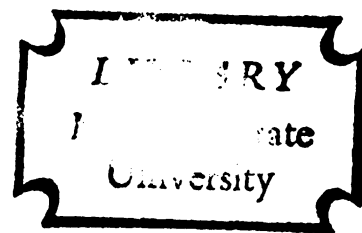




117  
418  
THS



This is to certify that the  
thesis entitled

Carbon Dioxide and Growth Limitation  
of a Submersed Aquatic Plant

presented by

John Richard Craig

has been accepted towards fulfillment  
of the requirements for

Master of Science degree in Fisheries & Wildlife

Major professor

Date January 6, 1978



6-113333

CARBON DIOXIDE AND GROWTH  
LIMITATION OF A SUBMERSED AQUATIC PLANT

By

John Richard Craig

A THESIS

Submitted to  
Michigan State University  
in partial fulfillment of the requirements  
for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

1978



# ABSTRACT

## CARBON DIOXIDE AND GROWTH LIMITATION OF A SUBMERSED AQUATIC PLANT

By

John Richard Craig

The net specific growth rate of *Ceratophyllum demersum* L. within a hypereutrophic wastewater pond in Michigan was controlled by the aqueous free CO<sub>2</sub> concentration in the following manner.

$$\mu = \mu_{\max} \frac{C - C_q}{(K_c - C_q) + (C - C_q)}$$

where  $\mu$  = net specific growth rate (day<sup>-1</sup>), determined from ash-free dry weights,  $\mu_{\max}$  = maximum specific growth rate (0.067 day<sup>-1</sup>),  $C$  = existing free CO<sub>2</sub> concentration (μmol/l),  $K_c$  = Michaelis constant (1.5 μmol/l), and  $C_q$  = threshold free CO<sub>2</sub> concentration required for net carbon fixation (1.3 μmol/l). Results suggest that of the inorganic carbon species, *C. demersum* responds in a kinetic fashion only to the free CO<sub>2</sub> concentration.

To my parents

## ACKNOWLEDGMENTS

I sincerely thank Dr. Clarence D. McNabb, my major professor, for his guidance, encouragement and ceaseless patience. I am grateful for his continuing interest in my personal and professional development.

I thank Dr. Niles R. Kevern and Dr. Bernard D. Knezek for serving on my graduate guidance committee and for their thoughtful review of my thesis.

I am deeply indebted to Dr. Darrell L. King for his invaluable advice and support during the writing of this thesis.

My appreciation is extended to those who assisted in the field and laboratory on the cooperative project of which this study is a part, especially Douglas Bulthuis, Jane Kotenko and Jerry Lisiecki.

Finally, I wish to express a special thank you to my fiancée, Helga, for her unending inspiration.

The research for this thesis was partially supported by funds provided by the U.S. Department of the Interior, Office of Water Resources Research under Project No. A-073-MICH of the Institute of Water Research at Michigan State University, the U.S. Environmental Protection Agency, Office of Water Programs under Training Grants WP-264 and T-900331, and the Michigan Agriculture Experiment Station under Project No. 1157.

## TABLE OF CONTENTS

|                            | Page |
|----------------------------|------|
| LIST OF TABLES . . . . .   | v    |
| LIST OF FIGURES . . . . .  | vi   |
| INTRODUCTION . . . . .     | 1    |
| METHODS . . . . .          | 4    |
| RESULTS . . . . .          | 11   |
| DISCUSSION . . . . .       | 25   |
| CONCLUSIONS . . . . .      | 42   |
| LITERATURE CITED . . . . . | 43   |
| APPENDIX . . . . .         | 47   |

# LIST OF TABLES

| Table                                                                                                                                                                                                                                                                                                                                      | Page |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| 1. The penetration of light in the hydrophyte-free portions of Pond 4 of the Belding, Michigan wastewater treatment system during the growing season, 1973 . . .                                                                                                                                                                           | 15   |
| 2. Means and ranges of dissolved oxygen concentrations in the water column of Pond 4 of the Belding, Michigan wastewater treatment system during the 1973 growing season . . . . .                                                                                                                                                         | 16   |
| 3. Changes in the pond standing crop biomass and the net specific growth rate, $\mu$ , over the 1973 growing season for <i>Ceratophyllum demersum</i> , <i>Potamogeton</i> spp., and <i>Lemna minor</i> in Pond 4 of the Belding, Michigan wastewater treatment system. The biomass is expressed on an ash-free dry weight basis . . . . . | 23   |
| 4. Minimum free $\text{CO}_2$ and $\text{HCO}_3^-$ concentrations reached during the daylight period in Pond 4 of the Belding, Michigan wastewater treatment system during the 1973 growing season . . . . .                                                                                                                               | 24   |
| A-1. Biomass of vascular hydrophytes over the 1973 growing season in Pond 4 of the Belding, Michigan wastewater treatment system . . . . .                                                                                                                                                                                                 | 47   |
| A-2. Percentage ash of dry weight in the vascular hydrophytes in Pond 4 of the Belding, Michigan wastewater treatment system during the growing season, 1973 . . . . .                                                                                                                                                                     | 48   |

## LIST OF FIGURES

| Figure                                                                                                                                                                                                                                                                                                                                                                                                         | Page |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| 1. The system of ponds receiving untreated wastewater from the city of Belding, Michigan . . . . .                                                                                                                                                                                                                                                                                                             | 5    |
| 2. Mean water temperatures and vertical extinction coefficients in Pond 4 of the Belding, Michigan wastewater treatment system during the 1973 growing season. The vertical lines show the range of temperatures . . . . .                                                                                                                                                                                     | 12   |
| 3. The pH values recorded during the daylight period (0800, 1200, 1600 and 2000 hrs.) in the water column (surface, 0.5, 1.0 and 1.5 m depth) at two stations in Pond 4 of the Belding, Michigan wastewater treatment system during the 1973 growing season. The solid line shows the median values. The range of values reflects the variability due to time of day, depth and location in the pond . . . . . | 18   |
| 4. The pond standing crop biomass of the dominant hydrophytes in Pond 4 of the Belding, Michigan wastewater treatment system over the 1973 growing season . . . . .                                                                                                                                                                                                                                            | 19   |
| 5. Plots of $\mu$ versus $\mu / [S]$ for $[S]$ expressed as the $CO_{2f}$ - $CO_{2q}$ concentration (upper figure) and as the corresponding $HCO_3^-$ - $HCO_{3q}^-$ concentration (lower figure) where $\mu$ is equal to the net specific growth rates for <i>C. demersum</i> in Pond 4 during the 1973 growing season . . . . .                                                                              | 31   |
| 6. Net specific growth rate ( $\mu$ ) of <i>C. demersum</i> as a function of the average minimum free $CO_2$ concentration in the water column reached during the daylight period. Dates refer to the midpoints of the sampling intervals . . . . .                                                                                                                                                            | 33   |
| 7. Net specific growth rate ( $\mu$ ) of <i>C. demersum</i> as a function of the average minimum $HCO_3^-$ concentration in the water column reached during the daylight period. Dates refer to the midpoints of the sampling intervals . . . . .                                                                                                                                                              | 34   |
| 8. The conditions of pH and carbonate-bicarbonate alkalinity at 24°C yielding various net growth rate limiting free $CO_2$ concentrations for <i>Ceratophyllum demersum</i> . . . . .                                                                                                                                                                                                                          | 35   |

9. The conditions of pH and carbonate-bicarbonate alkalinity at 24°C yielding various net growth rate limiting  $\text{HCO}_3^-$  concentrations for *Ceratophyllum demersum* . . . . . 36
10. The conditions of pH and carbonate-bicarbonate alkalinity at 24°C necessary to yield the  $\text{CO}_{2f}$  quit concentration and the corresponding equilibrium  $\text{HCO}_3^-$  quit concentration for *Ceratophyllum demersum* assuming net cellular uptake of each chemical species . . . . . 39

## INTRODUCTION

Submersed vascular hydrophytes are a conspicuous component of the biota of aerobic ponds in municipal wastewater lagoons systems in Michigan. In these ponds, relatively stable zooplankton populations develop in the absence of predation by planktivorous fish. Through their intense algal grazing activity, these zooplankton provide the necessary water clarity for development of high-density rooted hydrophyte populations (McNabb, 1976). Ramani (1976) suggests that carbon often becomes a limiting factor in the downstream polishing ponds of multi-staged lagoon systems treating domestic waste. King (1970, 1972) reports carbon limitation of algal species in algal-dominated aerobic and facultative lagoons of these systems. A characteristic pattern for many lagoons is for pH to increase and the corresponding equilibrium free  $\text{CO}_2$  concentration to decrease gradually with time from spring through summer (King, 1972, 1976). This indicates that atmospheric recarbonation and respiration of accrued organics can not keep up with the increasing daily carbon demand of the developing populations of primary producers. Botkin (1977) suggests that under conditions of non-limiting nitrogen, phosphorus or any other ion, the rate at which the maximum standing crop was reached would be controlled by the rate of infusion of  $\text{CO}_2$  from the atmosphere. In the time period of a growing season, carbon limits the rate of biomass accrual while the extent of biomass accrual is roughly a function of the amount of phosphorus, or possibly nitrogen.

In addition to controlling the rate at which an aquatic plant can reach its maximum standing crop, carbon availability has been suggested as a controlling factor in algal species dominance (King, 1970, 1972; Schindler and Fee, 1973). Klemovich (1973) showed that three different algal species differed significantly in their rate of free  $\text{CO}_2$  uptake and the minimum ambient concentration under which net uptake could proceed. The rate of uptake of essential ions appears to be a primary difference between plant species. On the basis of these differences, certain species might be predicted to out-compete others in nutrient-deficient environments (Gerloff, 1975). McNabb (1976) from observations of 30 oxidation pond systems in Michigan concluded that the pioneer species of hydrophytes in these ponds, such as *Potamogeton foliosus* and *P. berchtoldii*, are displaced over time by *Ceratophyllum demersum* and *Elodea canadensis*. The success of *C. demersum* and *E. canadensis* in displacing the early invading species may be due to their ability to continue net uptake and photosynthesis to very low free carbon dioxide concentrations.

The Monod (1949) formulation of the familiar Michaelis-Menten enzyme kinetics model has been applied to phytoplankton growth kinetics and the uptake of phosphorus (Fuhs, 1972), nitrate and ammonium (Eppley and Thomas, 1969; Eppley et. al., 1969) and free  $\text{CO}_2$  (Allen, 1972; King, 1976; Young and King, 1973). Application of this empirical function was based on the observation that the specific growth rate of an organism and the concentration of the assumed growth limiting nutrient could be related by a hyperbolic function described by the Monod equation. The hypothesis to be tested by this study was that the net specific growth rate of *Ceratophyllum demersum* is related to the free  $\text{CO}_2$  concentration

over the growing season and that this relationship can be described by the Monod formulation.

## METHODS

The city of Belding, Michigan, located in the southwest quadrant of the lower peninsula of the state, has a wastewater treatment system consisting of a series of five oxidation ponds. The series has a total area of approximately 23 hectares, has a maximum depth of 3 meters, and serves a primarily residential community of approximately 5000 persons. Untreated domestic waste enters the series at pond 1 and flows by gravity sequentially through the remaining four cells (Figure 1). The fifth cell serves as a seepage unit, discharging approximately 85% of its input through the sand and gravel materials on which it is perched (Bulthuis et al., 1974). The remaining effluent of pond 5 is used for spray irrigation on adjacent land, is released to the Flat River in the early spring and late fall, and is lost by evaporation.

There are three types of cells in the series: anaerobic, facultative, and aerobic. The anaerobic nature of pond 1 is exhibited by a lack of oxygen, apparent black color of the water, and the continuous production of gas, presumably methane, breaking the surface of the pond. Pond 2, a facultative cell, is characterized by a dense phytoplankton bloom throughout the summer, a high surface water dissolved oxygen content with a decreasing concentration with depth, and, finally, black gas-producing sediments. Ponds 4 and 5 are aerobic cells dominated by submersed hydrophytes, and have a high dissolved oxygen concentration throughout the water column during the growing season. The sediments of these ponds have a light reddish-brown coloration characteristic of

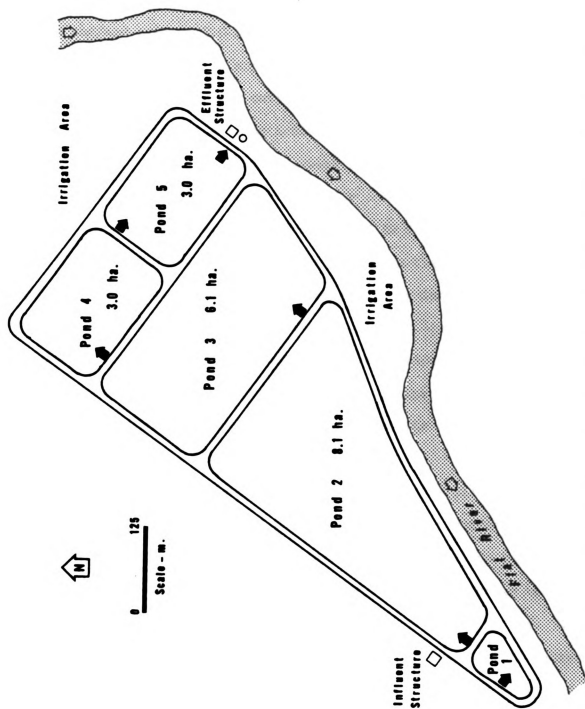


Figure 1. The system of ponds receiving untreated wastewater from the city of Belding, Michigan.

the aerobic oxidizing nature of the sediment-water interface. Pond 3 fluctuates between the facultative and aerobic state. The system began operations in 1966.

The fourth pond in this series was the site of this work. It has a surface area of 3.0 hectares and averaged 1.8 m in depth during the study. An average of approximately 12.2 million liters per week entered the pond, while the outflow averaged 11.7 million liters per week with the difference being accounted for by seepage and evaporation (Mahan, 1974). After an initial 4-week spring run-off period, the flow rate through the pond remained relatively constant. During the study period, pond 4 had a mean retention time of 32 days.

The pond was sampled regularly through the seasonal cycle of growth (14-day intervals) and senescence (21-day intervals) of the vascular hydrophytes (May 23 - September 27, 1973). Approximately 20 quadrats of the major hydrophyte species were randomly collected on each sampling date to estimate their standing crop biomass. At the time of biomass sampling, areal coverage of the hydrophyte was estimated for use in subsequent calculations.

The pleustonic *Lemna minor* was collected by placing a floating square 25 cm on a side (area =  $625 \text{ cm}^2$ ) on the water surface. All of the plants within the square were placed in a polyethylene bag for transport to the laboratory. The submersed *Ceratophyllum demersum* was collected in two ways, depending on its aspect in the water column. When anchored to the bottom, *C. demersum* was quantitatively sampled by gently dropping a weighted plastic cylinder with an area of  $0.114 \text{ m}^2$  to the pond bottom and collecting the hydrophytes within the cylinder. The rooted potamogetons were also sampled in this manner. The floating mat of *C. demersum* was sampled by raising an unweighted cylinder with an

area of  $0.094 \text{ m}^2$  from beneath the mat and collecting the plant material within. The plant material from each quadrat was placed in individual polyethylene bags. Upon return to the laboratory, the plants were washed and separated into species. The plant samples were dried to a constant weight in a forced draft oven at  $80^\circ\text{C}$  and dry weights determined. A subsample was ground to a powder in a Wiley mill and incinerated in a muffle furnace at  $500^\circ\text{C}$  for one hour. The percent of ash content was determined and ash-free dry weights calculated.

The replicated hydrophyte biomass data expressed as ash-free dry weight per sampler were transformed to base 10 logarithms to achieve homogeneity of variances. The transformed values for each species were curve-fitted with a polynomial regression and the simplest significant curve to adequately describe the biomass relationships over the growing season was chosen to express the data. The regression technique served to remove the non-significant sampling variability and to produce a logical mathematical continuum between sampling dates. This regression technique utilizes information from all the available samples in determining the value at any point in time. Estimated values for each of the sampling dates were taken from the curves and converted from the logarithmic scale to the original scale by the formula: arithmetic mean =  $\text{antilog}(\log \hat{Y} + \text{EMS}/2)$  where EMS is the error mean square value from the analysis of variance associated with the fitted curve. The biomass estimates were expressed as ash-free dry weight per square meter ( $\text{gm}/\text{m}^2$ ) and per pond (kg). Further details of the curve-fitting procedure are given in Lisiecki and McNabb (1976).

The pond hydrophyte standing crop biomass estimates were used to calculate the net specific growth rates after the method of Young and King (1973).

$$\mu = \frac{dM/dt}{M} \quad (1)$$

where  $\mu$  = net specific growth rate,  $\text{time}^{-1}$ ;

$M$  = biomass;

$t$  = time.

The net specific growth rate as used here is defined as the change of hydrophyte pond biomass per unit average hydrophyte biomass over the time interval between sampling dates.

The net specific growth rates were calculated for each hydrophyte over the growing season from the following relationship:

$$\mu_{\Delta t} = \frac{\Delta M / \Delta t}{m} = \frac{(M_t - M_{t_0}) / (t - t_0)}{(M_t + M_{t_0}) / 2} \quad (2)$$

where  $\mu_{\Delta t}$  = net specific growth rate over the sampling interval,  $\text{day}^{-1}$ ;

$M_{t_0}$ ,  $M_t$  = pond standing crop biomass at the beginning and the end of the sampling interval, kg;

$\Delta M$  = change in pond standing crop biomass, kg;

$t_0$ ,  $t$  = boundary parameters of the interval between sampling, day;

$\Delta t$  = sampling interval, day;

$m$  = average pond standing crop biomass during the sampling interval, kg.

The average standing crop biomass over the sampling period rather than the biomass at the beginning of the period was used to more closely approximate the integral growth rate over that period (Young and King, 1973). This will yield a slightly lower net specific growth rate but it is considered to be a better estimate of the true rate over the changing conditions in the pond during a given interval.

Percent transmittance of incident light was determined at weekly intervals throughout the growing season with a submarine

photometer connected to a low resistance galvanometer (Fred Schueler, Waltham, Massachusetts). Measurements, taken near mid-day in two hydrophyte-free portions of the pond, were made at 0.25 m intervals throughout the depth of the pond and expressed as a fraction of the surface incident light measured with the same photocell. The vertical extinction coefficients,  $\eta$ , were calculated from the percent transmittance measurements using the formula,

$$\eta z = \ln I_0 - \ln I_z \quad (3)$$

where  $z$  is depth in meters,  $I_0$  is the light intensity at the water surface, and  $I_z$  is the light intensity at depth  $z$  (Wetzel, 1975).

Vertical profiles at 0.5 meter intervals (surface, 0.5, 1.0, 1.5 m and bottom) of dissolved oxygen, temperature, and pH were taken in situ at 4-hour intervals (1200, 1600, 2000, 2400, 0400, 0800, and 1200 hrs.) over 24-hour cycles concurrently with biomass sampling. Dissolved oxygen and temperature were monitored with a YSI (Yellow Springs Instrument Company, Yellow Springs, Ohio) model 51 oxygen meter with a pressure-compensated Clark-type polarographic oxygen sensor. Integral thermistors permitted temperature readout and corrected for temperature-dependent membrane diffusion effects and for differential oxygen solubility with temperature. The dissolved oxygen probe was standardized against the azide modification of the Winkler method (American Public Health Association, 1971). The thermistor was checked for accuracy against two mercury thermometers. The pH was measured with a Sargent-Welch model PBX field pH meter using a glass electrode with a 3 m lead lowered through the water column and a calomel reference electrode floated at the surface of the pond. The pH meter was calibrated against pH 7 and pH 10 standard buffer solutions. These instruments were calibrated before each series of measurements.

The free carbon dioxide ( $\text{CO}_{2f}$ ) concentrations in the pond water were calculated from the pH and temperature data and the carbonate-bicarbonate alkalinity estimated to be 200 mg/liter as  $\text{CaCO}_3$  (4 milliequivalents/liter). Typical summer alkalinity values for Pond 4 are in the range of 175-225 mg/l (D. A. Bulthuis, pers. comm.). The equations of Harvey (1957) and Park (1969) were used to calculate the concentrations of free  $\text{CO}_2$  and  $\text{HCO}_3^-$  present in water at equilibrium.

$$\text{CO}_{2f} = a \left[ \frac{H^2}{K_1 (H + 2K_2)} \right] \quad (4)$$

and

$$\text{HCO}_3^- = a \left[ \frac{H}{H + 2K_2} \right] \quad (5)$$

where  $\text{CO}_{2f}$  = concentration of free carbon dioxide,  $\text{H}_2\text{CO}_3$  + free  $\text{CO}_{2(g)}$ , moles/liter;

$\text{HCO}_3^-$  = concentration of the bicarbonate ion, moles/liter;

$a$  = carbonate-bicarbonate alkalinity, equivalents/liter;

$H$  = hydrogen ion activity as determined by a pH meter, moles/liter; and

$K_1, K_2$  = temperature-dependent first and second dissociation constants of carbonic acid from Buch (1951) as listed by Harvey (1957).

## RESULTS

Sampling for this study began in late May, 1973, with the visual confirmation of germination of the vascular hydrophytes from overwintering tissues.

The conditions of water temperature and light penetration within hydrophyte-free portions of the pond are recorded in Figure 2. Pond water temperatures warmed from early May values of 10-12°C at mid-day to a mean value of 15.9°C and range of 15.0 - 17.5°C at the start of sampling on May 23. The mean water column temperature for the entire sampling period, May 23 - September 27, was 27.7°C. The recorded minimum was 15°C on May 23 and the maximum was 28°C on August 15. Due to wind mixing, the pond was generally homothermal with respect to depth throughout the day. The diurnal variation in temperature averaged 3.5°C for the entire season. It ranged from a minimum of 2°C on June 6-7 and progressed to a maximum of 5.6°C at the close of the study on September 26-27. From the onset of exponential growth of the submersed hydrophytes to their seasonal maximum biomass, the pond water had a daily mean temperature of 23.8°C and a range of 21.0 - 28.0°C. The daily difference between maximum and minimum values averaged 3.3°C during this period.

The light penetration in Pond 4 improved during May 17 - June 6, permitting at least 1% (1-6%) of the surface irradiance to reach the bottom. The plants began to grow from the bottom during this interval. Early in the year, the light transmission of Pond 4 expressed as vertical extinction coefficients,  $\eta$ , reflect the rapid attenuation of light in the

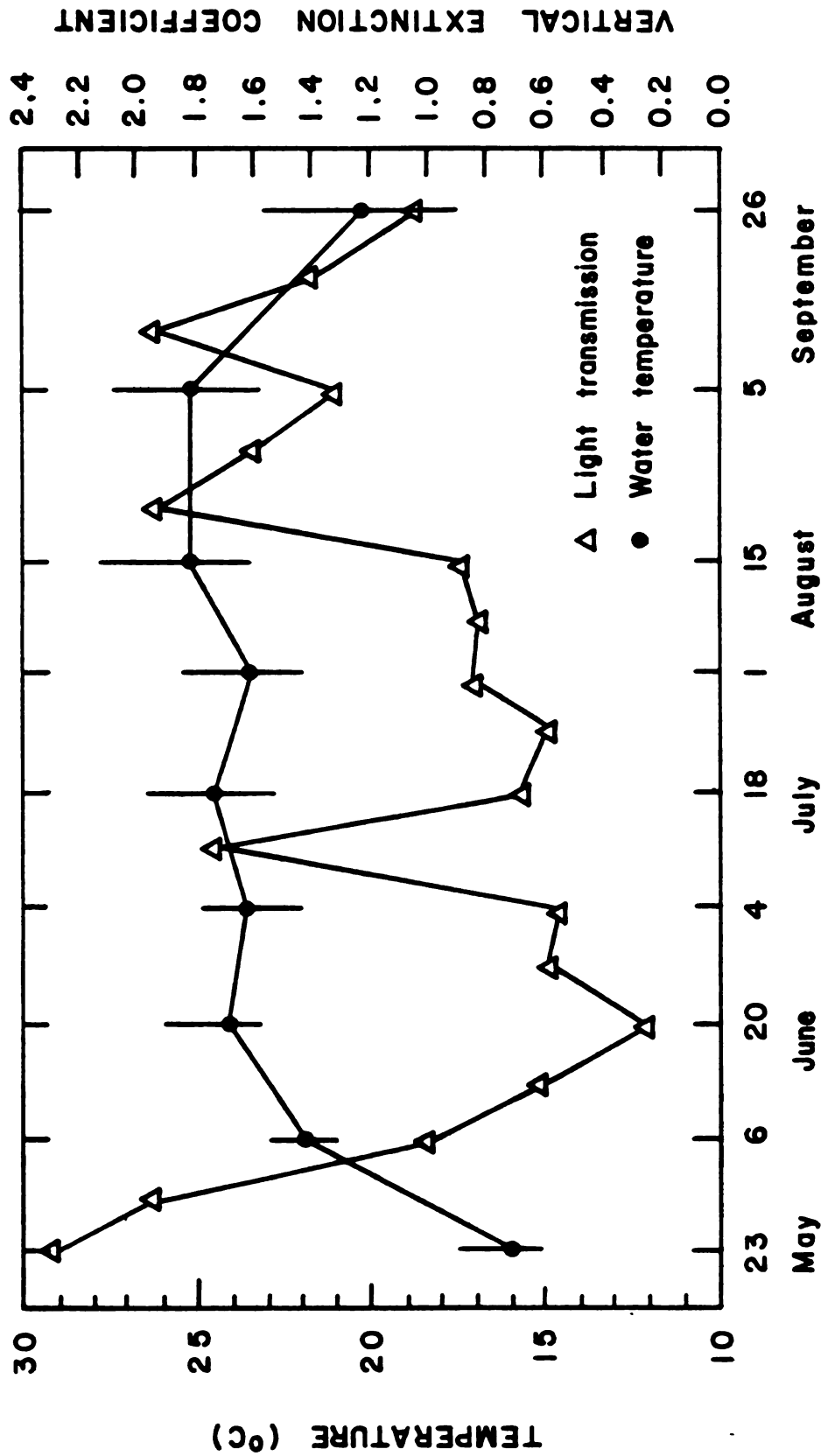


Figure 2. Mean water temperatures and vertical extinction coefficients in Pond 4 of the Belding, Michigan wastewater treatment system during the 1973 growing season. The vertical lines show the range of temperatures.

water column by a dense population of green algae (C. D. McNabb, pers. comm.). The improvement in water clarity prior to June 6 can be attributed to the rapid grazing of the early spring phytoplankton blooms by abundant zooplankton (Lisiecki, 1976). These zooplankton populations play a major role in maintaining excellent light penetration in the pond through much of the growing season (McNabb, 1976).

During the rapid development of the vascular hydrophyte maximum standing crop from June 6 through August 15, the median  $\eta$  was 0.69. This permitted 50% and 29% of the incident irradiance to reach the 1.0 m depth and bottom, respectively. The discrepant value for  $\eta$  recorded on July 12 can be attributed to turbidity caused by wind-induced re-suspension of sediment materials by storm conditions on that date. The period of hydrophyte senescence from August 23 through the close of sampling had a median  $\eta$  of 1.51. This value corresponds to approximately 7% of the incident light reaching the bottom and about 23% reaching the 1.0 m depth. This reduction in  $\eta$  corresponds to a developing population of the blue-green alga *Microcystis aeruginosa* Kuetz. from June 20 through the end of the study (C. D. McNabb, pers. comm.). During the period of rapid development of the vascular hydrophytes, the light transmission of the pond water remained consistently high, reaching the best values of the season; during their senescence, it declined. Thus, despite the variability over time, a trend in  $\eta$  can be seen related to the dynamics of the hydrophyte community.

The vertical extinction coefficients for Pond 4 can be compared with the range of values observed for natural lake waters. In very clear lakes, such as Crater Lake, Oregon, extinction coefficients ca. 0.2 per meter have been observed, while in highly stained lakes or lakes with dense phytoplankton blooms, values ca. 4.0 are common (Wetzel, 1975).

The degree of water clarity over the study period can be further assessed by calculating the depth at which 1% of the incident surface light would remain if the 1.8 meter deep pond were of sufficient depth. Table 1 records the  $\eta$  values and the calculated depths of 1% light penetration over the growing season. During the interval June 6 - August 15, there was adequate light for rooted hydrophyte growth to a depth of 6 meters (approximately 19.5 feet). McNabb (1976) reports that this degree of water clarity in these vascular hydrophyte-dominated ponds is considered excellent for recreational lakes in Michigan. Light transmission in these shallow ponds equals, if not exceeds, that of lakes in the region.

The quantity of total direct solar radiation decreases approximately 34% from June 15 to September 15, 14% from June 15 to August 15, and only 3% from June 15 to July 15 at the  $45^{\circ}\text{N}$  latitude (Perl, 1935). The maximum quantity of solar radiation occurs in mid-June. The absolute quantity of radiation falling on the Pond 4 surface may vary due to the slightly more southerly latitude ( $42^{\circ}\text{N}$ ) of the Belding system and to local climatic conditions. However, the general trend in distribution over time is considered to be the same. It can be assumed that the solar radiation input to the pond averaged to fairly uniform values over the 14 day intervals between biomass sampling during the June 6 - August 15 period.

The aerobic nature of Pond 4 is suggested in Table 2 by the dissolved oxygen concentrations recorded both during the daylight period and near the end of the non-photosynthetic period (0400 hours). The pond remained continually aerobic throughout the growing season. This provided a necessary condition for the persistent growth of vascular hydrophyte populations in the downstream ponds of municipal waste stabilization systems (McNabb, 1976).

Table 1. The penetration of light in the hydrophyte-free portions of Pond 4 of the Belding, Michigan wastewater treatment system during the growing season, 1973.

| Date | Vertical Extinction<br>Coefficient, $\eta$ , $\text{m}^{-1}$ | Calculated Depth of 1%<br>light penetration, in<br>meters |
|------|--------------------------------------------------------------|-----------------------------------------------------------|
| 3/29 | 3.75                                                         | 1.23                                                      |
| 4/17 | 6.38                                                         | 0.72                                                      |
| 4/24 | 7.93                                                         | 0.58                                                      |
| 5/2  | 3.52                                                         | 1.31                                                      |
| 5/17 | 1.52                                                         | 3.03                                                      |
| 5/23 | 2.30                                                         | 2.00                                                      |
| 5/31 | 1.96                                                         | 2.35                                                      |
| 6/6  | 1.02                                                         | 4.51                                                      |
| 6/14 | 0.64                                                         | 7.20                                                      |
| 6/20 | 0.26                                                         | 17.71                                                     |
| 6/28 | 0.59                                                         | 7.81                                                      |
| 7/4  | 0.55                                                         | 8.37                                                      |
| 7/12 | 1.75                                                         | 2.63                                                      |
| 7/18 | 0.69                                                         | 6.67                                                      |
| 7/26 | 0.60                                                         | 7.68                                                      |
| 8/1  | 0.86                                                         | 5.35                                                      |
| 8/9  | 0.83                                                         | 5.55                                                      |
| 8/15 | 0.89                                                         | 5.17                                                      |
| 8/23 | 1.96                                                         | 2.35                                                      |
| 8/30 | 1.61                                                         | 2.86                                                      |
| 9/5  | 1.33                                                         | 3.46                                                      |
| 9/13 | 1.96                                                         | 2.35                                                      |
| 9/20 | 1.41                                                         | 3.27                                                      |
| 9/26 | 1.06                                                         | 4.34                                                      |

Table 2. Means and ranges of dissolved oxygen concentrations in the water column of Pond 4 of the Belding, Michigan wastewater treatment system during the 1973 growing season.

| Date    | $O_2$ (mg/l)      |            |                   |            |
|---------|-------------------|------------|-------------------|------------|
|         | Daylight Period   |            | 0400 Hours        |            |
|         | Mean <sup>a</sup> | Range      | Mean <sup>b</sup> | Range      |
| 5/23-24 | 8.2               | 6.6 - 10.1 | 6.8               | 6.4 - 7.7  |
| 6/6-7   | 7.8               | 5.2 - 9.0  | 6.5               | 6.2 - 7.0  |
| 6/20-21 | 10.1              | 6.3 - 15.8 | 8.0               | 5.2 - 10.0 |
| 7/4-5   | 12.6              | 2.4 - 18.0 | 11.7              | 5.8 - 14.0 |
| 7/18-19 | 11.0              | 3.2 - 16.2 | 6.0               | 1.6 - 11.0 |
| 8/1-2   | 7.0               | 0.5 - 14.5 | 9.1               | 5.8 - 9.8  |
| 8/15-16 | 10.8              | 2.4 - 15.6 | 10.3              | 6.2 - 11.4 |
| 9/5-6   | 8.2               | 1.4 - 12.5 | 6.5               | 6.2 - 6.9  |
| 9/26-27 | 10.9              | 1.6 - 16.0 | 10.0              | 7.6 - 10.5 |

<sup>a</sup> Mean of surface, 0.5, 1.0, 1.5 m depth at 0800, 1200, 1600, 2000 hrs. at two stations.

<sup>b</sup> Mean of surface, 0.5, 1.0, 1.5 m depth at 0400 hrs. at two stations.

The pH of the pond over the study period was in a range characteristic of an active photosynthetic system. Figure 3 records the median pH and range during the daylight period through the growing season. The pH values plotted in Figure 3 were recorded at 0800, 1200, 1600, and 2000 hours, at the surface, 0.5, 1.0, and 1.5 meter depth, and at two locations in the pond. The range of pH values observed on each sampling date can be attributed to the variation of light intensity and concomitant photosynthetic activity with time of day, with depth and with location in the pond. The median pH and range on May 23 reflect an intense green algal bloom in the pond during early spring. The minimum median seasonal pH of 8.4 on June 6 reflects a respiratory event due to the disintegration and decomposition of much of the hydrophyte overwintering tissues and the green algal biomass of the early spring blooms. The temperature-dependent bacterial decomposition and subsequent recarbonation was accelerated by the rapid warming of the pond waters from mid-May values in the interval of May 23 - June 6. The pond pH values increase from June 6 through July 18 reflecting the photosynthetic consumption of CO<sub>2</sub> by the primary producers of the pond. The maximum median pH of 9.6 was reached on July 18. Despite the daily variation of pH during the light period, a trend can again be seen related to the population dynamics of the vascular hydrophytes.

Figure 4 records the population development of the dominant hydrophytes, *Ceratophyllum demersum* L., *Potamogeton foliosus* Raf., *P. berchtoldii* Fieber and *Lemna minor* L. The vegetative parts of *P. foliosus* and *P. berchtoldii* are taxonomically quite similar and were indistinguishable until mid-July when they could be accurately identified from reproductive structures. It was found that the *Potamogeton* spp. biomass consisted largely of *P. foliosus*. *Potamogeton zosteriformis*

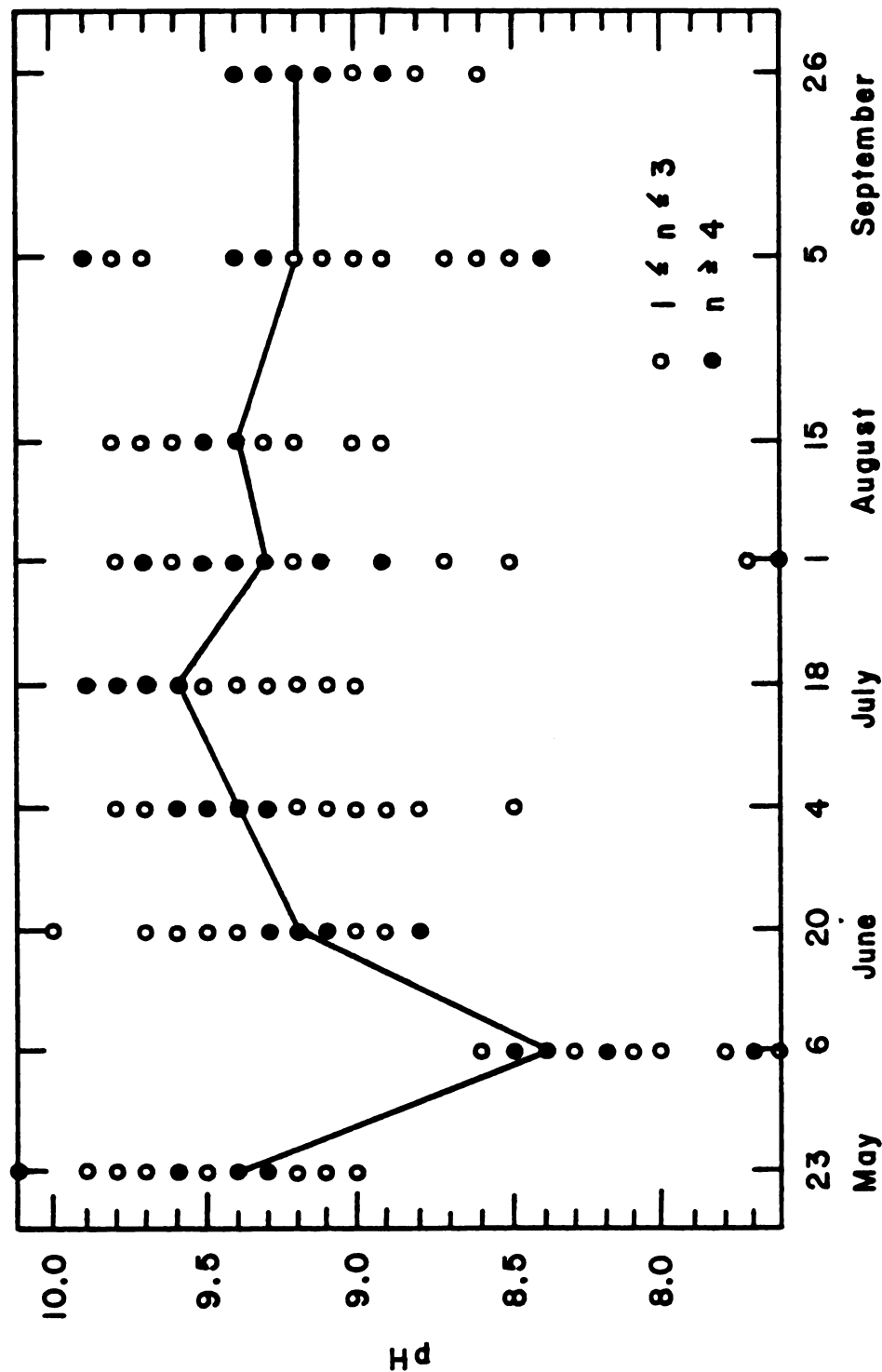


Figure 3. The pH values recorded during the daylight period (0800, 1200, 1600 and 2000 hrs.) in the water column (surface, 0.5, 1.0 and 1.5 m depth) at two stations in Pond 4 of the Belding, Michigan wastewater treatment system during the 1973 growing season. The solid line shows the median values. The range of values reflects the variability due to time of day, depth and location in the pond.

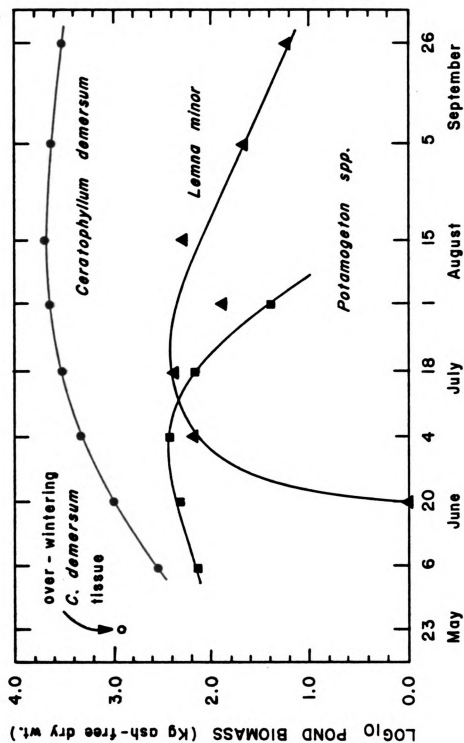


Figure 4. The pond standing crop biomass of the dominant hydrophytes in Pond 4 of the Belding, Michigan wastewater treatment system over the 1973 growing season.

Fernald, *Lemna trisulca* L. and the epipellic filamentous Chlorophycean alga, *Cladophora fracta* (Dillw.) Kuetzing were occasionally found in trace quantities. The populations of submersed hydrophytes covered an estimated 90-100% of the pond bottom during the period May 23 - August 15. Coverage during the remaining portion of the season was approximately 85% of the pond bottom. Areal coverage of the pond bottom averaged 94% on a seasonal basis. Biomass expressed as grams per square meter ash-free dry weight and the percent ash of the dominant hydrophytes are given in Appendix Tables A1 and A2, respectively.

*Ceratophyllum demersum* L., the dominant submersed aquatic angiosperm, began development from dormant winter apices. These vegetative propagules are dense clusters of dark green apical leaves of lateral shoots with greatly augmented starch reserves and a thickened cuticle (Sculthorpe, 1967). These perennating organs are formed by cessation of elongation in the apical regions of the stem in response to unfavorable environmental conditions such as nutrient deficiency, low temperatures, shortened photoperiod, and possibly, low light intensities (Sculthorpe, 1967). The apices may be liberated and sink to the substrate because of a specific gravity greater than water or remain attached to the parent plant between seasons of maximum growth depending on the rate of disintegration of the parent plant. The overwintering *C. demersum* apices in Pond 4 remained attached to significant portions of the biomass from the previous growing season (Figure 4). Terminal stem portions, 5 to 10 cm in length, were liberated with the decomposition of the basal portions of the stem and settled into the sediments during the interval of May 22 - June 6. Temperature and light intensity are considered to be the major physical factors influencing the germination of these apices (Sculthorpe, 1967). Ikusima (1965) reports that vegetative development

in *C. demersum* is restricted at temperatures below 15°C. The dormant apices responded to the rapid warming of the pond and the improvement in the light penetration from early May values in the May 17 - June 6 interval and growth commenced.

Shoots of *C. demersum* possess modified basal lateral branches called rhizoid-shoots whose leaves are extremely finely divided into whiteish thread-like segments. These rhizoids penetrate the substrate and aid in anchorage of this rootless plant. The hydrophyte grew from the pond bottom in this manner and eventually occupied the entire water column. Individual plants pulled free of the substratum and formed a floating mat during the period of most prolific production in the interval of June 6 - July 4. The mat began to disintegrate in the interval of August 1 - August 15 and this continued leaving senescent plants along the bottom at the end of the study in September.

On June 20, the mat biomass was 865 kg as ash-free dry weight and, through continued recruitment from the bottom, reached 1450 kg on July 4. The mat biomass remained relatively constant during the rest of its occurrence with 1385 kg recorded on July 18 and 1350 kg on August 1. The mat varied in position and size (0.6 - 0.8 hectare) on the pond surface depending on the wind direction and on the degree of wind-induced aggregation or dispersion of the floating plant tissue. After its initial formation, the mat biomass remained constant while the bottom-anchored tissues increased in biomass. During the growing season, *C. demersum* comprised between 72 and 99% of the total hydrophyte biomass. The hydrophyte began development from viable tissue estimated to be 3-7% of its maximum seasonal biomass. The maximum biomass was reached on August 15 with 5101 kg of plant tissue present in the pond. At the close of the study on September 27, 3368 kg of biomass remained.



The rooted hydrophytes, *P. foliosus* and *P. berchtoldii.*, began development in late May from seeds and small pieces of meristematic tissue produced during the previous growing season. They grew along the pond bottom in a carpet-like fashion and, at maximum development, grew to a height of 0.5 m. The *Potamogeton* spp. were overgrown by *C. demersum* early in the growing season. They made up a sizeable portion of the hydrophyte biomass early in the growing season, comprising 28% of the biomass on June 6, but completed a cycle of vegetative growth, seed setting, senescence, and disintegration by August 1. Their maximum seasonal biomass of 279 kg was recorded on July 4 (11% to total hydrophyte biomass on that date). The perennating seeds and stem fragments were estimated to be 4-7% of the seasonal maximum biomass.

From scattered fronds, the pleustonic *Lemna minor* expanded to cover the floating mat of *C. demersum* as it developed. Although it occasionally covered up to one hectare of the pond surface, *L. minor* never made up a large portion of the total hydrophyte biomass ( $\leq 6\%$ ). *Lemna minor* reached its maximum standing crop on July 18 with 238 kg present on the pond.

Table 3 records the pond standing crop estimates for the major hydrophyte species and the calculated net specific growth rates for the intervals of time between biomass sampling. In Table 4, the minimum free  $\text{CO}_2$  and  $\text{HCO}_3^-$  concentrations in the water column are reported for the photosynthetic (daylight) period.

Table 3. Changes in the pond standing crop biomass and the net specific growth rate,  $\mu$ , over the 1973 growing season for *Ceratophyllum demersum*, *Potamogeton* spp.<sup>a</sup>, and *Lemna minor* in Pond 4 of the Belding, Michigan wastewater treatment system. The biomass is expressed on an ash-free dry weight basis<sup>b</sup>.

| Date | <i>C. demersum</i>                |                               | <i>Potamogeton</i> spp. <sup>a</sup> |                               | <i>L. minor</i>                   |                               |
|------|-----------------------------------|-------------------------------|--------------------------------------|-------------------------------|-----------------------------------|-------------------------------|
|      | Pond Biomass <sup>c</sup><br>(kg) | $\mu$<br>(day <sup>-1</sup> ) | Pond Biomass <sup>c</sup><br>(kg)    | $\mu$<br>(day <sup>-1</sup> ) | Pond Biomass <sup>d</sup><br>(kg) | $\mu$<br>(day <sup>-1</sup> ) |
| 6/6  | 362                               |                               | 141                                  |                               | nil                               |                               |
|      |                                   | .066                          |                                      | .028                          |                                   |                               |
| 6/20 | 987                               |                               | 210                                  |                               | 1                                 |                               |
|      |                                   | .053                          |                                      | .020                          |                                   | .141                          |
| 7/4  | 2164                              |                               | 279                                  |                               | 159                               |                               |
|      |                                   | .032                          |                                      | -.041                         |                                   | .028                          |
| 7/18 | 3416                              |                               | 155                                  |                               | 238                               |                               |
|      |                                   | .017                          |                                      | -.103                         |                                   | -.070                         |
| 8/1  | 4336                              |                               | 25                                   |                               | 81                                |                               |
|      |                                   | .012                          |                                      | -.143                         |                                   | .059                          |
| 8/15 | 5101                              |                               | nil                                  |                               | 194                               |                               |
|      |                                   | -.005                         |                                      |                               |                                   | -.057                         |
| 9/5  | 4577                              |                               | 0                                    |                               | 49                                |                               |
|      |                                   | -.014                         |                                      |                               |                                   | 0.042                         |
| 9/26 | 3368                              |                               | 0                                    |                               | 19                                |                               |

<sup>a</sup> *P. foliosus* and *P. berchtoldii* combined.

<sup>b</sup> Ash-free dry weight is based on the seasonal average of 78% of dry weight for *C. demersum*, 80% of dry weight for *Potamogeton* spp., and 79% of dry weight for *L. minor*.

<sup>c</sup> Pond biomass based on the 94.4% seasonal average areal cover of pond bottom.

<sup>d</sup> Pond biomass based on areal cover of pond surface.

Table 4. Minimum free  $\text{CO}_2$  and  $\text{HCO}_3^-$  concentrations reached during the daylight period in Pond 4 of the Belding, Michigan wastewater treatment system during the 1973 growing season.

| Date | Mean water                          | Mean water                             |
|------|-------------------------------------|----------------------------------------|
|      | column <sup>a</sup>                 | column <sup>a</sup>                    |
|      | $\text{CO}_2$<br>(micromoles/liter) | $\text{HCO}_3^-$<br>(millimoles/liter) |
| 5/23 | 1.5                                 | 2.7                                    |
| 6/6  | 34.3                                | 3.9                                    |
| 6/20 | 2.2                                 | 3.1                                    |
| 7/4  | 1.7                                 | 2.9                                    |
| 7/18 | 1.3                                 | 2.8                                    |
| 8/1  | 1.5                                 | 2.8                                    |
| 8/15 | 1.9                                 | 3.0                                    |
| 9/5  | 2.1                                 | 3.1                                    |
| 9/26 | 4.3                                 | 3.4                                    |

<sup>a</sup> Mean of surface, 0.5, 1.0 and 1.5 m depths at two stations (n = 8).

## DISCUSSION

The distinction between standing crop limiting and growth rate limiting factors is an important one (O'Brien, 1972). The maximum standing crop of aquatic plants has been shown to be a function of the amount of phosphorus, or occasionally, nitrogen available (Gerloff, 1975). Carbon availability has been suggested as important in determining the rate at which the phosphorus or nitrogen determined standing crop is reached (King, 1970, 1972; Schindler and Fee, 1973). Botkin (1977) suggests that under non-limiting conditions of nitrogen, phosphorus and other essential nutrients, the rate at which the plant population would attain its maximum standing crop would be limited initially by carbon availability and eventually by light due to self-shading. The light limitation would also serve to limit the maximum standing crop as the optimum population density is exceeded (Botkin, 1977). Under luxury consumption of essential nutrients, the plants are able to fully exploit other environmental variables. From the data available to date, it appears that temperature, light and carbon are the primary variables interacting to control plant productivity in the downstream aerobic ponds of multistaged lagoon systems where vascular hydrophyte populations commonly develop (King, 1970; McNabb, 1976; Ramani, 1976). King (1976) suggests that long detention times relative to the alkalinity of the wastewater increase the probability of a carbon limitation of the primary producers of the lagoon.

Gerloff (1975) reports the critical concentrations of phosphorus and nitrogen for *C. demersum* as 0.10% and 1.30% of the dry weight, respectively. The critical concentration is defined as the minimum concentration of an element in plant tissues which will permit maximum plant yield and growth (Gerloff, 1975). Mahan (1974) in a concurrent study in Pond 4 reports *C. demersum* tissue concentrations of phosphorus in the range of 1.09 - 1.55% of the dry weight during the growing season. *C. demersum* showed luxury consumption of phosphorus throughout the growing season accumulating phosphorus to levels exceeding some of the highest reported in the literature (Mahan, 1974). Although seasonal nitrogen tissue concentrations for *C. demersum* are lacking, non-limiting levels of nitrogen through the growing season are suggested by the presence in the pond of a developing population of *Microcystis aeruginosa*. *M. aeruginosa*, a non-nitrogen-fixing blue-green alga, has a high nitrogen requirement (Gerloff et al., 1952). Comparison of the reported critical concentration of nitrogen for *M. aeruginosa* of 4.0% of the dry weight with that for *C. demersum* of 1.3% of the dry weight suggest abundant nitrogen available to *C. demersum* (Gerloff et al., 1952; Gerloff, 1975). McNabb (1976) reports a seasonal mean nitrogen tissue concentration of 4.2% of the dry weight for *Ceratophyllum* growing in hypereutrophic sewage lagoon systems. The tissue concentration was found to be independent of the mean ambient water concentrations in the range of 0.5 - 13.5 mg/liter inorganic-N. On the strength of these observations, it was assumed that neither phosphorus or nitrogen was limiting to the growth rate or maximum standing crop of *C. demersum* in Pond 4 during this study. Presumably, the concentrations of various trace nutrients required for hydrophyte photosynthesis were also more than adequate.

The relatively constant and high light regime of the shallow Pond 4 during the period of hydrophyte growth permits a reasonable estimate of *C. demersum* kinetic parameters obtained under near optimum conditions in a natural light cycle.

Among potential limiting nutrients, carbon dioxide has a special place in view of its immediate involvement in photosynthesis, its non-conservative nature and its large net uptake relative to such elements as nitrogen and phosphorus. On the average, 79 percent of the dry weight of the submersed vascular hydrophytes of Pond 4 is organic matter (Appendix Table A2). This carbonaceous material is derived from the light-dependent reduction of carbon dioxide by photosynthesis. The carbonate-bicarbonate alkalinity system serves as a significant reserve photosynthetic carbon source and is supported by variable  $\text{CO}_2$  exchange rates with the atmospheric  $\text{CO}_2$  reserve and by temperature-dependent rates of respiration of accrued organic matter by the biota. However, even in sewage lagoon systems, the bulk of the  $\text{CO}_2$  available for immediate plant uptake is from the alkalinity system (King, 1972). Although at a given temperature, the total dissolved inorganic carbon is a function of the carbonate-bicarbonate alkalinity, the partitioning of the carbon at equilibrium among the components of the system (free carbon dioxide,  $\text{CO}_2 + \text{H}_2\text{CO}_3$ ; bicarbonate,  $\text{HCO}_3^-$ ; and carbonate,  $\text{CO}_3^{=}$ ) is a function of the pH at that temperature (Stumm and Morgan, 1970). Photosynthetic consumption of carbon raises the pH, shifts the partitioning of the total inorganic carbon among its components, and reduces the chemical species involved in cellular uptake to rate-limiting concentrations.

Hutchinson (1975) considering the available literature suggests that *C. demersum* is able to use the bicarbonate ion as an additional source of carbon for photosynthesis. The complexity of the chemical

equilibria of the carbonate-bicarbonate alkalinity system does not permit unequivocal demonstration of  $\text{HCO}_3^-$  uptake by aquatic plants by the means employed to date. However, recent workers (Brown, et al., 1974; Carr, 1969; Van, et al., 1976) are not convinced of  $\text{HCO}_3^-$  uptake and offer alternative explanations for the phenomenon. Although bicarbonate uptake and utilization would be of significant competitive advantage, the morphological, biochemical and physiological adaptations which serve to increase the ease of net  $\text{CO}_2$  assimilation may be more than adequate to explain the competitive success of certain aquatic plants.

If the growth of an organism follows the empirical Monod (1949) application of nutrient limitation to entire organisms of the Michaelis-Menten enzyme kinetics formulation, a plot of the net specific growth rate of the organism versus a non-conservative substrate (nutrient) concentration yields a right rectangular hyperbola of the form

$$\mu = \mu_{\max} \frac{[S]}{K_S + [S]} \quad (6)$$

where  $\mu$  = net specific growth rate,  $\text{time}^{-1}$ ;

$\mu_{\max}$  = maximum net specific growth rate,  $\text{time}^{-1}$ ;

$K_S$  = half-saturation constant which is numerically equal to the substrate concentration at which  $\mu = \frac{1}{2} \mu_{\max}$ , mass per unit volume;

$[S]$  = substrate (nutrient) concentration, mass per unit volume.

The parameters which characterize this equation and which must be estimated from the observed data are the maximum net specific growth rate ( $\mu_{\max}$ ) which is theoretically obtainable with an infinite concentration of the substrate, and the Michaelis constant ( $K_S$ ) which is numerically equal to the concentration of the substrate corresponding to the half-maximal net specific growth rate.

The minimum  $\text{CO}_{2f}$  and  $\text{HCO}_3^-$  concentrations attained in the pond during the growing season can be designated the  $\text{CO}_2$  quit ( $\text{CO}_{2q}$ ) and the  $\text{HCO}_3^-$  quit ( $\text{HCO}_{3q}^-$ ) concentrations (Klemovich, 1973; King and King, 1974). Assuming cellular uptake of both  $\text{CO}_{2f}$  and  $\text{HCO}_3^-$ , these values represent the minimum  $\text{CO}_{2f}$  and  $\text{HCO}_3^-$  concentrations permitting net uptake by *C. demersum* under the near-optimum light and non-limiting nutrient conditions in Pond 4. The empirical  $\text{CO}_{2q}$  and  $\text{HCO}_{3q}^-$  concentrations are 1.3  $\mu\text{mol/l}$  and 2.7  $\text{mmol/l}$ , respectively. At the  $\text{CO}_{2q}$  or  $\text{HCO}_{3q}^-$  concentration, the rate of  $\text{CO}_{2f}$  or  $\text{HCO}_3^-$  extraction from the alkalinity and consequently the rate of gross production, equals the rate of respiration. Therefore, the rate of net production equals zero. At this point a finite concentration of both  $\text{CO}_{2f}$  and  $\text{HCO}_3^-$  remains. This suggests that the curves defined by Equation 6 do not go through the origin. Therefore, Equation 6 can be corrected for the  $\text{CO}_{2q}$  and  $\text{HCO}_{3q}^-$  values:

$$\mu = \mu_{\max} \frac{[S] - [S_q]}{(K_S - [S_q]) + ([S] - [S_q])} \quad (7)$$

where  $[S_q]$  = the empirically determined minimum substrate concentration at which  $P_G - R = P_N = 0$ .

The kinetic constants,  $\mu_{\max}$  and  $K_S$ , were calculated using the  $\mu$  versus  $\mu/[S]$  linear transformation of the original equation recommended by Dowd and Riggs (1965) rather than the more familiar Lineweaver-Burk (1934) double reciprocal transformation. The  $\mu$  versus  $\mu/[S]$  transformation is highly sensitive to departures from the linear relationship expected on the basis of Michaelis-Menten kinetics (Dowd and Riggs, 1965). Therefore, this transformation may be used as a test of the applicability of the Michaelis-Menten formulation to the uptake of either chemical species of carbon. Equation 7 becomes:

$$\mu = \mu_{\max} - (K_S - [S_q]) \left[ \mu / ([S] - [S_q]) \right] \quad (8)$$

In Figure 5, plots of  $\mu$  versus  $\mu / ([S] - [S_q])$  are given where  $\mu$  is equal to the net specific growth rate (Table 3) of the dominant hydrophyte, *C. demersum*, and where  $[S]$  is equal to both the average minimum free  $\text{CO}_2$  concentration and the average minimum  $\text{HCO}_3^-$  concentration (Table 4) over the time interval for which the  $\mu$  was determined. The average concentration over the interval was used rather than the concentration at the beginning of the interval to more closely approximate the changing conditions in the pond. The lines fitted to the observed data by the method of least squares have a slope of  $-(K_S - [S_q])$  and the intercept on the  $\mu$ -axis gives  $\mu_{\max}$ . The empirically determined constants for *C. demersum* under the light and nutrient conditions of Pond 4 for free  $\text{CO}_2$  are  $\mu_{\max} = 0.067 \text{ day}^{-1}$ ,  $K_S = 1.5 \text{ } \mu\text{mol/l}$  and  $\text{CO}_{2q} = 1.3 \text{ } \mu\text{mol/l}$ ; and for  $\text{HCO}_3^-$ , the constants are  $\mu_{\max} = 0.091 \text{ day}^{-1}$ ,  $K_S = 3.0 \text{ mmol/l}$ , and  $\text{HCO}_{3q}^- = 2.7 \text{ mmol/l}$ .

The considerably better fit for  $\mu$  as a function of the free  $\text{CO}_2$  concentration is reflected in the correlation coefficients ( $r = -0.99$  for  $\text{CO}_{2f}$  and  $r = -0.76$  for  $\text{HCO}_3^-$ ) for the fitted lines of Figure 5. Some degree of correlation of  $\mu$  with the  $\text{HCO}_3^-$  concentration is inevitable since above a pH of approximately 8.35 both the  $\text{CO}_{2f}$  and  $\text{HCO}_3^-$  concentrations are decreasing as defined by the equilibrium equations of the carbon dioxide system. A direct kinetic response to the  $\text{HCO}_3^-$  concentration at any pH reached during the growing season would appear as a significant deviation from linearity of the  $\text{CO}_{2f}$  data points. This strongly suggests that *C. demersum* is responding kinetically to the equilibrium  $\text{CO}_{2f}$  concentration rather than the  $\text{HCO}_3^-$  concentration and that the hydrophyte can assimilate and use only free  $\text{CO}_2$  as its

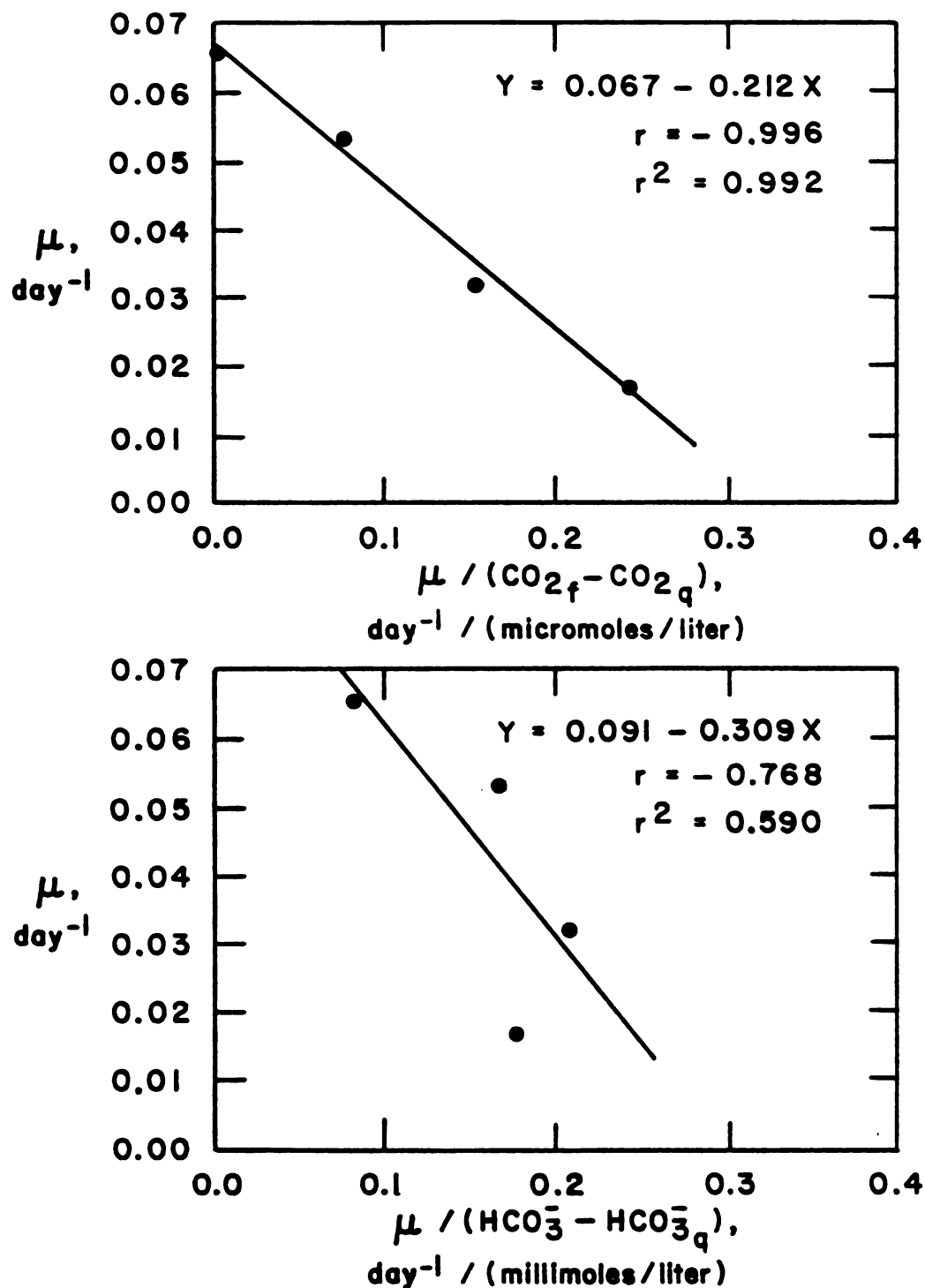


Figure 5. Plots of  $\mu$  versus  $\mu/[S]$  for  $[S]$  expressed as the  $\text{CO}_{2f} - \text{CO}_{2q}$  concentration (upper figure) and as the corresponding  $\text{HCO}_3^- - \text{HCO}_3^-_q$  concentration (lower figure) where  $\mu$  is equal to the net specific growth rates for *C. demersum* in Pond 4 during the 1973 growing season.

photosynthetic carbon source.

In Figure 6, the net specific growth rates for *C. demersum* are plotted against the average minimum free  $\text{CO}_2$  concentration. The equation for the fitted line is

$$\mu = 0.067 \frac{([\text{CO}_{2f}] - 1.3)}{0.2 + ([\text{CO}_{2f}] - 1.3)} \quad (9)$$

The line fitted to the data of Figure 7 is given by

$$\mu = 0.091 \frac{([\text{HCO}_3^-] - 2.7)}{0.3 + ([\text{HCO}_3^-] - 2.7)} \quad (10)$$

where the growth rates for *C. demersum* are plotted against the average minimum  $\text{HCO}_3^-$  concentrations in the pond.

Late in the growing season, from August 1 through September 26, the decline in the daily median pH and increase in  $\text{CO}_{2f}$  and  $\text{HCO}_3^-$  does not result in an increase in the net growth rate as expected. This can be attributed to the influence of some other limitation, presumably light. The seasonal decline in solar energy incident to the pond surface equal to approximately a 34% reduction of total radiation from June 15 to September 15, the general decline in light transmission of the pond water and self-absorption by the hydrophytes as they exceed their optimum population density contribute to the light limitation late in the growing season. For this reason, the point corresponding to the interval August 1 - August 15 (identified as 8/8 on Figures 6 and 7) was excluded from the computation of the kinetic constants.

Graphic presentation of the conditions of carbonate-bicarbonate alkalinity and pH at which the  $\text{CO}_{2f}$  and the  $\text{HCO}_3^-$  concentrations significantly limit the net specific growth rate of *C. demersum* are given in Figures 8 and 9, respectively. The basic chemical equilibrium

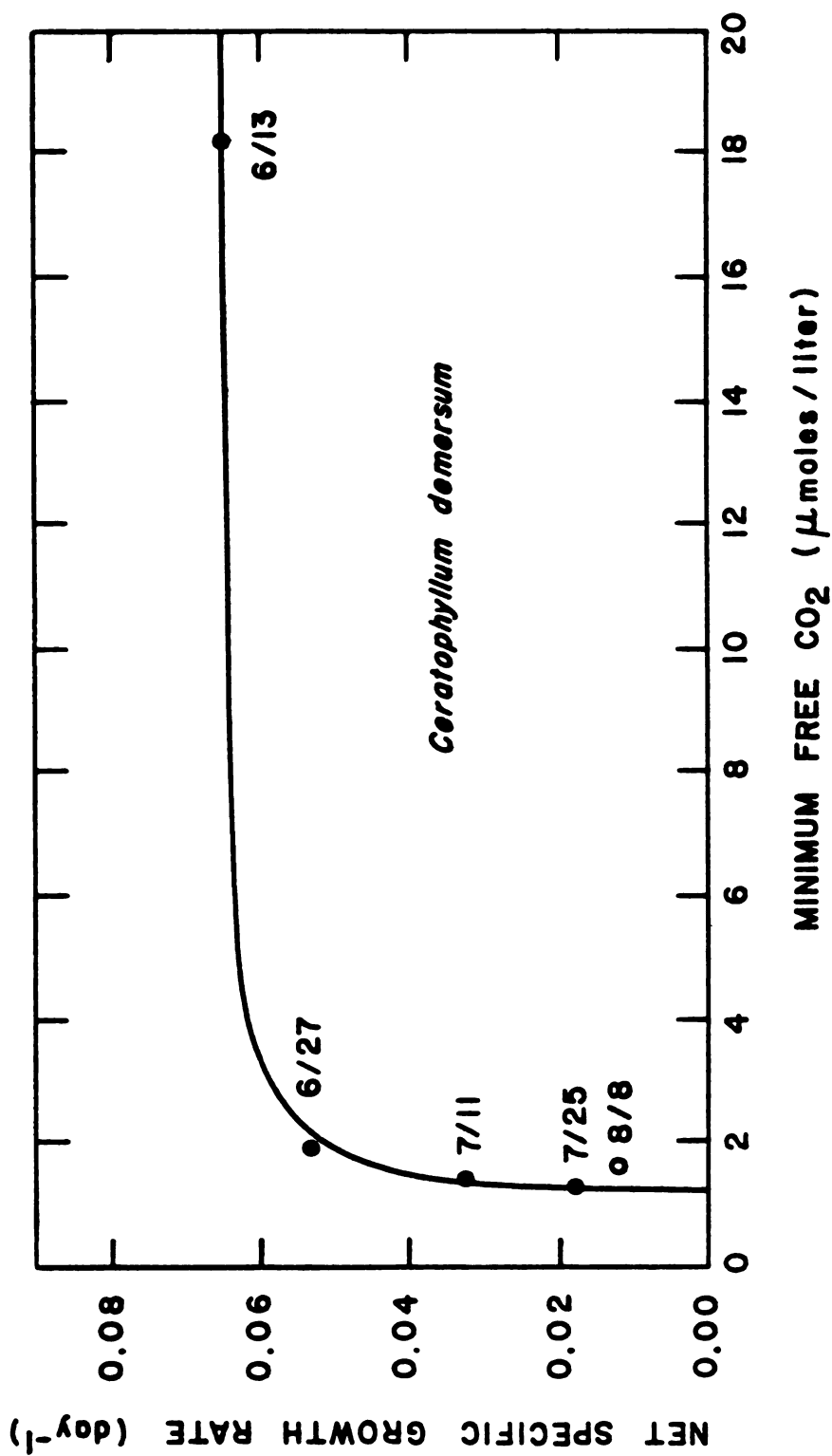


Figure 6. Net specific growth rate ( $\mu$ ) of *C. demersum* as a function of the average minimum free CO<sub>2</sub> concentration in the water column reached during the daylight period. Dates refer to the midpoints of the sampling intervals.

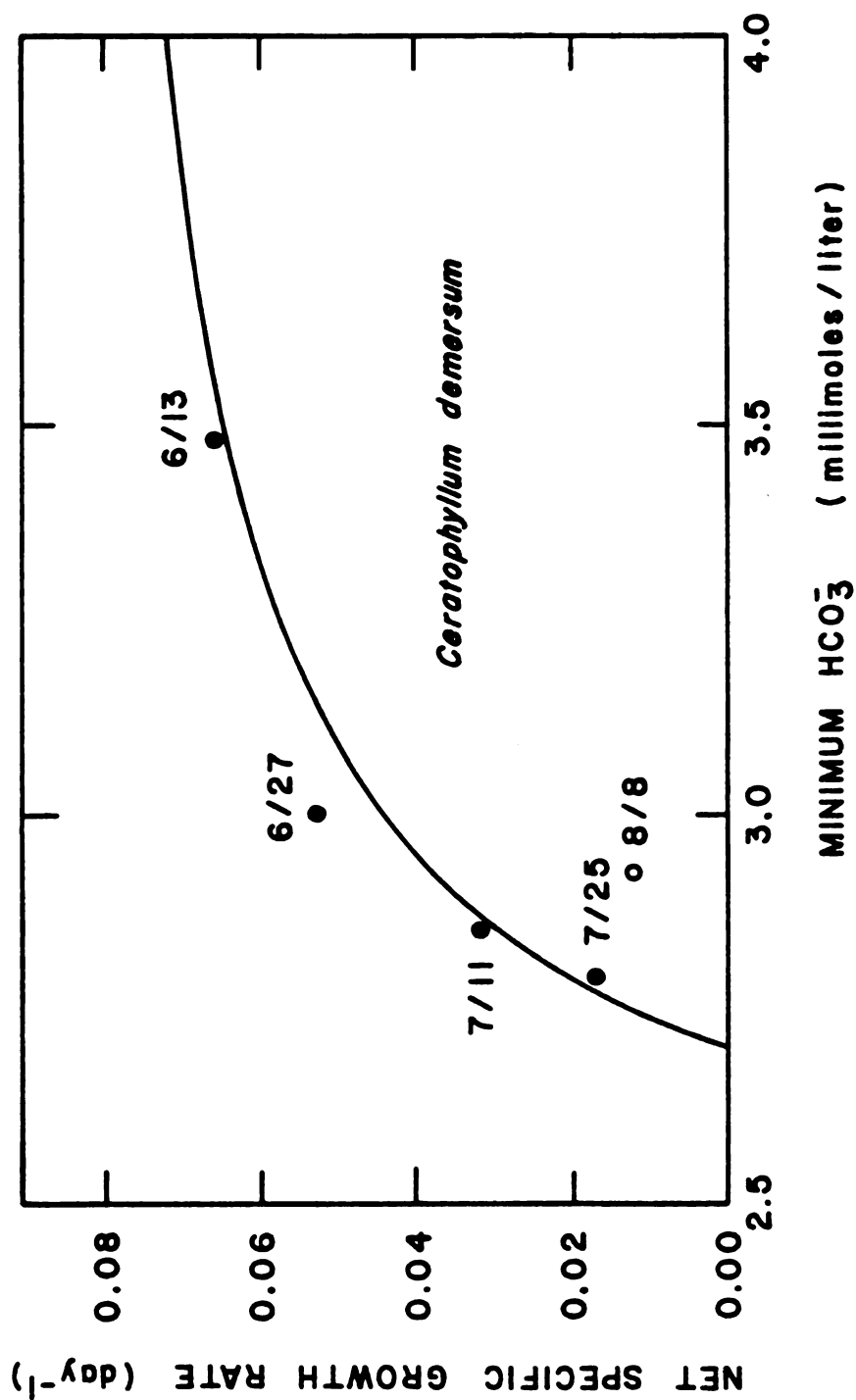


Figure 7. Net specific growth rate ( $\mu$ ) of *C. demersum* as a function of the average minimum  $\text{HCO}_3^-$  concentration in the water column reached during the daylight period. Dates refer to the midpoints of the sampling intervals.

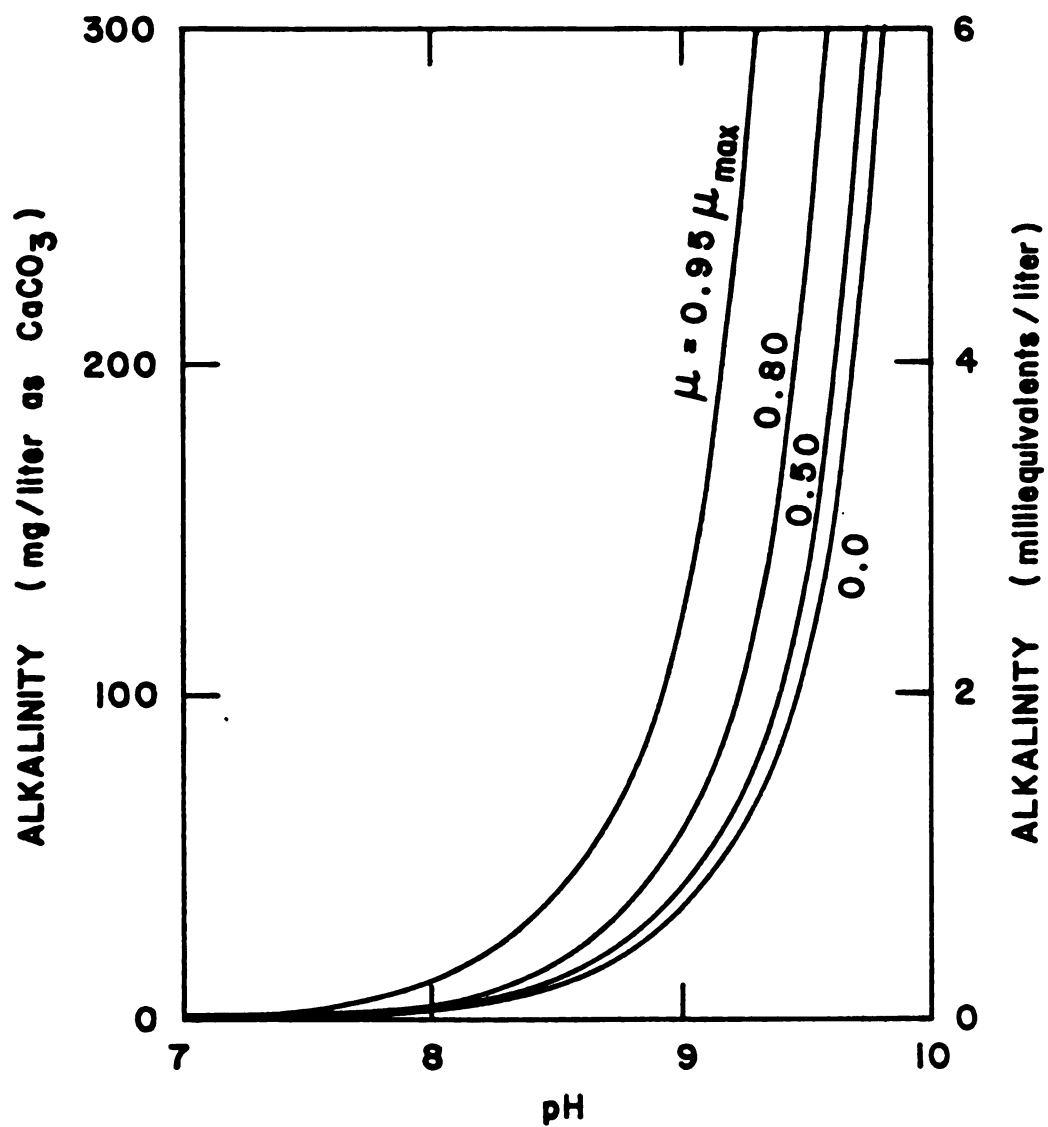


Figure 8. The conditions of pH and carbonate-bicarbonate alkalinity at  $24^\circ\text{C}$  yielding various net growth rate limiting free  $\text{CO}_2$  concentrations for *Ceratophyllum demersum*.

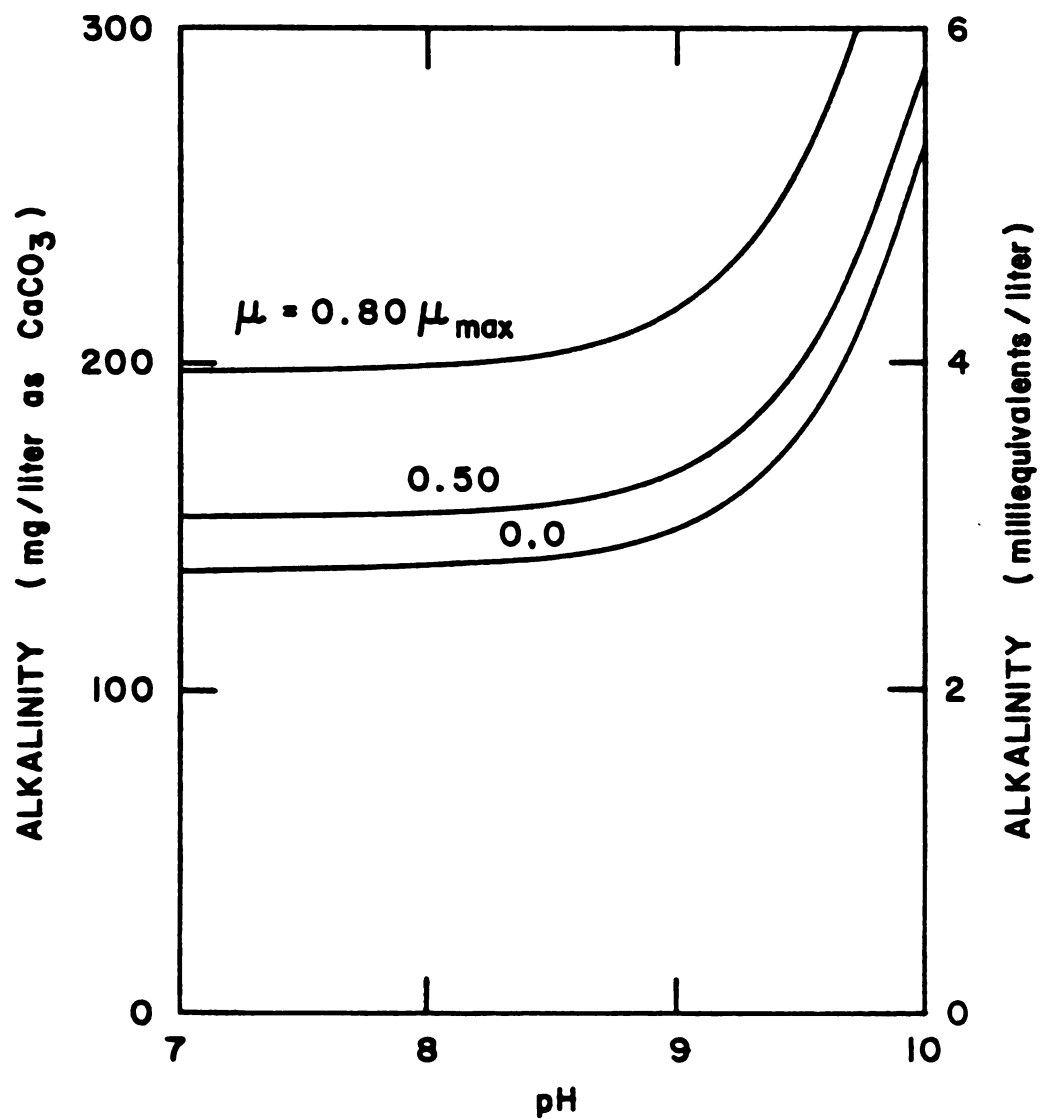


Figure 9. The conditions of pH and carbonate-bicarbonate alkalinity at 24°C yielding various net growth rate limiting  $\text{HCO}_3^-$  concentrations for *Ceratophyllum demersum*.

relationships of the carbonate-bicarbonate alkalinity system (Harvey, 1957; Park, 1969) and Equations 9 and 10 were used to calculate the isopleths of  $\text{CO}_{2f}$  and  $\text{HCO}_3^-$  at which net growth can proceed at some given proportion of the maximum rates determined for *C. demersum*. The calculations were based on  $K_1$  and  $K_2$  values at  $24^\circ\text{C}$  which corresponds closely to the mean water temperature during the plant growth phase. The effect of temperature is slight with a change of temperature of  $10^\circ\text{C}$  being equivalent to a change in pH of only 0.05 units.

In Figure 8, the curves identified as  $0.95 \mu_{\text{max}}$ ,  $0.80$ ,  $0.50$  and  $0.0$  correspond to  $\text{CO}_{2f}$  concentrations of 5.3, 2.5, 1.5 and 1.3  $\mu\text{mol/l}$ , respectively. In Figure 9, the curves identified as  $0.80 \mu_{\text{max}}$ ,  $0.50$  and  $0.0$  correspond to  $\text{HCO}_3^-$  concentrations of 3.9, 3.0 and 2.7  $\text{mmol/l}$ , respectively. The curve for  $0.95 \mu_{\text{max}}$ , corresponding to 8.0  $\text{mmol HCO}_3^-/\text{l}$ , lies outside the range of the figure.

As indicated in Figures 8 and 9, both the alkalinity and pH are of significance in determining the equilibrium free  $\text{CO}_2$  or  $\text{HCO}_3^-$  concentrations at any given temperature. For example in Figure 8, the net growth rate of *C. demersum* would be half-maximal at an alkalinity of 20  $\text{mg/l}$  and a pH of 8.8, while at an alkalinity of 175  $\text{mg/l}$ , half-maximal growth would be permitted at a pH of 9.5.

At a given alkalinity and temperature, continued uptake of  $\text{CO}_{2f}$  by the primary producers results in an increase in the pH and a corresponding decrease in the equilibrium  $\text{CO}_{2f}$  concentration. Although a water may contain a considerable amount of dissolved inorganic carbon in the alkalinity system, the amount of  $\text{CO}_{2f}$  available at any point in time is fixed by the equilibrium reactions. The increase in plant biomass and a resultant increase in the daily carbon demand, together with the inability of atmospheric recarbonation and respiratory  $\text{CO}_2$  to meet that demand,

should result in a gradual increase in the minimum, maximum and median pH during each successive daylight period. This would result in a higher pH and lower equilibrium  $\text{CO}_{2f}$  concentration for a longer portion of each successive daylight period. Figure 3 reflects this phenomenon in Pond 4 during the period of rapid plant growth from June 6 through July 18. Comparison of the data of Figure 6 for the Pond 4 alkalinity of 4 meq/l (200 mg/l as  $\text{CaCO}_3$ ) with the data of Figure 3 suggests that *C. demersum* spent increasingly longer portions of successive daylight periods at high pH values and the corresponding growth rate limiting free  $\text{CO}_2$  concentrations during the interval June 6 - July 18. At the Pond 4 alkalinity, *C. demersum* would be able to attain net specific growth rates of 0.50, 0.80 and 0.95  $\mu_{\text{max}}$  at pH values of 9.6, 9.5 and 9.2, respectively. At a pH of 9.7, *C. demersum* would be at its  $\text{CO}_{2q}$  value and its net specific growth rate would be equal to zero. Eventually, in the absence of some other limitation, carbon dioxide would not be available for a sufficient portion of each daylight period for net biomass accrual to continue on a daily basis. At this point, the hydrophyte has reached its seasonal maximum standing crop.

If an aquatic plant can use only free  $\text{CO}_2$ , photosynthesis will cease when the concentration of  $\text{CO}_{2f}$  becomes negligible. However, if the plant can utilize  $\text{HCO}_3^-$  as an additional carbon source, photosynthesis will continue until a minimum  $\text{HCO}_3^-$  concentration is reached. The maximum pH attainable is then a function of the minimum  $\text{HCO}_3^-$  value and the alkalinity as described by the equilibrium equations of the carbon dioxide system. Figure 10 records the conditions of alkalinity and pH common to natural waters necessary to yield these threshold  $\text{CO}_{2f}$  and  $\text{HCO}_3^-$  concentrations. The curves of Figure 10, drawn for a

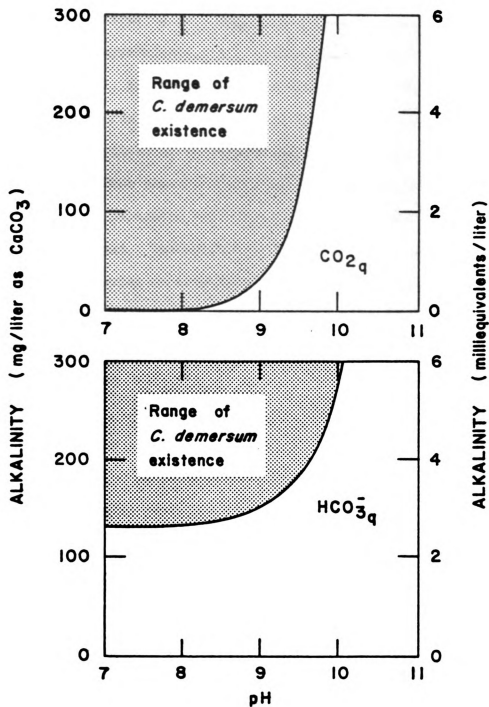


Figure 10. The conditions of pH and carbonate-bicarbonate alkalinity at  $24^\circ\text{C}$  necessary to yield the  $\text{CO}_{2q}$  quit concentration and the corresponding equilibrium  $\text{HCO}_3q$  quit concentration for *Ceratophyllum demersum* assuming net cellular uptake of each chemical species.

temperature of  $24^{\circ}\text{C}$ , are equal to the  $\text{CO}_{2\text{q}}$  concentration ( $1.3 \mu\text{mol/l}$ ) and the  $\text{HCO}_{3\text{q}}^{-}$  concentration ( $2.7 \text{ mmol/l}$ ) for *C. demersum*. At alkalinities less than approximately  $2.75 \text{ meq/l}$  ( $135 \text{ mg/l}$  as  $\text{CaCO}_3$  and pH in the range of 7-11, the  $\text{HCO}_3^{-}$  concentration would be insufficient as an alternate carbon source for net production of the hydrophyte. Under these conditions, typical of soft-water lakes, where both the total dissolved inorganic carbon concentrations and the equilibrium  $\text{CO}_{2\text{f}}$  concentrations are low, bicarbonate utilization would be a significant adaptive advantage to the plant. Examination of the literature suggests that *Ceratophyllum* does exist at alkalinities less than  $135 \text{ mg/l}$  and at pH values in the range of 7.0-9.0. Moyle (1945), based on extensive data from Minnesota lakes, reports *C. demersum* present at alkalinities from 22 to  $376 \text{ mg/liter}$  as  $\text{CaCO}_3$  ( $22$ - $448 \text{ mg/liter}$  as  $\text{HCO}_3^{-}$ ) and a pH in the range of 6.3 - 9.0. Livingston and Bentley (1970) report the distribution of *Ceratophyllum* in lakes of western Massachusetts with alkalinities of  $30$ - $114 \text{ mg/liter}$  and pH values of 7.5 - 8.1. Figure 10 suggests the potential range (shaded areas) of *C. demersum* as predicted by the empirically determined  $\text{CO}_{2\text{q}}$  and  $\text{HCO}_{3\text{q}}^{-}$  concentrations. The conditions of alkalinity and pH necessary to provide at least the minimum  $\text{CO}_{2\text{f}}$  concentration more adequately describe the distribution of the cosmopolitan hydrophyte, *C. demersum*, reported in the literature, than those conditions necessary for the minimum  $\text{HCO}_3^{-}$  concentration.

The rate of  $\text{CO}_{2\text{f}}$  uptake is one primary quantitative nutritional difference between plant species. King (1970, 1972) and Klemovich (1973) showed that different algal species significantly differ in both the rate of carbon fixation and the extent of carbon fixation, i.e., the minimum  $\text{CO}_{2\text{f}}$  concentration permitting net photosynthesis. Schindler et al. (1973)

and King (1972) suggest that carbon availability controls both the algal species dominance and the rate at which the dominant species reach their maximum standing crop. In  $\text{CO}_{2f}$  deficient environments, certain hydrophyte species might also be predicted to out-compete others. Examination of the Monod formulation suggests that the more efficiently a hydrophyte uses low substrate concentrations the smaller its  $K_S$  will be. The  $K_S$  value for a given substrate formally resembles the Michaelis-Menten constant of an enzyme and provides a yardstick for measuring the degree of affinity which the experimental organism has for the limiting substrate (Gerloff, 1975). This is the basis for competition under limiting substrate concentrations. Assuming no other limitation, aquatic plant species with low  $K_S$  values for  $\text{CO}_{2f}$  will outgrow those with higher  $K_S$  values at low  $\text{CO}_{2f}$  concentrations.

With the accelerating eutrophication of our lakes, the aquatic plants will be released from the constraints of standing crop limiting N and P concentrations. This will place greater emphasis on non-conservative parameters such as carbon and light, and their control of the rate at which the maximum standing crop is reached. The rate of population development may be important to management strategies of troublesome hydrophytes in recreational lakes or of hydrophyte-dominated ponds used for wastewater reclamation as described by King (1976) and McNabb (1976).

## CONCLUSIONS

1. Examination of the growth pattern of the aquatic hydrophyte, *Ceratophyllum demersum*, under conditions of non-limiting nutrients and relatively constant near optimum light intensities, suggests a close relationship between the free  $\text{CO}_2$  concentration of the water over the growing season and the net specific growth rate of the plant. The free  $\text{CO}_2$  concentration was shown to limit the net specific growth rate of the hydrophyte as described by the Monod formulation. The kinetic constants determined are  $\mu_{\text{max}} = 0.067 \text{ day}^{-1}$ ,  $K_S = 1.5 \text{ } \mu\text{mol/l}$ , and  $\text{CO}_{2q} = 1.3 \text{ } \mu\text{mol/l}$ . The rate at which the maximum seasonal standing crop was reached was therefore controlled by carbon availability.
2. *Ceratophyllum demersum* responded solely to the free  $\text{CO}_2$  concentration of the water for its photosynthetic carbon source. There was no evidence that  $\text{HCO}_3^-$  was utilized as an alternate carbon source.

#### LITERATURE CITED

- Allen, H. L. 1972. Phytoplankton photosynthesis, micronutrient interactions, and inorganic carbon availability in a soft-water Vermont lake. Pages 63-83. In G. E. Likens, ed. Nutrients and eutrophication: The limiting-nutrient controversy. Amer. Soc. Limnol. Oceanogr. Spec. Symp. 1.
- American Public Health Association. 1971. Standard methods for the examination of water and wastewater. 13th ed. American Public Health Association, Washington, D.C. 874 pp.
- Botkin, D. B. 1977. Forests, lakes, and the anthropogenic production of carbon dioxide. Bioscience 27:325-331.
- Brown, J. M. A., F. I. Dromgoole, M. W. Towsey, and J. Browse. 1974. Photosynthesis and photorespiration in aquatic macrophytes. Pages 243-249. In R. L. Bielecki, A. R. Ferguson, M. M. Cresswell, eds. Mechanisms of regulation of plant growth. Bull. 12, Royal Soc. New Zealand, Wellington.
- Buch, K. 1951. Das Kohlensäure Gleichgewichts-system im Meerwasser. Havsforskingstitutets Skrift Helsingfors, No. 151. 18 pp.
- Bulthuis, D. A., J. R. Craig, and C. D. McNabb. 1974. Metal dynamics in municipal stabilization ponds. Pages 127-135. In D. D. Hemphill, ed. Trace substances in environmental health-VII. A symposium. Univ. of Missouri, Columbia.
- Carr, J. L. 1969. The primary productivity and physiology of *Ceratophyllum demersum*. II. Micro primary productivity, pH, and the P/R ratio. Aust. J. Mar. Freshwat. Res. 20:127-142.
- Dowd, J. E. and D. S. Riggs. 1965. A comparison of estimates of Michaelis-Menten kinetic constants from various linear transformations. J. Biol. Chem. 240:863-869.
- Eppley, R. W. and W. H. Thomas. 1969. Comparison of half-saturation constants for growth and nitrate uptake of marine phytoplankton. J. Phycol. 5:375-379.
- Eppley, R. W., J. N. Rogers, and J. J. McCarthy. 1969. Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr. 14:912-920.

- Fuhs, G. W., S. D. Demmerle, E. Canelli, and M. Chen. 1972. Characterization of phosphorus-limited plankton algae. Pages 113-133. In G. E. Likens, ed. Nutrients and eutrophication: The limiting-nutrient controversy. Amer. Soc. Limnol. Oceanogr. Spec. Symp. 1.
- Gerloff, G. C. 1975. Nutritional ecology of nuisance aquatic plants. Ecol. Res. Series EPA 660/3-75-027. Nat. Environ. Res. Ctr., U. S. Environ. Protect. Agency, Corvallis, Oregon. 78 pp.
- Gerloff, G. C., G. P. Fitzgerald, and F. Skoog. 1952. The mineral nutrition of *Microcystis aeruginosa*. Amer. J. Bot. 39:26-32.
- Harvey, H. W. 1957. The chemistry and fertility of sea waters, 2nd Ed. Cambridge University Press, Cambridge, England. 234 pp.
- Hutchinson, G. E. 1975. A treatise on limnology. III. Limnological botany. John Wiley and Sons, Inc., New York. 660 pp.
- Ikusima, I. 1965. Ecological studies of the productivity of aquatic plant communities. I. Measurement of photosynthetic activity. Bot. Mag. Tokyo 78:202-211.
- King, D. L. 1970. The role of carbon in eutrophication. J. Water Pollut. Contr. Fed. 42:2035-2051.
- King, D. L. 1972. Carbon limitation in sewage lagoons. Pages 98-105. In G. E. Likens, ed. Nutrients and eutrophication: The limiting-nutrient controversy. Amer. Soc. Limnol. Oceanogr. Spec. Symp. 1.
- King, D. L. 1976. Changes in water chemistry induced by algae. Pages 73-84. In E. F. Gloyna, J. F. Malina, Jr., and E. M. Davis, eds. Ponds as a wastewater treatment alternative. Wat. Res. Symp. No. 9, Ctr. for Res. in Wat. Resources, Coll. of Engineering, Univ. of Texas, Austin.
- King, D. L., and R. E. King. 1974. Interactions between light and carbon dioxide availabilities as a control of algal species succession. Water Resources Res. Ctr. Completion Rep., Proj. No. A-071-MO, Univ. of Missouri, Columbia. 74 pp.
- Klemovich, R. M. 1973. The role of light intensity in algal carbon fixation. M.S. thesis, Univ. of Missouri, Columbia. 51 pp.
- Lineweaver, H., and D. Burk. 1934. The determination of enzyme dissociation constants. J. Amer. Chem. Soc. 56:658-666.
- Lisiecki, J. B. 1977. Copper and cadmium dynamics in a hypereutrophic pond. Ph.D. dissertation, Michigan State Univ., E. Lansing. 94 pp.
- Lisiecki, J. B., and C. D. McNabb. 1975. Dynamics of hazardous elements in wastewater ponds. Natl. Tech. Inform. Serv. PB-248-404. 88 pp.

- Livingston, R. B., and P. A. Bentley. [1970]. The role of aquatic vascular plants in the eutrophication of selected lakes in western Massachusetts. Wat. Resources Res. Ctr. Pub. No. 11, Univ. of Massachusetts, Amherst. 80 pp.
- Mahan, D. C. 1974. The participation of submersed vascular hydrophytes in the phosphorus budget of a waste stabilization lagoon. M.S. thesis, Michigan State Univ., E. Lansing. 61 pp.
- McNabb, C. D. 1976. The potential of submersed vascular plants for reclamation of wastewater in temperate zone ponds. Pages 123-132. In J. Tourbier and R. W. Pierson, Jr., eds. Biological control of water pollution. University of Pennsylvania Press, Philadelphia.
- Monod, J. 1949. The growth of bacterial cultures. Annu. Rev. Microbiol. 3:371-388.
- Moyle, J. B. 1945. Some chemical factors influencing the distribution of aquatic plants in Minnesota. Amer. Midl. Nat. 34:402-420.
- O'Brien, W. J. 1972. Limiting factors in phytoplankton algae. Their meaning and measurement. Science 178:616-617.
- Park, K. 1969. Oceanic CO<sub>2</sub> system: An evaluation of ten methods of investigation. Limnol. Oceanogr. 14:179-186.
- Perl, G. 1935. Zur Kenntnis der wahren Sonnenstrahlung in verschiedenen geographischen Breiten. Met. Z., 52:85-89. Cited by G. E. Hutchinson. 1957. A treatise on limnology. I. Geography, physics, and chemistry. John Wiley and Sons, New York. 1015 pp.
- Ramani, R. 1976. Design criteria for polishing ponds. Pages 159-170. In E. F. Gloyne, J. F. Malina, Jr., and E. M. Davis, eds. Ponds as a wastewater treatment alternative. Wat. Res. Symp. No. 9, Ctr. for Res. in Wat. Resources, Coll. of Engineering, Univ. of Texas, Austin.
- Schindler, D. W. and E. J. Fee. 1973. Diurnal variation of dissolved inorganic carbon and its use in estimating primary production of CO<sub>2</sub> invasion in Lake 227. J. Fish. Res. Board Can. 30: 1501-1510.
- Schindler, D. W., H. Kling, R. V. Schmidt, J. Prokopowich, V. E. Frost, R. A. Reid, and M. Capel. 1973. Eutrophication of Lake 227 by addition of phosphate and nitrate: The second, third, and fourth years of enrichment, 1970, 1971, and 1972. J. Fish. Res. Board Can. 30:1415-1440.
- Sculthorpe, C. D. 1967. The biology of aquatic vascular plants. Edward Arnold Ltd., London. 610 pp.
- Stumm, W., and J. J. Morgan. 1970. Aquatic chemistry. John Wiley and Sons, Inc., New York. 583 pp.

- Van, T. K., W. T. Haller, and G. Bowes. 1976. Comparison of the photosynthetic characteristics of three submersed aquatic plants. *Plant Physiol.* 58:761-768.
- Wetzel, R. G. 1975. *Limnology*. W. B. Saunders Co., Philadelphia. 743 pp.
- Young, T. C. and D. L. King. 1973. A rapid method of quantifying algal carbon uptake kinetics. *Limnol. Oceanogr.* 18:978-981.

## APPENDIX

Table A-1. Biomass of vascular hydrophytes over the 1973 growing season in Pond 4 of the Belding, Michigan wastewater treatment system.

| Species                              | Date | Ash-free Dry Weight in $\text{gm/m}^2$ |          |               |    |
|--------------------------------------|------|----------------------------------------|----------|---------------|----|
|                                      |      | Mean ( $\bar{Y}$ )                     | $s^2$    | $s_{\bar{Y}}$ | n  |
| <i>Ceratophyllum demersum</i>        | 5/23 | 26.03                                  | 144.69   | 2.92          | 17 |
|                                      | 6/6  | 17.57                                  | 320.45   | 4.00          | 20 |
| Open water                           | 6/20 | 17.91                                  | 249.47   | 3.62          | 19 |
|                                      | 7/4  | 80.49                                  | 3103.41  | 12.16         | 21 |
|                                      | 7/18 | 106.06                                 | 10499.04 | 23.51         | 19 |
|                                      | 8/1  | 159.40                                 | 19131.18 | 32.60         | 18 |
|                                      | 8/15 | 88.26                                  | 4564.54  | 15.11         | 20 |
|                                      | 9/5  | 129.32                                 | 12791.05 | 27.43         | 17 |
|                                      | 9/26 | 96.33                                  | 1714.09  | 10.04         | 17 |
| <i>Ceratophyllum demersum</i>        | 6/20 | 139.50                                 | 1993.82  | 14.12         | 10 |
|                                      | 7/4  | 230.83                                 | 31438.99 | 51.19         | 12 |
| Within Mat                           | 7/18 | 182.47                                 | 3132.40  | 17.70         | 10 |
|                                      | 8/1  | 177.51                                 | 14979.94 | 38.70         | 10 |
| <i>Potamogeton</i> spp. <sup>a</sup> | 5/23 | 10.27                                  | 197.80   | 3.41          | 17 |
| Open water                           | 6/6  | 11.39                                  | 133.39   | 2.58          | 20 |
|                                      | 6/20 | 10.11                                  | 105.85   | 2.43          | 18 |
|                                      | 7/4  | 18.35                                  | 604.97   | 5.97          | 17 |
|                                      | 7/18 | 20.45                                  | 999.01   | 10.00         | 10 |
|                                      | 8/1  | 6.32                                   | 82.25    | 3.70          | 6  |
| <i>Potamogeton</i> spp. <sup>a</sup> | 6/20 | 8.74                                   | 72.04    | 2.68          | 10 |
| Within Mat                           | 7/4  | 2.42                                   | 9.81     | 2.21          | 2  |
| <i>Lemna minor</i>                   | 7/4  | 18.38                                  | 298.08   | 3.86          | 20 |
| Pond surface                         | 7/18 | 24.78                                  | 451.55   | 4.75          | 20 |
|                                      | 8/1  | 20.10                                  | 590.00   | 5.43          | 20 |
|                                      | 8/15 | 60.58                                  | 3445.78  | 13.13         | 20 |

<sup>a</sup> *P. foliosus* and *P. berchtoldii* combined.

Table A-2. Percentage ash of dry-weight in the vascular hydrophytes in Pond 4 of the Belding, Michigan wastewater treatment system during the growing season, 1973.

| Species                              | Date  | Mean ( $\bar{Y}$ ) | $s_{\bar{y}}$ | $s^2$ | n |
|--------------------------------------|-------|--------------------|---------------|-------|---|
| <i>Ceratophyllum demersum</i>        | 5/23  | 17.43              | .67           | 2.22  | 5 |
|                                      | 6/6   | 18.45              | .34           | .59   | 5 |
|                                      | 6/20  | 29.68              | 1.97          | 19.46 | 5 |
|                                      | 7/4   | 26.10              | 1.62          | 13.07 | 5 |
|                                      | 7/18  | 22.38              | 1.72          | 14.75 | 5 |
|                                      | 8/1   | 21.60              | 1.14          | 6.65  | 5 |
|                                      | 8/15  | 18.78              | .73           | 2.64  | 5 |
|                                      | 9/5   | 20.46              | 2.66          | 35.27 | 5 |
|                                      | 9/26  | 20.85              | 1.31          | 8.62  | 5 |
|                                      | 12/13 | 21.71              | 1.72          | 14.83 | 5 |
| <i>Potamogeton</i> spp. <sup>a</sup> | 5/23  | 18.09              | 1.69          | 14.25 | 5 |
|                                      | 6/6   | 20.37              | 2.85          | 40.74 | 5 |
|                                      | 6/20  | 22.27              | 1.30          | 8.43  | 5 |
|                                      | 7/4   | 22.01              | 2.74          | 37.49 | 5 |
|                                      | 7/18  | 18.79              | 4.54          | 61.93 | 3 |
|                                      | 8/1   | 19.00              | 1.33          | 8.82  | 5 |
|                                      |       |                    |               |       |   |
| <i>Lemna minor</i>                   | 5/23  | 13.84              | -             | -     | 1 |
|                                      | 6/20  | 22.82              | 1.18          | 2.76  | 2 |
|                                      | 7/4   | 21.35              | 1.20          | 7.20  | 5 |
|                                      | 7/18  | 20.78              | .83           | 3.48  | 5 |
|                                      | 8/1   | 19.09              | .42           | .87   | 5 |
|                                      | 8/15  | 20.43              | .37           | .67   | 5 |
|                                      | 9/5   | 19.44              | .39           | .76   | 5 |
|                                      | 9/26  | 19.87              | .29           | .42   | 5 |

<sup>a</sup> *P. foliosus* and *P. berchtoldii* combined.

MICHIGAN STATE UNIV. LIBRARIES



31293010599250