THE PARTICIPATION OF SUBMERSED VASCULAR HYDROPHYTES IN THE PHOSPHORUS BUDGET OF A WASTE STABILIZATION LAGOON

Thesis for the Degree of M. S. MICHIGAN STATE UNIVERSITY DAVID CRAIG MAHAN
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ABSTRACT

THE PARTICIPATION OF SUBMERSED VASCULAR HYDROPHYTES IN THE PHOSPHORUS BUDGET OF A WASTE STABILIZATION LAGOON

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David Craig Mahan

In Pond 4 of the Belding, Michigan wastewater system submersed vascular hydrophytes aid in the removal of phosphorus from the water during the growing season of the plants. Through their uptake of this element the hydrophytes contained an estimated 40% of the phosphorus trapped by the pond at the time of their maximum biomass on August 15. As a result of their decline in biomass and release of phosphorus after this date, they contained only 20% of the total trapped phosphorus when the study was terminated in early fall.

The vascular hydrophyte community present during this study was dominated by <u>Ceratophyllum demersum</u>. This and the other plant species that were present showed luxury consumption of phosphorus, with levels in the plant tissues generally over 1.0% of the ash-free dry weight.

Due to their active growth and resulting large biomass, the macrophytes influenced the water chemistry of the pond. The oxidizing environment that they promoted enhanced the precipitation of inorganic phosphates. The presence of the plants also served to physically stabilize the water column which would promote sedimentation of both inorganic and organic phosphorus containing materials. Sedimentation accounted for 80% of the phosphorus removal during this study.

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BY

David Craig Mahan

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INTRODUCTION

In fresh waters that have not experienced gross pollution phosphorus is often the limiting factor for aquatic productivity (Bahr, et al., 1973; Vollenweider, 1968). This is due to its relatively high requirement by living things in relation to its low abundance in the environment. Man concentrates large amounts of phosphorus as a result of his cultural activities with much of this often dispersed into natural waters. The release of phosphorus into the aquatic environment has resulted not only in a loss of this valuable element to non-available storage but also in a deterioration of water quality. Today, this excessive fertilization of natural waters is one of the most significant causes of water quality problems in North America (Lee, 1970).

Various schemes are now being implemented to reduce this cultural enrichment, an example being waste stabilization ponds. Tertiary waste stabilization ponds are designed to function as manageable sinks for the objectionable material found in municipal effluents (McNabb and Tierney, 1972). These systems usually consist of a series of cells, progressing from anaerobic to facultative to aerobic units. The aerobic final cells are often dominated by various species of vascular hydrophytes with these plants having considerable influence on the physical-chemical milieu of the cell.

One of the objectives of these pond systems is to reduce the amount of phosphorus contained in the waste water. This reduction

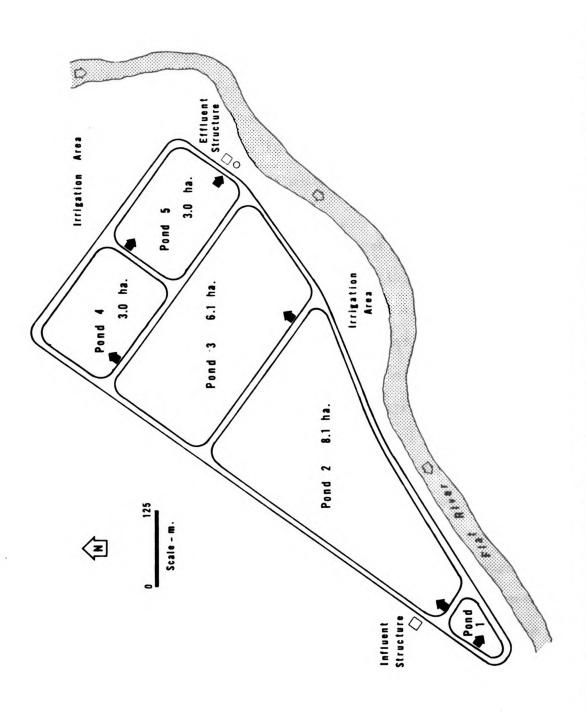
takes place mainly through the sedimentation of phosphorus containing organic material and inorganic chemical compounds and complexes and the accumulation of phosphorus in vascular plant tissues as a result of orthophosphate uptake by epidermal foliage and by the roots. In general, sediments serve as a phosphorus sink with the net flux of phosphorus from the water to the sediments (Lee, 1973). The overall effect of the plants on phosphorus in the water, however, is not so well known.

The primary objective of this study was to determine the degree to which submersed vascular hydrophytes participate in the phosphorus budget of an aerobic waste stabilization pond during the summer growing season.

DESCRIPTION OF STUDY SITE

This study was carried out in the fourth pond of a five pond series of waste stabilization lagoons located in the west-central Michigan community of Belding (Figure 1). Belding is a residential city of 5,000, discharging domestic and industrial waste to the treatment system. The cells in the series can be characterized as anaerobic (Pond 1), facultative (Ponds 2 and 3), and aerobic (Ponds 4 and 5) (Bulthuis, 1973). A flow of water through the system is promoted during the growing season by irrigation of adjacent land from Pond 5, and from the loss of water by seepage through the bottom of Pond 5 (Annett, et al., 1973). The ponds are ordinarily drawn down in the spring and fall for storage in subsequent months. This outflow is discharged to the nearby Flat River.

Pond 4 has an average surface area of approximately 3.0 hectares and a working depth of 2.0 meters. The sediment is composed of approximately 10 centimeters or less of organic matter underlain with native clay. The pond is dominated by large growths of submersed vascular plants with only occasional algal blooms occurring. The dominant hydrophyte species during the summer of 1973 was Ceratophyllum demersum. Potamogeton foliosus, Potamogeton berchtoldii, and Lemna minor were also present in relative abundances that were quantified. Three other species, Potamogeton zosteriformis, Lemna trisulca, and Cladophora fracta (a filamentous Chlorophycean alga) were occasionally found.



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

The water budget from this study is shown in Table 1. From the seasonal totals it can be seen that water entered the pond from the Pond 3 outflow and by rainfall. Pond 3 effluent provided 95% of the incoming water with rainfall being a relatively minor contribution. Seepage and transpiration accounted for the changes in pond volume not explained by the flow and rainfall data with the net result being a small loss from the pond due to these factors. Thus, it would appear that Pond 4 is well sealed at the bottom. This is well documented by the close agreement of the net inputs and outputs to the pond as shown in Table 1.

In looking over the hydrologic budget the most noticeable trend is one of uniformity. Average inflow was 12.2 million liters per week with a pond volume replacement time of 4.5 weeks. The outflow averaged 11.7 million liters per week, 96% of the inflow. Flow rates remained quite constant during the study, being somewhat higher during the first four weeks, probably the end of spring runoff. Since Belding is a residential city and the flow into Pond 4 is moderated by the earlier ponds, this rather constant flow might be expected.

A summary of the chemical and physical parameters that were monitored in this study is given in Table 2. Water temperatures responded rather quickly to air temperature changes though differences between surface and deeper water temperatures were quite small. As would be expected at this latitude, lower temperatures of the range were characteristic of the May and late September sampling dates while higher temperatures generally occurred in mid-June through early September.

The hydrogen ion concentration exhibited large fluctuations in the daily and seasonal readings. The median values show that pH levels

Hydrologic data (values x 10^3 liters) for Pond 4 of the Belding wastewater system during the growing season of 1973. Table 1.

| Date | Volume in Pond | Change in Pond Volume | Inflowa | Outflow ^a | Rain | Evaporation | Evapotranspiration and Seepage ^b |
|-----------------|-------------------|--------------------------|---------|----------------------|--------|-------------|--|
| 5/23 | 53,770 | C t | | | (| | |
| 9/9 | 58,559 | + 4 , (69 | 27,560 | 56,269 | 2,980 | 1,63/ | 4.2,149 |
| 00/9 | 000 25 | - 639 | 26,939 | 25,123 | 1,216 | 2,852 | - 819 |
| 0 / 1 | 136.63 | - 610 | 22,399 | 22,399 | 1,289 | 2,255 | + 806 |
| 1/ t | 71, 101 | - 2,714 | 20,280 | 18,161 | 363 | 2,697 | - 2,499 |
| 01/1 | 00°CC | - 639 | 25,426 | 22,701 | 1,848 | 2,209 | - 3,003 |
| T/0 | 004,40 | - 319 | 25,123 | 24,517 | 1,098 | 2,418 | + 395 |
| 8/15 | 74,089 0.89 | - 1,277 | 34,052 | 34,506 | 659 | 2,722 | + 1,270 |
| 97.5 | 52,971 | + 160 | 37,576 | 37,101 | 1,679 | 2,388 | + 394 |
| Season Total | | - 799 | 219,361 | 210,777 | 11,102 | 19,178 | - 1,307 |

Ror the interval given.

bwater not accounted for by other measurements. Note the season total is 0.6% of total seasonal inflow.

Table 2. Summary of chemical and physical measurements taken on nine twenty-four hour surveys of Pond 4, June-September, 1973.

| Depth | Mean ^a | Range | Mean | Range |
|---------|-------------------|--------------------------|------|-------------------------|
| | Te | mperature (°C) | | |
| | | 4AM ^b | | 4PM ^C |
| Surface | 22.3 | 15.0-26.5 | 24.1 | 16.5-28.0 |
| 1.0 m | 22.0 | 15.5-24.2 | 23.1 | 16.3-26.0 |
| Bottom | 21.1 | 15.3-24.2 | 21.6 | 15.3-25.0 |
| | | рН Ф М р | | 4PM ^C |
| Surface | 9.1 | 7.3-9.7 | 9.6 | 8.5-9.9 |
| 1.0 m | 9.0 | 6.3-9.6 | 9.6 | 8.5-9.7 |
| Bottom | 7.5 | 6.7-8.8 | 8.3 | 7.2 -9. 3 |
| | Disso | lved Oxygen (mg/l) | | |
| | | 8am ^b | | 8 PM^C |
| Surface | 8.7 | 5.2-12.4 | 12.4 | 8.4-17.8 |
| 1.0 m | 8.0 | 5.2-12.4 | 12.2 | 8.4-16.0 |
| Bottom | 1.9 | 0.2-5.4 | 2.9 | 0.8-4.8 |
| | | E ₇ (mv) | | |
| | | 8AM ^b | 1 | 2 Noon ^c |
| Surface | 284 | 102-514 | 483 | 292-744 |
| 1.0 m | 223 | 26-463 | 428 | 226-566 |
| Bottom | 86 | - 96 - 367 | 227 | 67-402 |

a The pH values are medians rather than means.

bLowest values occurred most consistently at this time.

^cHighest values occurred most consistently at this time.

were quite high which in natural waters is often indicative of a large plant biomass (Ruttner, 1963).

As with pH, the level of oxygen in Pond 4 was usually influenced by photosynthesis and respiration. During the first month of the study when plant biomass was relatively small, diurnal oxygen fluctuations at a given depth were not nearly as pronounced as those occurring later in the summer. The very high, supersaturated oxygen levels characteristic of mid summer were not present then either.

Redox potentials also experienced diurnal fluctuations due to the changes in pH and dissolved oxygen. With the exception of the sediment interface, redox measurements were generally above 200 mV, the critical level for the resolubilizing of the iron-phosphates (Graetz, et al., 1973).

Light intensity had a mean seasonal value of 75% and 13% of the surface light at 0.25 meter and 2.0 meters respectively. At 0.25 meter the percent light remaining was fairly stable throughout the season, however, at 2.0 meters light underwent a general decline after mid-June and was reduced to less than 20% remaining after late July. This reduction of light during the last two months of the study was a result of the large hydrophyte biomass and a bloom of Microcystis sp. which took place.

Aerobic lagoons are characterized by oxidation of organic materials and nutrients with this oxidizing environment maintained by the photosynthetic activities of the plants (Cooper, 1966). In view of the results in Table 2, this pond can be classified as an aerobic, oxidizing environment for the period of the study. Reducing conditions were consistently approached only at the sediment interface with low dissolved

oxygen and redox potential levels occurring for just a short time during the day.

METHODS

This study took place over an eighteen week period from May 23, 1973 to September 27, 1973 with nineteen sampling trips being made to the study site. Nine of the sampling trips were twenty-four hour sampling surveys and took place on the dates shown in Table 1. twenty-four hour trips consisted of: 1) measurements of dissolved oxygen, pH, redox potential, temperature, and light extinction; 2) water and plant samples; 3) measurements of flux of water in the pond. Water chemistry and temperature profiles were done every four hours, beginning on Wednesday at noon and ending Thursday at noon. Flow measurements and water samples of the influent and effluent of the pond were also taken at these four hour intervals. Sampling of the pond water and the macrophytes was done on Thursday afternoon just before returning to the laboratory. The remaining ten sampling trips took place on alternate Thursdays in this eighteen week period and consisted of the above mentioned measurements and samples with the exception of the macrophyte sampling.

Temperature and oxygen were measured with a Yellow Springs
Instrument Model 51 automatic compensating probe. Redox potential
and pH values were determined with a Beckman Model SBL and SBX,
respectively. Flow-rate measurements were made with a Gurley current
meter. Volume of input and output was calculated from the flow-rate
and the cross sectional area of the conduits. Light extinction was
measured with a submarine photometer.

Water sampling in the pond was done by lowering an acid washed one-liter bottle below the surface of the pond from a boat. Initially another sample was taken near the bottom but this additional sample was discontinued when phosphorus concentrations were found to be almost identical at the two locations. Samples from the inflow and outflow were taken from the bottom of the conduits between ponds with a sampler specially constructed to open in the stream of flow within the conduits. A single sample was taken on the short term trips while an approximately one-liter sample was composited from the four-hour samples on the basis of flow on the twenty-four hour trips.

A portion of the water samples was filtered through a 0.45 u Millipore filter for dissolved orthophosphate analysis upon return to the laboratory with the remainder of the sample refrigerated. The refrigerated samples were digested in a 4:1 mixture of concentrated nitricularity acid for total phosphorus analysis.

Sampling for submersed macrophytes was done at random locations in the pond but varied according to the occurrence of the species.

Most sampling was done by dropping a weighted cylinder (0.1135 m²) to the bottom and collecting the plants inside. When floating mats of the submersed plants occurred, the cylinder was raised into the mat from below. Lemna minor, a floating macrophyte, was collected after randomly placing a 25 cm² sampler in the mat of this species. The total amount of plant material from these samples was placed in individual plastic bags. Each sample was then washed and separated by species in the laboratory. The plants were dried in a forced-draft oven at 80°C and weighed. Five grams of plant tissue were digested in a Bethge distillation apparatus with a 5:1 nitric-perchloric acid

mixture. Total phosphorus measurements were made on diluted subsamples from this digestion with mean values for each species on a specific date established by replicate analyses.

All phosphorus determinations were done by the vanadomolybdophosphoric acid method as described in Standard Methods (APHA, AWWA, WPCF, 1971). Absorbance was measured on a Bausch and Lomb Spectronic 20 colorimeter with a blue filter at 400 mu.

In an attempt to quantify the effect of the macrophytes upon the chemical milieu and primary productivity of the pond specially constructed enclosures were used. These enclosures were made of 0.32 centimeter plexiglass with dimensions of 0.61 meter by 0.61 meter by 1.53 meters and had tight fitting lids made of the same material which floated on the water surface. They were placed at a 1.1 meter water depth in various locations in the pond with determinations of dissolved oxygen, pH, and redox potential made at dusk, dawn, and dusk for a twenty-four hour period. On the dates when the enclosures were put in the pond and monitored two of the structures were placed over a sample of the C. demersum population and two other structures were placed over sediments cleared of macrophytes. Gross oxygen production was estimated by addition of the oxygen lost from the enclosure from dusk to dawn to the oxygen gained in the enclosure from dawn to dusk of the following evening. Oxygen production by the vascular hydrophytes was taken as the amount remaining after oxygen production in the open enclosures was subtracted from that produced in the hydrophyte containing enclosures. Gross primary production in terms of g carbon/m²/day was estimated by multiplying the oxygen production by a factor of 0.312 (Vollenweider, 1969).

RESULTS

The results from the macrophyte sampling are shown for the different species of the plant community in Table 1. A one way analysis of variance was done for each species (<u>Potamogetons</u> were combined) with Duncan's Multiple Range Test run if treatment effects were present (Table A-8). Logarithmic transformations were carried out on all biomass data prior to statistical analysis since means were positively correlated with variances (Sokal and Rohlf, 1969). Variances were also checked with the F-max test to insure homogeneity before analysis of variance was done.

C. demersum (Table 3). It generally accounted for over 90% of the plant biomass. When vascular plant growth began, this species covered 95% of the pond bottom and it maintained this cover over the entire season. Wet growth rates were determined for the plant species by measuring changes in standing crop biomass (g/m²) with a maximum growth rate of 3.0 g/m²/day observed for C. demersum between July 4 and 18. Its maximum estimated biomass occurred on August 15 with 5100 Kg of organic weight present in the pond, a standing crop of 169 g/m². The results of the analysis of variance show that there was a highly significant change in biomass over the summer (Table A-8). A real change in biomass such as this is often a characteristic of rapidly expanding biological populations.

Table 3. Biomass and net productivity (ash free dry weight) of vascular hydrophytes in Pond 4 as estimated from curves fitted to the hydrophyte sampling data.

| Species: | | tophyllum mersum | | amogeton ^b | <u>Lemna</u> minor |
|----------|------|-----------------------|-----------------|-----------------------|-----------------------|
| Date | Kga | g/m ² /day | Kg ^a | g/m ² /day | Kg ^a |
| 6/6 | 362 | 3.5 | 141 | | |
| 6/20 | 987 | 1.5 | 210 | 0.2 | 1 |
| 7/4 | 2164 | 2.8 | 279 | 0.2 | 159 |
| 7/18 | 3416 | 3.0 | 155 | -0.5 | 238 |
| 8/1 | 4336 | 2.3 | 25 | -1.0 | 81 |
| 3/15 | 5101 | 1.9 | | | 194 |
| 9/5 | 4577 | -0.8 | | | 49 |
| 9/26 | 3368 | -1.8 | | | 18 |
| | | | | | |

^aAntilog of estimated mean + $s^2/2$ from ideal curve with exception of <u>L. minor</u> values which are field estimates.

 $b_{\underline{P}}$. foliosus and \underline{P} . berchtoldii combined.

Another way of expressing the biomass data which serves to better show the suitability of the environment for a plant species is biomass doubling time (Tierney, 1972). This involves comparisons of the time required for doubling plant biomass from the beginning of growth to the inflection point on the seasonal growth curve. It can be seen in Figure 2 that the <u>C. demersum</u> biomass doubled almost four times during the summer. As environmental conditions in the pond changed and growth slowed down, the doubling times became longer. The shortest doubling time represents the maximum unlimited rate of growth for a plant species in a particular environment (McNabb and Tierney, 1972). In Pond 4 the doubling time of 10 days between June 6 and 16 represents the maximum rate of growth for <u>C. demersum</u>.

Potamogeton foliosus and P. berchtoldii made up a sizable portion of the plant biomass early in the summer but were eliminated after August 1. Since the two pondweeds are quite similar and must be differentiated taxonomically by reproductive structures, they could not be accurately separated until they formed seeds, just prior to vegetative extinction of the populations. Therefore, for the purpose of analysis their material was lumped together. When identification was possible, it was found that most of the Potamogeton biomass consisted of P. foliosus. The pondweed biomass did not change significantly during its occurrence which is indicative of the small maximum growth of 0.2 g/m²/day being observed. The largest Potamogeton spp. biomass realized was on July 4 with 279.5 Kg organic weight found at 13.3 g/m². The doubling of biomass took approximately 28 days, indicating a poor coupling of the pondweeds to the environment present during this study.

The other important vascular plant species in this pond was Lemna minor, common duckweed. Although it occasionally covered up to

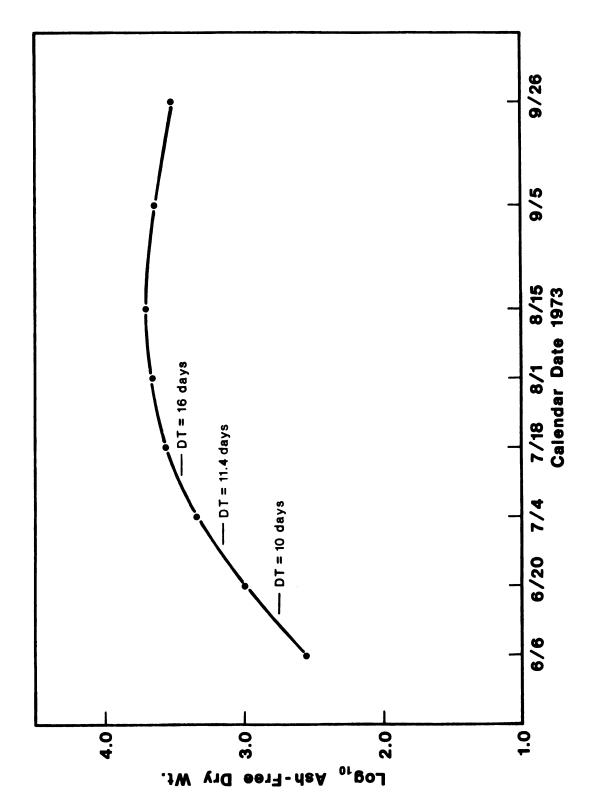


Figure 2. Rates of growth for Ceratophyllum demersum in Pond 4 of the Belding system.

l hectare of the surface of the pond, it never made up a large portion of the plant biomass. A maximum biomass of 237.6 Kg was achieved by <u>L. minor</u> on July 18. During its initial growth the duckweed biomass doubled rapidly with the pond apparently providing optimum habitat (Table 3).

As previously mentioned, plexiglass enclosures were used to obtain an estimate of the relative contribution to the primary production of the pond by the vascular hydrophytes and phytoplankton and to determine any effects of the hydrophytes on the water chemistry of the pond by looking at water chemistry differences between the two types of enclosures. Dissimilarities between the enclosures and the pond which should be kept in mind are the absence of mixing and gas exchange in the enclosures due to their structure.

The calculated gross production rates for each of the dates along with mean values for a seven week period are given in Table 4. The enclosure results show a higher primary production rate for the vascular plants during this time period, their average being almost 60% of the total production. Since relatively few areas of the pond were free of vascular plants, the enclosure without vascular plants may have over estimated the phytoplankton primary production in the pond. If that is true, the subtraction of this amount from the production in the C. demersum containing enclosure to determine vascular plant production would have resulted in an under estimation of submersed vascular plant production. Therefore, from the results obtained, vascular hydrophytes appear to be the largest contributor to primary production in Pond 4 at this time of year.

Water temperature differences were slight between the two types of enclosures as would be expected, however, there were slightly

Table 4. Gross primary production for vascular hydrophytes and phytoplankton as determined with plexiglass enclosures.

| | Vascular Hyd | | Phytoplankton |
|------|--|-------------------------|--|
| Date | g O ₂ /m ² /day ^b | g C/m ² /day | g O ₂ /m ² /day ^b |
| 7/19 | 4.78 | 1.49 | 7.53 |
| 8/2 | 9.10 | 2.84 | 3.92 |
| 8/16 | 8.73 | 2.72 | 8.21 |
| 9/5 | 17.34 | 5.41 | 8.05 |
| Mean | 9.99 | 3.12 | 6.93 |

^aProduction estimate in terms of the standing crop biomass of \underline{c} . $\underline{demersum}$ on the given data within the pond.

bMean value for two enclosures.

higher temperatures in the enclosures as compared to the pond around them (Table A-2). Although pH was similar in the two types of enclosures down to 0.5 meter, it was higher in the vascular hydrophyte enclosures at the bottom. In the pond, pH was lower than that in the enclosures. Dissolved oxygen changes were quite different in the two kinds of enclosures with higher oxygen levels at dusk in the vascular plant enclosures and higher levels at dawn in the phytoplankton enclosures. These differences are a result of the greater productivity in the vascular hydrophyte enclosures. The oxygen concentrations were alike in the pond and in the vascular plant enclosures at dusk indicating a similarity of the two environments. At dawn the pond water oxygen content was similar to that in the phytoplankton enclosures. If the C. demersum containing enclosures did approximate the pond environment, rates of respiration should have been nearly alike. However, oxygen could not diffuse from the atmosphere into the enclosures at night due to their covers, so lower values would have resulted. Redox potentials were quite similar in all three situations with the exception of the dawn pond readings which were higher than those of the enclosures.

When considering phosphorus uptake by vascular plants, it is important to establish the phosphorus status of the water in which the plants were growing. As will be more fully explained in the following section on the phosphorus budget, the phosphorus concentrations in the water were subjected to a regression analysis in order to establish lines and/or curves best estimating phosphorus in the water over the summer (Table 5). The best fits for influent phosphorus (both total phosphorus as P and dissolved orthophosphate as P) were

Table 5. Phosphorus concentrations (mg/l as P) as taken from curves fitted to the data of Table A-3.

| Sample | Date | Total P | Dissolved Ortho-P |
|---------|------|---------|----------------------|
| Inflow | 6/6 | 3.49 | 3.14 |
| | 6/20 | 3.27 | 2.87 |
| | 7/4 | 3.05 | 2.59 |
| | 7/18 | 2.83 | 2.31 |
| | 8/1 | 2.62 | 2.03 |
| | 8/15 | 2.40 | 1.75 |
| | 9/5 | 2.07 | 1.34 |
| | 9/26 | 1.74 | 0.92 |
| Pond | 6/6 | 2.65 | 2.12 |
| | 6/20 | 2.02 | 1.60 |
| | 7/4 | 1.46 | 1.16 |
| | 7/18 | 1.06 | 0.84 |
| | 8/1 | 0.81 | 0.64 |
| | 8/15 | 0.73 | 0.56 |
| | 9/5 | 0.91 | 0.66 |
| | 9/26 | 1.45 | 1.04 |
| Outflow | 6/6 | 3.14 | 2.39 |
| | 6/20 | 2.87 | 1.82 |
| | 7/4 | 2.59 | 1.37 |
| | 7/18 | 2.31 | 1.03 |
| | 8/1 | 2.03 | 0.82 |
| | 8/15 | 1.75 | 0.72 |
| | 9/5 | 1.34 | 0.81 |
| | 9/26 | 0.92 | 1.16 |

straight lines of negative slope showing a gradual decline in incoming phosphorus over the summer. The actual value for total phosphorus in the influent was 3.66 mg/l on May 23 and declined to 1.66 mg/l on September 27, a reduction of 55%. Dissolved orthophosphate was 3.41 mg/l at the beginning of the study and ended up at 0.91 mg/l, a 73% reduction. Over the duration of the study, 501 Kg of phosphorus came into the pond.

The effluent total phosphorus and dissolved orthophosphate levels almost mirrored one another over the season. However, their curves were very different from those of the inflow indicating a marked effect on phosphorus in the water as it passed through the pond. The outflow values actually measured at the beginning of the summer (3.35 mg/l total-P, 3.15 mg/l ortho-P) and at the end (1.65 mg/l total-P, 1.16 mg/l ortho-P) were close to influent values, but over the majority of the study they were quite different. From June 6 to September 5 total phosphorus in the outflow was at least 0.90 mg/l less than the inflow with the greatest differences on July 18 and August 1 of 1.60 mg/l. The differences between influent and effluent dissolved orthophosphate were similar over this time period also since the curve was similar to that of total phosphorus as mentioned above. A total of 386 Kg of phosphorus left the pond during the study. This was 77% of the total influent.

In the pond water the phosphorus concentrations were similar to those found in the effluent water. Total phosphorus in the pond was 2.65 mg/l on June 6 and 0.73 mg/l on August 15. Dissolved orthophosphate was 2.12 mg/l and 0.56 mg/l on the same respective dates. Generally the pond water values were somewhat lower than those of the outflow.

Phosphorus levels in the plant tissues expressed as a percentage of the ash-free dry weight were determined over the summer as shown in Table 6. The results were handled statistically like those for biomass sampling, with the exception of transformations which were not required.

The mean total phosphorus in <u>C</u>. <u>demersum</u> varied significantly over the summer (Table A-8), ranging from 1.37 to 2.24% phosphorus.

The highest mean came on June 20 during the species exponential growth phase while the lowest value was found when the plant biomass was declining at the end of the season. A plant sample taken from the pond on December 13 during winter senescence had the lowest phosphorus content of 1.09%. In Figure 3 a significant linear regression (p < .05) of phosphorus in the plant tissue on the dissolved orthophosphate concentration in the water is shown. With the exception of the first date when seasonal growth was beginning and the last date when biomass was quickly declining, these points fit a straight line quite well. These changes in plant phosphorus in accord with changes in water concentrations suggest a relationship which will be discussed in a following section.

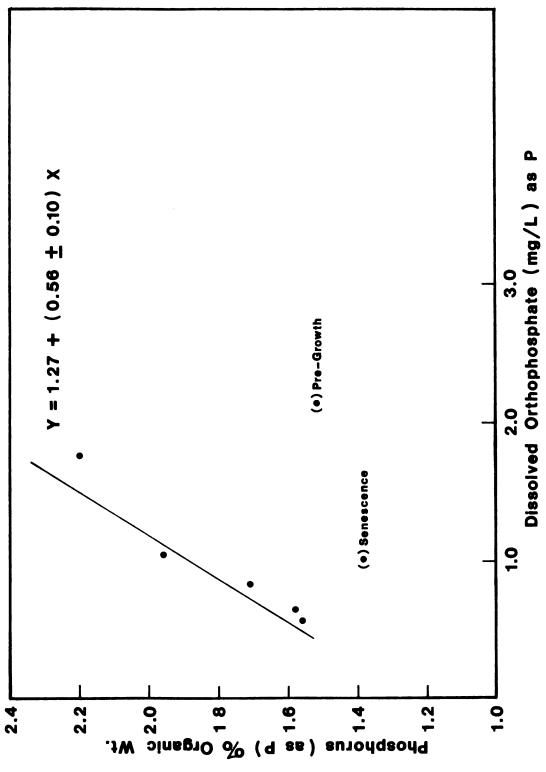
In <u>Potamogeton spp.</u>, phosphorus means ranged from 1.45 to 1.81% with significant differences being found between sampling dates (Table A-8). These means appear to be related to the state of activity of the plants and the changes in water concentrations in a manner similar to <u>C</u>. demersum. Phosphorus in <u>L</u>. minor also changed significantly over the summer with means ranging from 0.63 to 1.34%. The highest phosphorus level was found on June 20 when the duckweed was growing most rapidly while the lowest value came on September 5. Again there appears to be a relationship between phosphorus in the plant and that

Table 6. Total quantity of phosphorus in the vascular hydrophytes of Pond 4 as derived from curves fitted to the data for ash-free biomass and tissue concentration (Table A-5) (also given) for dates of sampling in 1973.

| Date | P as % Ash-Free Dry Wt. | P Held in Kg | P Uptake ^a |
|-------------|-------------------------------|--------------------|-----------------------|
| Ceratophyl. | lum demersum | | |
| 6/6 | 1.51 | 5.5 | 26.7 |
| 6/20 | 2.24 | 22.1 | 16.1 |
| 7/4 | 1.90 | 41.1 | 11.7 |
| 7/18 | 1.63 | 55 .7 | 19.2 |
| 8/1 | 1.69 | 73.3 | 15.2 |
| 8/15 | 1.66 | 84.7 | |
|)/5 | 1.59 | 72.8 | |
| 9/26 | 1.37 | 46.1 | |
| Potamogeton | n spp.b | | |
| 6/6 | 1.70 | 2.4 | 20.3 |
| 5/20 | 1.81 | 3.8 | 15.4 |
| 7/4 | 1.75 | 4.9 | |
| 7/18 | 1.60 | 2.5 | |
| 3/1 | 1.45 | 0.4 | |
| Lemna mino | <u>r</u> | | |
| 5/20 | 1.33 | 0.1 | 10.8 |
| 7/4 | 1.06 | 1.7 | 5.1 |
| 7/18 | 0.86 | 2.0 | |
| 3/1 | 0.71 | 0.6 | |
| 8/15 | 0.63 | 1.2 | |
| 9/5 | 0.62 | 0.3 | |
| 9/26 | 0.75 | 0.1 | |

 $^{^{\}mathbf{a}}\mathbf{P}$ uptake during species growth expressed in mg P/g ash-free dry weight.

 $^{^{}b}\underline{P}$. foliosus and \underline{P} . berchtoldii combined.



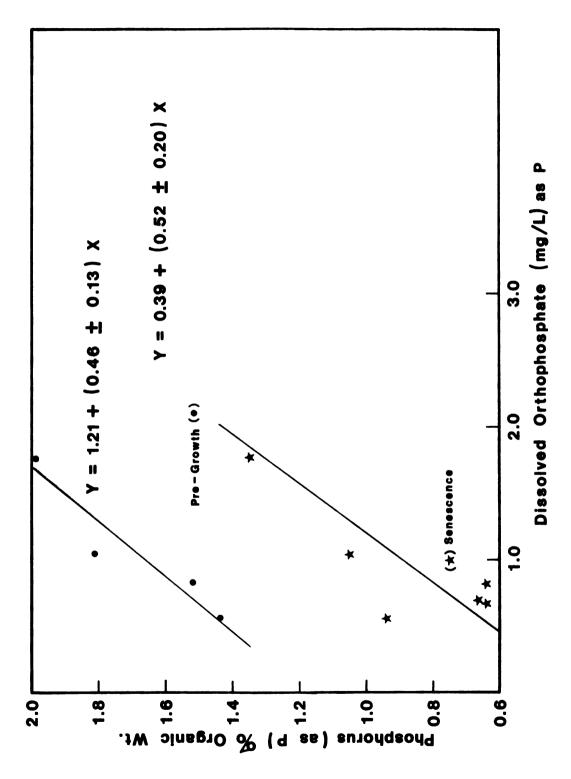
Regression of tissue-P on ambient concentration of orthophosphate for Ceratophyllum demersum during growth. Figure 3.

in the water with the tissue phosphorus declining rapidly with decline of phosphorus in the pond water. The linear regressions for <u>Potamogeton spp.</u> and <u>L. minor</u> shown in Figure 4 were both significant (.05 < p < .10).

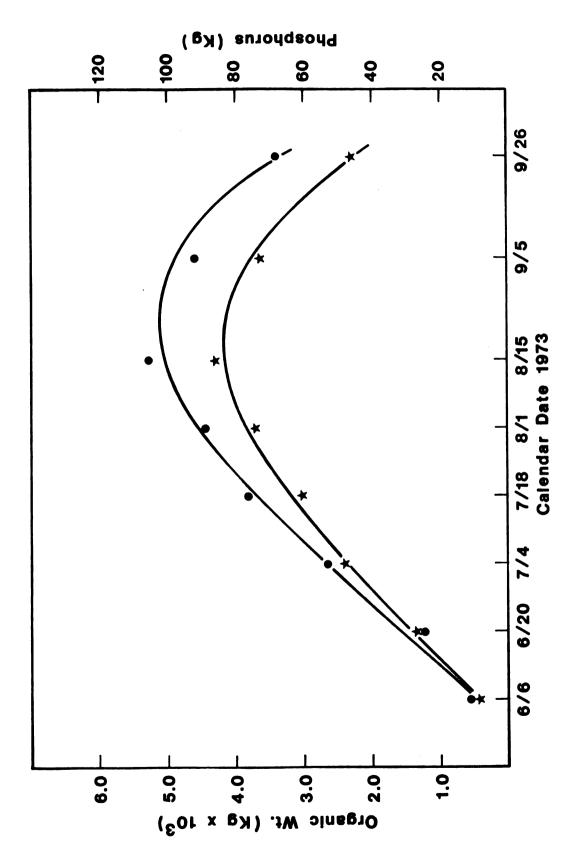
In Table 6 the total estimated phosphorus content of the vascular plant species is shown. Due to its large biomass and relatively high concentration in its tissues, <u>C. demersum</u> was the dominant plant species in terms of total kilograms of phosphorus in the plant biota. With the exception of the first month of the study, the <u>Potamogeton spp.</u> contained only a small part of the vascular plant phosphorus.

<u>L. minor never contained a significant amount of phosphorus.</u>

Figure 5 is a representation of the total plant community biomass and its phosphorus content during this study. On August 15 the maximum amount of 86 Kg of phosphorus in the plants corresponded with the maximum plant biomass of 5300 Kg. The relative amount of increase in these two curves is similar, phosphorus increased seven times while biomass increased nine times. As the plants were growing, they took up phosphorus from their environment. It was noted earlier that with each individual species phosphorus uptake was greatest in the early part of the seasonal growth as phosphorus content per unit weight of plant tissue gradually declined after this high rate of uptake. This decreased uptake is shown by the somewhat smaller relative increase in phosphorus content as compared to biomass. Since the two curves are not parallel, these two parameters are not directly related. decline in phosphorus uptake relative to organic weight increase may have been due to the decreased phosphorus in the water which would have resulted in less phosphorus being available to the plants.



Regression of tissue-P on ambient concentration of orthophosphate for Lemna minor (*) and Potamogeton spp. (o) during growth. Figure 4.



Biomass (o) of the plant community and total amount of phosphorus (*) held by the community during the growing season. Figure 5.

As shown by the water budget (Table 1), changes in the pond volume are mainly due to the inflow from Pond 3 with rainfall, evaporation, seepage, and transpiration causing only minor changes. Therefore, the phosphorus entering Pond 4 is mainly from Pond 3 and this phosphorus either remains in the pond or passes out in the effluent. The amounts of phosphorus in the system components on a given date are not actual field determined values. For the purpose of formulating a phosphorus budget and looking at general trends in the system, lines of best fit were determined with a polynomial regression program on a CDC 6500 computer. Curves were fitted with increasingly higher degree polynomials until a reasonable approximation of the data was obtained (Table A-7). The amounts of change in phosphorus in a system component between two sampling dates are denoted as differences. These

Referring to Table 7, column 2, the change in Kg of phosphorus in the plants over a time interval was determined by multiplying the estimated biomass of the plants in Kg ash-free dry weight by the percent phosphorus in the ash-free dry weight and subtracting the previous estimate from it. Column 4, the change in total phosphorus in the pond water, was found by multiplying the mg/l concentration of phosphorus by the total volume of the pond and subtracting this from the previous estimate. Column 5, total phosphorus trapped in the pond over time, was calculated by subtracting the average effluent amount multiplied by concentration from the average influent amount multiplied by concentration. Column 6, total phosphorus going to the plants or sediments over time, was found by subtracting the change in total phosphorus in the pond (column 4) from the amount of phosphorus

Table 7. Phosphorus budget (in Kg P) for Pond 4 established with values from curves fitted to the data for phosphorus levels in the water and vascular hydrophytes.^a

| Date | Plants | Diff. | Pond Water | Diff. | Inflow- Outflow | Plants & Sediments | Sedi- ments |
|--|---|--|--|--|---|--|---|
| Column | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 6/6 6/20 7/4 7/18 8/1 8/15 9/5 9/26 | 7.9 25.9 47.7 60.2 74.2 85.9 73.1 46.3 | 18.0 21.8 12.5 14.0 11.7 -12.8 -26.8 | 155.2 177.0 84.3 58.4 44.1 39.5 48.1 76.8 | -38.2 -32.7 -25.9 -14.3 - 4.6 8.6 28.7 | 15.6 9.6 15.1 20.0 16.7 22.8 15.4 | 53.8 42.3 41.0 34.3 21.3 14.2 | 35.8 20.5 28.5 20.3 9.6 27.0 |
| Study Total | | 38.4 | | -78.4 | 115.2 | 193.6 | 155.2 |

^aBudget calculations explained on previous page.

trapped in the pond (column 5). Column 6 minus column 2 gave column 7, the total phosphorus going to the sediments between two dates.

The total phosphorus content (column 1) and the estimated amount of net phosphorus flux (column 2) in the vascular hydrophyte community are shown in Table 7. Over the time of this study there was a net accumulation of phosphorus in the plants; approximately 20% of the total phosphorus trapped by either plants or sediments. If the large release of phosphorus by the vascular plants during the last six weeks had not occurred, this amount would have been substantially greater.

Columns 3 and 4 show the change in total phosphorus in the pond water during the study. This part of the system showed a trend opposite to that of the plants with a net phosphorus loss from this compartment over the season. Only during the last six weeks when the plant release of phosphorus was taking place was there any increase in the amount of phosphorus contained in the pond water. Between June 6 and August 15, phosphorus in the water decreased 75% (from 155.2 Kg to 39.5 Kg).

The difference between influent and effluent phosphorus is shown in column 5. The percent of the phosphorus coming into the pond that went out through the outflow decreased over the summer (Table A-4). The important function of this column, however, is in combination with column 4 to give column 6, the amount of phosphorus trapped in the pond by the other two components (plants and sediments) over time. Just by itself column 5 is ambiguous since it does not account for changes in the pond water.

The amount of phosphorus going to the plants and sediments (column 6) was a positive quantity until the final three weeks of the study.

The net amount was almost 40% of the 501 Kg of phosphorus that came into the pond during the study. The amount trapped during each time interval decreased throughout the summer with this decrease related most closely to the changes of phosphorus in the pond water (column 4).

Column 7 is an estimate of the total phosphorus being sedimented over time. No clear trends seem evident except there is a net deposition of phosphorus to the sediments, not only over the entire study period but also over each individual time interval. On September 26, of the cumulative total phosphorus going to the sediments and plants, the sediments accounted for 80%.

To aid in an explanation of the results a second table was constructed with the data. Table 8 is a listing of the cumulative totals added over each time period. In this way the net changes in the various components of the budget over time can be observed. The amount of phosphorus trapped or released by the sediments and plants can be used as a divisor for the cumulative amounts of phosphorus trapped in each of these two components (columns 2 and 7). Over the first 70 days of the sampling period, 40% of the phosphorus trapped in the plants and sediments went to the plants. Looking at the totals for the entire period, this percentage changed markedly with only 20% of this trapped phosphorus in the plants.

Another way of looking at the budget is shown by Table 9. Partitioning coefficients express all the components in terms of the total influent phosphorus with this total coming into the pond identified as one unit. Phosphorus processed in this system can be thought of as the total amount of phosphorus handled and is equal to influent phosphorus minus stored phosphorus (the change in the pond water phosphorus

Table 8. Cumulative totals of differences (in Kg P) from Table 5.

| Date | Plants | Pond Water | Inflow- Outflow | Plants & Sediments | Sedi- ments | • | in Sediments ^a |
|-------------|--------|-----------------|--------------------|--------------------|----------------|--------------|------------------------------|
| Column | 2 | Ţŧ | 5 | 6 | 7 | | |
| 6/6 6/20 | 18.1 | -38.2 | 15.6 | 53.8 | 35.7 | 33.6 | 66.4 |
| 7/4 | 39.8 | - 70.9 | 25.2 | 96.1 | 56.2 | 41.5 | 58.5 |
| 7/18 | | -96.8 -111.1 | 40.3 60.4 | 137.2 171.5 | 84.8 | 38.2 38.7 | 61.8 61.3 |
| 8/1 8/15 | 78.0 | -115.7 | 77.1 | 192.8 | 114.7 | 40.5 | 59.5 |
| 9/5 | | -107.2 | 99.9 | 207.0 | 141.8 | 31.5 | 68.5 |
| 9/26 | 38.4 | -78.4 | 115.3 | 193.6 | 155.2 | 19.8 | 80.2 |

^aColumns 2 and 7 were divided by column 6 to determine these percentages.

bColumn numbers from Table 5.

Table 9. Coefficients of partitioning of phosphorus in Pond 4.ª

| Time | Processed | Sedi- ments | Plants | Outflow | % in Plants | % in Sediments ^c |
|-------------------|-----------|----------------|--------|---------|----------------|--------------------------------|
| 6/6 - 7/3 | 1.44 | 0.35 | 0.25 | 0.84 | 17 | 24 |
| 6/6 - 8/14 | 1.33 | 0.32 | 0.22 | 0.78 | 17 | 24 |
| 6/6 - 9/26 | 1.16 | 0.31 | 0.08 | 0.77 | 7 | 27 |

^aThese coefficients were calculated by taking the total input to the pond over the time period as 1.00 unit.

bProcessed equals inflow minus storage with storage being the change in total Kg phosphorus in the pond water.

c% in plants equals the percent of processed phosphorus contained by the plants. % in sediments equals the percent of processed phosphorus in the sediments.

over time). An amount of phosphorus processed greater than that coming in results when phosphorus is lost from the water column. Since there was a net loss of phosphorus from the water over the summer, processed phosphorus was greater than unity. The effect of the phosphorus loss from the plant tissue over the final six weeks is shown by the substantial decrease in the amount of processed phosphorus in the plants. The sediments seemed to be a rather constant factor over the entire study as demonstrated by the similar amounts of processed phosphorus that was sedimented over the three time intervals.

DISCUSSION

Phosphorus is absorbed by submersed hydrophytes in relation to its availability with concentrations in the plant tissue often far above the critical level needed for maximum growth (Gerloff and Krombholtz, 1966). Luxury consumption of the element is especially evident in highly enriched environments such as Pond 4. The highest means reported in this study are at least twice as high as the maximum values ordinarily observed in field studies. In a review of aquatic plant mineral composition, Boyd (1967) reports phosphorus levels ranging from 0.1 to 0.6% by dry weight. Other investigators have also indicated phosphorus tissue levels generally within this range (Boyd, 1970b; Caines, 1965; Gerloff and Krombholtz, 1966; Stake, 1968). Only Adams et al. (1971), Fish and Will (1966), and Tierney (1972) have observed phosphorus levels above 1.0% of the dry weight with all three of these studies being conducted in nutrient-rich environments.

In looking over different studies describing phosphorus levels in vascular hydrophyte tissues it is evident that differences between species occur. As seen in Table 4 this was true for the species occurring in Pond 4 in 1973. These differences suggest alternate ways of obtaining phosphorus among the plant species. Two obvious dissimilarities of the plants in this study are their locations of growth and anatomical structure. Since Lemna minor floats on the surface with short, unattached roots, its source of nutrition (other than CO_2) must be the pond water. With submersed macrophytes, nutrients are

obtained from both the water and the substrate, though there is considerable disagreement on this. Den Hartog and Segal (1964), for example, consider that submerged plants are almost completely dependent on the aquatic medium for their nutrition, absorbing nutrients through the epidermis of their foliage. According to Bristow and Whitcombe (1971), however, the substrate (via the roots) is most important in plant nutrition. DeMarte and Hartman (1974) and McRov and Barstow (1970) have noted ³²P uptake in both the roots and the plant shoots. Denny (1972) suggests a range among aquatic plants from emergent plants that rely entirely on the roots for nutrient supply to rootless plants that rely solely on their shoots for absorption. He also suggests that the absorption site may alter, depending on relative nutrient levels in substrate and solution. Since C. demersum is rootless, its major source of phosphorus nutrition is probably the ambient water. The two Potamogetons sampled have well developed roots (the roots of P. foliosus are not very extensive), therefore they obtain nutrients from both the water and substrate. As indicated in Figure 4 the association between tissue and ambient phosphorus is such that much of the pondweed's luxury consumed phosphorus may have come from the water.

In the context of this study the importance of phosphorus accumulation and fluctuation in the plant tissues is the effect this has on phosphorus in the other two main components of the pond and the effect on the budget as a whole. A net uptake of phosphorus by the hydrophytes will result in lower amounts in the water and sediments while a net release will have the opposite effect. Phosphorus concentrations in the water underwent a gradual decline over the summer resulting

in a net loss of phosphorus from the pond water. Total phosphorus in kilograms in the water was negatively correlated (r = -.97) to the plant biomass. This would indicate that the uptake of phosphorus by the developing plant biomass may have influenced the decline of phosphorus in the water.

Although phosphorus levels in all plant species decreased after their early, high levels, the total phosphorus in the plant community increased to a maximum on August 15. The increase in plant biomass overshadowed the decrease in tissue content with the total kilograms of phosphorus in the plants highly correlated (r = .99) to the total plant biomass. Though uptake (mg P/g plant) was higher at the beginning of the summer, Table 9 shows that the relative amount of processed phosphorus in the plants was almost identical for the first and second time periods. Apparently, the plants maintained a constant rate of phosphorus uptake in relation to the concentration of phosphorus in the water. The season's total of only 7% of the processed phosphorus in the plant tissues shows the marked effect on the water and sediment components of the phosphorus release during the last six weeks. If sampling had continued, the amount of processed phosphorus in the plants would have been expected to decline even further with the sediments and the water being virtually the only reservoirs for net trapped phosphorus.

By looking at the cumulative totals in the phosphorus budget

(Table 6) it can be seen that the plants maintained a hold on about

40% of the trapped phosphorus up to August 15, containing an estimated

78 kilograms. Since most of the phosphorus in the major plant species,

C. demersum, probably came from the water, much of the phosphorus in

the plants must have come from the current growing season inputs.

Thus, while the submersed plants were actively growing in Pond 4, a large amount of phosphorus trapped in the pond was trapped solely as a result of its absorption into their tissues.

The role of submersed hydrophytes in the phosphorus balance in fresh waters has not received much attention in limnological writing. Various scientists have noted decreasing soluble phosphorus levels in the water due to phytoplankton uptake (Ruttner, 1963), however, this association with ambient phosphorus and vascular plants has seldom been expressed. In a study of Linsley Pond, Conneticut, Cowgill (1968) noted a sharp decline in total phosphorus in the water coincident with the developing submersed vegetation and suggested this decline was due to the vegetation and that this was probably a normal occurrence in the pond. Rigler (1956), in attempting to account for ³²P added to the littoral zone of an experimental lake, concluded that the major loss was to the littoral zone rather than precipitation to pelagic sediments. This ³²P loss to the littoral zone was presumably due to vascular hydrophytes there. In a one year study of the Belding pond system, Annett et al. (1973) observed a mean phosphorus level of .59 ppm at the system outflow from July 21 to October 12, 1969. reported that the concentrations and variability during this time were considerably lower than the rest of the year. This would roughly coincide with the time of year when the submersed hydrophytes most strongly influence the system.

Although the total kilograms of phosphorus did increase in the plants through August 15, this amount would have been substantially larger if the percent of phosphorus in the plant tissues had not

declined. Since the major source of the plant phosphorus was probably the pond water, this decline in phosphorus in the plant tissues is reasonable due to the decline in concentration in the water.

Another reason for phosphorus decline in the plant tissues is the possible relationship between tissue phosphorus and metabolic activity. Coinciding with decreased tissue levels was a slowdown in growth rate as seen not only by the decreased production rates (g/m²/day) but also by the increased doubling times. Relative to the critical levels established by Gerloff and Krombholtz (1966), tissue phosphorus was more than adequate for maximum growth. Thus, it was not a phosphorus limitation which caused this decrease in growth. In several similarly enriched environments, Tierney (1972) observed that the rate of phosphorus accrual by C. demersum and Elodea canadensis was fairly close to the rate of biomass synthesis.

Seasonal changes in tissue phosphorus have been noted in the literature indicating a decline in submerged and emergent plant with aging (Boyd, 1969, 1970a; Stake, 1968; Caines, 1965) or indiscriminant variation (Gerloff and Krombholtz, 1966). Caines (1965) and Boyd (1969) found the greatest net absorption of phosphorus during the early growth of the plants with much of luxury consumed phosphorus being later translocated to new growth. Phosphorus levels in vascular hydrophytes are generally associated with fertility of the habitat (Adams et al., 1971; Caines, 1965; Gerloff and Krombholtz, 1966), however, a relationship between the tissue and water has seldom been demonstrated. Tierney (1972) and McNabb and Tierney (1972) have noted a significant high correlation between tissue phosphorus and ambient soluble phosphorus concentrations among several study sites. They also found the plants

grown in an environment high in external phosphorus had higher seasonal variations than those from less fertile habitats.

Besides the reduction of phosphorus by tissue uptake, the vascular hydrophytes may be expected to exert other influences on phosphorus cycling in the pond. One influence which has been briefly mentioned is that of the plants on the water chemistry, specifically the dissolved oxygen, pH, and redox potential. Where they are the dominant plant form, macrophytes are closely associated with the diurnal oxygen changes in lakes and ponds (Sculthorpe, 1967). During the day plants are actively engaged in photosynthesis and release oxygen, thereby increasing the dissolved oxygen in the water. At night though, no oxygen is produced and dissolved oxygen in the water decreases due to plant (and other community members) respiration. Macrophytes store some of the oxygen produced in their lacunal air spaces for use in respiration as night (Hartman and Brown, 1966) which does serve to moderate the amount of diurnal fluctuation, especially on days of low light intensity. The presence of these diurnal fluctuations is evident from the high and low means shown in Table 2. Since macrophytes were such a major part of the pond biota, these fluctuations may have been largely due to their presence. In small and/or shallow lakes, the effect of vascular hydrophytes on diel oxygen fluctuations can be very great (Davies, 1970; Wetzel, 1965). As plant biomass in a system is increased more pronounced oxygen fluctuations may be expected. In general, greater dissolved oxygen changes did occur in mid summer when the biomass was largest with conditions of over 200% saturation occasionally found in dense macrophyte stands.

The addition of oxygen to the water of Pond 4 by the plants would directly affect the solubilities of many of the phosphorus

compounds in the water. Most phosphorus not organically held exists in cationic compounds and complexes (Frink, 1967). These compounds are relatively insoluble in oxygenated waters at pH and redox potential ranges normally encountered, but under anaerobic reducing conditions they become more soluble. Since significant concentrations of Fe, Mm, and other metal species are found in this pond (Bulthuis, 1973; Lisiecki, 1974), dissolved oxygen levels are important in considering available phosphorus in the system.

It has been reported by Mortimer (1971) that at dissolved oxygen concentrations at the sediment surface of over 2.0 mg/l little nutrient release takes place, however, below this level major amounts of phosphorus may be released. From the study results, dissolved oxygen was always above 2.0 mg/l in the water column with the exception of some measurements at the sediment-water interface. Even at the interface the consistently lowest time of the day (8 AM) had a mean of 1.9 mg/l. Due to the generally high oxygen levels in this pond it seems reasonable to conclude that sedimentation of phosphorus compounds was aided by this factor.

Since all but an average of 24% of the plant biomass decomposes, macrophyte decomposition does exert considerable pressure on the dissolved oxygen content of the water (Jewell, 1971). Although some decomposition occurs throughout the growing season, it is most pronounced in the fall when the plants die or return to a small overwintering biomass. A large drop in dissolved oxygen could resolubilize much phosphorus at this time, both in the upper few centimeters of the sediments and much of that in the decomposing plant tissue. Although plant biomass was declining in the pond over the last six weeks of the

study, pronounced drops in dissolved oxygen were not found. However, phosphorus levels did increase in the pond water during this period possibly indicating a release of phosphorus from the macrophytes.

Abundant plant growth in the water has a definite effect on the hydrogen ion concentration. Plants often use up all of the free CO₂ in the water and much of the bicarbonate resulting in a fairly high alkalinity. In extreme conditions, with luxuriant submersed vegetation, the decomposition of bicarbonate can proceed up to the formation of hydroxide with the pH being raised up to about 11 (Ruttner, 1963). Free CO₂ is returned to the system at night due to respiration, resulting in a diurnal change in pH values. From the median values given in Table 2, it is evident that diurnal changes occurred in the pond, although these fluctuations are shown more clearly in daily values (see Lisiecki, 1974). That relatively high pH values occurred is also apparent with both the diurnal changes and high values considerably influenced by the plant biomass.

Besides the organically bound phosphorus, probably the other major amount of phosphorus precipitation was through the loss of Fe, Al, Ca, and other cation-phosphate complexes and compounds. In Wisconsin lakes, Armstrong et al. (1971) found that the amount of inorganic phosphorus in the sediments was most closely related to the amount of hydrated Fe oxides and hydrous oxides. The solubility dependence of these cation-phosphate complexes and compounds on pH was mentioned earlier. Above pH 7 their solubilities are generally increased while their ability to bind phosphate ions is decreased (Stumm and Morgan, 1970). This means that the high pH values in Pond 4 may have aided in maintaining the relatively high phosphorus levels

in the water. The crucial question, though, is the phosphorus/cation ratios which existed in the pond since high cation levels would have resulted in large inorganic phosphorus sedimentation, even at these high pH levels. Bulthuis (1973) found a mean level of 0.1 mg/l Fe and 0.03 mg/l Mn in Pond 3 water. Since phosphorus in Pond 4 was at least six times greater than this Fe concentration in 1973, high levels of inorganic phosphorus would tend to remain in the water.

It is likely that, in natural environments, the pH of microenvironments such as those around macrophytic surfaces is raised well
above pH 9 with photosynthetic activity (Otsuki and Wetzel, 1972).
This would cause coprecipitation of phosphates and carbonates thus
reducing phosphorus levels in the water. Although alkalinity levels
are moderately high in Pond 4 (McNabb, unpublished data), the macrophyte
surface appeared to have very little marl on them when microscopically
examined. Thus, this coprecipitation probably did not have a large
effect on phosphorus removal from the water.

Closely associated with the changes in pH and dissolved oxygen in the water are the changes taking place in the redox potential. It has been shown that vascular hydrophytes do influence redox potentials (Wium-Andersen and Andersen, 1972) and this could be expected to exert control on the metal-phosphate complexes and compounds in the water. Generally, redox levels in the pond were such that an oxidizing environment was found. The high levels of oxygen in the pond water as a result of macrophyte photosynthesis would serve to maintain this oxidizing environment. The oxidized forms of Fe and Mn which are more insoluble than the reduced forms would predominate in the pond water resulting in phosphate precipitation. Below 200 mv, the ferric compounds dissolve due to reduction (Graetz, 1973) with phosphate being

released. Redox potentials were quite constant over the summer with mean values at the sediment interface of 86 mv at 8 AM (consistent low) and 227 mv at 12 noon (consistent high). In the water column above the interface, values were generally over 200 mv.

The surfaces of macrophytes may provide a means for phosphorus to be taken out of the water. Many phosphorus containing compounds and complexes adsorb to surfaces quite readily and macrophytes provide an increased surface area where this adsorption could take place. The surface of vascular plants also provides an ideal substrate for epiphytic algae. The algae may attain such biomass that they can obscure the nutritional response of the macrophytes (Fitzgerald, 1969). The epiphyte cover, in general was very sparse on the macrophytes during this study. This scarcity of epiphytes is consistent with observations on the macrophytes in this wastewater system from previous years (McNabb and Tierney, 1972).

Another effect of the vascular hydrophytes on the phosphorus in the pond was the greater stability of the water column resulting from the presence of the plants. Due to its openness, the pond is quite subject to wind action. Somewhat indicative of this added stability of the water column is the occurrence of <u>L. minor</u> throughout the final two-thirds of the study. According to Duursma (1967), agitation of sediments can serve to increase phosphorus release from the sediments since diffusion coefficients are at least a factor of ten lower for mud than solutions. Phosphorus containing seston and the insoluble cation-phosphate compounds and complexes mentioned earlier would tend to precipitate out easier in calmer water. Since 80% of the phosphorus trapped in the pond during this study went to

the sediments, the stabilizing effect and the aid in maintenance of an oxidizing environment in the pond may have been the most important function of the submersed hydrophytes (as far as phosphorus removal) in the pond.

Until recently, it has generally been assumed that the exchange of nutrients across the sediment-water interface is the only significant pathway of nutrient recycling between these two compartments. Vascular hydrophytes were thought to contribute to this exchange only after their death when they had become part of the sediment compartment. The studies of DeMarte and Hartman (1974), McRoy and Barsdale (1970), Bristow and Whitcombe (1971), and McRoy et al. (1972) have shown that these plants play an important role in nutrient cycling in aquatic systems by releasing elements through the shoots to the surrounding water. According to Foehrenbach (1969), Zostera released substantial amounts of inorganic phosphate to the water. McRoy and Barsdale (1970) found that 33% of the phosphate absorbed by the roots of eelgrass (Zostera) was excreted by the foliage into the surrounding sea water. DeMarte and Hartman (1974) have also reported substantial release of ³²P by the shoots of Myriophyllum exalbescens and that the amount released could be increased by injury to the test plants. These studies do not report whether the amount of phosphorus release would vary according to phosphorus levels in the plant tissue or phosphorus concentrations in the ambient water.

From the studies of these scientists, it seems appropriate to hypothesize that phosphorus was released by the vascular hydrophytes to the water of Pond 4. The overall magnitude of this release and its effect on phosphorus levels in the other system components cannot

be determined. It is possible that through their release of phosphorus to the water, the plants maintain some kind of equilibrium between the phosphorus level in the water and their tissues. The apparent response of tissue phosphorus to changes in concentrations in the water may be evidence for this.

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CONCLUSIONS

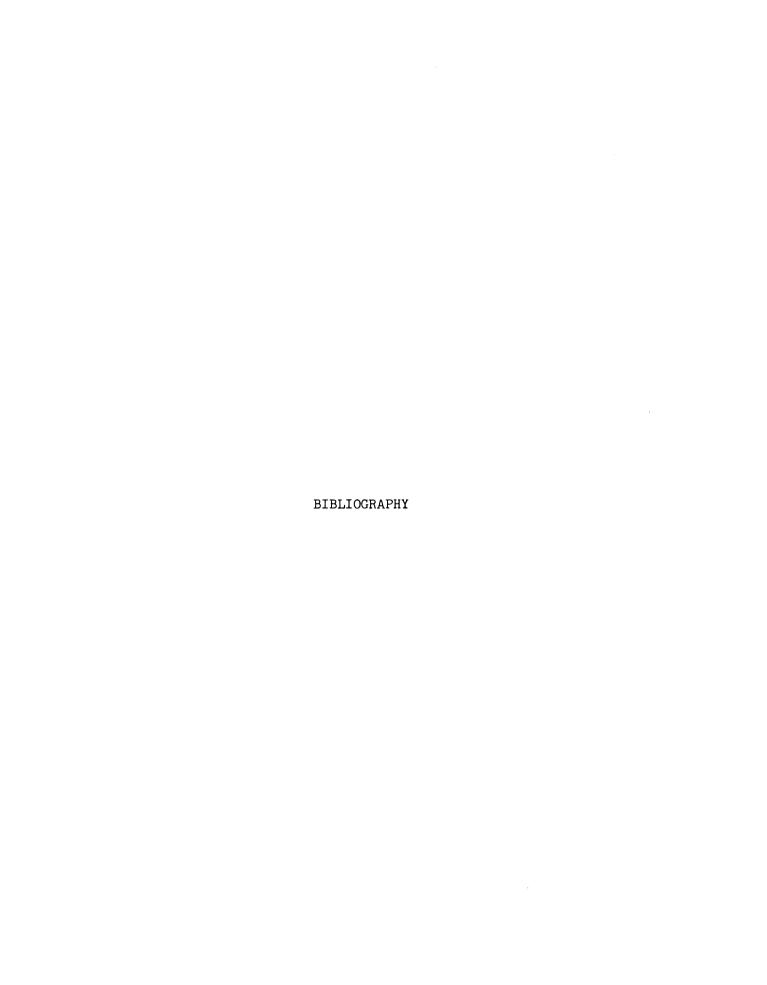
The habitat of the pond during this study was best suited to

C. demersum since this species virtually dominated the vascular hydrophyte community. All the macrophyte species which were quantitatively sampled showed luxury consumption of phosphorus with levels in the plant tissues being some of the highest reported in the literature.

In general the submersed species contained almost twice the amount of phosphorus on an ash-free dry weight basis as the floating species (L. minor). The access of the submersed hydrophytes to the sediments may explain this difference.

In Pond 4 of the Belding wastewater system submersed vascular hydrophytes aid in the removal of phosphorus from the water during the growing season of the plants. Through their active uptake of the element the hydrophytes contained an estimated 40% (17% processed phosphorus) of the phosphorus trapped by the pond at the time of their maximum biomass on August 15. As the biomass declined after this date much of the accumulated phosphorus was re-introduced to the sediments and the water. When the study was terminated the plants contained only 20% (7% processed phosphorus) of the phosphorus trapped by the plants and sediments.

Due to their active growth and resulting large biomass, the hydrophytes influenced the water chemistry of the pond. The oxidizing environment present in the pond enhanced the precipitation of inorganic cation-phosphate compounds and complexes. The presence of the submersed vascular plants also served to physically stabilize the water column which would promote sedimentation of both inorganic and organic phosphorus containing materials. Sedimentation accounted for 80% of the phosphorus removed by the pond during this study.



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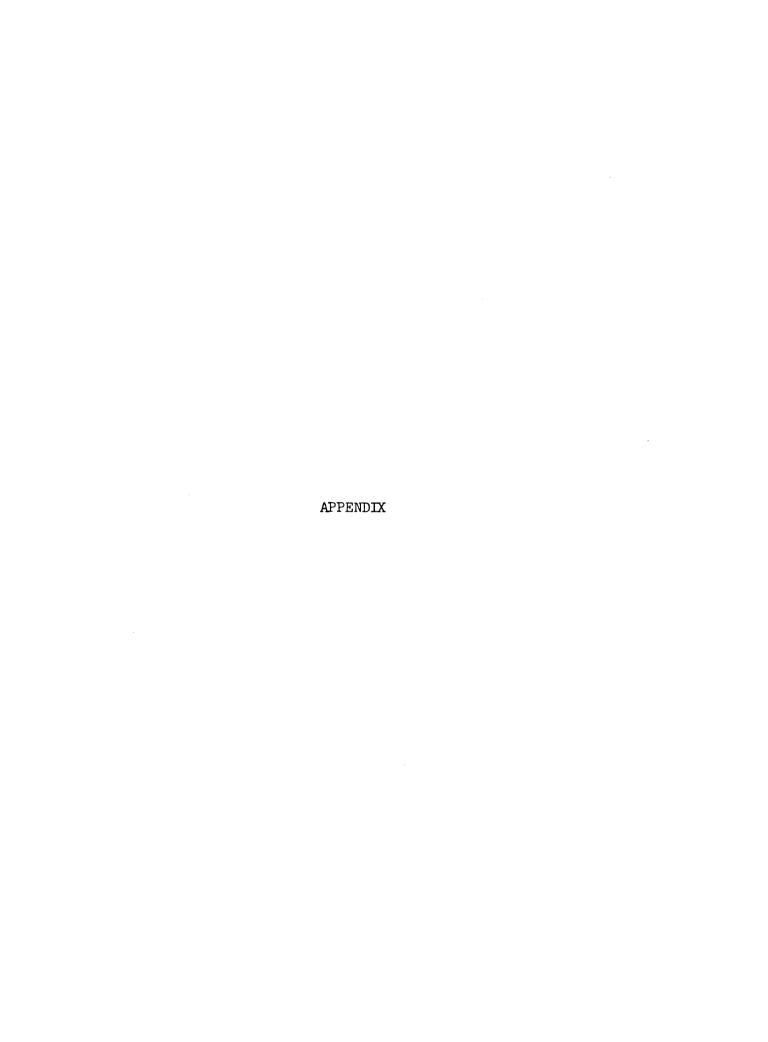
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Summary of chemical and physical measurements in plexiglass enclosures on the dates shown in Table 2. The dawn values are shown first followed by those at dusk. Table A-2.

| | Temp. | (00) | | На | D.0. | D.O. (mg/1) | 臣7 | (mv) |
|---------|------------------------|------------------------------------|------------|---|--------------|-------------|--------------------------------------|---------|
| Depth | Mean | Range | Median | Range | Mean | Range | Mean | Range |
| Enclos | Enclosures with | vascular plants | | | | | | |
| Surface | 23.9 | 22.8-24.8 | 6.0 | 9.1-9.7 | 9.0 | 3.2-12.0 | 261 | 88-589 |
| Bottom | 23.8 | 23.1-24.2 | 8.7 7.8 | 7.3-9.1 | 7.7 | 0.3-4.0 | 102 | 244-08- |
| 90 ob | ر کر | L 20-0 10 | c C | α σ · · · · · · · · · · · · · · · · · · | ر د ر | ט אר ט רר | 1,20 | 812,610 |
| 0.5 m | 25.3 | 24.8-26.0 | . o. | 8.4-9.6 | 13.5 | 10.0-18.3 | 1 1 1 1 1 1 1 1 | 230-672 |
| Bottom | 2,42 | 23.4-25.0 | 8.2 | 7.5-8.8 | 4.5 | 0.7-7.4 | 265 | 2-560 |
| Enclos | ures withou | Enclosures without vascular plants | | | | | | |
| Surface | 23.8 | 22.8-24.7 | 9.3 | 8.8-9.7 | 7.6 | 5.4-11.0 | 236 | 60-564 |
| 0.5 m | 24.1 | 23.0-24.8 | 9.5 | 8.8-9.5 | 7.4 | 5.4-11.0 | 241 | 55-578 |
| Bottom | 24.0 | 22.8-24.5 | 7.7 | 0.6-9.9 | 5. 6 | 0.4-7.0 | 83 | -53-209 |
| Surface | 26.2 | 24.4-27.0 | 9.5 | u v | 12.1 | 9.1-15.2 | 244 | 223-542 |
| 0.5 B | 25.3 | 24.3-26.0 | 6.6 6.9 | 8.5-9.5 | 11.9 | 8.8-15.5 | ካተተ | 250-536 |
| Bottom | 24.2 | 23.2-25.0 | 9.7 | ⊸ | 5.6 | 0.5-5.8 | 235 | 45-347 |
| Pond n | Pond nearby enclosures | osures | | | | | | |
| Surface | 23.3 | 22.0-23.6 | 8.8 | 8.5-9.7 | 7.6 | 3.1-11.4 | 310 | 169-518 |
| 0.5 m | 23.6 | 22.5-24.0 | 8.9 | 7.7-9.6 | 7.8 | 3.0-12.2 | 58 58 | 157-467 |
| Bottom | 23.5 | 22.5-24.1 | 7.7 | 5.1-7.9 | 2.2 | 0.7-5.7 | 158 | 6-370 |
| Surface | 25.0 | 23.9-26.2 | 8.9 | 7.9-9.8 | 13.2 | 11.8-14.6 | 756 | 153-538 |
| 0.5 m | 24.7 | 24.0-25.5 | 8.7 | 8.0-9.7 | 13.5 | 12.8-14.5 | 407 | 134-533 |
| Bottom | 24.0 | 23.2-25.0 | 7.3 | 6.3-8.3 | 4.2 | 0.8-10.2 | 259 | 48-351 |
| | | | | | | | | |

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Table A-3. Total phosphorus and dissolved orthophosphate concentrations (both as mg/l P) in the water expressed as three-point means.

| | Tot | al Phosphoru | เร | Dissol | ved Orthopho | sphate |
|------|---------------------|--------------|-------------------|---------|--------------|-------------------|
| Date | Inflow ^b | Outflowb | Pond ^c | Inflowb | Outflow | Pond ^c |
| 5/23 | 3.47 | 3.16 | | 3.04 | 2.92 | |
| 5/31 | | | | | | |
| 6/6 | 3.29 | 3.05 | 2.65 | 2.96 | 2.79 | 2.12 |
| 6/14 | | | | | | |
| 6/20 | 3.31 | 1.94 | 2.18 | 3.00 | 1.69 | 1.76 |
| 6/28 | | | | | | |
| 7/4 | 4.06 | 1.33 | 1.35 | 3.60 | 1.22 | 1.04 |
| 7/12 | | | | | | |
| 7/18 | 2.85 | 1.06 | 0.77 | 2.48 | 0.91 | 0.56 |
| 7/26 | | | | | | |
| 8/1 | 2.34 | 1.08 | 0.97 | 1.76 | 0.92 | 0.82 |
| 8/9 | | | | | | |
| 8/15 | 2.00 | 0.99 | 0.79 | 1.22 | 0.74 | 0.67 |
| 8/23 | | | | | | |
| 8/30 | | | | | | |
| 9/5 | 1.85 | 1.37 | 1.02 | 1.14 | 0.94 | 0.68 |
| 9/13 | | | | | | |
| 9/20 | | | | | | |
| 9/26 | 2.01 | 1.54 | 1.36 | 1.17 | 1.07 | 0.99 |

^aTabled values are means of samples taken on date listed, preceding date, and following date, except for the first and last in the series which are two point means.

^bFrom samples composited over twenty-four hours.

^cFrom surface samples taken randomly in the pond at noon.

Table A-4. Total quantities of water and phosphorus flowing in and out of Pond 4 over the time periods shown and total pond volume and phosphorus in the pond on the given dates.

| | Tota | l Volume (10 | ^S L) | Tota | al Amount (K | (g) |
|-------|--------|--------------|-----------------|-------------|--------------|-------|
| Date | Inflow | Outflow | Pond | Inflow | Outflow | Pond |
| 6/6 | | | 58.6 | | | 155.2 |
| 6/20 | 26.9 | 25.1 | 57.9 | 91.1 | 75.5 | 117.0 |
| 6/20 | 22.4 | 22.4 | 51.9 | 70.8 | 61.2 | 117.0 |
| 7/4 | 00.0 | 70.0 | 57.8 | 50 (|).) | 84.3 |
| 7/18 | 20.3 | 18.2 | 55.1 | 59.6 | 44.5 | 58.4 |
| , | 25.4 | 22.7 | | 69.3 | 49.3 | - |
| 8/1 | 25.1 | 24.5 | 54.4 | 63.1 | 46.3 | 44.1 |
| 8/15 | 27.1 | 24.7 | 54.1 | 03.1 | 40.5 | 39.5 |
| , | 34.1 | 34.5 | 0 | 76.1 | 53.3 | |
| 9/5 | 37.6 | 37.1 | 52.8 | 71.6 | 56.2 | 48.1 |
| 9/26 | 21.0 | ۲۰۱۲ | 53.0 | 12.0 |)U•L | 76.8 |
|), =0 | | | 75.0 | | | 10.0 |

Table A-5. Phosphorus as percent of the ash-free dry weight in the vascular hydrophytes.

| Date | Mean (\overline{Y}) | s _y | s ² | n |
|------------|-----------------------|----------------|----------------|----|
| Ceratophyl | lum demersum | | | |
| 6/6 | 1.51 | .0200 | .0040 | 7 |
| 6/20 | 2.20 | .0970 | .0630 | 7 |
| 7/4 | 1.% | .0750 | .0560 | 10 |
| 7/18 | 1.56 | .0750 | .0560 | 10 |
| 8/1 | 1.71 | .1100 | .0900 | 10 |
| 9/5 | 1.58 | .0734 | .0550 | 10 |
| 9/26 | 1.38 | .0351 | .0900 | 10 |
| 12/13 | 1.09 | .0200 | .0400 | 10 |
| Potamogeto | n spp.a | | | |
| 5/23 | 1.38 | .0447 | .0100 | 5 |
| 6/6 | 1.50 | .0685 | .0240 | 5 |
| 6/20 | 1.99 | .0860 | .0370 | 5 |
| 7/4 | 1.81 | .1212 | .0733 | 5 |
| 7/18 | 1.43 | .0721 | .0260 | 5 |
| 8/1 | 1.51 | .0632 | .0200 | 5 |
| Lemna mino | <u>r</u> | | | |
| 6/20 | 1.34 | .1319 | .0694 | 4 |
| 7/4 | 1.04 | .1014 | .0517 | 5 |
| 7/18 | 0.93 | .0300 | .0046 | 5 |
| 8/1 | 0.63 | .0223 | .0025 | 5 |
| 8/15 | 0.63 | .0173 | .0015 | 5 |
| 9/5 | 0.65 | .0141 | .0010 | 5 |
| 9/26 | 0.74 | .0173 | .0013 | 5 |

 $^{{}^{\}mathbf{a}}\underline{P}$. foliosus and \underline{P} . berchtoldii combined.

Table A-6. Percentage ash of dry-weight in the vascular hydrophytes.

| Species | Date | Mean (\overline{Y}) | $s_{\overline{\mathbf{y}}}$ | s ² | n |
|------------------------|---|--|---|--|--|
| Ceratophyllum demersum | 5/23 6/6 6/20 7/4 7/18 8/1 8/15 9/5 9/26 12/13 | 17.43 18.45 29.68 26.10 22.38 21.60 18.78 20.46 20.85 21.71 | .67 .34 1.97 1.62 1.72 1.14 .73 2.66 1.31 1.72 | 2.22 .59 19.46 13.07 14.75 6.65 2.64 35.27 8.62 14.83 | 55555555555555555555555555555555555555 |
| Potamogeton spp. | 5/23 6/6 6/20 7/4 7/18 8/1 | 18.09 20.37 22.27 22.01 18.79 19.00 | 1.69 2.85 1.30 2.74 4.54 1.33 | 14.25 40.74 8.43 37.49 61.93 8.82 | 5 5 5 5 5 5 5 5 |
| Lemna minor | 5/23 6/20 7/4 7/18 8/1 8/15 9/5 9/26 | 13.84 22.82 21.35 20.78 19.09 20.43 19.44 19.87 | 1.18 1.20 .83 .42 .37 .39 | 2.76 7.20 3.48 .87 .67 .76 | 1 2 5 5 5 5 5 5 5 5 5 5 |

 $a_{\underline{P}}$. foliosus and \underline{P} . berchtoldii combined.

Table A-7. Y-intercept and coefficients for X-variable to n-powers of polynomial regression curves fit to growing season data (6/6-9/26) for components of Pond 4.

| Y- | Y-intercept | x | x ² | х3 | х | x5 |
|---|-------------|---------|----------------|--------|-------|--------|
| Total P in Inflow Water (mg/1) | 3.81937 | 10946 | | | | |
| Dissolved Ortho-P in Inflow Water $(mg/1)$ | 3.55931 | 13885 | | | | |
| Total P in Outflow Water $(mg/1)$ | 3.78132 | 44327 | .01747 | | | |
| Dissolved Ortho-P in Outflow Water $(mg/1)$ | 3.46922 | 40451 | .01488 | | | |
| Total P in Pond Water $(mg/1)$ | 4.13508 | 52304 | .02099 | | | |
| Dissolved Ortho-P in Pond Water $(mg/1)$ | 3.23332 | -,40095 | .01503 | | | |
| Loglo C. demersum biomass (g ash-free dry weight) | 95743 | .37262 | .02056 | .00033 | | |
| Loglo Potamogeton spp. a bio- mass (g ash-free dry weight) | .70426 | 67400 | .13455 | 00765 | | |
| P in C. demersum (% ash-free dry weight) | -7.35560 | 5.79067 | -1.27778 | .12764 | 00591 | .00103 |
| P in Potamogeton spp. (% ash- free dry weight) | .99979 | .36907 | 05081 | .00191 | | |
| P in <u>L. minor</u> (% ash-free dry weight) | 2.28163 | 22856 | 62400. | | | |

Table A-7 (cont'd)

| | Y-intercept | x | x ² | х3 | ħΧ | x5 |
|---------------------------------------|-------------|----------|----------------|---------|----|----|
| Whole Pond Plant Biomass (Kg) | -888.3068 | 301.2582 | 47.0756 | -2.7029 | | |
| P in Whole Pond Plant Biomass (Kg) | -17.4641 | 6,9869 | .5759 | 0405 | | |
| | | | | | | |

Table A-8. Ranked means and results of Duncan's multiple range test $(p=0.05)^a$ for biomass measurements and phosphorus in vascular hydrophytes.

| Log ₁₀ C. demersum biomass (g ash-free dry weight/sampler) b | | | | | | | | | |
|---|-------------|----------------|--------------|--------------|----------------|--------------|--|--|--|
| AOV = 12.63*** | | | | | | | | | |
| | | 1.01747 9/5 | | | .43109 6/20 | | | | |
| Phosphorus in C. demersum (% ash-free dry weight) AOV = $12.22***$ | | | | | | | | | |
| 2.20 6/20 | 1.96 7/4 | 1.71 8/1 | 1.58 9/5 | 1.56 7/18 | 1.51 6/6 | 1.38 9/26 | | | |
| Phosphorus in Potamogeton spp. c (% ash-free dry weight) AOV = 8.90*** | | | | | | | | | |
| | | 1.51 8/1 | | | | | | | |
| Phosphorus in <u>L</u> . minor (% ash-free dry weight) AOV = $18.52***$ | | | | | | | | | |
| | 1.04 7/4 | 0.93 7/18 | 0.74 9/26 | 0.65 9/5 | 0.63 8/1 | 0.63 8/15 | | | |

aLines connect means not significantly different at the 95% level. AOV values are the analysis of variance mean square statistics.

*** = significant differences at the 99.9% level.

bSize of vascular hydrophyte sampler was 0.1135 m².

 $^{{}^{\}mathbf{C}}\underline{P}$. foliosus and \underline{P} . berchtoldii combined.

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