlorella.

The data in the second investigation for Pediastrum would seem to indicate that varying the alkalinity would also have some effects on Figure 27. Varying the alkalinity from 2 meq/liter would move the three dimensional curve up or down depending on the initial carbonate-bicarbonate alkalinity concentration and the light intensity. The data would suggest that with an alkalinity of 3 meq/liter the curve would be slightly above at a light intensity of 130 footcandles and hence at an alkalinity of 1 meq/liter the curve would fall slightly below at a light intensity of 130 footcandles. The curve in Figure 27 would not vary at a light intensity of 42 footcandles.

A plot of the final CO_{2_q} for Scenedesmus, Phormidium, Anabaena, and Pediastrum is shown as a function of light intensity in Figure 23 for the range of light intensities considered. The CO_{2_q} concentrations shown in Figure 23 were obtained from the previous figures relating CO_{2_f} concentration with time. The Anacystis data presented in Figure 23 were taken from a similar study by Young (3) and the Nostoc and Chlorella data were taken from Klemovich (1).

An alga is considered to be more efficient if it is able to continue to extract carbon from the carbonate-bicarbonate alkalinity to a lower CO_{2_f} concentration when compared to another algae. Upon inspection of Figure 23, Anacystis is able to continue photosynthetic activity to lower CO_{2_f} concentrations than all other algae studied, regardless of light intensity.

Over the entire range of light intensities used in these studies Nostoc, shown in Figure 23, is not able to actively compete for the carbon in the carbonate-bicarbonate alkalinity and would be dominated by any of these other species if they were competing for carbon in the same microcosm and if carbon was in short supply.

Figure 23 shows how the relative competitiveness for carbon will play a role in algal succession. As can be seen, the curves cross-over at different CO_{2_q} concentrations if light intensity is varied. Beyond this intersection the alga that has the ability to reach a lower CO_{2_q} concentration has a competitive advantage for carbon from the carbonate-bicarbonate alkalinity and domination by that algal species would be expected.