THE EFFECTS OF THE AQUATIC ANGIOSPERM *CERATOPHYLLUM DEMERSUM* L. ON PHOSPHORUS DYNAMICS IN A LABORATORY SYSTEM

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
Russell J. Erickson
1976

ABSTRACT

THE EFFECTS OF THE AQUATIC ANGIOSPERM

CERATOPHYLLUM DEMERSUM L. ON PHOSPHORUS

DYNAMICS IN A LABORATORY SYSTEM

By

Russell J. Erickson

Theoretical considerations and results of investigations reported in the literature suggest that aquatic macrophytes have both direct and indirect effects on the dynamics of phosphorus in aquatic systems. Direct effects are defined as resulting from the uptake or release of phosphorus by the plants and indirect effects are those resulting from effects of the macrophytes on other properties of the system, such as pH, which in turn affect the behavior of phosphorus. The integrated effect of all involved factors has never been directly measured on any system and cannot be inferred from the existing body of knowledge.

In this study, the effects of the aquatic angiosperm *Ceratophyllum demersum* L. on the behavior of phosphorus in simple water/sediment systems in 20-gallon aquaria were studied. Phosphorus distribution between water and sediment was found to be significantly affected both by the presence of this plant and by changes in photoperiod which caused the plants to either grow and accumulate phosphorus or to senesce and release phosphorus. Direct effects of *C. demersum* were clearly present and exerted such a large influence on phosphorus levels that indirect effects were obscured and could be only tentatively established with the use of a parameter, the weighted mean rate of phosphorus removal, which was designed to compensate for all or most of the direct effect of the plants.

To better establish the existence and nature of indirect effects of C. demersum, a model of phosphorus dynamics in the aquaria was developed and used to conduct simulations of the aquarium experiment, with data partly from the aquarium experiment and partly from a series of beaker experiments in which phosphorus removal from water was measured as a function of pH, alkalinity, hardness, and orthophosphorus concentration in small water/sediment systems comparable to those in the aquaria. These simulations roughly duplicated the values of phosphorus observed in the aquarium experiment, but a significant lack of fit occurred. Sensitivity analysis of these simulations substantiated the large, direct effect of C. demersum on phosphorus behavior, and also showed slight effects on phosphorus dynamics due to decreases in evaporation caused by plants and major effects due to plant-induced changes in pH. It was concluded that these indirect effects most likely were also present in the actual aquarium experiment, though probably to different extents and in the company of effects from other factors that could not be included in the model. Testing of the weighted mean rate of phosphorus removal in these simulations showed that this parameter had some shortcomings, most importantly being influenced by direct effects of plants, but that, if used judiciously, it could serve as a useful indicator of indirect effects of plants on phosphorus dynamics, at least in systems comparable to the ones described here.

THE EFFECTS OF THE AQUATIC ANGIOSPERM *CERATOPHYLLUM DEMERSUM* L. ON PHOSPHORUS DYNAMICS IN A LABORATORY SYSTEM

By

Russell J. Erickson

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

1976

ACKNOWLEDGMENTS

I would like to express my gratitude to E. W. Roelofs for his guidance and friendship as my advisor during my years as both an undergraduate and graduate student at Michigan State and to C. D. McNabb and F. M. D'Itri for their time and help in reviewing my research and this thesis. I would also like to thank all the faculty and students that I have been privileged to be associated with at Michigan State, who have in no small way contributed to the thoughts expressed in this thesis. I would like to especially thank Chuckie for all the good times.

The research discussed here was made possible through support from a National Science Foundation graduate fellowship and from training grant T900-331 of the Environmental Protection Agency.

TABLE OF CONTENTS

Table

1

2

3

4

5

6

7

8

9

10

11

12

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Due to its role in limiting productivity, the distribution and dynamics of phosphorus in aquatic systems have been the subjects of much research. In particular, the exchange reactions of inorganic phosphorus between dissolved and solid states (e.g., precipitation, coprecipitation, adsorption) have received considerable attention since they account for a significant part of the phosphorus removed to the tropholytic zone of lakes and to sediments. Even more importantly, these reactions control the retention of phosphorus in sediments, including not only the inorganic phosphorus originally deposited in an inorganic form, but also the inorganic phosphorus released from decomposing, sedimented organic matter. Because of this, these exchange reactions exert a tremendous influence on the amount of phosphorus available to primary producers and thus affect the productivity of all components of aquatic systems. The following discussion will consider (1) the forms of inorganic phosphorus found in water and sediments (indicative of the exchange reactions occurring), (2) the chemical and physical factors which are known to affect the exchange reactions of inorganic phosphorus, (3) the indirect effects of aquatic macrophytes on phosphorus because of their effects on these chemical and physical factors, and (4) the direct effects of aquatic macrophytes on phosphorus dynamics through their uptake, retention, and release of phosphorus.

FORMS OF INORGANIC PHOSPHORUS IN WATER AND SEDIMENT

Except where large inputs of synthetic inorganic phosphorus compounds occur, dissolved inorganic phosphorus exists almost exclusively as orthophosphate ($H_iPO_4^{i-3}$, i=0...3) and its various complexed forms, such as $FeHPO_4^+$ (Stumm and Morgan, 1970). Orthophosphate is quite insoluble in water in the presence of a variety of cations and minerals and can be removed by adsorption to clays and metal hydroxides, especially ferric hydroxide (Stumm and Morgan, 1970), coprecipitation with calcium carbonate (Zicker et al., 1956; Otsuki and Wetzel, 1972), and precipitation as a phosphate mineral. Stumm and Morgan (1970) suggest that the phosphorus-containing minerals of significance in natural waters include various apatites ($(Ca,H_2O)_{10}(F,OH)_2(PO_4,CO_3)_6$), brushite ($CaHPO_4 \cdot 2H_2O$), variscite ($AlPO_4 \cdot 2H_2O$), strengite ($FePO_4 \cdot 2H_2O$), and wavellite ($Al_3(OH)_3(PO_4)_2$). In addition, vivianite ($Fe_3(PO_4)_2 \cdot 8H_2O$) has been reported in strongly reduced sediments (Mackereth, 1966; Rosenqvist, 1970).

The formation of these phosphate minerals appears to be especially important in sediments for retaining phosphorus that was initially deposited in forms unstable under sediment conditions. For example, much of the apatite found in sediments is apparently formed via conversion of deposited calcium carbonate and orthophosphate released from organic matter decomposing in the sediment (Stumm and Morgan, 1970; Williams and Mayer, 1972). Similarly, vivianite would be formed from orthophosphate released from decaying organic matter and from ferrous iron derived from reduction of precipitated ferric iron (Williams and Mayer, 1972).

Very few data exist regarding actual quantities of the above forms of solid inorganic phosphorus present in natural systems. The removal of orthophosphate from water and its retention in sediments have been

variously attributed to precipitation and coprecipitation with ferric iron (Einsele, 1936, 1938; Mortimer, 1941, 1942, 1971; Mackereth, 1966), manganese (Mackereth, 1966), and calcium (Hepher, 1958; Zicker et al., 1958; Wentz and Lee, 1969), but these studies relied mainly on laboratory data, theoretical calculations, and correlations in field data, with little or no direct measurement of forms of phosphorus in and above sediments. The work by Mortimer (1941, 1942), though, did provide convincing evidence of the role of hydroxides and hydrated oxides of ferric iron at the surface of oxidized sediments in removing orthophosphate from the water and preventing its escape from lower, reduced layers of sediments.

More recent work on Lake Erie and Wisconsin lake sediments, using extraction techniques with some selectivity for forms of phosphorus, suggests that inorganic phosphorus is present in sediments associated with aluminum, iron, and calcium, with apatite and an amorphous hydrated iron oxide-orthophosphate complex being predominant (Williams et al., 1971a, 1971b, 1971c; Shukla et al., 1971; Li et al., 1972; Williams and Mayer, 1972). Additionally, data from Lake Erie suggest that, while surface sediments contain appreciable quantities of apatite, organic phosphorus, and sorbed (Al and Fe bound) orthophosphate, the values of the last two decline quickly with depth in the sediment, presumably in part due to conversion to apatite, which increases with depth (Williams and Mayer, 1972). Nelson (1967), using similar extraction techniques, reported the presence of strengite and variscite in quantities exceeding apatite in fresh-water sediments, but his results are questionable since these techniques do not actually distinguish these minerals from other forms of aluminum and iron bound phosphorus (Williams et al., 1971a). As mentioned above, vivianite has also been found in some sediments, but its occurrence

is infrequent and erratic, presumably due to the extremely low redox potentials required for its formation and to competing reactions (Rosenqvist, 1970).

EFFECTS OF CHEMICAL AND PHYSICAL FACTORS ON PHOSPHORUS DYNAMICS

The rate and extent of removal of dissolved inorganic phosphorus from water have been correlated with several chemical and physical factors, including redox potential, pH, dissolved organic matter, multivalent cations, sediment properties, temperature, and turbulence. In most cases, the effects of these factors are easily understandable when they are considered in relation to the forms of inorganic phosphorus discussed above.

Redox Potential

A large concentration of inorganic phosphorus is typically found in the hypolimnion of lakes with extreme clinograde oxygen curves. Einsele (1936, 1938) correlated this to the release of ferrous iron from the sediment, suggesting that phosphorus is held in oxidized sediments in association with ferric iron ($FePO_4$, $Fe(OH)_3$-P) and is released upon reduction of the ferric iron to ferrous iron, the phosphates and hydroxides of the latter being much more soluble. Subsequent work by Mortimer (1941, 1942, 1971) established more precisely the mechanisms involved in this phenomenon and is the basis for the following discussion.

When oxygen is present in adequate quantities (1 mg/l O_2 or more) in water overlying sediment, there exists a thin surface layer in the sediment in which oxygen penetrates in amounts sufficient to maintain the redox potential (E_7) at greater than 200 mv. This layer, the oxidized microzone, can vary from less than 1 mm to greater than 2 cm in thickness, depending on actual oxygen concentration and turbulence, and is characterized by

iron being almost exclusively in the ferric form, primarily as ferric hydroxide. Orthophosphate present in the overlying water is removed in large part by adsorption to this ferric hydroxide, as is orthophosphate released from decomposing organic matter deposited on the sediment. More importantly, the oxidized microzone prevents the passage of phosphorus to the water from lower layers of sediment, where large concentrations of dissolved phosphorus and ferrous iron exist due to reducing conditions. Virtually all upward diffusing orthophosphate will be precipitated or adsorbed when it reaches this layer. While other solid inorganic forms of phosphorus certainly participate in this exchange between sediment and water, the correlation between the release of ferrous iron and phosphorus is so strong that iron must be considered the key factor in this process, at least where it is reasonably abundant.

As oxygen drops to lower levels, the oxidized microzone becomes thinner and may eventually disappear, greatly reducing the ability of the sediment to remove phosphorus from the overlying water and allowing dissolved phosphorus to diffuse from the sediment in large quantities. Once in the water of the hypolimnion, some of this phosphorus may be transported into the trophogenic zone and result in increased primary productivity. Also, inorganic phosphorus levels will be higher at overturn, at least temporarily, and will likewise stimulate primary productivity. This is especially true if redox levels during stratification fall low enough to cause large scale reduction of sulfate to sulfide, which readily precipitates ferrous iron. After reoxygenation, this results in ferric iron levels too low to remove much of the phosphorus, at least initially.

pH

Several investigations with sediments from a variety of locations

have demonstrated a high degree of uniformity in the pH dependence of adsorption and desorption of phosphorus by sediments. Macpherson et al. (1958) tested sediments and their ashes from eight lakes of widely varying productivity and, though the absolute rates of phosphorus release and uptake by these sediments varied as to lake type, they all showed the same general trends with pH. Maximum adsorption (minimum desorption) occurred at a slightly acidic (5-6) pH, adsorption decreasing at lower (3-5) and slightly higher (6-8) pH values and increasing again at somewhat basic (8-9) pH values. The same trends were observed by Gumerman (1970) for sediments from Lakes Superior and Erie, while Jitt (1959) only observed maximum adsorption at pH 5 and declining adsorption on either side of this value, without an upturn in adsorption at pH values greater than eight (apparently due to a washing out of the sediment of all but insoluble silt particles).

Except for the adsorption at higher pH, the trends described above are remarkably close to those for ferric hydroxide and magnesium and aluminum silicates (Macpherson et al., 1958), suggesting that these or similar substances are responsible for orthophosphate removal by sediments at pH values below eight. The pH dependence of these substances is most likely due to their amphoteric nature, with the presence of sites suitable for phosphorus adsorption depending on the exchange of hydrogen and hydroxide ions with the substance (Stumm and Morgan, 1970). Also, the maximum adsorption at slightly acidic pH values corresponds well with the maximum mole fraction of dihydrogen phosphate ($H_2PO_4^-$), a form that might be more amenable to adsorption to the minerals in sediment. Above pH 8, the increased adsorption is consistent with precipitation or coprecipitation of phosphorus with calcium (Stumm and Morgan, 1970).

The above studies dealt with sediments shaken with distilled water to which only potassium dihydrogen phosphate, hydrochloric acid, and sodium hydroxide were added for pH and phosphate concentration adjustments. Consequently, they have limited applicability to real situations and give no indication of the pH dependence of phosphorus-containing precipitates formed in the water overlying sediments. The number of protons on a phosphate ion is certainly pH-dependent and should have a marked influence on the precipitation and coprecipitation of the species mentioned above. Additionally, the precipitation of metal hydroxides and calcium carbonate, with which phosphate is coprecipitated, is definitely affected by pH. Otsuki and Wetzel (1972) showed the coprecipitation of phosphorus on calcium carbonate to be strongly pH-dependent, being greater at higher pH values, mostly due to increased calcium carbonate formation.

Dissolved Organic Matter

Concentrations of ionic iron and other metals are often found in water far exceeding equilibrium levels, which has led to the belief that they form complexes or chelates with dissolved or colloidal organic matter. Shapiro (1966) has shown ferric iron to form a colloidal association with naturally-occurring aliphatic, polyhydroxy, carboxylic acids (MW 200-400), possibly by chelation or complexation, but more likely by peptization. Regardless of the exact forms, this iron will remain in true or colloidal solution instead of being precipitated out, so that, especially if phosphate is bound to it (Golterman, 1967), phosphorus removal to the sediments is inhibited.

Further evidence of the role of organic acids comes from the work of Harrison *et al.* (1972), who demonstrated the solubilization of $FePO_4$, $Ca_3(PO_4)_2$, $CaHPO_4$, $Al_2(PO_4)_2$, and $Mg_3(PO_4)_2$ by certain bacterial isolates

from lake sediments. They attributed this to chelation by organic acids secreted by the bacteria and showed that such acids were extremely effective in solubilizing these phosphate salts under sterile conditions.

Sediment Properties

As discussed above, the presence of ferric hydroxide in the surface layer of sediments has a pronounced effect on the ability of sediments to remove and retain phosphorus. In general, iron and aluminum hydroxides, oxides, and silicates are quite effective in adsorbing orthophosphate, so that the presence of any of these minerals would improve the ability of sediments to remove phosphorus. Work by Jitts (1959), Gorham and Swaine (1965), Livingstone and Boykin (1962), Li et al. (1972), and Williams et al. (1971a, 1971b, 1971c) supports this statement.

The effect of calcium carbonate in sediments appears less certain. Li et al. (1972) report that the calcium fraction of sediments does not appear to be related to the phosphorus exchange capacity, at least over a short time span, which is consistent with Otsuki and Wetzel (1972), who concluded that coprecipitation of phosphorus with calcium carbonate relies more on mechanical inclusion or the formation of a solid solution rather than strong surface adsorption. Zicker et al.(1958), though, present data suggesting that calcium carbonate in sediments increases their ability to remove and retain orthophosphate, presumable due to surface adsorption, which had been demonstrated by Cole et al.(1953).

Organic matter in sediments, though it represents a significant portion of the phosphorus removed into and retained by sediments, appears to have a negative impact on the ability of sediment to remove orthophosphate from water. Jitts (1959) demonstrated a decrease in phosphorus removal by sediments in proportion to their organic content.

He also found sediment particle size to increase with organic matter. He suggested that organic matter either competes with phosphorus for available sorption sites or physically masks the sites, in part by cementing sediment particles together. Work by Macpherson et al. (1958) also demonstrated an interference by organic matter with phosphorus removal by sediments in that ashed sediments generally removed more phosphorus from water than whole sediments, even though ashing would have released additional inorganic phosphorus.

Multivalent Cations

As the above discussions suggest, multivalent cations (Fe^{+3}, Mn^{+4}, Ca^{++}, Mg^{++}, Al^{+3}) are quite important in the exchange of orthophosphate between dissolved and solid states. It should then be expected that additions of such cations would enhance phosphorus removal, a principle that is recognized in lake rehabilitation and in waste treatment with the use of such substances as ferric chloride, alum, aluminate, and lime (Rohlich and Uttormark, 1972). Conversely, a lowering of cation levels may have a negative impact on phosphorus removal, even if, as in marl formation, some phosphorus is removed during the removal of the cations. Overall, it is not unreasonable to expect soft water lakes to tolerate less phosphorus loading than hard water lakes before significant productivity increases occur, assuming phosphorus is limiting production.

Temperature and Turbulence

Rates and extents of reactions are well known to depend on temperature, and reactions involving phosphorus exchange should not be exceptions to this, but studies regarding this are few. Gumerman (1970) found greater equilibrium concentrations of phosphorus at 22°C than at 4°C in sediments from Lakes Superior and Erie. Otsuki and Wetzel (1972) also found

increased temperature to increase phosphorus coprecipitation with calcium carbonate at pH values less than 9.5. This was attributed to increased calcium carbonate formation rather than increased phosphorus inclusion in a given amount of precipitate.

Due to the slow rates of diffusion in sediments, stirring of reduced sediments should increase phosphorus export. Also, in oxidized sediments, disruption of the oxidized microzone should allow at least a temporary loss of phosphorus and reduced materials from newly exposed reduced sediment layers before they are oxidized. Zicker et al. (1958) found phosphorus release from bog lake muds to double with 'vigorous stirring.' His results, though, shed little light on the effect of natural turbulence on the release of phosphorus from lake sediments, especially in the littoral zone, where turbulence is highest and the sediments are in close proximity to primary producers that can, perhaps, use released phosphorus before it is returned to the sediments.

INDIRECT EFFECTS OF AQUATIC MACROPHYTES ON PHOSPHORUS DYNAMICS

Due to metabolic activities and, sometimes, just to their physical presence, aquatic macrophytes have been shown to affect the chemical and physical factors discussed above. Because of this, it can be inferred that aquatic macrophytes will exert some influence on the dynamics of inorganic phosphorus indirectly by their effect on such things as dissolved oxygen, pH, dissolved organic matter, sediment characteristics, multivalent cations, temperature, and water circulation.

Redox Potential

The oxygen produced by aquatic macrophytes during photosynthesis is, in the long run, nearly or completely balanced by oxygen consumed during respiration and decomposition. Because these processes are separated in

time and space, aquatic macrophytes can either cause oxygen depletion or accretion, depending on time and location, and thus either raise or lower the redox potential. More often than not, the depletion of oxygen by macrophytes will be more critical with respect to phosphorus dynamics since (1) the redox potential and its effects on inorganic phosphorus exchange only change markedly at very low oxygen levels and (2) macrophyte production of oxygen usually occurs in places where oxygen levels are not critically low, while consumption of oxygen resulting from macrophytes is often in areas already partly depleted of oxygen.

Severe oxygen depletions (to less than 0.5 mg/l O_2) have been reported in a stand of the emergent Glyceria aquatica (Dvorak, 1970), at the bottom of a stand of Elodea canadensis (Buscemi, 1958), at sediment level under a mat of Ceratophyllum demersum (Goulder, 1969), and under a mat of Nuphar sp. and Hydrocharis sp. (Straskraba, 1965). In the last three of these studies, oxygen was found to be at or over saturation in the upper part of the plant beds, which was at most two meters above the anoxic zone. In Dvorak's study, oxygen was uniformly low throughoutthe plant bed, climbing markedly at the margins to levels of 6 mg/l O_2 just outside the bed.

In extreme situations, the decomposition of heavy growths of macrophytes can deplete oxygen in an entire pond or small lake, not only under ice cover, but also during calm, late summer days. Additionally, macrophytes can contribute to hypolimnetic oxygen depletion when decomposing macrophytes are are exported from the littoral to the profundal zone. The overall contribution of macrophytes to consumption of oxygen in hypolimnia is not well established, but it may be quite significant in small lakes, due to relatively larger littoral zones and to the fact that much of the decomposition of phytoplankton occurs in upper waters, while the more

resistant tissue of vascular plants would decompose to a greater extent at sediment level.

pH

Associated with oxygen production and consumption are, respectively, a raising and lowering of pH, often of enough magnitude to significantly affect inorganic phosphorus dynamics. The data of Dvorak (1970) on pH parallel his oxygen data, with pH dropping from approximately 8 just outside the bed of Glyceria aquatica to about 6 within most of the bed. Straskraba (1965) observed a pH drop from 8.2 at the top to 7.0 at the bottom of a one meter water column under Nuphar sp. and Hydrocharis sp. Goulder (1969) found pH values to exceed 10 in a Ceratophyllum demersum mat, with values dropping to below 8 just outside the mat.

The pH values observed by Goulder are certainly high enough to result in marl precipitation if hardness and alkalinity are sufficiently high; in fact, he did observe drops in alkalinity in the C. demersum beds, indicative of carbonate precipitation. In extreme cases, the elevation of pH by macrophyte photosynthesis can cause massive encrustation of marl on the plants and the deposition of carbonate rich sediments, presumable with significant phosphorus removal by coprecipitation.

Dissolved Organic Matter

Significant excretion of dissolved organic matter is known to occur in some aquatic macrophytes (Wetzel, 1969), but as yet the released compounds have not been characterized, so that the quantities of cation-chelating or peptizing organic acids released by these plants are unknown. In addition to this excretion while alive, macrophytes contribute dissolved organic matter to the water upon death and decomposition (Nichols and Keeney, 1973), both by release of cell contents and by bacterial

degradation of their tissues. Particularly important is the fact that the humic acids associated with iron in the work by Shapiro (1966) are, in general, products of microbial action on resistant types of organic matter, associated more with vascular plants than algae. Therefore, aquatic macrophytes may contribute to the dissolved organic matter associated with cation solubilization in amounts out of proportion to their contribution to total production of organic matter in a basin.

Sediment Properties

Aquatic macrophytes can have a marked effect on both the mineral and organic content of sediments. Marl deposition, in large part due to macrophytes, has an impact on the nature of sediments and their phosphorus relations, as discussed above. In extreme cases, littoral sediments can be composed almost entirely of marl as a result of macrophyte activity.

The large, stable biomasses and resistant vascular tissue produced by macrophytes (as compared to phytoplankton) can cause significant increases in the organic content of sediments, not only in the plant beds, but also in other portions of a lake due to export of plant material by currents and turbulence. Ulehlova (1970) observed wide variations in the organic content of sediments (from less than 10% to greater than 90%) that he related to the types and abundance of macrophytes above the sediments.

Multivalent Cations

Due to high availability of calcium, magnesium, manganese, aluminum, and, usually, iron in relation to the physiological requirements of macrophytes for these elements (Boyd, 1969, 1970), aquatic macrophytes have relatively little direct impact on the levels of cations of importance to inorganic phosphorus exchange reactions. A major exception to this is the removal of massive amounts of cations by Sphagnum spp. mats, resulting in

the typically soft waters of bogs and bog lakes. Also, macrophyte-mediated production of marl can significantly reduce the concentrations of some cations.

Temperature and Turbulence

The physical presence and photosynthetic activity of macrophytes would logically be expected to alter light absorption and water circulation patterns, thus affecting both temperature and turbulence. Dvorak (1970) observed markedly lower temperatures in a bed of Glyceria aquatica, presumably due to shading. Also, the low oxygen levels he reported are indicative of little surface turbulence and mixing. Straskraba (1965) found a temperature differential of 9.8°C in a one-meter water column under Nuphar sp. and Hydrocharis sp., indicating effects on both temperature and circulation. Goulder (1969) also observed marked stratification of chemical parameters in water too shallow (3 m) to support such stratification without the stabilizing effect of a C. demersum mat.

DIRECT EFFECTS OF AQUATIC MACROPHYTES ON PHOSPHORUS DYNAMICS

Phosphorus uptake by aquatic macrophytes can occur from the water or sediments, or both (Sculthorpe, 1967). The impact of this uptake on water and sediment phosphorus levels can be great due to (1) large accumulations of biomass, (2) high physiological requirements for phosphorus in relation to its availability, and (3) assimilation of phosphorus in excess of immediate requirements, or so-called 'luxury consumption' (Boyd, 1969, 1970; Gerloff, 1969; Gossett and Norris, 1971; Kosek, 1971; McNabb, Tierney, and Kosek, 1972; Tierney, 1972; Wilson, 1970).

Obviously, when phosphorus is removed from the water by macrophytes, it is unavailable for other primary producers and for removal into the sediment. Retention of phosphorus in macrophytes is not permanent, though,

and all phosphorus taken up by macrophytes will eventually be released to the water in excreted matter and products of decomposition or be deposited in the sediments in an organic form. Nichols and Keeney (1973) demonstrated that, under oxidized conditions, phosphorus released from decomposing Myriophyllum exalbescens in laboratory sediment/water systems is rapidly removed into the sediment, though some is temporarily available for uptake by other primary producers. Uptake of phosphorus from water and its subsequent release by macrophytes will therefore markedly alter the distribution of phosphorus in time and space, though what total effect this has on primary productivity, removal of phosphorus to sediments, and the flux of phosphorus through aquatic systems is largely unknown.

Uptake of phosphorus from sediments by aquatic macrophytes is, perhaps, of greater significance than uptake from water in that it constitutes a major reversal of the general tendency of phosphorus to be removed into the sediment under oxidized conditions. Besides providing nutrients necessary for growth of rooted macrophytes, this uptake can result in greater production by free-floating macrophytes and algae when the phosphorus is excreted into the water or released upon decomposition. In fact, certain estuarine macrophytes have been shown to, in effect, pump inorganic phosphorus from the sediments to the water (McRoy and Barsdate, 1970; McRoy et al., 1972; Reimold, 1972). The net phosphorus pumped from sediment to water can even exceed the amount assimilated by the plants and thus has a marked influence on the phosphorus flux through the estuary and on the phosphorus available to other primary producers. In fresh-water, budgets of phosphorus for littoral and pelagic zones have suggested that export of phosphorus from littoral areas, presumably due in large part to macrophyte activity, helps maintain phytoplankton production in the pelagic waters

for a significant portion of the growing season (Wetzel, 1975).

STATEMENT OF PROBLEM AND PLAN OF RESEARCH

The following major points have thus far been made:

(1) The exchange of inorganic phosphorus between solid and dissolved states is critical to the productivity of many aquatic systems.

(2) Inorganic phosphorus exists as dissolved orthophosphate and a variety of solid forms, the relative importance of which are poorly known.

(3) The removal of inorganic phosphorus from water and its deposition and retention in sediments depend on a variety of chemical and physical factors, including redox potential, pH, dissolved organic matter, multivalent cations, sediment properties, temperature, and turbulence.

(4) Aquatic macrophytes can significantly alter the above factors and therefore can presumably affect phosphorus dynamics indirectly.

(5) Aquatic macrophytes can directly affect phosphorus dynamics through uptake, retention, release, and deposition of phosphorus.

This summary suggests an important and complex set of interactions between aquatic macrophytes and phosphorus distribution, but the present level of knowledge is insufficient to allow integration of all the discussed factors into a realistic scheme that will describe the overall effects of macrophytes on phosphorus cycling. In addition, few studies have even established empirically the net effect of a macrophyte on phosphorus, even under simple, restricted circumstances.

The work to be reported here therefore attempted to (1) establish the integrated effect of the submerged angiosperm, Ceratophyllum demersum, on the distribution of phosphorus, through time, in small, controllable laboratory systems and (2) to determine the major mechanisms affecting phosphorus dynamics in these systems and how C. demersum affects them.

C. D

to s

(2)

gene

appr

anal

labo

firs

and

gall

of (1

hour

resul

at le

plant

of th

twenty

ortho

(3) th

(5) th

replic

series

renova

in the

be dra

of phos

C. demersum was selected as the experimental plant because (1) it is known to significantly alter such water parameters as pH and dissolved oxygen, (2) it is an important component of many small lakes and ponds, (3) it is generally free floating in lab culture and therefore does not obtain an appreciable amount of nutrients directly from sediments, which makes analysis of phosphorus dynamics easier, and (4) it is amenable to laboratory rearing.

With regard to the above goals, two experiments were devised. The first experiment (Aquarium experiment) consisted of monitoring phosphorus and other relevant factors in water/sediment systems established in twenty-gallon aquaria with metered flow-through of water. Treatments consisted of (1) the presence and absence of plants and (2) twelve- and fourteen-hour photoperiods, with three replicates each. The different photoperiods resulted in declining and increasing plant biomass, respectively, that were at least somewhat representative of stages in the annual cycle of this plant. The second experiment (Beaker Experiment) involved the determination of the rate of orthophosphate removal from water in one-liter beakers over twenty-four hours. Treatments included (1) three levels of initial orthophosphate concentration, (2) three levels of initial hardness, (3) three levels of initial alkalinity, (4) five levels of initial pH, and (5) the presence and absence of sediment, in a factorial design with one replicate of most of the treatment combinations. From this experiment, a series of regression equations for phosphorus, alkalinity, and hardness removal were developed and used to simulate the behavior of these parameters in the aquarium experiment. Such a simulation allowed some conclusions to be drawn regarding how and why Ceratophyllum demersum affects the dynamics of phosphorus in these aquarium systems.

MATERIALS AND METHODS

AQUARIUM EXPERIMENT

Experimental Apparatus

Experiments were conducted in a row of six 20-gallon aquaria (numbered 1 to 6) under a bank of twenty-four 40-watt Gro-lux fluorescent lights. At water level, light intensity varied from approximately 550 foot-candles in the end aquaria (1 and 6) to 650 foot-candles in the middle aquaria (3 and 4). Because of this gradient of light, aquaria were paired when treatments were assigned (i.e., if aquarium 5 received plants, aquarium 2 did not). Photoperiod was controlled with a timer switch through which all lights were circuited.

For the initial experimental run (12-hr photoperiod), flow through the aquaria was controlled by a gravity/siphon system. Influent was delivered through glass and vinyl tubing from a head tank in which a constant water level was maintained by continual pumping of water into the tank from a reservoir and overflow into a standpipe which returned water to the reservoir. Influent to each aquarium was restricted to approximately 100 ml/hr by a screw clamp at the exit of each delivery tube. Effluent from each aquarium was maintained by a siphon which delivered overflow to a two-gallon sampling bottle. An additional sampling bottle received water directly from the head tank for influent water chemistry determinations. Circulation in the aquaria resulted not only from flows caused by these influents and effluents, but also from air forced over the

aquaria at 5 to 10 fps by a large fan.

This gravity influent system proved to be incapable of delivering sufficiently constant flows, resulting in poor estimates of influent volumes and in quite different volumes delivered to each aquarium. Therefore, for the second experimental run (14-hr photoperiod), this system was replaced with a variable-flow, seven-channel tubing pump, which delivered a constant and equal flow directly from the floor reservoir to each aquarium and to the influent sampling bottle, which then served for determination of influent volume as well as water chemistry.

Plants, Water, and Sediment

A large quantity of Ceratophyllum demersum was obtained from the waste stabilization ponds at Belding, Michigan on June 21, 1974. A portion of this was cleaned of filamentous algae and placed in two 2' x 8' x 1' holding tanks through which water was continually circulated, overflow draining into a reservoir in which it was aerated and pumped back to the tanks. The water in these tanks was from the same source as that used in the experimental runs in the aquaria and was renewed periodically. Light conditions were also made as near as possible to experimental conditions.

All of the water used in the holding tanks and the aquaria was obtained from Pond 2 of the Michigan State University Water Quality Management Project, with the exception of the initial filling of tanks and aquaria for the first experimental run, when well-water from the Fisheries Research Laboratory was used in part (ca. 30%). Results of chemical analyses of this pond for the duration of the experiments were obtained from C. Annett of the Institute of Water Research (Table 1).

The sediment used in all experiments was a dried, air-filtered, native kaolinite clay, obtained through a ceramics supplier. This clay was found

Table 1. Summary of water chemistry of pond 2 of the Michigan State University Water Quality Management Project for the summer of 1974.

Date	6/17/74	7/8/74	8/1/74	8/21/74
Alkalinity (mg/l $CaCO_3$)	175	186	-	173
Hardness (mg/l $CaCO_3$)	273	234	197	-
Dissolved Oxygen (mg/l O_2)	10.6	8.5	-	-
pH	8.75	8.20	10.1	-
Calcium (mg/l Ca)	110	93	-	-
Magnesium (mg/l Mg)	17	18	-	-
Total Iron (mg/l Fe)	1.0	1.1	-	-
Potassium (mg/l K)	5.9	6.4	-	-
Sodium (mg/l Na)	79	76	-	-
Chloride (mg/l Cl)	95	100	-	-
Sulfate (mg/l SO_4)	76	74	85	-
Ammonia (mg/l N)	0.26	0.31	-	0.14
Nitrate (mg/l N)	0.32	0.32	-	0.04
Nitrite (mg/l N)	0.09	0.07	-	0.01
Total Phosphorus (mg/l P)	0.72	0.70	0.28	0.38
Soluble Phosphorus (mg/l P)	0.60	0.69	0.23	0.37

to contain less than 0.5% organic matter, as determined by ashing oven-dried clay at 350°C for four hours. Testing of the settling of this clay in water showed that it formed a non-compacted sediment with a water content of approximately 42%, by weight.

Experimental Procedure

A mixture of 5000 g of dry clay and 4000 ml of water was sieved through a twenty mesh screen and spread evenly on the bottom of each aquarium. This was allowed to settle at least twelve hours, resulting in about 200 ml of clear water overlying the sediment and in no size stratification of the clay as would occur if a greater volume of water had been used. Measured volumes of water were then siphoned or pumped into each aquarium as fast as possible without appreciably disturbing the sediment until the depth was sufficient for the effluent siphon to hold water. A large quantity of plants was then removed from the holding tanks, drained, and blotted to constant weight. A portion of these plants (400 g wet-weight for the initial run and 200 g wet-weight for the second run) was weighed and placed in a randomly selected member of each pair of aquaria. A fourth portion (ca. 100 g wet-weight) was also weighed and oven-dried (70°C for at least 72 hr) for dry-weight determination and phosphorus analysis. After the addition of plants, timed to occur at 'midday', water was added to each aquarium until the effluent siphons began to drip. A flow of approximately 100 ml/hr was then started in the influent system. Finally, temperature, pH, dissolved oxygen, hardness, alkalinity, total phosphorus, dissolved phosphorus (second run only), and orthophosphorus (second run only) determinations were made on the water in each aquarium or on the stock of water added to the aquaria.

Periodically throughout the runs, usually every two days, measurements

of pH, temperature, and dissolved oxygen were made in the aquaria and the reservoir at midday. At the same time, effluent and influent bottles were emptied and determinations of volume, alkalinity, hardness, total phosphorus, dissolved phosphorus (second run only), and orthophosphorus (second run only) were made. In the initial run only, influent rates were measured and adjusted daily. In the second run only, plant wet-weights were measured and part of the biomass was harvested for dry weight determinations and phosphorus analysis on day seven. Also in the second run only, influents and effluents were terminated on day twelve to allow independent estimates of evaporation rates from the aquaria. Additionally, samples were taken over a twenty-four hour period once during each experimental run.

Both experimental runs were terminated on the fifteenth day, at which time determinations of hardness, alkalinity, and forms of phosphorus were made on water drawn from each aquarium in additon to those determinations described above for intermediate days. Plants were removed, rinsed, drained and blotted, weighed, and oven-dried for dry-weight measurement and phosphorus analysis. Volumes within the aquaria were then determined and, finally, sediment samples were taken for moisture content and phosphorus analysis.

BEAKER EXPERIMENT

Treatments

Treatments consisted of the presence or absence of sediments in the experimental beakers and of adjustments to pH, alkalinity, hardness, and phosphorus concentration in the water added to the beakers. This water was prepared from deionized distilled water by the addition of various compounds (Table 2) in amounts determined by the desired treatment levels (Table 2), the analysis of Pond 2 water (Table 1), and the results of the

Table 2. Water chemistry treatment levels for beaker experiment.

I. Initial Alkalinity

Level	Alkalinity	Compounds and concentrations used
1	ca. 0	Sufficient NaOH and HCl for pH adjustment
2	1.8 meq/l	0.2 meq HCl; 2.0 meq NaOH + $NaHCO_3$/l
3	3.6 meq/l	0.4 meq HCl; 4.0 meq NaOH + $NaHCO_3$/l

II. Initial pH

Level	pH	Ratios of normalities of HCl : $NaHCO_3$: NaOH
1	7.7 - 8.1	1 : 10 : 0
2	8.3 - 8.8	1 : 9 : 1
3	8.8 - 9.3	1 : 8 : 2
4	9.3 - 9.7	1 : 7 : 3
5	9.5 - 10.0	1 : 6 : 4

III. Initial Hardness

Level	Hardness	Compounds and concentrations used
1	0.0 meq/l	5.0 meq NaCl/l; 1.6 meq Na_2SO_4/l
2	3.1 meq/l	2.5 meq $CaCl_2$/l; 0.8 meq $MgSO_4$/l; 2.5 meq NaCl/l; 0.8 meq Na_2SO_4/l; 0.27 meq $Fe(NO_3)_3$/l
3	6.2 meq/l	5.0 meq $CaCl_2$/l; 1.6 meq $MgSO_4$/l; 0.54 meq $Fe(NO_3)_3$/l

IV. Initial Orthophosphorus

Level	(PO_4^{-3})	Compound and concentration used
1	0.1 mg P/l	0.4393 mg KH_2PO_4/l
2	0.5 mg P/l	2.1966 mg KH_2PO_4/l
3	1.0 mg P/l	4.3932 mg KH_2PO_4/l

aquarium experiment (see Results and Discussion). Proportions of calcium, magnesium, and ferric iron were not varied independently due to the lack of data on their individual levels in the aquarium experiments. Sulfate concentration, ionic strength, and dissolved oxygen were kept relatively constant, while sodium, chloride, potassium, and nitrate were varied as required by the levels of the other species and were assumed unimportant to the phosphorus exchange reactions. Combinations of the above water chemistry treatments were run with and without the presence of sediments in order to evaluate what portion of orthophosphate removal was due to precipitation or coprecipitation with substances in the water and what portion was due to adsorption by the sediment. Factorially arranged, these treatments comprise 270 combinations, of which only 165 were run, the omitted ones being almost entirely those with low alkalinity and hardness values. Also, 25 of the combinations were repeated to provide better estimates of variation.

Experimental Procedure

Eight series of experiments were conducted in a set of twenty-four 1000 ml beakers, to which treatment combinations were randomly assigned. Sediment was prepared as in the aquarium experiment, except that deionized distilled water was used, and 50 ml of this sediment were added to those beakers selected for treatment combinations calling for sediment. Appropriate amounts of phosphate and acid stock solutions were added to deionized distilled water which had been vigorously aerated for at least two hours and 860 ml of this solution were transferred to each beaker, taking care not to disturb the sediments. Other stock solutions were then added to each beaker in amounts appropriate for the water chemistry required in each beaker and for a final volume of 900 ml. Finally, pH and

temperature were measured in each beaker and all beakers were covered with watch glasses and placed in a shaded area. Initial values for hardness, alkalinity, and orthophosphorus were measured on duplicate beakers for each combinations of water chemistry treatments. After twenty-four hours, pH and temperature were again measured in each beaker and a water sample was with drawn from each beaker for determination of hardness, alkalinity, and orthophosphate concentration.

RESULTS AND DISCUSSION

AQUARIUM EXPERIMENT

Plant Growth and Phosphorus Content

Photoperiod had a marked influence on the growth of Ceratophyllum demersum during the experimental runs (Table 3), the shorter photoperiod resulting in a decline of 30% to 40% in dry weight, as opposed to an increase of 40% to 50% under the longer photoperiod. Growth of plants in the holding tanks showed a similar difference in rates due to photoperiod and further demonstrated that the density of the plants did not contribute to this difference. The slowdown of growth in the later part of the second experimental run (Table 3) was apparently due to nutrient limitation.

Changes in plant phosphorus content were also quite different between experimental runs (Table 3), largely due to the different growth rates, but also attributable to differences in plant condition and ambient phosphorus concentration. During the first experimental run, phosphorus equal to as much as 80% of that originally in the plants was released due to declining biomass and tissue concentration, the latter probably a result of both poor condition of the plants and lower concentrations of phosphorus in the aquaria than in the holding tanks. On the other hand, the positive growth rates during the second experimental run resulted in increases in phosphorus contained in the plants, even though moderate decreases in tissue phosphorus concentration occurred due to declining ambient phosphorus levels. As discussed below, this uptake and release of phosphorus by C. demersum had

Table 3. Summary of plant weights and phosphorus contents in aquarium experiment.

First Experimental Run (Aquaria 1, 4, 5 with plants, 12-hr photoperiod)				
Aquarium		1	4	5
Wet Weight (g)	- Day 0	400.0	400.0	400.0
	Day 15	321.2	316.3	329.5
Dry Weight (g)	- Day 0	21.25	21.25	21.25
	Day 15	13.20	14.34	12.17
Phosphorus (mg)	- Day 0	202.8	202.8	202.8
	Day 15	80.8	98.0	78.5
Second Experimental Run (Aquaria 2, 4, 6 with plants, 14-hr photoperiod)				
Aquarium		2	4	6
Wet Weight (g)	- Day 0	200.0	200.0	200.0
	Day 7			
	Preharvest	264.4	294.2	273.8
	Postharvest	200.0	200.0	200.0
	Day 15	236.4	236.4	203.8
Dry Weight (g)	- Day 0	16.14	16.14	16.14
	Day 7			
	Preharvest	19.95	22.17	20.11
	Postharvest	15.09	15.07	14.69
	Day 15	17.26	17.81	16.17
Phosphorus (mg)	- Day 0	72.45	72.45	72.45
	Day 7			
	Preharvest	86.27	102.51	87.93
	Postharvest	65.26	69.69	64.23
	Day 15	63.88	65.64	68.06

a great impact on the phosphorus levels in sediment and water during both experimental runs.

Water Budget

Volumes of water in the aquaria at the start and end of each run and volumes of influents, effluents, and evaporation are summarized in Table 4. For the second experimental run, all quantities were measured directly except evaporation, which was calculated by difference and was found to be significantly lower in aquaria with plants. Conversion of light energy to chemical energy in photosynthesis by C. demersum would account for an insignificant portion of the observed differences in evaporation, so the physical presence of the plants is presumed here to be the cause of these differences, most likely through effects on water circulation and surface turbulence. The latter was much reduced in the aquaria with plants. Since evaporation was found to be lower in aquaria with the macrophyte, effluent volumes from those aquaria were necessarily greater than in aquaria without plants. Together, these differences would obviously result in proportionately greater export of materials in aquaria with plants and greater accumulation of materials in aquaria without plants, with consequences on phosphorus distribution that will be evident below.

For the initial experimental run, influent volumes as well as evaporation were unknown due to the unreliable delivery system. Because of this, the evaporation rates from the second run were applied to the appropriate aquaria in the first run and influent rates were then computed by difference. This is a major assumption with potentially a significant impact on the conclusions drawn from the experimental results. Because of this, data analysis on the first experimental run was repeated with a wide range of evaporation rates to insure that any trends observed were real and

Table 4. Volumes (liter) of aquaria, influents, effluents, and evaporation in aquarium experiment.

First Experimental Run (Aquaria 1, 4, 5 with plants, 12-hr photoperiod)						
Aquarium	1	2	3	4	5	6
Initial Aquarium Volume	49.4	50.2	50.6	48.5	48.9	51.9
Influent Volume	46.1	48.6	50.2	42.0	41.7	38.7
Evaporation	11.6	14.2	13.8	10.3	9.7	13.5
Effluent Volume	34.5	34.5	36.4	31.7	32.0	25.2
Final Aquarium Volume	49.5	50.2	50.6	48.6	49.0	51.9
Second Experimental Run (Aquaria 2, 4, 6 with plants, 14-hr photoperiod)						
Aquarium	1	2	3	4	5	6
Initial Aquarium Volume	51.0	50.8	51.0	50.8	51.0	50.8
Influent Volume	28.9	28.4	28.2	29.0	29.1	28.2
Evaporation[a]	13.5	9.7	13.8	10.3	14.2	11.6
Effluent Volume	17.1	20.3	16.6	21.8	16.7	19.6
Final Aquarium Volume	49.3	49.1	48.8	47.7	49.2	47.8

[a] Mean effect of plants on evaporation = -0.22 liter/day; significant at the 0.04 level, based on a paired t-test.

not artifacts of this assumption.

Twenty-four Hour Sampling

Representative values of samples taken over a twenty-four hour period during the first experimental run are listed in Table 5. Temperature, pH, and dissolved oxygen in the aquaria showed definite diel cycles, the magnitude of which was similar in aquaria with and without plants, so that any conclusions drawn on data at midday were valid for the entire day. Interestingly, values of these parameters taken at midday are quite close to the means over the twenty-four hour period, a fact which was of significance in the simulations discussed later.

Alkalinity, hardness, and phosphorus in the effluent did not demonstrate any significant differences between light and dark periods, but this does not preclude cycling that might cancel itself out within these periods, as approximately occurs in the cases of pH and dissolved oxygen. However, additional measurements of orthophosphorus in the aquaria during the second experimental run did show that there was no significant deviation of orthophosphorus concentration over a light/dark cycle from the general trends observed in the run.

Temperature, Dissolved Oxygen, and pH

Midday values for temperature, dissolved oxygen, and pH are plotted for all aquaria in both experimental runs on Figures 1, 2, and 3, respectively. Mean values of these and other parameters over the duration of each run are listed in Table 6, along with the mean effects of plants on each parameter and the significance levels of these effects, based on a paired t-test. Of these parameters, temperature is the only one to fail to show any significant effects due to plants on any of the days or in the means. Consistent differences in temperature were only found

Table 5. Results of twenty-four hour sampling during first run of aquarium experiment (day 14 to day 15).

Aquarium		1	2	3	4	5	6
pH	- Midday	8.64	8.79	8.60	9.00	9.30	8.92
	Lights Out	9.08	9.04	8.81	9.25	9.45	9.13
	Midnight	8.55	8.75	8.51	8.83	9.09	9.06
	Lights On	8.47	8.60	8.40	8.75	8.81	8.68
	Midday	8.60	8.74	8.51	8.91	9.15	8.81
Dissolved Oxygen ($mg/l\ O_2$)	- Midday	10.2	8.0	8.5	12.6	13.3	10.4
	Lights Out	17.5	13.4	10.6	16.9	18.6	14.5
	Midnight	9.2	10.7	8.6	10.3	13.4	11.8
	Lights On	6.8	8.1	6.6	7.0	9.8	8.6
	Midday	10.4	10.7	8.5	12.1	11.8	11.0
Temperature (^{o}C)	- Midday	22.8	22.8	22.8	23.0	23.0	22.8
	Lights Out	23.4	23.7	23.8	24.0	23.7	23.4
	Midnight	22.7	22.8	22.8	22.7	22.7	22.6
	Lights On	22.6	22.6	22.7	22.6	22.6	22.5
	Midday	23.4	23.6	23.7	23.7	23.6	23.4
Hardness ($mg/l\ CaCO_3$)	- Light Period	280	162	188	282	269	167
	Dark Period	279	163	185	278	266	166
Alkalinity ($mg/l\ CaCO_3$)	- Light Period	293	180	205	292	280	182
	Dark Period	294	182	206	293	281	183
Total Phosphorus ($mg/l\ P$)	- Light Period	.912	.058	.070	.723	.511	.055
	Dark Period	.858	.082	.087	.680	.481	.060

Table 6. Mean values of water chemistry parameters during aquarium experiment.

First Experimental Run (Aquaria 1, 4, 5 with plants, 12-hr photoperiod)								
Aquarium	1	2	3	4	5	6	Mean Effect of Plants	Alpha Level[a]
Midday Temperature (°C)	22.8	22.9	23.0	23.0	22.9	22.8	0.0	1.000
Midday pH	8.38	8.61	8.56	8.66	8.79	8.61	+0.02	0.887
Midday Dissolved Oxygen (mg/l O_2)	7.96	10.45	9.81	9.72	10.75	10.80	-0.88	0.471
Alkalinity (mg/l $CaCO_3$)	280	228	238	282	278	234	+47	0.008
Hardness (mg/l $CaCO_3$)	293	248	255	295	291	251	+41	0.001
Total Phosphorus (mg/l P)	1.000	0.308	0.305	0.824	0.684	0.307	+0.529	0.033
Second Experimental Run (Aquaria 2, 4, 6 with plants, 14-hr photoperiod)								
Aquarium	1	2	3	4	5	6	Mean Effect of plants	Alpha Level[a]
Midday Temperature (°C)	20.3	20.5	20.6	20.7	20.7	20.6	+0.1	0.712
Midday pH	8.52	9.10	8.63	9.31	8.63	9.05	+0.55	0.015
Midday Dissolved Oxygen (mg/l O_2)	10.65	13.94	10.96	14.86	10.90	14.13	+3.47	0.008
Alkalinity (mg/l $CaCO_3$)	168	114	155	101	159	113	-51	0.007
Hardness (mg/l $CaCO_3$)	268	209	252	193	258	208	-56	0.007
Total Phosphorus (mg/l P)	0.361	0.117	0.315	0.114	0.319	0.133	-0.210	0.004
Dissolved Phosphorus (mg/l P)	0.341	0.102	0.286	0.094	0.286	0.106	-0.204	0.009
Orthophosphorus (mg/l P)	0.286	0.073	0.236	0.067	0.240	0.077	-0.181	0.009

[a] Alpha significance level of plant effect, based on a paired t-test.

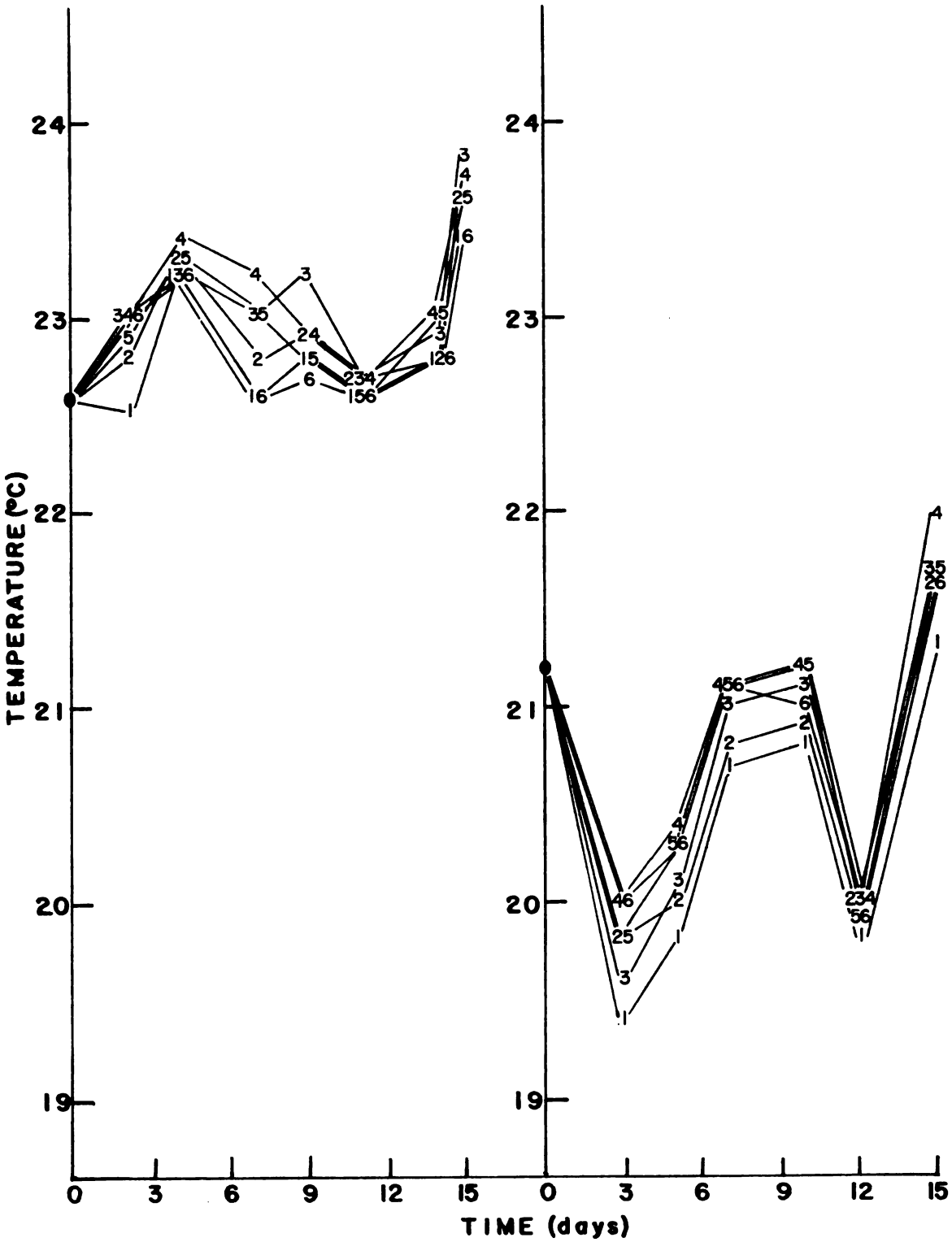

Figure 1. Midday temperature vs. time for all aquaria during experimental runs one (left, aquaria 1, 4, 5 with plants) and two (right, aquaria 2, 4, 6 with plants).

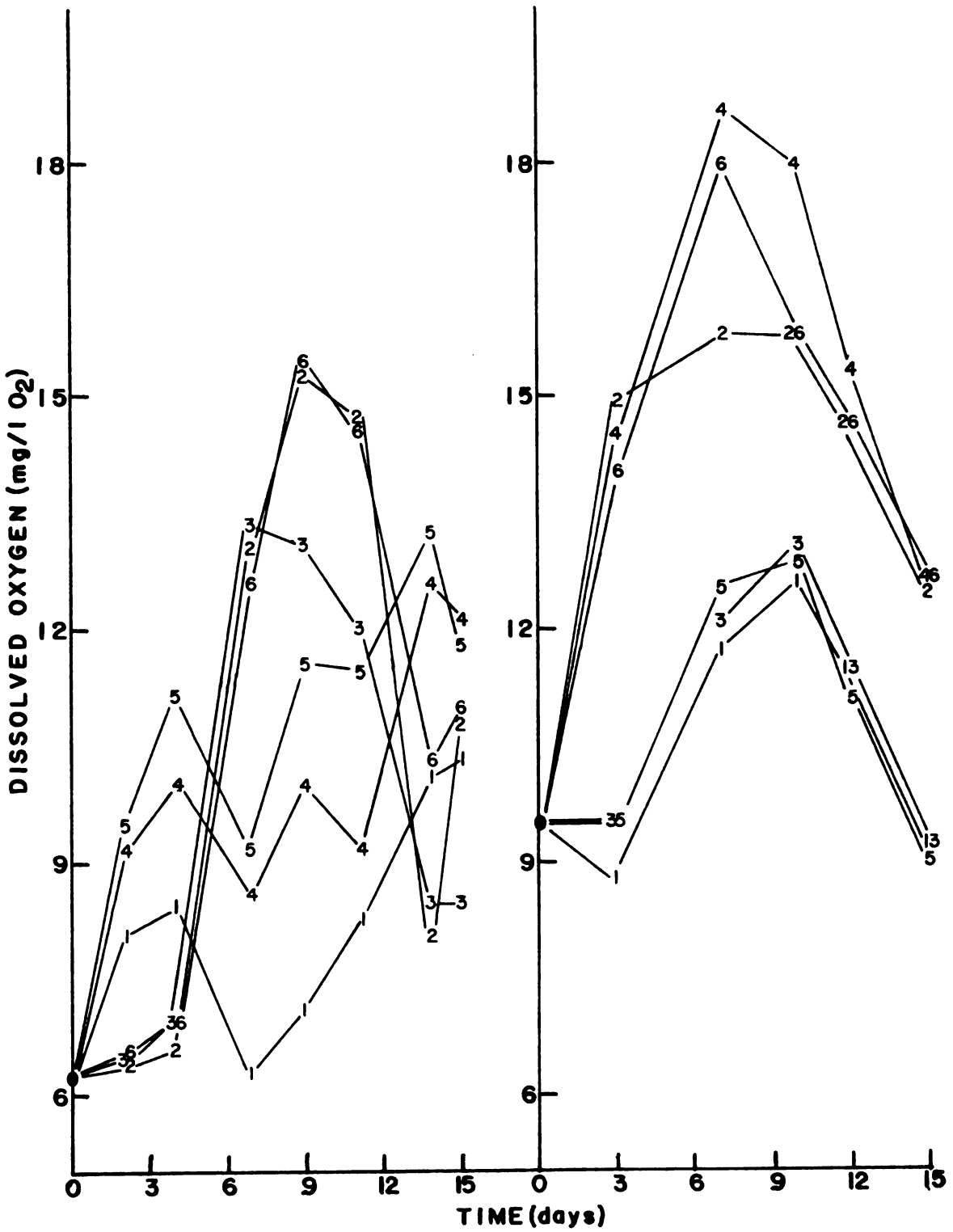

Figure 2. Midday dissolved oxygen concentration vs. time for all aquaria during experimental runs one (left, aquaria 1, 4, 5 with plants) and two (right, aquaria 2, 4, 6 with plants).

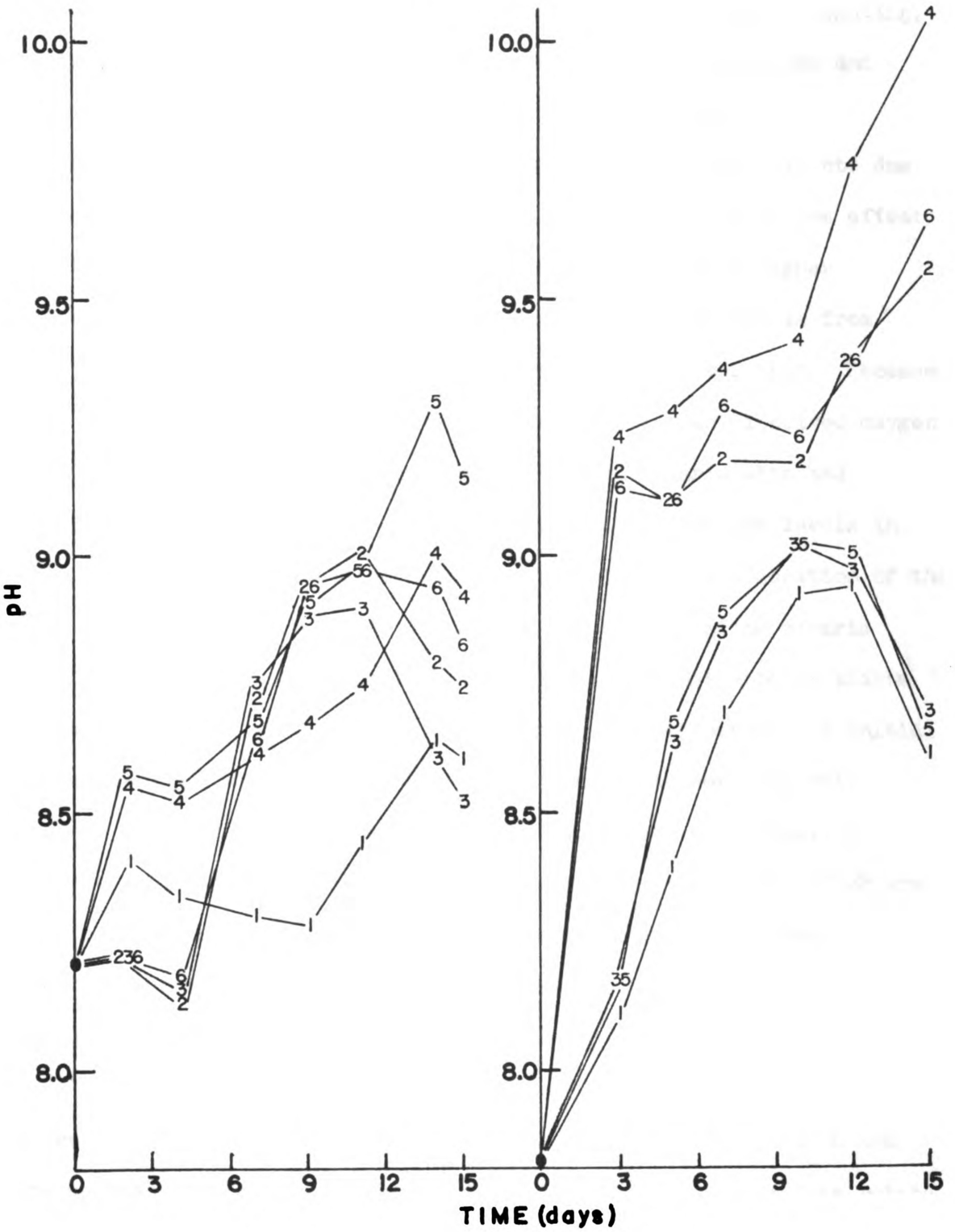

Figure 3. Midday pH vs. time for all aquaria during experimental runs one (left, aquaria 1, 4, 5 with plants) and two (right, aquaria 2, 4, 6 with plants).

between end and middle aquaria, attributable to the gradient of lighting, and between experimental runs, attributable to lower temperatures and humidities in the experimental room during the second run.

Dissolved oxygen, on the other hand, showed significant effects due to C. demersum in both experimental runs. In the initial run, the effect varied through time, with aquaria containing plants showing higher dissolved oxygen levels during the first five days, lower levels from then to approximately day 12, and higher levels again after that. Because of this time dependence of the effect of the plants, mean dissolved oxygen levels did not show consistent differences between aquaria with and without plants. In the second run, however, dissolved oxygen levels in aquaria with plants were consistently higher for the entire duration of the run and had significantly higher means than the corresponding aquaria without plants. Comparing aquaria between runs, the ones without plants showed a high degree of similarity, considering the differences in initial dissolved oxygen concentration, whilethose with plants were markedly different. As would be expected since both parameters are largely a function of photosynthetic and respiratory activity, the trends of pH are quite close to those described above for dissolved oxygen and show comparable mean effects and significance levels.

Hardness and Alkalinity

Values for hardness and alkalinity for all aquaria in both experimental runs are plotted on Figures 4 and 5. Points at days 0 and 15 are as measured in the aquaria on those days, while intermediate points are those determined on effluent water, plotted at the midpoints of the periods over which effluent was collected. These parameters behaved quite similarly to each other and show comparable mean effects due to plants

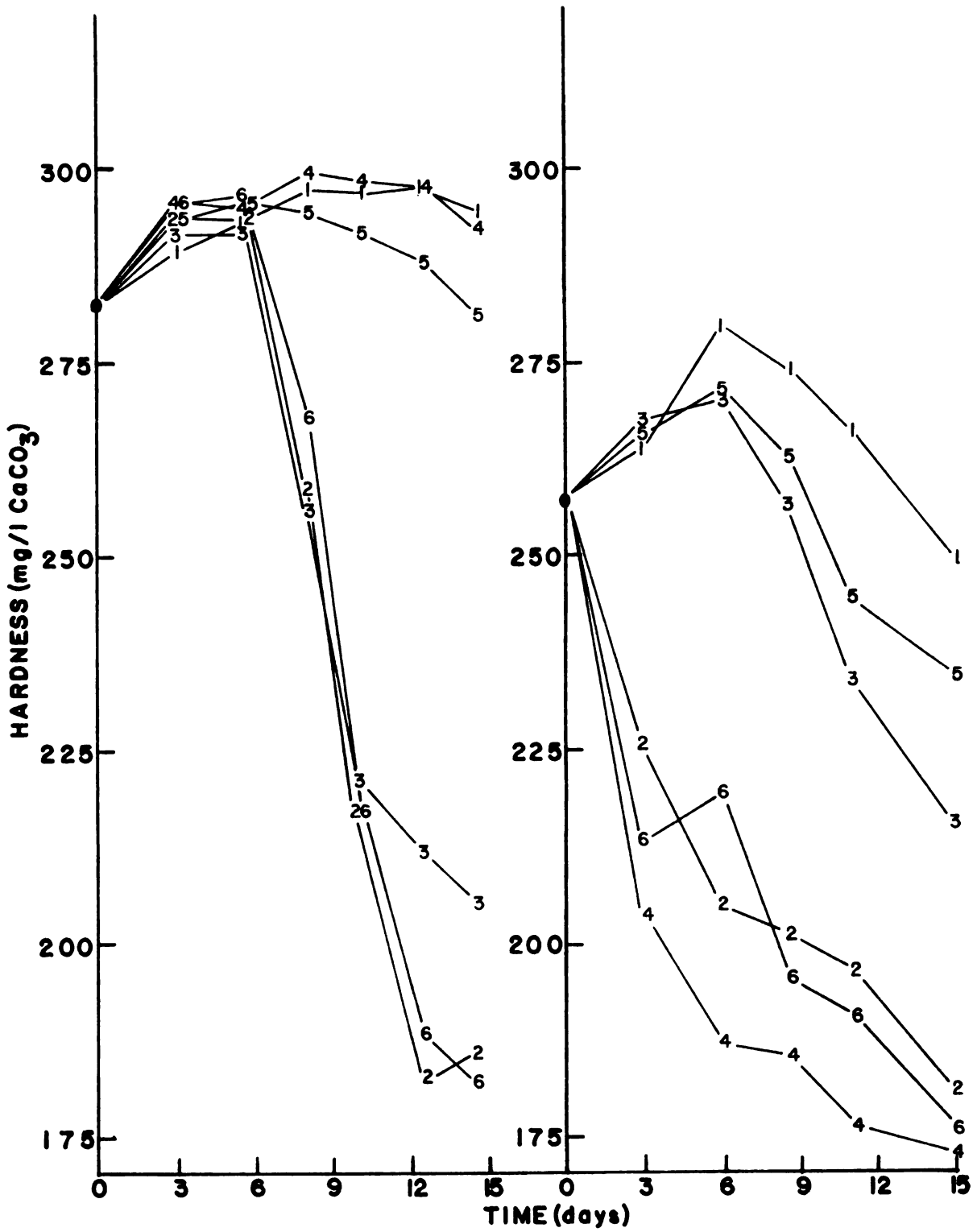

Figure 4. Hardness vs. time for all aquaria during experimental runs one (left, aquaria 1, 4, 5 with plants) and two (right, aquaria 2, 4, 6 with plants).

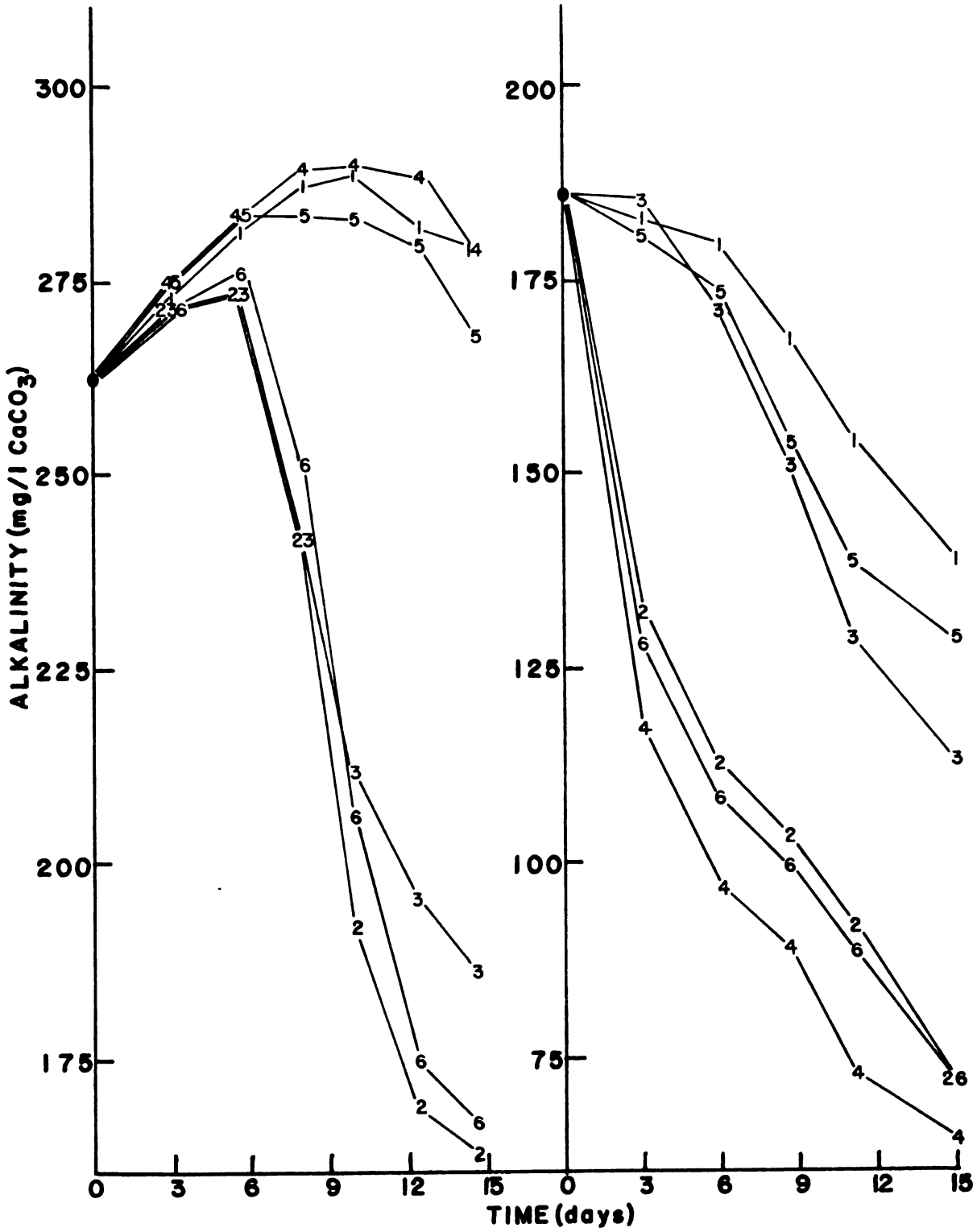

Figure 5. Alkalinity vs. time for all aquaria during experimental runs one (left, aquaria 1, 4, 5 with plants) and two (right, aquaria 2, 4, 6 with plants).

(Table 6).

In the first experimental run, aquaria with plants showed a slight increase in both hardness and alkalinity through day 10, presumably due to concentration by evaporation, followed by a slight decline which left final values at or near initial ones. Aquaria without plants, meanwhile, showed similar slight increases through day 5, at which time precipitous declines occurred, with values leveling out in the final days of the run. The declines in hardness and alkalinity were of similar magnitude and corresponded well to increasing pH, suggestive of carbonate precipitation. As would be expected, mean values for both hardness and alkalinity were significantly lower in aquaria with plants (Table 6).

In the second experimental run, hardness and alkalinity in aquaria without plants behaved qualitatively similar to the first run, with major differences in the magnitude of the decline after day 5 being attributable to the different initial values of hardness and alkalinity. The aquaria with plants, however, behaved markedly different from both the aquaria without plants and the aquaria with plants in the first run, showing large, immediate declines in both hardness and alkalinity and lesser declines that continued throughout the run. Again this is best attributed to the rise in pH and suggests carbonate precipitation, deposits being observed on the plants starting on day 1. Hardness and alkalinity were significantly lower in aquaria with plants throughout this run and showed correspondingly lower means (Table 6), an effect opposite to that observed in the first run.

Phosphorus

Total phosphorus values for all aquaria in both experimental runs are plotted on Figure 6. Dissolved phosphorus and orthophosphorus values for the second experimental run are included in Figure 7. Even more so than

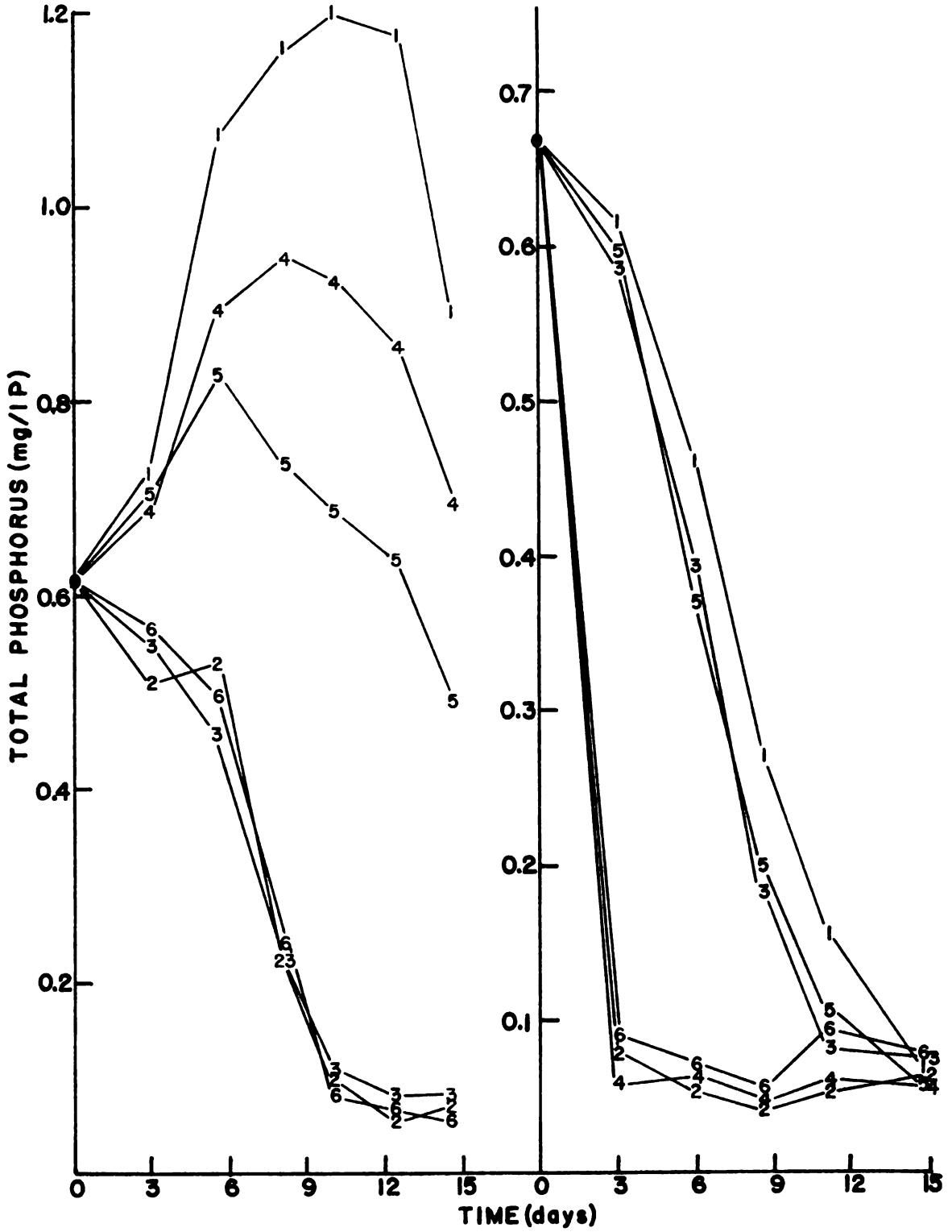

Figure 6. Total phosphorus vs. time for all aquaria during experimental runs one (left, aquaria 1, 4, 5 with plants) and two (right, aquaria 2, 4, 6 with plants).

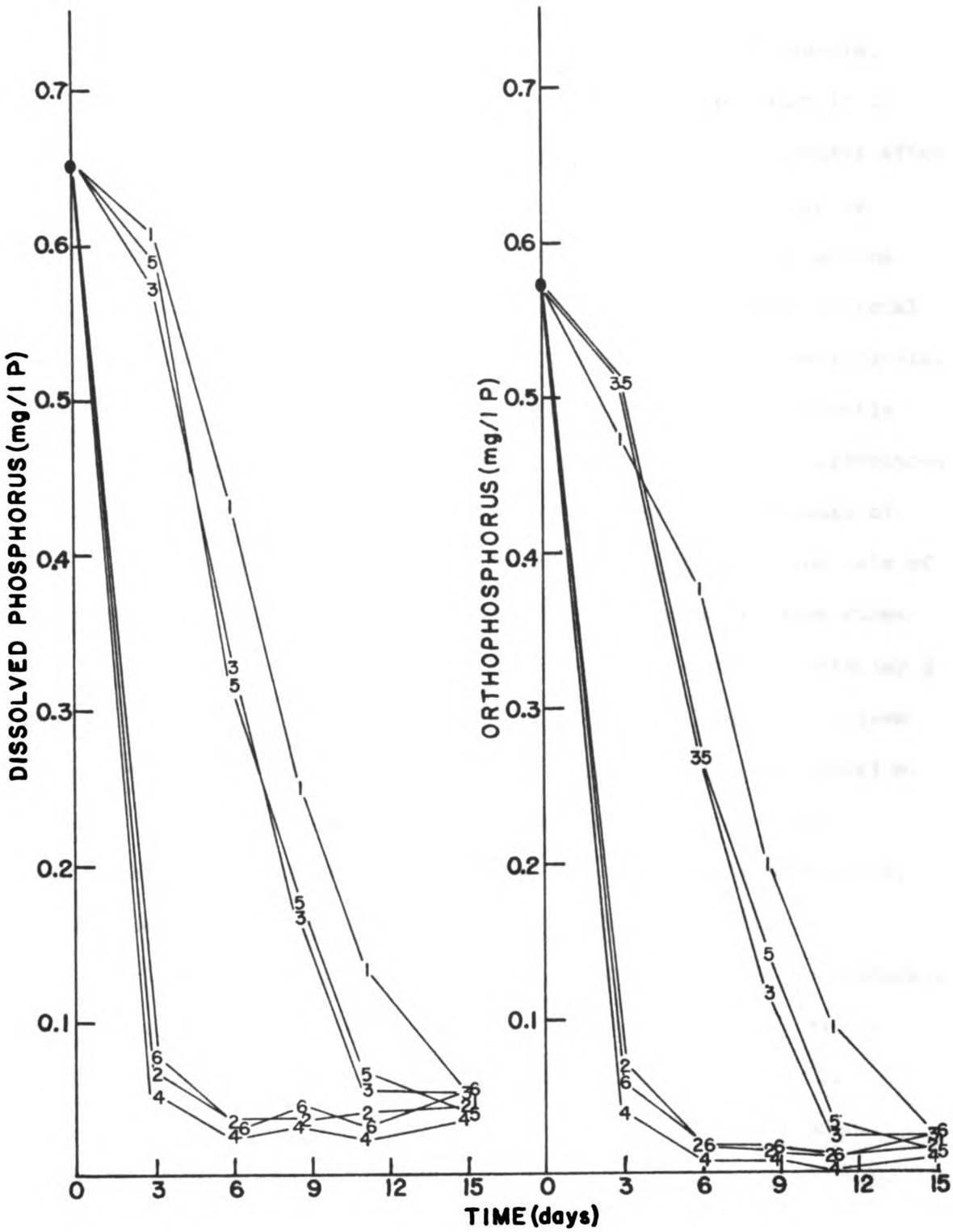

Figure 7. Dissolved phosphorus (left) and orthophosphorus (right) vs. time for all aquaria during experimental run two (aquaria 2, 4, 6 with plants).

for the other parameters, aquaria without plants showed a high degree of similarity between runs with respect to phosphorus. In these aquaria, total phosphorus declined slowly through day 5, then dropped sharply to approximately 0.1 mg/l P by day 10 or 11, and showed slight decreases after that. Not surprisingly, this sharp drop was correlated with drops in hardness and alkalinity and with rises in pH. Dissolved phosphorus and orthophosphorus in the second experimental run behaved similarly to total phosphorus, merely starting at and dropping to proportionately lower levels.

The behavior of phosphorus in aquaria with plants differed greatly between experimental runs and, in both runs, showed significant differences from aquaria without plants. In the first experimental run, release of phosphorus from the senescing plant tissues initially exceeded the rate of reactions removing phosphorus to the sediments, resulting in large rises in total phosphorus levels in the water. These rises peaked between day 5 and day 10, after which moderate declines occurred, leaving final values still quite high, being less than the initial value in only one aquarium. After day 1, aquaria with plants showed significantly higher total phosphorus levels than those without plants for the duration of the run, which resulted in drastically different means (Table 6).

In the second experimental run, large, immediate declines in phosphorus concentrations in aquaria with plants were observed, presumably due to both plant uptake and removal by inorganic reactions, especially by coprecipitation with the carbonates mentioned above. Total phosphorus levels declined to below0.1 mg/l P by day 3 and remained there till the end of the run, dropping to or below 0.05 mg/l P at day 8 or 9, with slight rises afterward. Dissolved phosphorus and orthophosphorus were correspondingly lower, with the latter being at the limits of detectability

(0.01 mg/l P) in some instances. Aquaria with plants in this run showed significantly lower levels of phosphorus than those without plants from day 1 to day 13, after which all aquaria were at roughly the same, low level. Means for all forms of phosphorus measured reflect this effect of the plants (Table 6).

The collective behavior of total phosphorus, dissolved phosphorus, and orthophosphorus in the second experimental run showed that the decline in phosphorus levels in the water in all aquaria was predominantly from the dissolved orthophosphorus fraction. This fact and the observed abrupt declines in phosphorus levels suggest that the removal of phosphorus to the sediments was primarily due to inorganic phosphorus exchange reactions, rather than uptake and deposition by seston. Supporting this statement was observation of very little deposition of organic matter, as opposed to significant deposition of inorganic precipitates. However, attempts to measure directly the amounts of phosphorus deposited into the sediment in inorganic and organic forms proved impossible due to high initial levels of phosphorus in the sediment masking any changes during the experimental period.

Budgets for total phosphorus in each aquarium were computed for both experimental runs, with the amount of phosphorus removed into the sediments being computed by difference (Tables 7 and 8). Various parameters that are useful in summarizing the behavior of phosphorus in the aquaria were then calculated from these budgets, the simplest of these being the proportion of the phosphorus initially in the aquaria and the phosphorus added in the influent that was removed by plants and sediment combined (Parameter A) and by sediments alone (Parameter B), these two parameters being equal in aquaria without C. demersum. The effect of plants on these

Table 7. Total phosphorus budget for first run of aquarium experiment (12-hr. photoperiod, aquaria 1, 4, 5 with plants).

Aquarium	1	2	3	4	5	6
Initial phosphorus in aquarium (mg)	28.46	31.49	30.92	30.63	30.68	31.61
Phosphorus in influent (mg)	35.65	37.99	38.92	32.03	32.13	29.99
Plant uptake of phosphorus (mg)	-122.07	0.00	0.00	-104.81	-124.32	0.00
Phosphorus in effluent (mg)	33.75	11.86	12.17	25.98	22.63	8.53
Final phosphorus in aquarium (mg)	40.43	3.70	4.07	31.78	22.66	2.88
Phosphorus removed into sediment (mg)	112.00	53.92	53.60	109.71	141.84	50.19
Derived Parameters:						
A. % of initial and influent phosphorus removed by sediments and plants combined.[a]	-15.7	77.6	76.7	7.8	27.9	81.5
B. % of initial and influent phosphorus removed by sediments alone.[b]	174.7	77.6	76.7	175.1	225.8	81.5
C. Mean removal rate of phosphorus into sediments, weighted by division by mean orthophosphorus concentration (day^{-1}).[c]	8.68	15.38	15.43	10.42	16.46	14.37

Significance levels of plant effects based on paired t-test = [a]0.040, [b]0.021, and [c]0.281.

Table 8. Total phosphorus budget for second run of aquarium experiment (14-hr. photoperiod, aquaria 2, 4, 6 with plants).

Aquarium	1	2	3	4	5	6
Initial phosphorus in aquarium (mg)	33.83	33.70	33.83	33.70	33.83	33.70
Phosphorus in influent (mg)	30.99	30.41	30.22	31.12	31.21	30.26
Plant uptake of phosphorus (mg)	0.00	12.44	0.00	26.01	0.00	19.32
Phosphorus in effluent (mg)	6.26	1.04	4.97	1.84	5.20	1.59
Final phosphorus in aquarium (mg)	3.51	3.25	3.66	2.92	3.08	3.70
Phosphorus removed into sediment (mg)	55.05	47.37	55.42	34.05	56.77	39.36
Derived Parameters:						
A. % of initial and influent phosphorus removed by sediments and plants combined.[a]	84.9	93.3	86.5	92.7	87.3	91.7
B. % of initial and influent phosphorus removed by sediments alone.[b]	84.9	73.9	86.5	52.5	87.3	61.5
C. Mean removal rate of phosphorus into sediments, weighted by division by mean orthophosphorus concentration (day^{-1}).[c]	12.83	42.41	15.66	33.87	15.76	33.92

Significance levels of plant effects based on paired t-test = [a]0.003, [b]0.062, and [c]0.014.

parameters was tested using paired t-tests, transforming the proportions to arcsines except where any exceeded 1.0. In the first experimental run, the release of phosphorus by plants caused the combined removal of phosphorus by plants and sediment (Parameter A) in aquaria with plants to be greatly lower than in those without plants, while the removal of phosphorus by sediments alone (Parameter B) was quite higher because much more phosphorus was available for removal. In the second experimental run, on the other hand, the reverse of this effect occurred due to the large uptake of phosphorus by plants, with the combined removal by plants and sediment being higher in the aquaria with plants than in those without, while the sediments alone removed a lesser proportion since they were, in effect, competing with the plants for available phosphorus.

Thus far it is apparent that Ceratophyllum demersum had a marked influence on the dynamics of phosphorus in the experimental aquaria and that a major cause of this impact was the release or uptake of phosphorus by the plants, which greatly altered the concentration of this element in the water and made more or less phosphorus available for removal in the effluent and into the sediments. This direct effect, though, is not necessarily the only effect of the plants on phosphorus dynamics, with the alteration of pH, for example, being likely to have an impact on phosphorus in the manners discussed earlier. Any such indirect effects are effectively masked by the large amounts of phosphorus released or taken up by the plants. To penetrate this masking, an additional parameter (Parameter C) was calculated from the budget of each aquarium, this being the mean rate of removal of total phosphorus, on a concentration basis, from the water into the sediments, weighted by dividing by the mean concentration of orthophosphorus. With this parameter, any change in the

rate of removal of phosphorus into the sediments due to release or uptake of phosphorus by plants would be compensated for by division by the altered orthophosphorus concentration. This compensation is complete only if removal of phosphorus into the sediments if first order with respect to orthophosphorus. If this is not true, differences in this parameter between aquaria could still be attributed to lower or higher phosphorus levels caused directly by plants or to changes in rates of deposition of particulate organic matter due to plants. Therefore, without further evidence to establish the validity of this parameter, it will be used only as a preliminary indicator of indirect effects of plants.

For the initial experimental run, plants were found to have mixed effects on this parameter (Table 7), while in the second run, they were observed to cause marked increases (Table 8). Besides strongly indicating indirect plant effects, this combination of results is correlated well with mean pH (Table 6) and, along with results and theory discussed earlier, suggests that pH is critical to the manner in which C. demersum alteredphosphorus dynamics in the aquaria. This parameter was also correlated well with dissolved oxygen, but this is unlikely as an important factor since levels in all aquaria were far above the values considered critical to phosphorus dynamics. Other factors of possible significance to the indirect effects of C. demersum on rates of phosphorus removal include dissolved and particulate matter, evaporation rates, and hardness and alkalinity levels. Of these, organic matter cannot be considered since measurements were not made, though deposition of particulate organic matter appeared slight. Also, hardness and alkalinity are predominantly functions of pH and evaporation, so that these two latter factors are the only ones that need to be considered with respect to indirect effects of

C. demersum on phosphorus. The testing of these factors requires a separation of their individual effects from each other and from the direct effects of the plants, which in turn requires the simulation of phosphorus dynamics in the aquaria as a function of all significant factors. Such a simulation was the reason for the beaker experiment described earlier, the discussion of which follows.

BEAKER EXPERIMENT

Regression Analysis

The series of beaker experiments resulted in 188 data points, each with initial and final values for temperature, pH, hardness, alkalinity, and orthophosphorus. These data were used to develop regression equations for the changes over twenty-four hours in orthophosphorus concentration, hardness, and alkalinity, both in the presence and absence of sediments, as a function of the means of the initial and final values of temperature, pH, hardness, alkalinity, and orthophosphorus. All regression analyses were conducted using the multiple linear regression programs of the Michigan State University computer laboratory STAT package.

The regression analysis for orthophosphorus removal was based on the following expression, which is analogous to a simple chemical rate equation with temperature dependence according to the Arrhenius expression:

Change in orthophosphorus concentration =

$$K \times e^{k_T/T} \times (\text{alkalinity})^{k_A} \times (\text{hardness})^{k_H} \times (\text{ortho-P})^{k_P} \times 10^{k_{pH} \times pH}$$

A logarithmic transformation of this equation was made to allow linear regression techniques to be used. Appropriate interactions and squared terms were then permitted to be added where significant. The resultant equations (Appendix B) included all the basic terms plus four two-way interactions, and accounted for 82% and 92% of the data variability in the

absence and presence of sediment, respectively. Significant differences due to the presence of sediment were found in several of the coefficients of these equations (Appendix B).

The response surfaces for phosphorus removal as a function of mean orthophosphorus and pH at hardness = 250 mg/l $CaCO_3$ and alkalinity = 150 mg/l $CaCO_3$ are plotted on Figure 8, both for the presence and absence of sediment. Similar surfaces were found at other levels of alkalinity and hardness. Without sediments, phosphorus removal showed stong pH dependence, with as much as a five-fold increase from pH 8.0 to 9.5. This increase was undoubtedly at least in part due to coprecipitation of orthophosphorus with carbonates, which were observed in significant amounts at pH 9.0 and above. Precipitation of phosphorus with various of the cations present and coprecipitation with their hydroxides would also contributed in various ways to the pH dependence of the response surface. With sediments present, similar high amounts of phosphorus removal were found at high pH, but the decline in removal at lower pH was not nearly as great as in the absence of sediments. In fact, a slight increase in phosphorus removal with decreasing pH sometimes occurred in the presence of sediments, though this increase was usually not statistically significant. This response of phosphorus removal to the presence of sediments suggests that adsorption of phosphorus by the sediment is much greater at lower pH, consistent with the properties of clay minerals discussed earlier.

As would be expected, phosphorus removal from the water increases with orthophosphorus concentration in both response surfaces, but this increase is usually not proportional, as would occur if the kinetics of phosphorus removal were first order with respect to orthophosphorus. Estimates of the actual order of these kinetics varied from 1.33 at pH 8.0

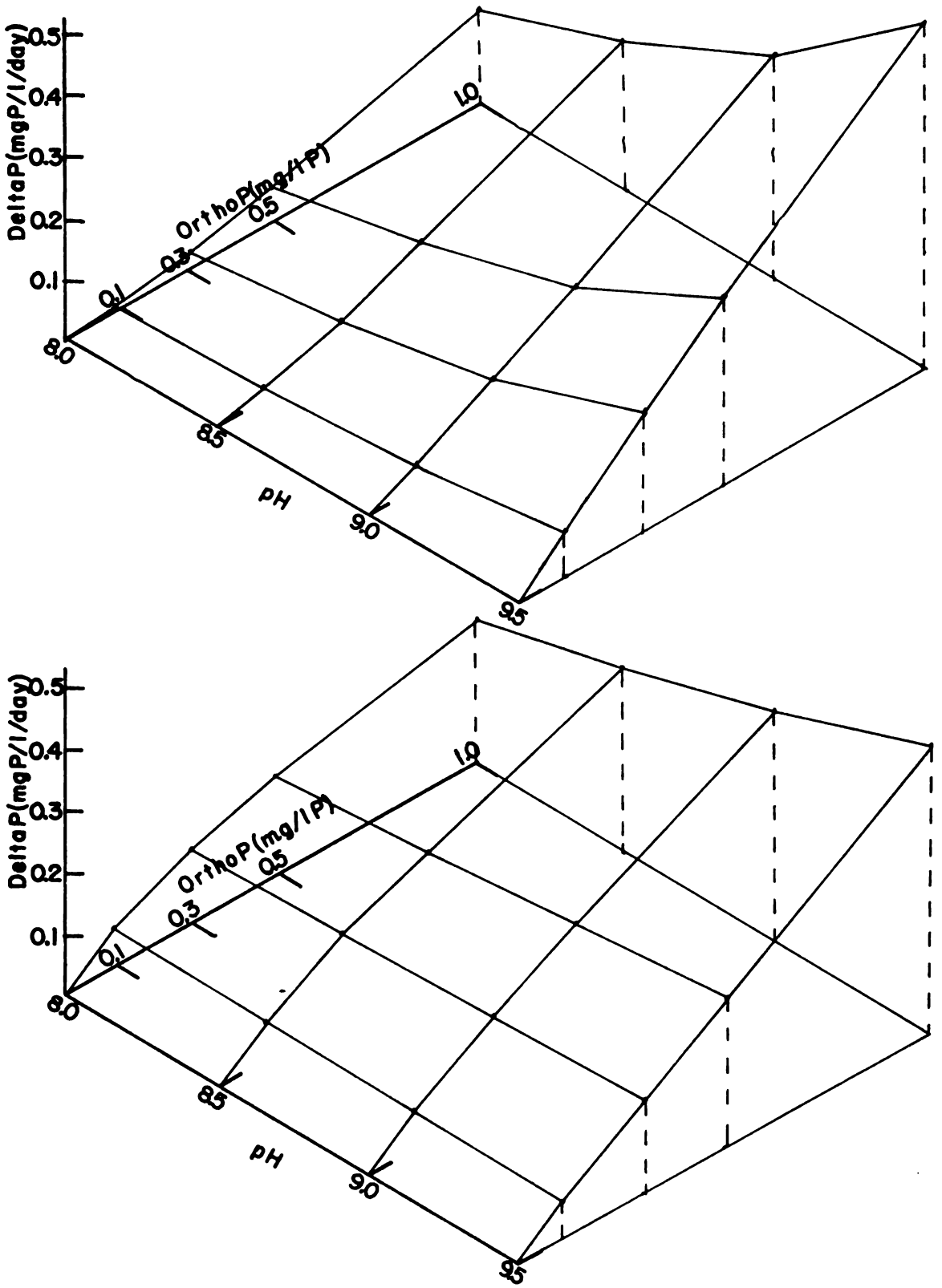

Figure 8. Response surfaces of the removal of orthophosphorus from water (DeltaP) vs. mean pH and mean orthophosphorus concentration at hardness = 250 mg/l $CaCO_3$ and alkalinity = 150 mg/l $CaCO_3$ in beakers without (top) and with (bottom) sediments.

to 0.87 at pH 9.5 in the absence of sediments and from 0.54 at pH 8.0 to 0.93 at pH 9.5 in the presence of sediments. The differences in these estimates are indicative of removal of phosphorus by different reactions as a function of pH and sediment. Further computations showed that, under the conditions of the aquarium experiment, the removal of orthophosphorus would have had an order that varied from 0.85 to 0.95. The consequences of the order being at these values on the use of Parameter C in the analysis of the aquarium experiment will be seen in the results of the simulations below.

The changes in hardness and alkalinity over twenty-four hours were regressed on mean temperature, hardness, alkalinity, and pH, the squares of mean pH and hardness, and all possible two and three-way interactions of these variables. No attempt was made to eliminate insignificant terms. The equations so computed accounted for 72% and 78% of the variation in the removal of alkalinity and hardness, respectively, in the absence of sediment, and 77% and 86% in the presence of sediment (Appendix B).

Simple regression equations of total phosphorus on orthophosphorus were computed from the data for each aquarium from the second run of the aquarium experiment. These equations were found not to be significantly different, so a combined regression equation was computed that accounted for 98% of the variation in the data (Appendix B).

Modeling and Simulation of Aquarium Experiment

The regression equations discussed above were used in a simple model to simulate the behavior of phosphorus during the aquarium experiment. Calculations were made at twenty-four hour intervals, starting and ending at midday. For each interval, computations started with initial values for hardness, alkalinity, orthophosphorus, total phosphorus, pH, and

temperature. Inputs included final pH and temperature for the interval, the volume, hardness, alkalinity, and total phosphorus concentration of the influent during the interval, and daily evaporation from the aquarium being simulated. If plants were present, uptake or release of phosphorus by the plants during the interval was also an input, this value being computed from curves of plant phosphorus content vs. time, which for the first experimental run were based on exponential decay between initial and final values (Table 3), while for the second experimental run the curves were of quadratic equations fitting the initial, intermediate, and final values of plant phosphorus content.

The regression equations used in this model were functions of the means of the initial and final values of the various parameters for the computational interval, which, since they were midday values were reasonable estimates of the actual means over the interval. Since the final values of hardness, alkalinity, and phosphorus were unknown, the use of an iterative procedure was necessary, in which the final values of these parameters were first assumed to be equal to the initial values. Means were then calculated and removal rates of alkalinity, hardness, and orthophosphorus to the sediment were computed using linear combinations of the regression equations for these parameters in beakers with and without sediments. The coefficients of these linear combinations were based on the sediment area to water volume ratio in the aquaria and beakers, assuming that removal varied linearly between beakers with and without sediment as a function of this ratio. The computed rates of removal to the sediments were then used in mass balance equations with the rates of influent, effluent, and uptake by plants of the various parameters to compute new estimates of the final values for total phosphorus, alkalinity,

and hardness for the computational period. Estimates of the final level of orthophosphorus were then calculated using the regression equation of this parameter with total phosphorus. The entire procedure, starting with the calculation of the mean values of the parameters, was then repeated until final estimates remained unchanged between computation cycles to the desired number of significant digits, at which time computations proceeded to the next twenty-four hour period.

This model was first used to simulate the behavior of alkalinity, hardness, and phosphorus in the aquarium experiment with unmodified data on influents, pH, temperature, evaporation, and plant phosphorus content. The results of these simulations are summarized in plots of hardness (Figure 9), alkalinity (Figure 10), and total phosphorus (Figure 11) and in budgets of total phosphorus (Tables 9 and 10), comparable to those presented earlier for the actual results of the aquarium experiment. To an extent, the simulations were successful with respect to phosphorus behavior in that the general effects of plants and photoperiod were duplicated, but, since these effects are predominantly functions of the large amounts of phosphorus taken up or released by the plants, the gross similarities between simulation and reality are hardly indicative of the success of all components of the model. Similarly, simulations of hardness and alkalinity were moderately successful, but did show significant differences from reality, especially in the aquaria with plants.

Several consistent differences in phosphorus behavior exist between the simulations and the actual experiments. Simulations of aquaria without plants are similar for both runs and show less deviation from reality than those for aquaria with plants, but they do exhibit faster initial declines in phosphorus and level off at higher concentrations of phosphorus than

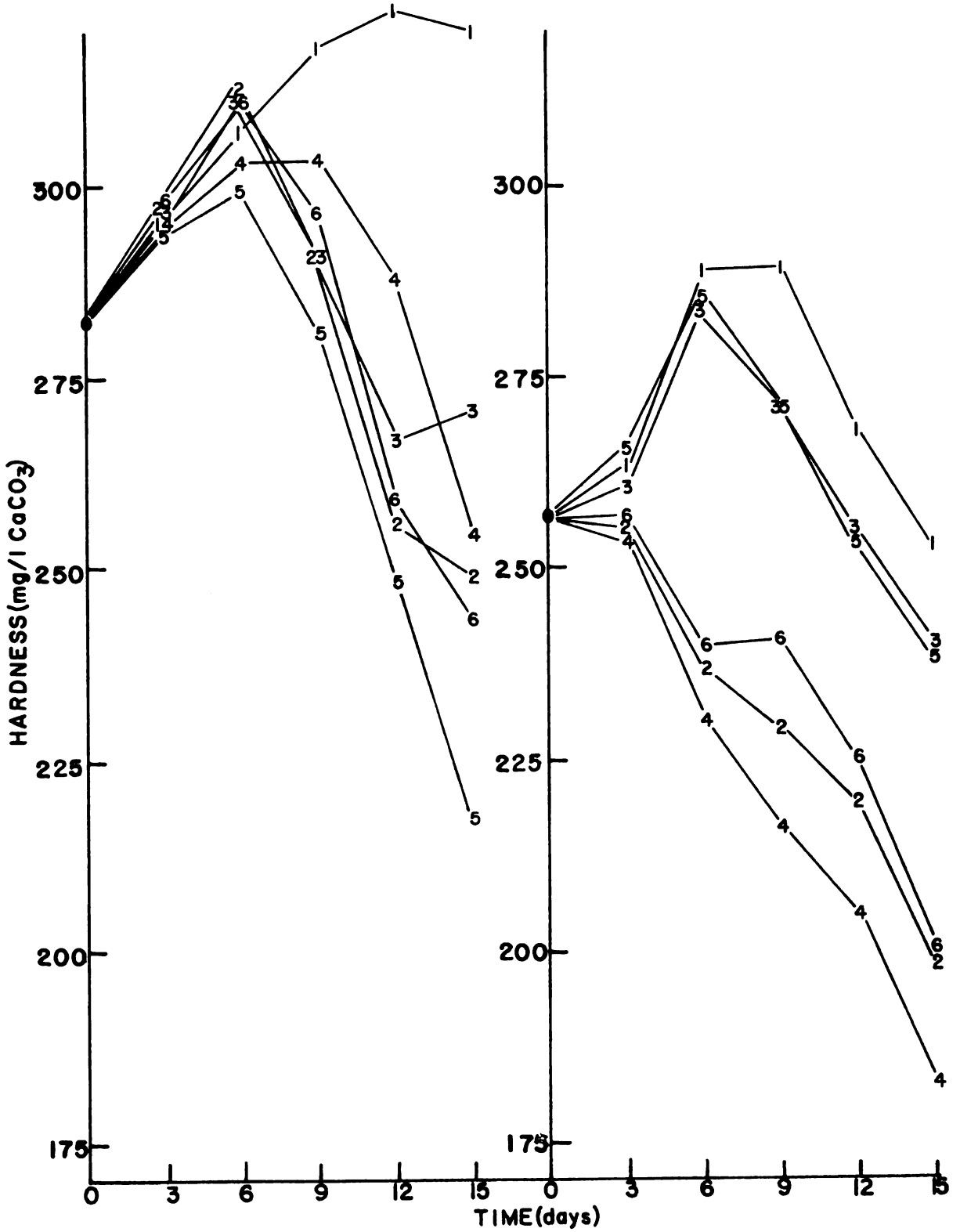

Figure 9. Unmodified simulations of hardness vs. time for all aquaria during experimental runs one (left, aquaria 1, 4, 5 with plants) and two (right, aquaria 2, 4, 6 with plants).

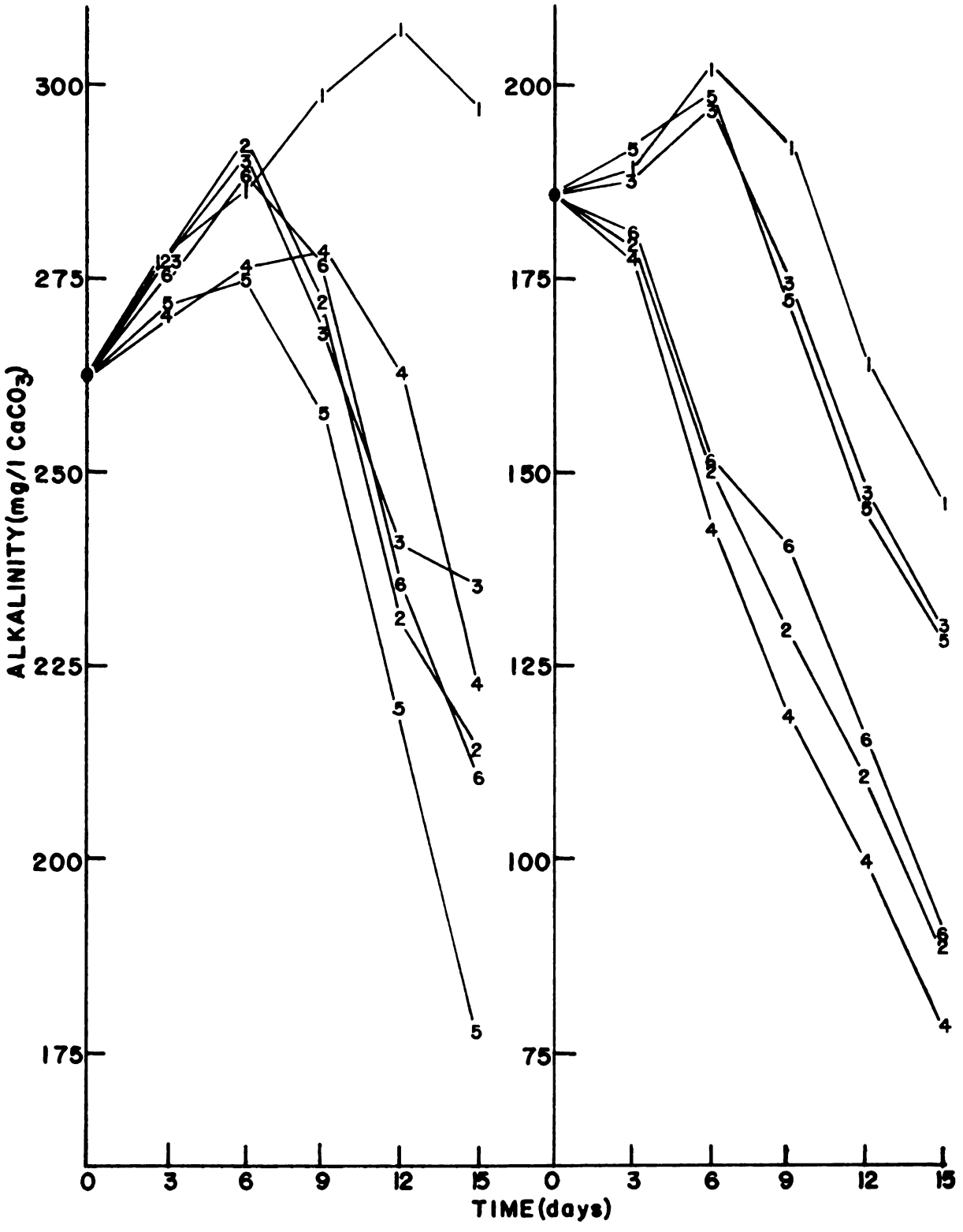

Figure 10. Unmodified simulations of alkalinity vs. time for all aquaria during experimental runs one (left, aquaria 1, 4, 5 with plants) and two (right, aquaria 2, 4, 6 with plants).

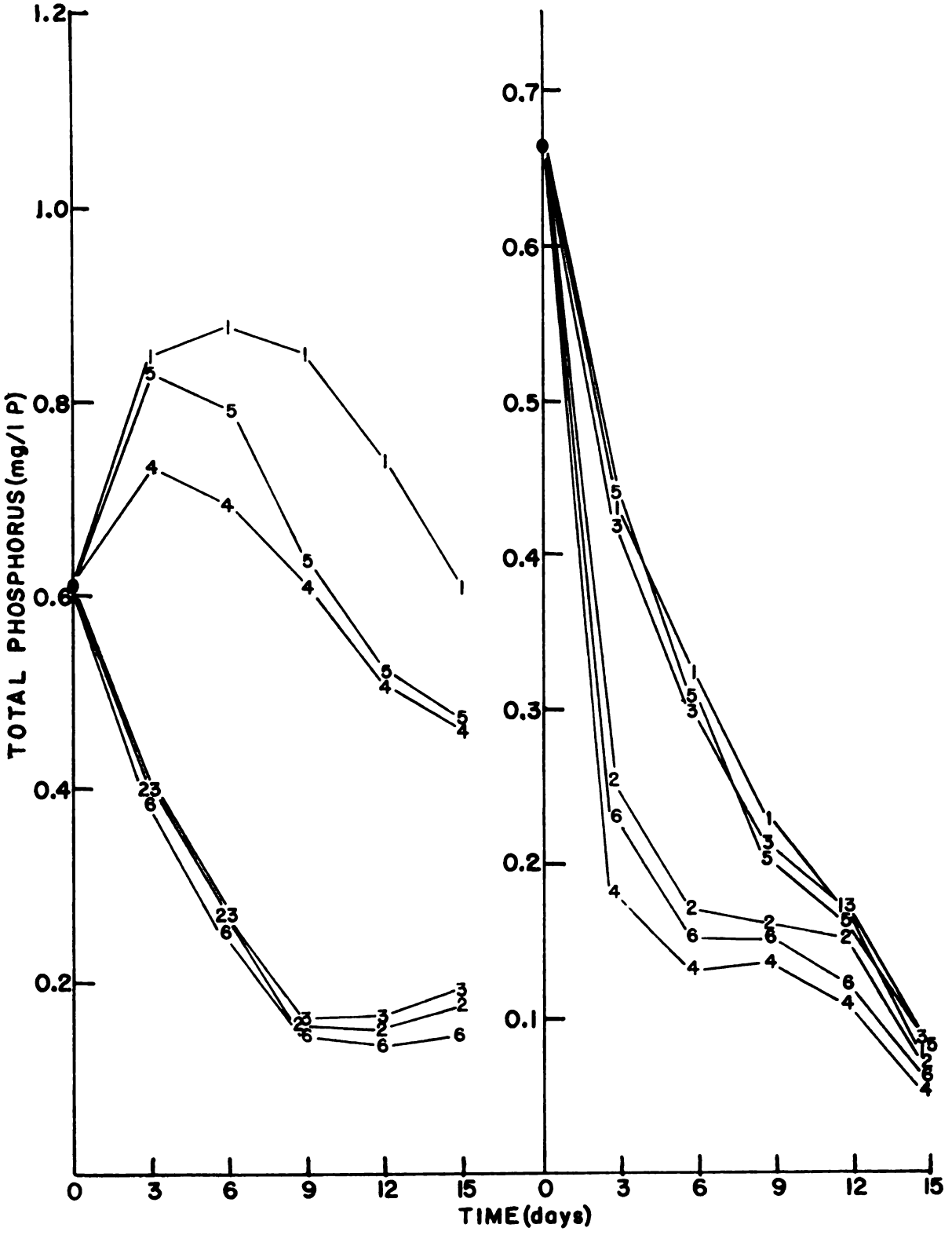

Figure 11. Unmodified simulations of total phosphorus vs. time for all aquaria during experimental runs one (left, aquaria 1, 4, 5 with plants) and two (right, aquaria 2, 4, 6 with plants).

Table 9. Total phosphorus budget for unmodified simulations of first run of aquarium experiment (12-hr. photoperiod, aquaria 1, 4, 5 with plants).

Aquarium	1	2	3	4	5	6
Initial phosphorus in aquarium (mg)	28.46	31.49	30.92	30.63	30.68	31.61
Phosphorus in influent (mg)	35.65	37.99	38.92	32.03	32.13	29.99
Plant uptake of phosphorus (mg)	-122.07	0.00	0.00	-104.81	-124.32	0.00
Phosphorus in effluent (mg)	27.51	10.02	10.37	20.69	22.75	6.58
Final phosphorus in aquarium (mg)	30.20	9.89	10.03	22.27	22.96	8.07
Phosphorus removed into sediment (mg)	128.47	49.57	49.44	124.51	141.42	46.95
Mean total phosphorus (mg/l P)[a]	0.787	0.269	0.269	0.621	0.669	0.253
Mean ortho phosphorus (mg/l P)[b]	0.668	0.199	0.199	0.518	0.561	0.184
Derived Parameters:						
A. % of initial and influent phosphorus removed by sediments and plants combined.[c]	10.0	71.3	70.8	31.4	27.2	76.2
B. % of initial and influent phosphorus removed by sediments alone.[d]	200.4	71.3	70.8	198.7	225.2	76.2
C. Mean removal rate of phosphorus into sediments, weighted by division by mean orthophosphorus concentration (day^{-1}).[e]	12.82	16.60	16.58	16.03	16.81	17.01

Significance levels of plant effects based on paired t-tests = [a&b]0.017, [c]0.030, [d]0.008, and [e]0.387.

Table 10. Total phosphorus budget for unmodified simulations of second run of aquarium experiment (14-hr. photoperiod, aquaria 2, 4, 6 with plants).

Aquarium	1	2	3	4	5	6
Initial phosphorus in aquarium (mg)	33.83	33.70	33.83	33.70	33.83	33.70
Phosphorus in influent (mg)	30.99	30.41	30.22	31.12	31.21	30.26
Plant uptake of phosphorus (mg)	0.00	12.44	0.00	26.01	0.00	19.32
Phosphorus in effluent (mg)	4.97	3.70	4.53	3.51	4.68	3.60
Final phosphorus in aquarium (mg)	4.28	3.95	4.38	2.78	4.42	2.94
Phosphorus removed into sediment (mg)	55.57	44.02	55.14	32.52	55.94	38.10
Mean total phosphorus (mg/l P)[a]	0.306	0.220	0.295	0.176	0.298	0.196
Mean ortho phosphorus (mg/l P)[b]	0.232	0.155	0.223	0.115	0.225	0.133
Derived Parameters:						
A. % of initial and influent phosphorus removed by sediments and plants combined.[c]	85.7	88.1	86.1	90.3	86.0	89.8
B. % of initial and influent phosphorus removed by sediments alone.[d]	85.7	68.7	86.1	50.2	86.0	59.6
C. Mean removal rate of phosphorus into sediments, weighted by division by mean orthophosphorus concentration (day^{-1}).[e]	15.94	18.93	16.48	18.85	16.51	19.01

Significance levels of plant effects based on paired t-tests = a&b0.016, c0.040, d0.043, and e0.009.

actually observed. The results of these differences on the phosphorus budgets are relatively minor and include lower amounts of phosphorus in the effluent, higher final phosphorus levels in the aquaria, lower amounts of phosphorus removed into the sediments, lower mean concentrations of phosphorus, and higher weighted rates of phosphorus removal (Parameter C).

For aquaria with plants, the differences between simulation and reality are greater. In the first experimental run, the simulations uniformly predict lower phosphorus concentrations than actually observed, with lower amounts of phosphorus in the effluents and in the final aquarium water, higher amounts of phosphorus removed to the sediments, lower mean phosphorus concentrations, and higher weighted mean rates of phosphorus removal. Only the simulation of aquarium 5 is close to the experimental data. For the second experimental run, the simulations show a much slower rate of phosphorus removal and much higher levels of phosphorus than actually observed for most of the run. Consequently, the budget shows higher amounts of phosphorus in the effluent, lower amounts removed into the sediment, higher mean phosphorus concentrations, and much lower values of Parameter C.

In general, the simulations predict less difference between aquaria with and without plants than was actually observed. This is particularly true for the average concentrations of orthophosphorus and total phosphorus and for the weighted mean rates of phosphorus removal. A contributing factor to this and a particularly disturbing aspect of the model is its apparent inability to sustain phosphorus removal at low levels of hardness and alkalinity, a phenomenon that was quite pronounced in the simulations of aquaria without plants in the first experimental run. Such a phenomenon did not appear to any significant degree during

the actual experiments, even at lower levels of hardness and alkalinity than occurred in the simulations. This is, perhaps, the most serious deficiency of the model.

The lack of fit between the simulations and reality could be the result of a variety of factors, the ones of most probable importance including:

(1) Exclusion from the model of such factors as dissolved organic matter, dissolved oxygen concentration, and deposition of phosphorus in particulate organic matter,

(2) Calculations on a twenty-four hour basis, ignoring diel fluctuations of such parameters as pH,

(3) Treatment of the aquaria and beakers as similar with respect to water circulation, homogeneity of materials, transport processes, etc.,

(4) Lack of data on the actual phosphorus in plants through time,

(5) Different water chemistry (e.g., cation ratios) in beakers and aquaria,

(6) Lack of data on evaporation and the forms of phosphorus in the first experimental run,

(7) Exclusion from the model of simulation of plant growth and phosphorus content,

(8) Errors in data from both aquarium and beaker experiments and lack of fit in regression equations.

With this impressive array of shortcomings of the model, the validity of further analysis and comparison is somewhat questionable, but the similarities between model and reality do suggest that certain common factors are operating, though perhaps to different extents. Therefore, it is at least worthwhile to identify the factors most important to the behavior of the model and consider their likely importance in the actual

aquarium experiment.

Sensitivity Analysis

One of the values of the kind of model described above is that, once a reasonable simulation is achieved, various factors can be manipulated to determine to which ones the behavior of the model, and presumably of the system being modeled, is most sensitive. This is especially important when certain factors cannot be independently manipulated experimentally. For example, for the model discussed here, pH and plant uptake of phosphorus were independently vaired in a series of simulations to establish their separate effects on the behavior of phosphorus. Also, use of the model can compensate for random differences among experimental units, such as the different influent volumes among aquaria in the aquarium experiment (Table 4), which might confuse results. Of course, this type of analysis is meaningless unless there is good reason to believe that the factors that influence the model are also operative, to at least some extent, in the system being modeled.

As discussed earlier, evaporation, pH, and plant uptake and release of phosphorus are the only factors of significance to phosphorus dynamics that are directly influenced by C. demersum and that can be considered within the framework of the above model. Of these, simulations with altered evaporation showed only slight changes in phosphorus behavior. A decrease in evaporation was found to increase export of phosphorus from an aquarium and thus to decrease the concentration of phosphorus left in the aquarium, but this was in part compensated for by slightly decreased removal of phosphorus into the sediment. Overall, the phosphorus was little altered by moderate changes in evaporation, and Parameter C, the weighted mean rate of phosphorus removal into the sediments, was left

essentially unchanged.

On the other hand, major alterations in phosphorus behavior were found in simulations in which changes were made in pH or plant uptake of phosphorus, or both. These changes consisted simply of switching the data on pH and plant phosphorus between paired aquaria. For example, aquaria that did not contain plants would be subject to the following simulations:

(1) All inputs unmodified,

(2) Inputs on plant uptake or release of phosphorus taken from the corresponding aquarium with plants,

(3) Inputs on pH taken from the corresponding aquarium with plants, and

(4) Inputs on pH and plant uptake or release of phosphorus taken from the corresponding aquarium with plants.

Of course, such a procedure does not exactly represent what would happen with respect to pH and uptake of phosphorus if plants had been introduced to the aquaria without plants, but there is reason to believe that this is a good approximation of that event.

Within each experimental run, the above simulations showed consistent effects in all aquaria. Because of this, results of simulations on only one randomly selected aquarium from each experimental run are summarized here in plots of total phosphorus (Figure 12) and phosphorus budgets (Tables 11 and 12).

In simulations of the first experimental run, the release of phosphorus by C. demersum obviously exerted a tremendous influence on phosphorus dynamics, increasing phosphorus levels far above those expected when no release of phosphorus by the plants was assumed, regardless of the level of pH (Figure 12). This influence was expressed in the phosphorus budget (Table 11) in obvious manners that have been discussed above.

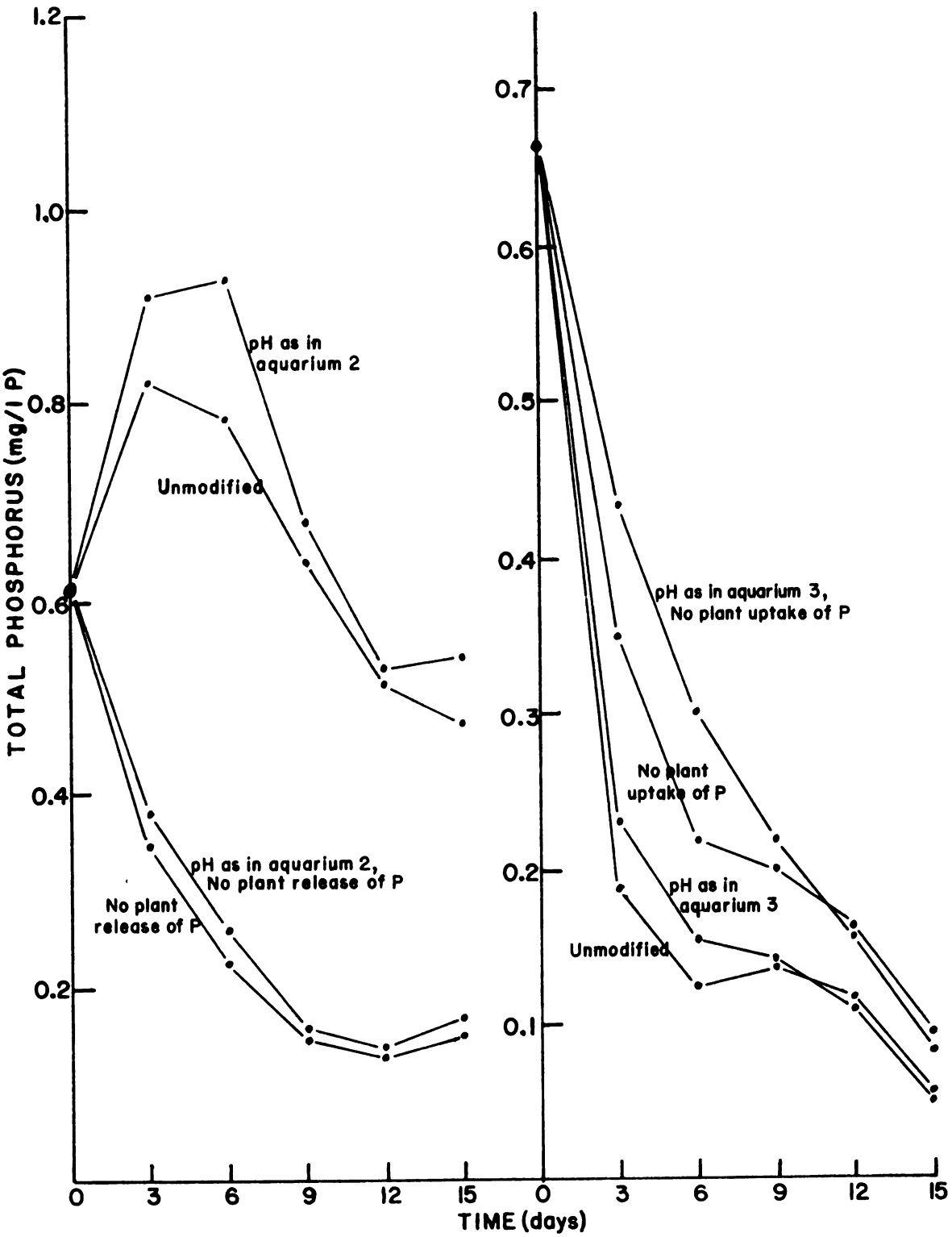

Figure 12. Modified simulations of total phosphorus vs. time for aquarium 5 of experimental run one (left) and aquarium 4 of experimental run two (right).

Table 11. Total phosphorus budgets for modified simulations of aquarium 5 of first run of aquarium experiment (12-hr. photoperiod).

Modification of simulation	Unmodified	Plant uptake data from aquarium 2	pH[a] data from aquarium 2	Plant and pH[a] data from aquarium 2
Initial phosphorus in aquarium (mg)	30.68	30.68	30.68	30.68
Phosphorus in influent (mg)	32.13	32.13	32.13	32.13
Plant uptake of phosphorus (mg)	-124.32	0.00	-124.32	0.00
Phosphorus in effluent (mg)	22.75	8.14	25.18	8.88
Final phosphorus in aquarium (mg)	22.96	7.67	26.34	8.18
Phosphorus removed into sediment (mg)	141.42	47.00	135.61	45.75
Mean total phosphorus (mg/l P)	0.669	0.241	0.730	0.258
Mean ortho phosphorus (mg/l P)	0.561	0.173	0.616	0.189
Derived Parameters:				
A. % of initial and influent phosphorus removed by sediments and plants combined.	27.2	74.8	18.0	72.8
B. % of initial and influent phosphorus removed by sediments alone.	225.2	74.8	215.9	72.8
C. Mean removal rate of phosphorus into sediments, weighted by division by mean orthophosphorus concentration (day^{-1}).	16.81	18.10	14.67	16.16

[a]Mean pH 0.18 units higher in aquarium 5 than in aquarium 2.

Table 12. Total phosphorus budgets for modified simulations of aquarium 4 of second run of aquarium experiment (14-hr. photoperiod).

Modification of simulation	Unmodified	Plant uptake data from aquarium 3	pH[a] data from aquarium 3	Plant and pH[a] data from aquarium 3
Initial phosphorus in aquarium (mg)	33.70	33.70	33.70	33.70
Phosphorus in influent (mg)	31.12	31.12	31.12	31.12
Plant uptake of phosphorus (mg)	26.01	0.00	26.01	0.00
Phosphorus in effluent (mg)	3.51	5.61	4.04	6.87
Final phosphorus in aquarium (mg)	2.78	4.70	2.56	4.06
Phosphorus removed into sediment (mg)	32.52	54.51	32.21	53.89
Mean total phosphorus (mg/l P)	0.176	0.262	0.190	0.297
Mean ortho phosphorus (mg/l P)	0.115	0.193	0.127	0.225
Derived Parameters:				
A. % of initial and influent phosphorus removed by sediments and plants combined.	90.3	84.1	89.8	83.1
B. % of initial and influent phosphorus removed by sediments alone.	50.2	84.1	49.7	83.1
C. Mean removal rate of phosphorus into sediments, weighted by division by mean orthophosphorus concentration (day^{-1}).	18.85	18.83	16.85	15.99

[a]Mean pH 0.68 units higher in aquarium 4 than in aquarium 3.

Worthy of special mention is the decrease in the weighted mean rate of phosphorus removal (Parameter C) due to the release of phosphorus by the plants. This decrease is apparently due to the increased orthophosphorus concentrations, consistent with the phosphorus removal reactions being less than first order with respect to orthophosphorus. The changes in this parameter via this direct effect of C. demersum are slight, though, compared to the changes in the phosphorus concentrations.

The effect of pH on these simulations was relatively minor compared to the effect of the release of phosphorus by the plants. Higher mean pH invariably resulted in lower phosphorus levels throughout the simulations (Figure 12), with the budgets (Table 11) showing lower amounts of phosphorus in the effluent and final aquarium water, greater amount removed into the sediment, and, of course, lower mean phosphorus concentrations. Because of this, all three derived parameters showed increases with higher mean pH, especially Parameter C, the weighted mean rate of phosphorus removal. The effects of pH observed here are especially impressive considering the slight (0.18) difference in mean pH between aquaria 2 and 5.

In the simulations of the second experimental run, the direct effect of plants, this time due to uptake of phosphorus rather than release, was again of predominant importance. Uptake of phosphorus by plants resulted in phosphorus levels being lower for the entire simulation than when plant uptake was assumed to be zero (Figure 12), with corresponding effects on the phosphorus budgets (Table 12). The weighted mean rate of phosphorus removal (Parameter C) was again found to be decreased at increased concentrations of orthophosphorus, though this decrease was even less than that observed for the simulations of the first experimental run since the differences in means of orthophosphorus were less. The effects of pH on

the simulations of the second experimental run were similar to those found for the first run, except that (1) the overall impact of the plant-induced pH changes was somewhat greater in the second run simulations due to greater differences in pH between paired aquaria (0.68 difference in means of aquaria 3 and 4) and (2) final phosphorus levels in the second run were lower at lower pH, this being due to the already discussed inability of the simulations to maintain high rates of phosphorus removal at low hardness and alkalinity, which resulted from the higher pH.

Overall, therefore, the direct effects of plants were shown in this analysis to be of major importance in the behavior of phosphorus in the simulations of the aquarium experiment, as was expected from the direct analysis of experimental data. More importantly, the indirect effect of plants on phosphorus behavior through their alteration of pH, which was not apparent in the actual results of the aquarium experiment, was shown to be quite significant in the simulations, with increases in mean pH having positive effects on the mean rate of phosphorus removal into sediments and negative effects on the mean phosphorus concentrations. Decreases in the rate of evaporation due to plants were also shown to slightly decrease the rate of phosphorus removal to the sediment and the mean concentrations of phosphorus by increasing export of this element from the tank somewhat.

Additionally, the effects of the various simulations on the weighted mean rate of phosphorus removal (Parameter C) suggest that this parameter is affected to some extent by the direct uptake or release of phosphorus by C. demersum since it is inversely correlated to mean orthophosphorus concentration. This has serious implications to the use of this parameter in detecting indirect effects of plants on phosphorus dynamics, especially when such effects are minor and direct effects are great, as in the first

experimental run of the aquarium experiment. Other shortcomings of this parameter include its sensitivity to deposition of phosphorus in a particulate organic form, which is in part a direct effect of plants, and its insensitivity to changes in phosphorus behavior due to changes in evaporation rate, which is an indirect effect of plants, but both of these shortcomings are either minor or can be relatively easily compensated for, or both. On the other hand, this parameter functions well in that it is rather sensitive to changes in phosphorus dynamics due to plant-induced pH changes, and would presumably would be sensitive to other, similar factors that affect phosphorus exchange reactions, such as dissolved oxygen and dissolved organic matter.

In sum, the weighted mean rate of phosphorus removal into sediments has limited utility for detecting indirect effects of macrophytes on phosphorus behavior, but can be used if one or more of the following criteria are met:

(1) only tentative indications of indirect effects are desired,

(2) the above-mentioned shortcomings are minor or can be compensated for,

(3) the changes in this parameter attributable to plants are very large.

With these criteria in mind, the changes in this parameter for the second run of the aquarium experiment (Table 8) can be considered to demonstrate real indirect effects of C. demersum on phosphorus dynamics. Also, with the results of the various simulations in mind, part of these indirect effects can be attributed to elevations of pH by the plants. For the first experimental run, less can be said, but the mixed effect of plants on this parameter corresponds well to the mixed effect of plants on pH and with the effect of pH on this parameter in the simulations, especially if the effects of elevated phosphorus levels on this parameter are taken into consideration.

SUMMARY AND CONCLUSIONS

The purpose of this research was to establish the extent and nature of the effects of Ceratophyllum demersum on phosphorus dynamics in a simple aquarium system. Under the experimental conditions, this plant clearly had a significant impact on the levels of phosphorus in the aquarium water, in the effluent from the aquarium, and in the sediments. Furthermore, the experimental data demonstrated that the major reason for this impact was the release and uptake of phosphorus by the plant, resulting, respectively, in more and less phosphorus removed in the effluent, deposited in the sediment, and left in the water. Photoperiod influenced this direct effect in that the plant declined in biomass (releasing phosphorus) under a twelve-hour photoperiod and grew well (taking up phosphorus) under a fourteen-hour photoperiod. Also, experimental data and observations suggested that the deposition of phosphorus into the sediment was predominantly through inorganic exchange reactions and that the direct effect of the plants should be explained primarily through such reactions.

Indirect effects of C. demersum on phosphorus dynamics were less obvious in the results of the aquarium experiment, but the magnitude of effect of the plant on the weighted mean rate of removal of phosphorus into the sediment during the second experimental run and the correlation of this parameter with pH in both runs suggested that indirect effects did exist and that plant-induced pH changes were a major factor in these

effects. From elementary mass balance considerations, an indirect effect on phosphorus dynamics was also concluded to result from decreases in evaporation rates due to C. demersum. Other indirect effects were of unlikely importance or could not be considered due to data limitations.

The use of a model of phosphorus dynamics to simulate the aquarium experiment, with the rates of removal of phosphorus, alkalinity, and hardness into the sediments estimated from the results of the beaker experiment, permitted further consideration of the above conclusions regarding direct and indirect effects of C. demersum. While the fit of the simulation to experimental data was not good, certain similarities existed that suggested the operation of common factors. Direct effects were again shown to be of predominant importance, but plant-induced pH changes were seen to account for a substantial amount of the total plant effect, with evaporation effects being very minor. This model also allowed the evaluation of the weighted mean rate of phosphorus removal, which was found to be quite sensitive to pH-induced changes in phosphorus behavior, but also was affected to some extent by direct plant effects, which naturally interferes with its value in detecting indirect plant effects. Further research is warranted to establish the validity of such a parameter and to set criteria for its use.

Evidence from this research therefore strongly supports the existence of direct and indirect effects of Ceratophyllum demersum on phosphorus dynamics in the described system. The behavior of C. demersum in this system was not atypical, so the existence of similar effects in natural systems would be expected, but is hardly certain. More significant findings regarding the mechanisms controlling phosphorus dynamics in aquatic systems should be possible with additional development and better use of the techniques and approaches discussed here.

APPENDICES

APPENDIX A - ANALYTICAL TECHNIQUES

(1) Temperature - Temperatures were measured with a -10°C to +50°C mercury-in-glass thermometer (calibrated only at 0°C).

(2) pH - pH determinations were made with a Beckman Chem-mate pH meter with a combination glass electrode. This meter was standardized using commercially prepared buffers with pH of approximately 4, 7, and 10.

(3) Dissolved Oxygen - Measurements of dissolved oxygen in the aquaria and the reservoir were made with a Precision oxygen meter with a galvanic oxygen probe. This meter was standardized by determining dissolved oxygen in the reservoir using the azide modification of the Winkler method (APHA, 1971).

(4) Hardness - Hardness determinations were made by titration of samples with EDTA in the presence of Eriochrome Black T (APHA, 1971).

(5) Alkalinity - Alkalinity determinations were made by potentiometric titration of samples with H_2SO_4 (APHA, 1971).

(6) Orthophosphorus - Orthophosphorus was determined colorimetrically on untreated samples using the ascorbic acid method for color development (APHA, 1971) and measuring color on a Spectronic 20 spectrophotometer in either $\frac{1}{2}$" or 1" cells, depending on concentration.

(7) Total Phosphorus - For total phosphorus, a 100-ml water sample was digested with persulfate (APHA, 1971) in a 200-ml volumetric flask in a pressure cooker under 20 lbs of pressure. After dilution, phosphorus was measured as orthophosphorus, as described above.

(8) Dissolved Phosphorus - For dissolved phosphorus, a water sample was first filtered through a 0.45-um membrane filter (APHA, 1971), after which a 100-ml aliquot was treated as for total phosphorus.

(9) Plant Tissue Digestion - Plant tissue was digested using procedures developed in the chemical laboratory of the Institute of Water Research of Michigan State University and further modified for this study. An oven-dried (80°C for at least 72 hr) plant sample was ground to 20 mesh in a Wiley mill, oven-dried again, and stored in a desiccator. Approximately 0.5 g of this sample was weighed (to four significant digits; in standardized time to avoid errors due to absorption of atmospheric water) and placed in a 500-ml round-bottomed boiling flask. Five ml of deionized, distilled water were then added to wet the sample, after which 3 mg of NH_4VO_3 (a catalyst for the digestion) and 5 mg of $K_2Cr_2O_7$ (an indicator for completion of the digestion) were added. Finally, 10 ml of 1:1 conc. HNO_3 : conc. $HClO_4$ were added. The flask was then placed in a heating mantle and a reflux apparatus was mounted atop the flask. The sample was then digested for 30 min at 100°C, after which the temperature was gradually increased (over 1 to 1½ hr) to approximately 195°C, at which point the dichromate indicator turned orange. Temperature was maintained at this point for ten minutes, after which the flask was removed and cooled in ice water. Fifty ml of deionized, distilled water were then quickly added. The digestate was transferred to a 200-ml volumetric flask and the boiling flask was rinsed repeatedly with distilled water and 0.1 N hydrochloric acid, adding the rinses to the volumetric flask. After dilution to volume in this flask, 10 ml of the digested sample was neutralized to pH 8.3 with HCl and NaOH, diluted to 500 ml, and measured for orthophosphorus.

APPENDIX B - SUMMARY OF REGRESSION EQUATIONS

(1) Change in Orthophosphorus Concentration in Beakers Without Sediments

Dependent Variable: $\log_{10}$(change in orthophosphorus conc (mg P/l/day))

Independent Variable	Regression Coefficient	Standard Error	Significance Level
(1) Constant	23.88521	13.76231	0.087
(2) Mean pH	-4.20018	1.26676	0.001
(3) 1.0/(273.2+Mean Temp)	3721.05153	2319.24552	0.113
(4) $\log_{10}$(Mean Alkalinity)	-17.47826	5.12719	0.001
(5) $\log_{10}$(Mean Hardness)	-2.63735	1.65978	0.116
(6) $\log_{10}$(Mean Phosphorus)	3.80165	2.27123	0.098
(7) (2) x (4)	1.79332	0.55908	0.002
(8) (2) x (5)	0.27715	0.14116	0.053
(9) (2) x (6)	-0.30864	0.26298	0.244
(10) (4) x (5)	0.77419	0.52928	0.148

(2) Change in Orthophosphorus Concentration in Beakers With Sediments

Dependent Variable: $\log_{10}$(change in orthophosphorus conc (mg P/l/day))

Independent Variable	Regression Coefficient	Standard Error	Significance Level
(1) Constant	-0.21519	3.22418	0.947
(2) Mean pH	-0.23791	0.31271	0.449
(3) 1.0/(273.2+Mean Temp)	-104.91248	541.23144	0.847
(4) $\log_{10}$(Mean Alkalinity)	-0.45012	1.31010	0.732
(5) $\log_{10}$(Mean Hardness)	0.12265	0.38189	0.749
(6) $\log_{10}$(Mean Phosphorus)	-1.54419	0.44057	0.001
(7) (2) x (4)	0.10901	0.14137	0.443
(8) (2) x (5)	0.07874	0.03267	0.018
(9) (2) x (6)	0.26080	0.05087	0.0005
(10) (4) x (5)	-0.16540	0.12273	0.181

(3) Orthophosphorus vs. Total Phosphorus Concentrations in Aquaria

Dependent Variable: Orthophosphorus concentration (mg/l P)

Independent Variable	Regression Coefficient
(1) Constant	-0.049398
(2) Total Phosphorus	0.90552456

(4) Change in Alkalinity in Beakers With and Without Sediment

Dependent Variable: Change in alkalinity (mg $CaCO_3$/l/day)

Independent Variable	Regression Coefficients	
	Beakers Without Sediment	Beakers With Sediment
(1) Constant	-96.15828	144.15471
(2) Mean Temperature	0.01648	0.29908
(3) Mean Alkalinity	0.45138	-1.94633
(4) Mean Hardness	-3.44938	-5.89237
(5) Mean pH	20.65088	-34.47854
(6) (3) x (4)	0.053374	0.079621
(7) (3) x (5)	-0.09187	0.47782
(8) (4) x (5)	0.88536	1.41783
(9) Mean Hardness Squared	0.034179	0.037167
(10) (3) x (9)	-0.00045945	-0.00050209
(11) (5) x (9)	-0.0083338	-0.0089486
(12) Mean pH Squared	-1.09726	2.01962
(13) (3) x (12)	0.0045345	-0.029727
(14) (4) x (12)	-0.056113	-0.085313
(15) (9) x (12)	0.00050521	0.00053766
(16) (3) x (4) x (5)	-0.013446	-0.019250
(17) (3) x (4) x (12)	0.00083968	0.0011612
(18) (3) x (5) x (9)	0.00011241	0.00012150
(19) (3) x (9) x (12)	-0.0000068447	-0.0000073343

(5) Change in Hardness in Beakers With and Without Sediment

Dependent Variable: Change in hardness (mg $CaCO_3$/l/day)

Independent Variable	Regression Coefficients	
	Beakers Without Sediment	Beakers With Sediment
(1) Constant	-260.72834	498.90109
(2) Mean Temperature	-0.14923	-0.0097602
(3) Mean Alkalinity	1.51798	-2.02482
(4) Mean Hardness	-0.33256	-1.62737
(5) Mean pH	62.73916	-114.54458
(6) (3) x (4)	0.044987	-0.010019
(7) (3) x (5)	-0.35687	0.49919
(8) (4) x (5)	0.11767	0.54504
(9) Mean Hardness Squared	0.025458	0.011767
(10) (3) x (9)	-0.00046032	-0.00015611
(11) (5) x (9)	-0.0061933	-0.0034046
(12) Mean pH Squared	-3.67396	6.70200
(13) (3) x (12)	0.020843	-0.029843
(14) (4) x (12)	-0.0095003	-0.041397
(15) (9) x (12)	0.00037559	0.00023516
(16) (3) x (4) x (5)	-0.011201	0.00051948
(17) (3) x (4) x (12)	0.00069521	0.000070069
(18) (3) x (5) x (9)	0.00011195	0.000044439
(19) (3) x (9) x (12)	-0.0000067899	-0.0000030491

LITERATURE CITED

LITERATURE CITED

APHA, AWWA, AND WPCF. 1971. Standard methods for the examination of water and wastewater. 13 ed. APHA, New York. 874p.

Boyd, C. E. 1969. Production, mineral nutrient absorption, and biochemical assimilation by Justicia americana and Alternanthera philoxeroides. Arch. Hydrobiol. 66:139-160.

Boyd, C. E. 1970. Production, mineral accumulation, and pigment concentrations in Typha latifolia and Scirpus americanus. Ecology 51: 285-290.

Buscemi, P. A. 1958. Littoral oxygen depletion produced by a cover of Elodea canadensis. Oikos 9:239-245.

Cole, C. V., S. R. Olsen, and C. O Scott. 1953. The nature of phosphate sorption by calcium carbonate. Soil Sci. Soc. Am. Proc. 17:352-356.

Dvorak, J. 1970. Horizontal zonation of macrovegetation, water properties, and macrofauna in a littoral stand of Glyceria aquatica (L.) Wahlb. in a pond in south Bohemia. Hydrobiologia 35:17-30.

Einsele, W. 1936. Über die Beziehung des Eisenkreislaufs zum Phosphatkreislauf im eutrophen See. Arch. Hydrobiol. 29:664-686.

Einsele, W. 1938. Über chemische und kolloidchemische Vorgänge in Eisen-Phosphat-Systemen unter limnochemischen und limnogeologischen Gesichtspunkten. Arch. Hydrobiol. 33:361-387.

Gerloff, G. G. 1969. Evaluating nutrient supplies for the growth of aquatic plants in natural water. In Eutrophication: causes, consequences, correctives. National Academy of Sciences, Washington, D. C. 661p.

Golterman, H. L. 1967. Influence of the mud on the chemistry of water in relation to productivity. In H. L. Golterman and R. S. Clymo (ed.), Chemical environment in the aquatic habitat. N. V. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam. 322p.

Gorham, E. and D. J. Swaine. 1965. The influence of oxidizing and reducing conditions upon the distribution of some elements in lake sediments. Limn. Ocean. 10:268-279.

Gossett, D. R. and W. E. Norris, Jr. 1971. Relationship between nutrient availability and content of nitrogen and phosphorus in tissues of the aquatic macrophyte, Eichornia crassipes (Mart.)Solms. Hydrobiologia 38:15-28.

Goulder, R. 1969. Interactions between the rates of production of a freshwater macrophyte and phytoplankton in a pond. Oikos 20:300-309.

Gumerman, R. C. 1970.Aqueous phosphate and lake sediment interaction. Proc. Conf. Great Lakes Res. 13: 673-682.

Harrison, M. J., R. E. Pacha, and R. Y. Morita. 1972. Solubilization of inorganic phosphates by bacteria isolated from Upper Klamath Lake sediment. Limn. Ocean. 17:50-57.

Hepher, B. 1958. On the dynamics of phosphorus added to fishponds in Israel. Limn. Ocean. 3:84-100.

Jitts, H. R. 1959. The adsorption of phosphate by estuarine bottom deposits. Aust. J. Mar. Freshw. Res. 10:7-21.

Kosek, S. R. 1971. Elodea nuttallii as a component of a managed wastewater treatment system. MS thesis, Michigan State University, East Lansing, Mi. 104p.

Li, W. C., D. E. Armstrong, J. D. H. Williams, R. F. Harris, and J. K. Syers. 1972. Rate and extent of inorganic phosphate exchange in lake sediments. Soil Sci. Soc. Am. Proc. 36:279-285.

Livingstone, D. A. and J. C. Boykin. 1962. Vertical distribution of phosphorus in Linsley Pond mud. Limn. Ocean. 7:57-62.

Mackereth, F. J. H. 1966. Some chemical observations on postglacial lake sediment. Phil. Trans. Royal Soc. London (Ser. B) 250:165-213.

Macpherson, L. B., N. R. Sinclair, and F. R. Hayes. 1958. Lake water and sediment. III. The effect of pH on the partition of inorganic phosphate between water and oxidized mud or its ash. Limn. Ocean. 3:318-326.

McNabb, C. D., D. P. Tierney, and S. R. Kosek. 1972. The uptake of phosphorus by Ceratophyllum demersum growing in wastewater. Tech. Rep. #24, Inst. Water Res., Mich State U., East Lansing, Mi. 23p.

McRoy, C. P. and R. J. Barsdate. 1970. Phosphate absorption in eelgrass. Limn. Ocean. 15:6-13.

McRoy, C. P., R. J. Barsdate, and M. Nebert. 1972. Phosphorus cycling in an eelgrass (Zostera marina L.) ecosystem. Limn. Ocean. 17:58-67.

Mortimer, C. H. 1941. The exchange of dissolved substances between mud and water in lakes (Parts I and II). J. Ecol. 29:280-329.

Mortimer, C. H. 1942. The exchange of dissoved substances between mud and water in lakes (Parts III and IV). J. Ecol. 30:147-201.

Mortimer, C. H. 1971. Chemical exchanges between sediments and water in the Great Lakes - speculations on probable regulatory mechanisms. Limn. Ocean. 16:387-404.

Nelson, B. W. 1967. Sedimentary phosphate method for estimating paleosalinities. Science 158:917-919.

Nichols, D. S. and D. R. Keeney. 1973. Nitrogen and phosphorus release from decaying water milfoil. Hydrobiologia 42:509-525.

Otsuki, A. and R. G. Wetzel. 1972. Coprecipitation of phosphate with carbonates in a marl lake. Limn. Ocean. 17:763-767.

Reimold, R. J. 1972. The movement of phosphorus through the salt marsh cord grass, Spartina alterniflora Loisel. Limn. Ocean. 17:606-611.

Rohlich, G. A. and P. D. Uttormark. 1972. Wastewater treatment and eutrophication. In G. E. Likens (ed.) Nutrients and eutrophication: The limiting nutrient controversy. Allen Press, Lawrence, Kansas. 328p.

Rosenqvist, I. T. 1970. Formation of vivianite in holocene clay sediments. Lithos 3:327-334.

Sculthorpe, C. D. 1967. The biology of aquatic vascular plants. St. Martin's, New York. 610p.

Shapiro, J. 1966. The relation of humic color to iron in natural water. Proc. Inter. Assoc. Limn. 16:477-484.

Shukla, S. S., J. K. Syers, J. D. H. Williams, D. E. Armstrong, and R. F. Harris. 1971. Sorption of inorganic phosphate by lake sediments. Soil Sci. Soc. Am. Proc. 35:244-249.

Straskraba, M. 1965. Contributions to the productivity of the littoral region of pools and ponds. I. Quantitative study of the littoral zooplankton of the rich vegetation of the backwater Labicko. Hydrobiologia 26:421-443.

Stumm, W. and J. J. Morgan. 1970 Aquatic chemistry. Wiley-Interscience, New York. 583p.

Tierney, D. P. 1972. Some aspects of the ecology of naturally occurring populations of submerged vascular hydrophytes in municipal wastewater lagoons. PhD thesis, Michigan State University, East Lansing, Mi. 104p.

Ulehlova, B. 1970. An ecological study of aquatic habitats in North-West Overijssel, the Netherlands. Acta Bot. Neerl. 19:830-858.

Wentz, D. A. and G. F. Lee. 1969. Sedimentary phosphorus in lake cores - observations on depositional pattern in Lake Mendota. Env. Sci. Tech. 3:754-759.

Wetzel, R. G. 1969. Factors influencing photosynthesis and excretion of dissolved organic matter by aquatic macrophytes in hard-water lakes. Proc. Inter. Assoc. Limn. 17:72-85.

Wetzel, R. G. 1975. Limnology. W. B. Saunders, Philadelphia. 743p.

Williams, J. D. H. and T. Mayer. 1972. Sediment diagenesis and regeneration of phosphorus. In H. E. Allen and J. R. Kramer (ed.), Nutrients in natural waters. Wiley-Interscience, New York. 457p.

Williams, J. D. H., J. K. Syers, D. E. Armstrong, and R. F. Harris. 1971. Characterization of inorganic phosphate in calcareous lake sediments. Soil Sci. Soc. Am. Proc. 35:556-561.

Williams, J. D. H., J. K. Syers, R. F. Harris, and D. E. Armstrong. 1971. Fractionation of inorganic phosphate in calcareous lake sediments. Soil Sci. Soc. Am. Proc. 35:250-255.

Williams, J. D. H., J. K. Syers, S. S. Shukla, and R. F. Harris. 1971. Levels of inorganic and total phosphorus in lake sediments as related to other sediment parameters. Env. Sci. Tech. 5:1113-1120.

Wilson, D. O. 1972. Phosphate nutrition of the aquatic angiosperm, Myriophyllum exalbescens Fern. Limn. Ocean. 17:612-616.

Zicker, E. L., K. C. Berger, and A. D. Hasler. 1956. Phosphorus release from bog lake muds. Limn. Ocean. 1:296-303.