PHOSPHORUS UPTAKE AND RELEASE IN AN AQUATIC MACROPHYTE (*VALLISNERIA SPIRALIS*) IN RELATION TO ALGAL PRODUCTIVITY

By

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ABSTRACT

PHOSPHORUS UPTAKE AND RELEASE IN AN AQUATIC MACROPHYTE (*VALLISNERIA SPIRALIS*) IN RELATION TO ALGAL PRODUCTIVITY

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Phosphorus uptake, translocation to and release from leaves were found in the submerged aquatic angiosperm *Vallisneria spiralis* L. when $^{33}PO_4$ was injected into the root environment. Rates of translocation and release were related to the concentration of PO_4-P in the medium and to root biomass. Autoradiographs indicated that both acropetal and basipetal transport occurred in the plant. Epiphytic and planktonic algae rapidly absorbed $^{33}PO_4$ released, and contained up to 37 percent of the amount released to the foliage medium.

Net productivity of phosphorus-starved *Scenedesmus* cultures increased when given phosphorus released from the *Vallisneria* foliage. It appears that phosphorus can be transported from the sediments through the plants and released into the water where it can subsequently be utilized by surrounding algae.

ACKNOWLEDGMENTS

My sincere thanks goes to my major professor, Dr. Peter Murphy. His constant help, suggestions and encouragement were extremely beneficial and fully appreciated. I would also like to thank my committee members, Drs. Robert Wetzel and Clarence McNabb for their critical reading and reviewing of my thesis and for use of equipment. Dr. Edward Farnworth and Carolyn Vann also read the thesis and offered constructive comments. Special thanks go to Dr. Hans Kende for his help and use of equipment while I was using radioisotopes and to Dr. Gary Mills for introducing me to some analytical procedures. I would also like to acknowledge and thank the graduate students with whom I shared a lab for useful conversations and for helping to make my studies at MSU well worthwhile. Finally, I wish to express my gratitude to my parents for their unending support in all my endeavors.

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INTRODUCTION

The importance of phosphorus in the aquatic environment is major because it is a limiting element and an essential nutrient for growth. Whereas the water is generally low in available phosphorus, the sediments usually contain much greater concentrations (Hepher 1966). Sediments rich in available nutrients may contribute to the productivity of rooted aquatic plants by ion uptake through roots. Various studies have demonstrated that the roots of aquatic plants serve a function in nutrient uptake by absorbing nutrients through the root and root hairs (Lundegardh 1966) and are not present only as a means of support in the substratum as has been argued previously (Sutcliffe 1962, den Hartog and Segal 1964).

This study investigates the extent to which phosphorus confined in the sediments can be absorbed by the roots of a submerged aquatic plant, translocated through the foliage and released into the water. The effect of phosphorus concentration on translocation and release is considered. Upon release from the plant, phosphorus movement is traced to observe the response of algal and epiphytic populations surrounding the plant. If phosphorus can be recycled from the sediments through plants to plankton, a phosphorus source otherwise unavailable for metabolism becomes accessible.

Literature Review

In early investigations Pond (1905) found that if the roots of submerged aquatic plants were prevented from entering the substratum,

optimal growth was not achieved. Plants rooted in sand grew better than plants in tap water but not nearly as well as plants rooted in a soil with a higher organic content. Correlations between the distribution of certain aquatic macrophytes and chemical properties of the substratum indicated that plants largely depend on nutrient uptake from the soil via the roots (Misra 1938).

Denny (1972) planted floating-leaved and submerged-leaved plants in artificial ponds where the sediment conditions ranged from nutrient-rich to nutrient-poor. The overlying water was similar in all ponds. Results showed that all plants grew faster on mud than sand, with differential species response to substrate type. Potamogeton thunbergii had a growth rate four times greater in mud than sand. Signs of nutrient deficiency were observed when the nutrient concentration available to the roots was suboptimal even though the foliage portion of the plant was in a mesotrophic water environment. Denny concluded that nutrients could be absorbed from either the substrate or water and that sometimes both could be sources of nutrient uptake.

Reviewing studies concerning the role of roots and leaves in nutrient uptake, Sculthorpe (1967) noted that as long as oxygen was available to root tips, roots were capable of absorbing and accumulating ions. Bristow and Whitcombe (1971) used radioactive tracers in nutrient uptake studies to determine the relative importance of root and foliage absorption. With species of Myriophyllum, they examined the plant's ability to translocate radioactive phosphorus (^{32}P) from the roots to the growing regions of the stem. Normally, roots absorb phosphate from the soil which then enters the xylem tissue to be rapidly transported to other plant parts. A two-compartment chamber was

constructed that partitioned root environment from shoot environment, and ^{32}P was injected into the lower root environment. After a ten-day growth period, the specific activity of ^{32}P in the shoots was determined for three species. Upward translocation of phosphate from the roots to the growing portions of the stem occurred in Myriophyllum brasiliense, M. spicatum and Elodea densa. With E. densa, the phosphate concentration in the foliage environment had no effect on the upward translocation. When ^{32}P was added to the foliage environment, downward translocation to the roots was minimal. Also axillary shoots absorbed a very low amount of the total phosphate present in the medium. Bristow (1975) noted that when nutrients are made available to both roots and shoots, root uptake exceeds foliage uptake in most cases. The availability of nutrients to either compartment had no immediate influence on the uptake ability of the other. In all experiments with Elodea and the Myriophyllum species, labelled phosphorus was not recycled from the substrate into the water by the plant unless plant decay occurred.

It has been demonstrated that submergent plants play a passive role in releasing nutrients upon death and decomposition at which time nutrient availability to surrounding algae increases. Recently, however, it has been sugested that during times of active growth, rooted submerged plants can serve as nutrient pumps. Uptake of trace metals has been found to occur in aquatic macrophytes by Mayes and McIntosh (1977). Different levels of the metals cadmium and lead in water and soil resulted in significantly different amounts in the plants. Also, the authors suggested that release of these metals from the stem and leaves occurs, implying a role of macrophytes in trace metal cycling.

Studies of nutrient absorption in vascular plants have demonstrated that phosphorus is absorbed by the roots, translocated to the foliage and released into the water. McRoy and Barsdate (1970) set up apparatus similar to Bristow et al. (1971) to observe ^{32}P movement through Zostera marina (eelgrass). In situ experiments in natural eelgrass habitats were also performed. Phosphate absorbed through roots was translocated through the eelgrass and often accumulated in areas of active growth and cell division. In experiments in light where phosphate absorption was greatest, 33 percent of the phosphate absorbed was eventually released from the eelgrass beds into the open water. McRoy et al. (1972) further evaluated the role of phosphorus transfer in a lagoon ecosystem dominated by eelgrass. Reactive phosphorus translocation rate through eelgrass was estimated to be 62.4 mg P/m^2-day. Of the reactive phosphorus excreted, three metric tons P/day was exported from the lagoon into the sea. This study gave further support to the importance of littoral vegetation in phosphorus cycling in lakes.

Using ^{32}P, ^{59}Fe, and ^{45}Ca to trace the movement of ions in Myriophyllum exalbescens Fernald, DeMarte and Hartman (1974) constructed a two-chambered apparatus. Elodea canadensis Michx. was placed in a tray that contained the foliage portion of Myriophyllum, and radioactive material was put into a beaker which contained the Myriophyllum roots. Under different conditions of light and substrate, experiments were carried out to determine if translocation and excretion of ions occurred. With autoradiography they observed that Elodea absorbed labelled ions released from the leaves of Myriophyllum. When a rooted plant was injured due to broken offshoots, the amount of ^{32}P released into the water increased significantly.

Spartina alterniflora Loisel was studied by Reimold (1972) to determine pathways of phosphorus in a salt marsh ecosystem. When tidal inundations occurred, labelled phosphorus previously injected into the sediments was released from Spartina leaves into the water approximately 10 to 15 days after its introduction into sediments. Maximum uptake of phosphorus was greatest when productivity was at a maximum. Twilley et al. (1977) observed absorption, translocation and secretion of labelled phosphorus in Nuphar luteum (L.) Sibthorpe and Smith. Secretion by submersed leaves was found only in the summer. Of the phosphorus transported from roots and rhizomes, 86 percent was released into the water.

Studies concerning the cycling of phosphorus have been widespread, reflecting its acknowledged status as a major limiting factor in the growth of algae. The concentration of inorganic or total phosphorus in water gives little indication of the amount available overall. The amount of phosphorus in the sediment in comparison with the phosphorus in the water may differ by several orders of magnitude (Wetzel 1975). High phosphorus concentrations of 7000 µg/g in the sediment were several orders of magnitude greater on a volume basis than the phosphorus in the overlying water in some oligotrophic lakes (Williams and Mayer 1972).

Based on the previously cited studies it is concluded that rooted aquatic plants take up phosphorus and release this nutrient to the surrounding water. Few studies, however, have dealt with the effect of phosphorus on surrounding organisms following release from rooted plants. McRoy and Goering (1974) showed that labelled nitrogen was transferred from Zostera to an algal epiphyte with the ^{15}N content being greater in the epiphyte than in any part of the plant. McRoy cites a

study where it was found that ^{14}C and ^{32}P could also be transferred from Zostera to epiphytes. Wetzel (1969 a,b) monitored dissolved organic compound excretion in the macrophyte Najas flexilis (Willd.) Rosth. and Schmidt. noting probable utilization of these compounds by epiphytic populations. Freshwater algal communities are often limited in their growth by phosphorus availability (Schindler 1977). Studies of the mechanisms of phosphorus transport that result in phosphorus availability to plankton are clearly warranted.

The forms of phosphorus that can be utilized by algal species vary as does the amount. Some algal species possess the ability to take up phosphorus in forms initially different from inorganic orthophosphate as a result of their enzymatic activity (Kuhl 1974). Chlorella can deacylate phospholipids on its surface and then absorb the phosphorus released (O'Kelley 1973). The macrophytic alga, Chara, can utilize glycerophosphate to obtain phosphate. Some marine algae are capable of using glucose-6-phosphate by splitting the compound and using the phosphate (Kuenzler 1965). Kuenzler and Perras (1965) found phosphatases bound to the cell surface of some marine algae that enabled the algae to obtain phosphorus from phosphorylated compounds.

Nutrient requirements for algae differ (Fogg 1973, Fuhs and Canelli 1970), and any phosphorus released from submersed rooted plants can subsequently affect algal growth providing that the phosphorus is in a form that can be assimilated. Bacteria and algae have been found to take up significant amounts of phosphorus from solution. Rigler (1956) found that rapid turnover of phosphate in a small lake may be due to bacteria that are competing effectively with algae. Paerl and Lean (1976) used autoradiography combined with tracer kinetics to demonstrate that

initial uptake of phosphorus was by algae and bacteria.

Jordan and Bender (1973) found that the interaction of other nutrients with phosphorus could increase the phytoplankton response to phosphorus assimilation. Growth was enhanced when EDTA, a chelating agent, and nitrogen were added to cultures containing phosphorus. Half of the algal species studied were stimulated by the treatments, but others showed little difference in productivity. These results reflected those of Lean and Nalewajko (1976). Differences in growth were noted in axenic cultures of Navicula, Chlorella, Anabaena and Scenedesmus. Both Chlorella and Scenedesmus could assimilate phosphorus rapidly, however neither culture increased significantly in biomass. In Anabaena, 20 percent of the phosphate present was absorbed before an increase in growth was noted. No direct correlation between phosphorus uptake and growth occurred. In Scenedesmus, a lag phase of six to eight days between phosphorus uptake and cell volume increase was observed. Fogg (1973) noted that if growth is measured after addition of phosphorus, a significant increase in population may take several days during which time changes in the chemistry and flora of the sample may occur.

Faust and Correll (1976) compared the utilization of orthophosphate between bacteria and algae by differential filtering of cells. Depending on the season of the year, assimilation of phosphorus varied between the two organisms. Bacteria absorbed more phosphorus per cell than did algae. When concentrations of orthophosphate in water were relatively low, phosphorus uptake by bacterial cells was not significantly greater than phosphorus uptake by algae, which indicated that phosphorus uptake per unit biomass and phosphorus concentration in the water were independent of each other. In this study no competitive advantage was noted in uptake by bacteria over algae at low phosphorus levels, which

suggested that any release of phosphorus from a particular source could have a nearly equal chance of being taken up by algae or bacteria in phosphorus limiting waters.

METHODS AND MATERIALS

The submerged rooted aquatic angiosperm Vallisneria spiralis L. was used for all uptake experiments. This species was chosen since it has a substantial root system, is small enough for laboratory experiments and is readily available in pet fish stores. A closely related species, V. americana Michx. occurs as a nuisance plant in many Michigan lakes and streams. The vascular system of Vallisneria is not well-developed. A central lacuna which results from degeneration of xylem conducting elements is present, as are conducting elements of the phloem (Bristow 1975). The stele of the root is surrounded by a well-developed endodermis. In terrestrial plants the endodermis may regulate lateral movement of ions and water in the roots (Sculthorpe 1967). Although a reduced vascular system is not directly related to root uptake, the conspicuous endodermis indicates the importance of roots in ion transport.

A two-compartment experimental chamber similar to that described by Bristow and Whitcombe (1971) was used in studies of phosphorus movement. The intact plant Vallisneria was partitioned into root and foliage components so that phosphorus could be introduced into either compartment (Figure 1). The foliage was contained in a 500 ml graduated cylinder. A 25 ml Erlenmeyer flask painted black to exclude light fit into the graduated cylinder and held the roots. By placing the plant through a hole in a rubber stopper and sealing the area around the hole with silicon grease seal and with Parafilm, the roots and root environment was assumed to be sealed from the foliage environment. Two side holes were

Figure 1. Experimental apparatus partitioning the Vallisneria foliage and roots

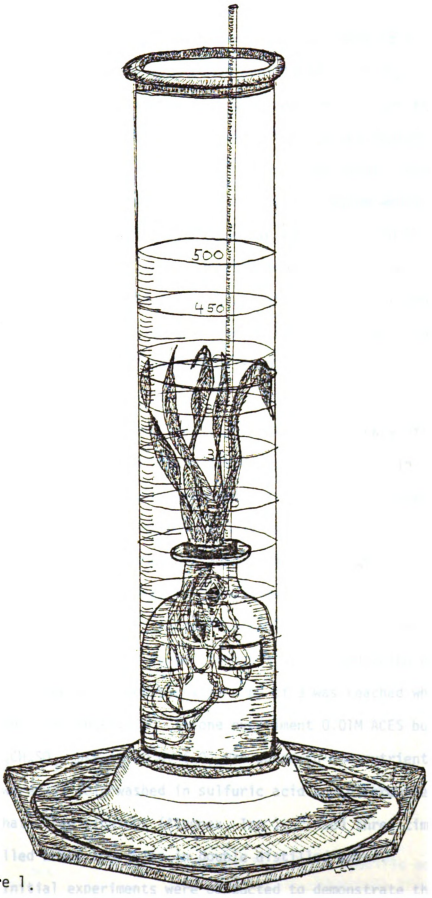


Figure 1.

blown into the flask and covered with septum seals to allow for injection of phosphorus. A glass tube was placed in another hole in the stopper to relieve pressure. As an added measure, 0.1 percent of the inert blue dye, Evan's blue was placed into the rooting medium. This dye is not toxic to the plant and is not taken up by plant roots (H. Kende, pers. comm.); any blue color in the foliage medium would have indicated leakage. The presence of the blue dye was determined by placing aliquot samples of the medium in a spectrophotometer at 620 nm.

Light was provided for 16 hours each day by four Gro-lite fluorescent bulbs and four 100-watt incandescent bulbs with an average light intensity at the water surface of 5380 lux. Temperature was kept at $25^{o}C \pm 2$ degrees.

For all experiments involving uptake and release of phosphorus, 1/10 strength modified Gaudet's medium (Gaudet 1963) in distilled water was used to meet daily requirements of the plants. However, no supplemental addition of vitamins that may have been essentjal for growth was given to the plants. Unlabelled phosphorus as K_2HPO_4 was added to the root or shoot compartment at various concentrations: 0, 0.001, 0.01, 0.1, 1.0, or 10 mg/l. Two to five replications per concentration were used. The pH normally ranged from 6.8 at the beginning of an experiment to 8.5 at the end. Occasionally a pH of 9 was reached when experiments extended long intervals. In one experiment 0.01M ACES buffer (H_2NCOCH_2-$NH_2CH_2CH_2SO_3$, pKa at $20^{o}C$, 6.9) was added to the nutrient medium. All glassware was acid washed in sulfuric acid and dichromate, washed with phosphate-free Liquinox (Alconox, Inc.), rinsed three times in single distilled water and twice in double distilled water.

Initial experiments were conducted to demonstrate that Vallisneria

could release phosphorus to the surrounding medium. After the plants were incubated for three days in the nutrient medium, 10 μCi of $^{33}PO_4$ (hereafter referred to as ^{33}P) in one ml as $K_2H^{33}PO_4$[1], was injected into the root environment. Three rooting environments were initially used: liquid nutrient medium only, autoclaved silica sand and nutrient medium and autoclaved Lake Lansing sediment and nutrient medium with four replications for each substrate type. One mg/l PO_4-P and 10 μCi ^{33}P were injected into each substrate. Subsequent experiments used liquid nutrient medium. Release of labelled phosphorus into the foliage environment was monitored over time by radioassaying aliquot samples by liquid scintillation. Where the growth medium was changed, counts from the previous sample were included. Upon termination of the experiment, plants were washed twice in distilled water, sectioned into foliage and root, dried for 48 hours at 80°C and weighed. The remaining nutrient medium was filtered using a Buchner filter and flask with Whatman paper #1 to measure the amount of ^{33}P in the algae that had colonized in the medium and placed directly into scintillation vials for counting.

Foliage and root samples were homogenized, subsampled and digested in 0.5 ml 10N H_2SO_4 and 0.2 ml 30% H_2O_2, and counted in a liquid scintillation spectrometer (Packard Tri-Carb Model). Scintillation fluid cocktail consisted of Toluene:Triton-X 100 (2:1 v/v) with 0.55% 2,5-diphenyloxazole and 0.01% 1,4-bis-(2-(5-phenyloxyazolyl))-benzene (Allamong and Abrahamson 1973) as the fluors. Counting efficiency for ^{33}P was 79% to 89%, and corrections for background and decay were made. Quenching

[1] $^{33}PO_4$ (E_{max} - 0.248 MeV, half-life 25.2 days, specific activity 50mCi/mm)

did not affect readings of the samples. Phosphorus uptake and release was quantified by monitoring activity in foliage medium, in root and leaves of plants, in rooting medium, in epiphytes that had been scraped from the glass and plants and filtered algae surrounding the plant, and by calculating the initial specific activity of ^{33}P injected into the medium. Plant growth was not measured.

Total phosphorus in the plant and in the medium was determined by using modified Fiske-Subbarow reagent (Fiske-Subbarow 1925). After the digestion procedure, ammonium molybdate was added followed by the reagent made of 0.25 grams 1-amino-2 napthol-1 sulfonic acid with 15% sodium bisulfite and 0.5 grams sodium sulfite. Samples were read at 620 nm in a Beckman DB Spectrophotometer.

Autoradiography was performed using Kodak X-ray film. Labelled phosphorus was injected into the rooting nutrient medium, and plants were removed after 30 seconds, 2, 5, and 10 minutes to observe translocation of ^{33}P. Plants were washed twice in double distilled water, sectioned, numbered and dried in a plant press to avoid diffusional translocation. Plants were placed on top of the film and kept in a folder press in the dark for seven days. Film was developed according to Kodak specifications. Whole plants were also autoradiographed from 20 minutes to 24 hours following injection of phosphorus into either the rooting or foliage medium to observe acropetal or basipetal translocation.

To determine the influence of released phosphorus on algal populations, aliquot samples were taken from two samples of Vallisneria foliage medium in which the roots of one plant had been given a nutrient medium with K_2HPO_4 while the other had received a nutrient medium without phosphorus in the root compartment. When phosphorus release was assumed

to be maximal, the aliquot samples were injected into separate cultures of Scenedesmus quadricauda (Turp.) Breb. Scenedesmus was used because it has a high tolerance to phosphorus (Fogg 1973) and can be easily cultured. Scenedesmus was found to occur normally in the macrophyte medium. Epiphytes present included Oscillatoria sp., other filamentous forms and diatoms. These algae may have been difficult to culture and remove due to their adhesion to surfaces.

Orthophosphate (PO_4-P) in the Vallisneria medium was measured using a Hach kit Stanna-Ver packet. This particular method is not very accurate; however, it was only necessary to know that phosphorus was being released from the plant that was given phosphate. Cultures of Scenedesmus were grown in modified Bold's Basic Medium (James 1974). Culture medium was autoclaved before addition of the algae. For experiments, cultures were phosphorus-starved, and KCl was substituted for NaCl to satisfy the potassium requirement that previously was met by K_2HPO_4. Two ml of suspended phosphorus-starved algae were placed into each of 12 Erlenmeyer flasks with 100 ml of nutrient solution. Twenty-five ml from the foliage medium of the growing plant was injected into six flasks. Aliquot samples of phosphorus-starved Vallisneria injected into the other six flasks were used as controls. Net productivity over time was measured as increases in organic carbon using a Beckman Total Organic Carbon Analyzer (Model 915A). Two samples from each culture, one of which was filtered with a 5μm Nucleopore filter to trap algae and let most bacteria and dissolved compounds pass, were analyzed for total carbon. Prior to analysis, the samples were acidified with 10N HCl to pH 2 to stop all reactions and drive off CO_2 and bubbled with nitrogen for five minutes immediately before running through the analyzer. By subtracting the

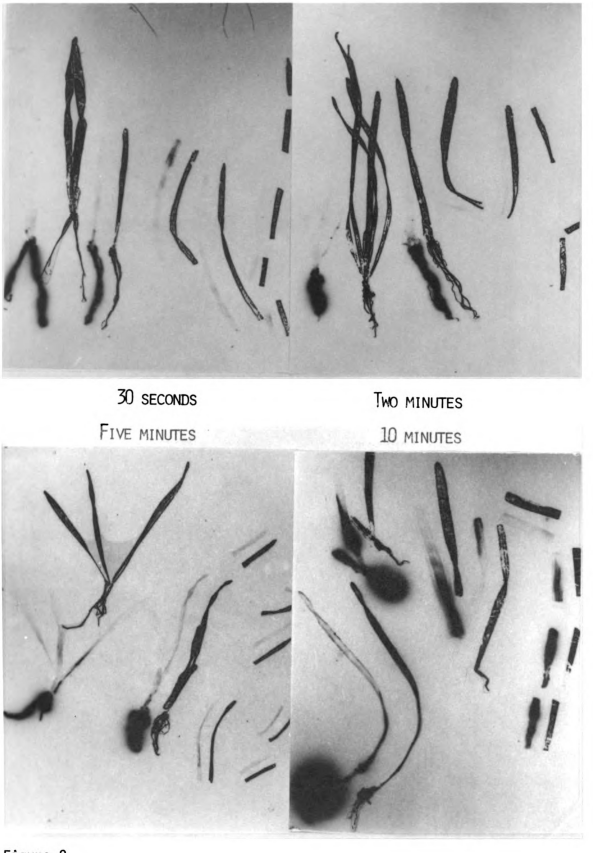
filtered from the non-filtered sample, the amount of carbon present in the algal cells was determined. Standards of 100, 75, 50 and 25 mg/l were made from a stock solution of anhydrous potassium biphthalate dissolved in CO_2-free water, which was obtained by boiling distilled water for 20 minutes. A CO_2 trap using an ascarite drying tube fitted to a holed stopper was used to seal the stock solution. A plastic tube was attached to the stopper for use as a siphon. Sampled cultures were maintained at a temperature of $25^{o}C \pm 2$ under fluorescent lights at 4304 lux, and were swirled manually three times per day. Cultures were also placed on a shaker in a growth chamber to observe possible differences in growth. The pH of the algal cultures was also monitored over time as an indicator of growth.

RESULTS

Roots of Vallisneria spiralis absorbed and translocated phosphorus rapidly to foliage. This was first observed by autoradiography. Autoradiographs showed that within 30 seconds of initial injection, some labelled phosphorus (^{33}P) could be found in the foliage (Figure 2). Since leaves were sectioned immediately after removal from the labelled source translocation to the upper parts of the plant must have occurred rapidly. A notable difference can be seen in root absorption among the four plants placed in the ^{33}P medium for 30 seconds, two, five or 10 minutes, respectively. Precautions, as previously mentioned, were taken to prevent and detect leakage. In order to assure that the small amount of phosphorus found in the foliage medium after a short time was from foliage release and not from leakage, the two-minute treated plant was not replaced in the foliage medium. After injection, a plastic bag was placed over the plant to prevent dessication. Only roots were submerged in the nutrient medium. After two minutes, the plant was removed from the flask, sectioned, dried and mounted on Kodak X-ray film. Labelled phosphorus was found in the leaves, indicating that translocation had occurred. Some leakage, however, was detected in the foliage medium of the 10-minute treated plant which could explain the heavier labelling seen in the foliage. Little difference in translocation to foliage was found for the other three treatments.

Whole plants were also autoradiographed over time to observe areas of accumulated ^{33}P (Figure 3). Higher activity appeared in the midvein

Figure 2. Autoradiographs of plants, whole and sectioned, given ^{33}P to the root medium for 30 sec, 2, 5, and 10 minutes showing rapid initial uptake. A photograph is to the right of each autoradiograph



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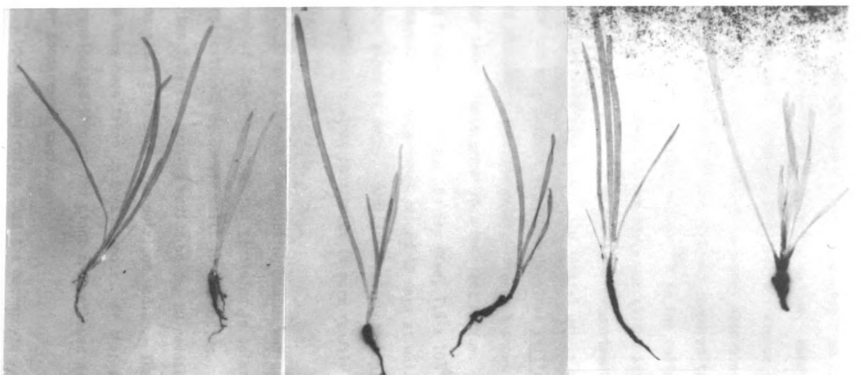

FIGURE 3. AUTORADIOGRAPHS OF WHOLE PLANTS GIVEN ^{33}P TO THE ROOT MEDIUM FOR (L-R) 20 MIN., 40 MIN., ONE, FIVE 10, AND 24 HOURS INDICATING LITTLE DIFFERENCE IN FOLIAGE TRANSLOCATION AND A SIGNIFICANT DIFFERENCE IN ROOT ABSORPTION.

region of the foliage in all samples. Little difference in foliage absorption was seen from times ranging from 20 minutes to 24 hours when ^{33}P was injected in the rooting medium. When ^{33}P was injected into the foliage compartment a significant difference in phosphorus movement could be detected relative to plants absorbing phosphorus by roots. Plants exposed to ^{33}P for 10 hours compared to those exposed for one hour had significantly higher ^{33}P in the foliage tissue (Figure 4). Basipetal movement to the roots was slight after 10 hours.

Release of ^{33}P into the upper compartment from the rooting medium was monitored over time. The initial uptake experiment was performed for the sole purpose of testing whether Vallisneria could release absorbed phosphorus into the water. Figure 5 shows the average ^{33}P release for seven plants grown in a nutrient medium. After one day, 1.0×10^{-4} µg P/mg was released. By day five, 1.5×10^{-4} µg P/mg dry weight was released. The experiment lasted 19 days at which time plants had begun to show signs of decay. Additional aliquot samples taken at that time were questionable since phosphorus is more readily released from decomposing plants (Wetzel 1975).

After six days, the rooting medium was sampled to determine the percent of labelled phosphorus remaining in solution. Based upon three replications, 86.5% was still in the medium. Aliquot samples that were taken from the rooting medium on day 20 revealed that 74.7% of ^{33}P was still present in the nutrient medium. The other 25.3% was assumed to have been taken up by the roots. Of that amount taken up, 1.7% was translocated through the plant and released by the foliage into the upper compartment medium.

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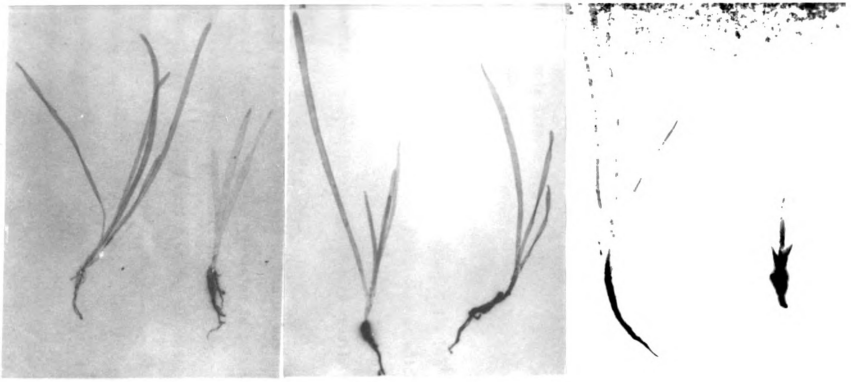

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Figure 4. Autoradiograph negative of plant given ^{33}P to foliage medium for one and 10 hours (1-r) showing significant difference in foliage absorption and little translocation to roots

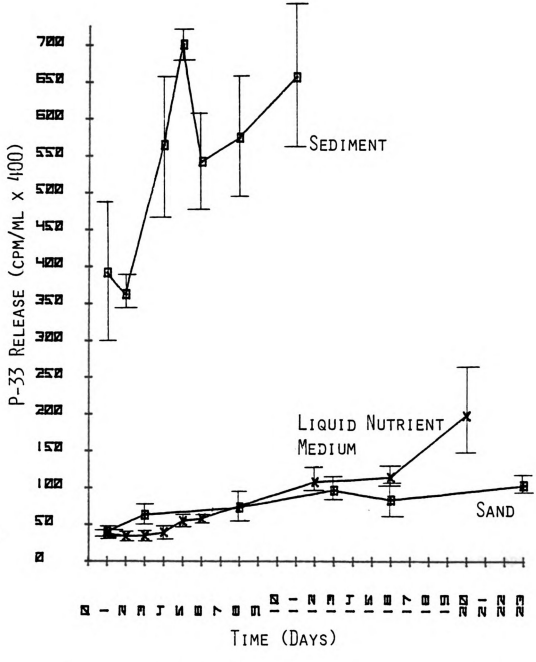

Figure 5. Average ^{33}P release to foliage medium from Vallisneria grown in three substrates. Vertical bar represents ± one standard error of the mean

take up ^{33}P by its roots, translocate it through its foliage and eventually release it into the upper compartment, a series of experiments were carried out to determine if sediment type or varying concentrations of phosphate influenced phosphorus translocation and release. Figure 5 shows results concerning ^{33}P release when the plant was rooted in nutrient medium, as described previously, autoclaved silica sand and nutrient medium, or Lake Lansing sediment and nutrient medium. The nutrient medium contained 10 mg/l unlabelled phosphorus as K_2HPO_4. Total phosphorus content of Lake Lansing sediment was 200 mg/l. After one day, plants rooted in the sediment showed a marked increase in release of ^{33}P over those in sand or water. After four days, those plants rooted in the sand showed little further release, while those in liquid nutrient medium continued to release ^{33}P to the upper compartment.

Fluctuations in the daily amount of ^{33}P released were detected, which was attributed to reabsorption of phosphorus by leaves of plants, and partially by uptake of epiphytic and planktonic algae that had colonized the incubatior apparatus. Epiphytes that were scraped from the plant were found to contain 0.5% of the ^{33}P in the foliage medium, which was an underestimate since epiphytes could not be completely removed. Planktonic algae filtered from the medium contained 1.3% of the ^{33}P in the foliage medium. Quantitative analysis of epiphytic uptake was not made because the filter paper was placed directly into the scintillation fluid; epiphytes were partially eluted while in the fluid. In one sample with a sediment substrate, a 2 ml sample of algae was assayed for ^{33}P in their cells. The upper compartment contained the equivalent of 0.08 µg PO_4-P. Of the ^{33}P released by the plant, 8.6% was taken up into the 2 ml sample of algae.

An analysis of variance was performed to compare daily ^{33}P release from plants growing on different substrates. A significant difference at the 5% level ($p < 0.05$) was found among the three substrates. An LSD test (Snedecor and Cochran 1967) showed that the release of ^{33}P from the plants rooted in Lake Lansing sediment was significantly greater at the 1% level ($p<0.01$) from those rooted in sand and nutrient medium only. No significant difference between sand and liquid nutrient medium treatments was found. Using the Lake Lansing sediment for further study created a problem. A standard phosphorus concentration in the sediment could not be predicted, adsorption of labelled phosphorus to sand was likely, and adequate mixing of the isotope could not be attained. Also, since Lake Lansing sediment is sandy, it seemed likely that increased phosphorus release was due to the high concentration of phosphorus and not to the ability of the plant to take up ions more successfully in that type of substrate. Therefore, liquid nutrient medium was used for all further experiments.

Two experiments were performed to determine if $^{P}O_4$-P concentration had an effect on ^{33}P uptake and release. In one experiment, concentration was varied in the compartment with roots, while in the other experiment, foliage compartment concentration was varied. In the latter, concentrations of 0, 0.001, 0.01, 0.1, 1.0, and 10 mg/l unlabelled PO_4-P were used with two replications. An equal amount of labelled phosphorus (10 μCi/ml) was injected into the rooting environment of each plant. Table 1 shows the amount of labelled and total phosphorus in the plant and labelled phosphorus in the foliage medium at the end of the experiment for the five concentrations. High variability within treatments was observed. Plants having similar weights were used but differences

Table 1. Comparison of phosphorus in Vallisneria compartments at day 11 for six concentrations

PO_4-P Concentration added along with 10 μCi ^{33}P to root compartment (mg/l)	^{33}P in Medium - (cpm)	% Translocated from foliage	^{33}P in Foliage (cpm/mg dry wt)	Total P in Foliage (μg P/mg dry wt)
0	10828	0.6	26108	2.0
0.001	7828	0.1	57798	0.8
0.01	8711	0.0	38556	1.0
0.1	16202	1.2	24060	0.7
1.0	12877	0.6	27075	0.8
10.0	31620	5.2	14542	1.8

could have occurred in root biomass, number of leaves or leaf size, all factors contributing to possible increased variability.

Linear regression analysis indicated that the only significant difference between treatments was in the amount of ^{33}P in the leaves and the foliage medium ($p<0.01$), where a negative correlation of $r = -0.58$ was observed. There were indications that one of the treatments using 10 mg/l unlabelled PO_4-P had leaked to the foliage medium. When this compartment was excluded from analysis, a greater negative correlation of $r = -0.74$ was found (Figure 6). No significant correlation was found between ^{33}P in the medium and total phosphorus in the plant ($r = -0.41$) or ^{33}P in the foliage and total phosphorus in the foliage ($r = -0.47$).

Differences in concentration in the foliage medium did not have a significant effect on release of ^{33}P from the foliage through the first six days of the experiment (Figure 7). Following that time, phosphorus release was significantly greater ($p<0.01$) in the plant having the highest concentration (10 mg/l) as compared to the other concentrations (LSD, $p<0.05$). These results exclude the one replication where leakage was noted. Concentrations from 0 mg/l to 1 mg/l showed no significant differences at the 5 percent level. The nutrient medium was changed frequently, and epiphytes did not colonize the containers. Measurements of the amount of phosphorus released from the plant were accumulated over time to account for changing of the medium.

Translocation of ^{33}P in roots and foliage varied according to the concentration of unlabelled phosphorus injected into the rooting medium. Three concentrations, 0, 0.01, and 10 mg/l PO_4-P were placed into the various rooting media along with 10 μCi $K_2H^{33}PO_4$ in one ml. Table 2 shows the amount of ^{33}P in each compartment at the end of the

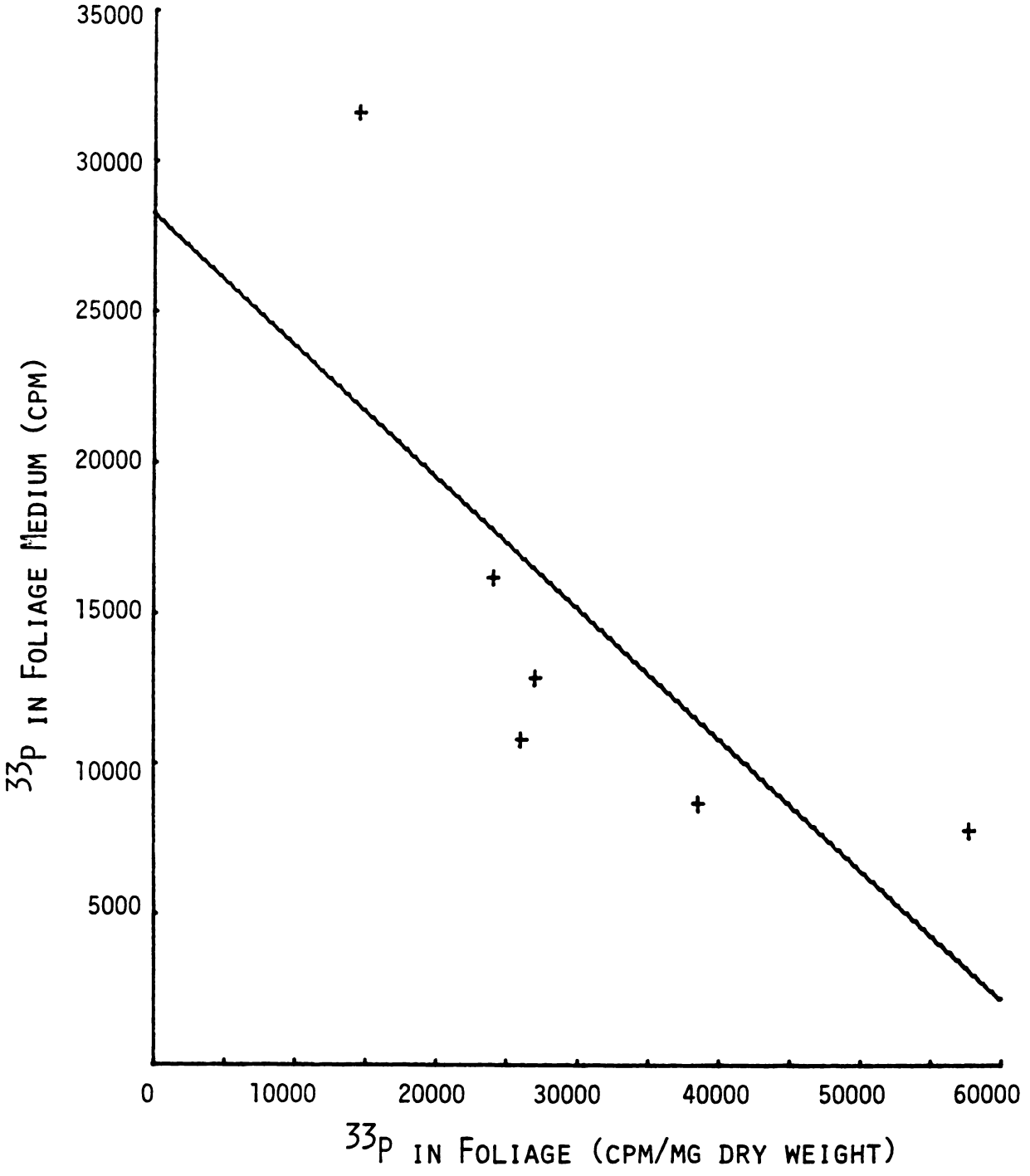

FIGURE 6. REGRESSION OF LABELLED P PRESENT IN VALLISNERIA LEAVES AND IN THE NUTRIENT MEDIUM IN WHICH IT GREW.

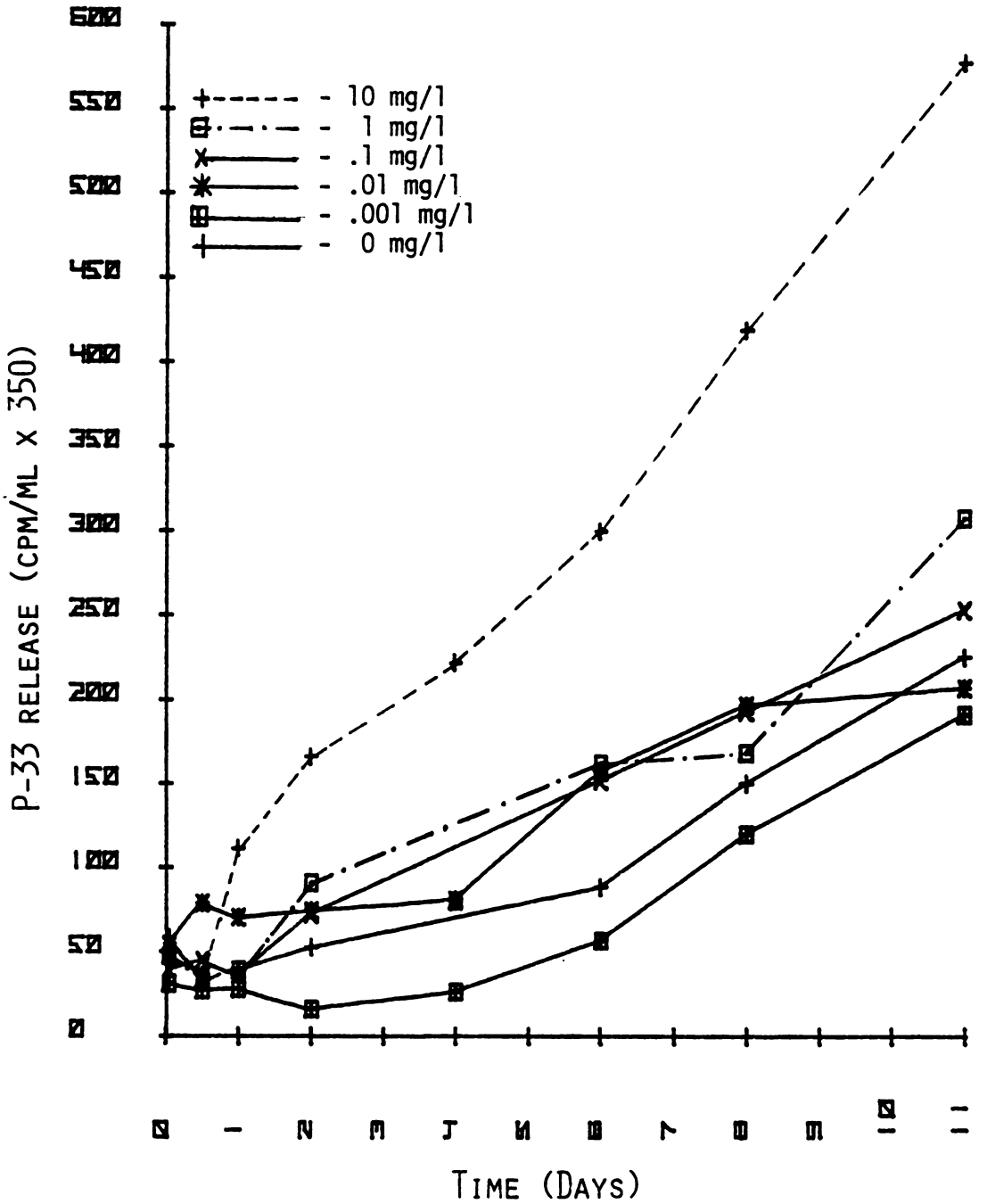

FIGURE 7. AVERAGE ^{33}P RELEASE FROM VALLISNERIA FOLIAGE TO NUTRIENT MEDIUM WITH VARYING P CONCENTRATIONS IN THE FOLIAGE COMPARTMENT.

Table 2. Percent Phosphorus in Vallisneria and the growing medium at day 12*

PO_4-P added to Root Compartment (mg/l)	Root Biomass (mg dry wt.)	$\%^{33}P$ in Root Medium	$\%^{33}P$ in Roots	$\%^{33}P$ in Foliage	$\%^{33}P$ in Foliage Medium
0	2.1	7.7	1.8	51.7	0.6
0.01	18.4	19.3	55.5	5.75	1.0
0.01	51.5	5.5	66.5	1.75	1.1

* Totals less than 100% due to loss of ^{33}P from unscraped epiphytes on glass and glass adsorption

Table 2 (cont'd).

PO_4-P added to Root Compartment (mg/l)	Total P in Foliage (F) and Roots (R) (μg/mg dry weight)	$\%^{33}P$ in Foliage translocated from roots	$\%^{33}P$ in Foliage Medium translocated from foliage	$\%^{33}P$ in Epiphytes-% Translocated from foliage medium
0	F - 3.1×10^{-4} R - 1.4×10^{-4}	96.6	1.10	0.06 - 9.5
0.01	F - 9.4×10^{-5} R - 2.2×10^{-4}	9.4	14.70	0.13 - 11.6
0.01	F - 4.9×10^{-5} R - 2.5×10^{-4}	2.6	37.7	0.49 - 31.6

experiment (12 days) for two concentrations. Most of the ^{33}P injected into the rooting medium was translocated after 12 days. The amount of phosphorus in the root compared with the phosphorus in the foliage differed significantly. At 0 mg/l PO_4-P, 51.7% of the ^{33}P injected into the rooting medium was found in the foliage, while only 1.8% was found in the roots. At 0.01 mg/l PO_4-P, 55.5 and 66.5% ^{33}P was found in roots with 5.75 and 1.75% found in the foliage, respectively, which indicated that total phosphate was limiting.

Overall, little ^{33}P was released to the foliage medium. On a percentage basis, the amount of phosphorus released to the medium from that translocated through the foliage ranged from 1.1% in the treatment with zero phosphate added to 37.7% of ^{33}P released from a sample treated with 0.01 mg/l PO_4-P.

Figure 8 shows the average amount of ^{33}P released per ml of media to the foliage medium for the three treatments based on four replications per treatment when ^{33}P and PO_4-P were added to root compartments. In individual samples, reabsorption of phosphorus by the leaves may have occurred. A significant difference in release was seen at days 11 and 12 ($p<0.05$). The amount of ^{33}P released from the treatment with 10 mg/l PO_4-P was equivalent to 0.05 µg PO_4-P. Epiphytes and plankton colonized all cylinders and incorporated ^{33}P. Table 2 shows that 31.6% and 11.6% of ^{33}P released to the foliage medium was taken up by epiphytes for the two treatments that were given a concentration of 0.01 mg/l PO_4-P. Nine percent of the ^{33}P released from the 0 mg/l treated sample was taken up by epiphytes.

A fairly high percentage of labelled phosphate was unaccounted for in all cases, ranging from 18 percent to 38 percent, which could have

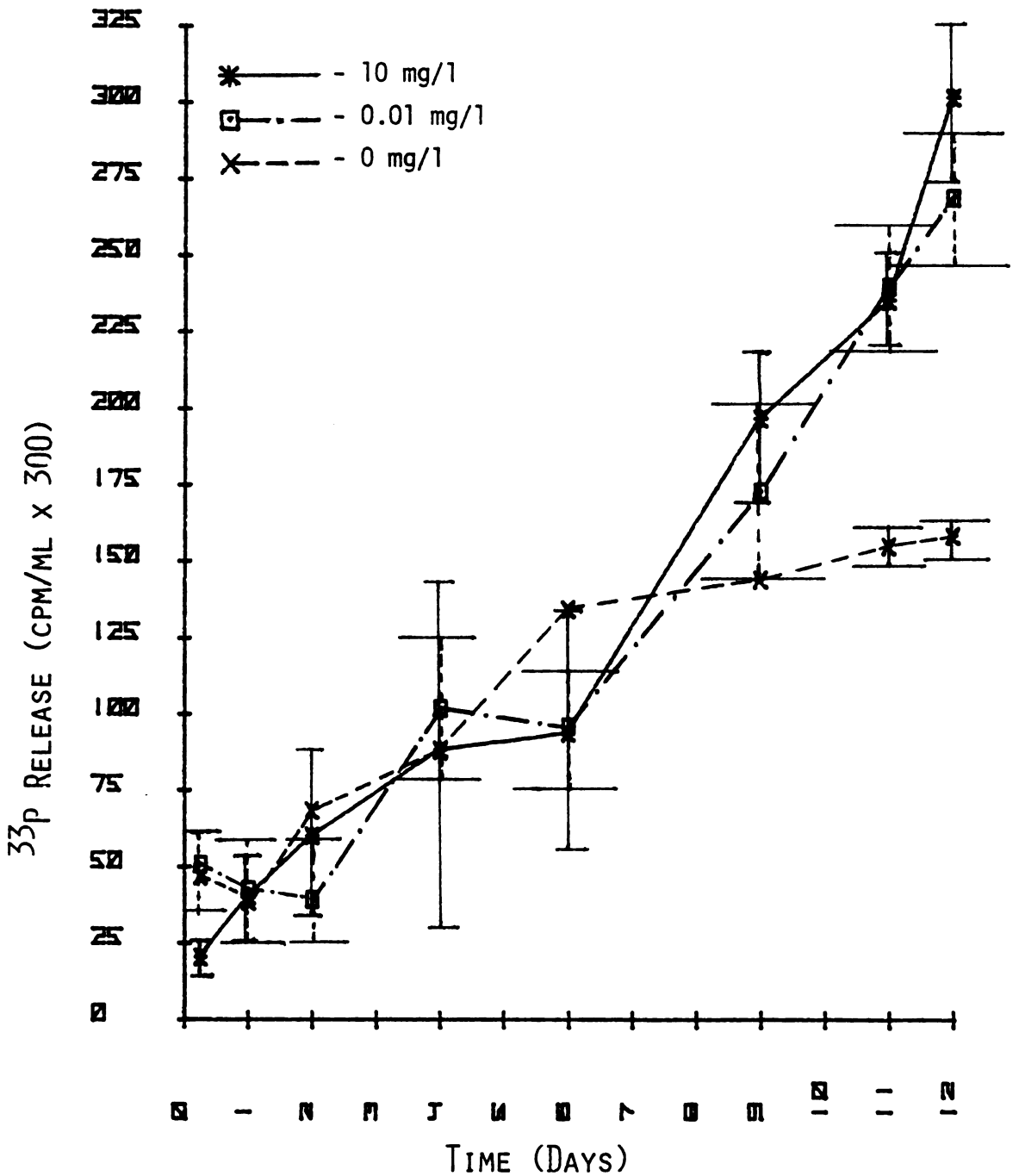

Figure 8. Release of ^{33}P from Foliage to Surrounding Medium with Three Concentrations of PO_4-P in Root Compartment. Vertical bar represents ± one standard error of the mean

resulted from absorption of phosphorus by epiphytes on the glass container and flask. Much of the algae could not be successfully scraped from the walls and counted, and consequently, the amount of ^{33}P in epiphytes was an underestimate of the actual amount of ^{33}P they incorporated. No quantitative measurement of phosphorus per cell was made. Table 3 shows the results of an experiment similar to the previous one but with some variations. Five replications per concentration were used. Concentrations were 0, 0.01, and 0.1 mg/l PO_4-P with the addition of 5 μCi of $K_2H^{33}PO_4$ in one ml. Little ^{33}P was translocated to the foliage from the roots for all concentrations. For this experiment, chewing gum rather than a grease seal was used as a sealant (Twilley et al. 1977) and packed extremely tight into the holes of the stopper. This may have caused some damage to the delicate plant leaves preventing optimal translocation. Also a buffer, ACES (Norman Good, pers. comm.) was added to the nutrient medium. It has been noted in algae that pH may change the rate of PO_4 absorption in several ways (Wetzel 1975) which may hold true for submersed macrophytes as well. Plants began to senesce before epiphytes colonized the container. A similar trend, however, was seen for average replicates of ^{33}P in the foliage for three concentrations as in the previous experiment. As the concentration of unlabelled PO_4-P was increased from 0 to 0.1 mg/l in the root compartment, the amount of ^{33}P translocated to the foliage decreased. When unlabelled PO_4-P was not added, translocation of ^{33}P was greatest. Differences between treatments, however, were not significant ($p>0.10$). In contrast, similar trends in ^{33}P root absorption were not found. Most of the ^{33}P should have been found in the roots for the treatment given 0.1 mg/l PO_4-P with a percentage significantly higher than 46.4, however, this was not

Table 3. Percent Phosphorus at day 7 in different compartments at three concentrations

PO_4-P concentration with 5 μCi ^{33}P added to the root compartment (mg/l)	$\%^{33}P$ in Root Medium	$\%^{33}P$ in Roots	$\%^{33}P$ in Foliage	$\%^{33}P$ in Foliage Medium
0	66±8.8	27.1±10.9	7.1±3.3	0.07±0.01
0.01	55.1±8.3	40.2±7.3	4.8±2.4	0.10±0.02
0.1	81±2.9	17.1±6.2	0.98±0.53	0.20±0.05

observed. Less than 19% of the ^{33}P injected into the root compartment was taken up by the roots. The remaining 81% was still in the root medium. Although a similar trend did not emerge for all treatments, there was some evidence that concentration variation in the root compartment had an effect on ^{33}P translocation and release.

To better understand the effect of released ^{33}P on surrounding epiphytic and planktonic algae, cultures of the planktonic green alga, Scenedesmus quadricauda were grown in separate flasks. The results of added aliquot samples of Vallisneria medium to the net primary productivity of the algae is presented in figure 9. The upper curve represents those algae given foliage media from Vallisneria with PO_4-P added to the root compartment. The lower curve represents cultures given foliage media from Vallisneria without PO_4-P added to the root compartment. After six days, a significant difference was observed ($p<0.01$) between the algal cultures. While total organic carbon as a measure of net productivity had increased to 29.5 mg/l total carbon in the phosphorus-enriched one, net productivity in phosphorus-starved cultures fell to 9.5 mg/l carbon. The decrease in carbon was likely due to cell lysis of the algae. In individual samples, five of the six phosphorus-starved cultures crashed by day six. Only one of the phosphorus-enriched cultures showed a decrease in total organic carbon at this time.

Single cultures, one phosphate-enriched and one phosphate-starved, were grown on a shaker in a growth chamber to observe if constant shaking of the culture altered the results. It was indicated (Figure 10) that constant shaking had no significant effect on cultures as compared to intermittant shaking.

Supplemental experiments were done using pH as an indicator of

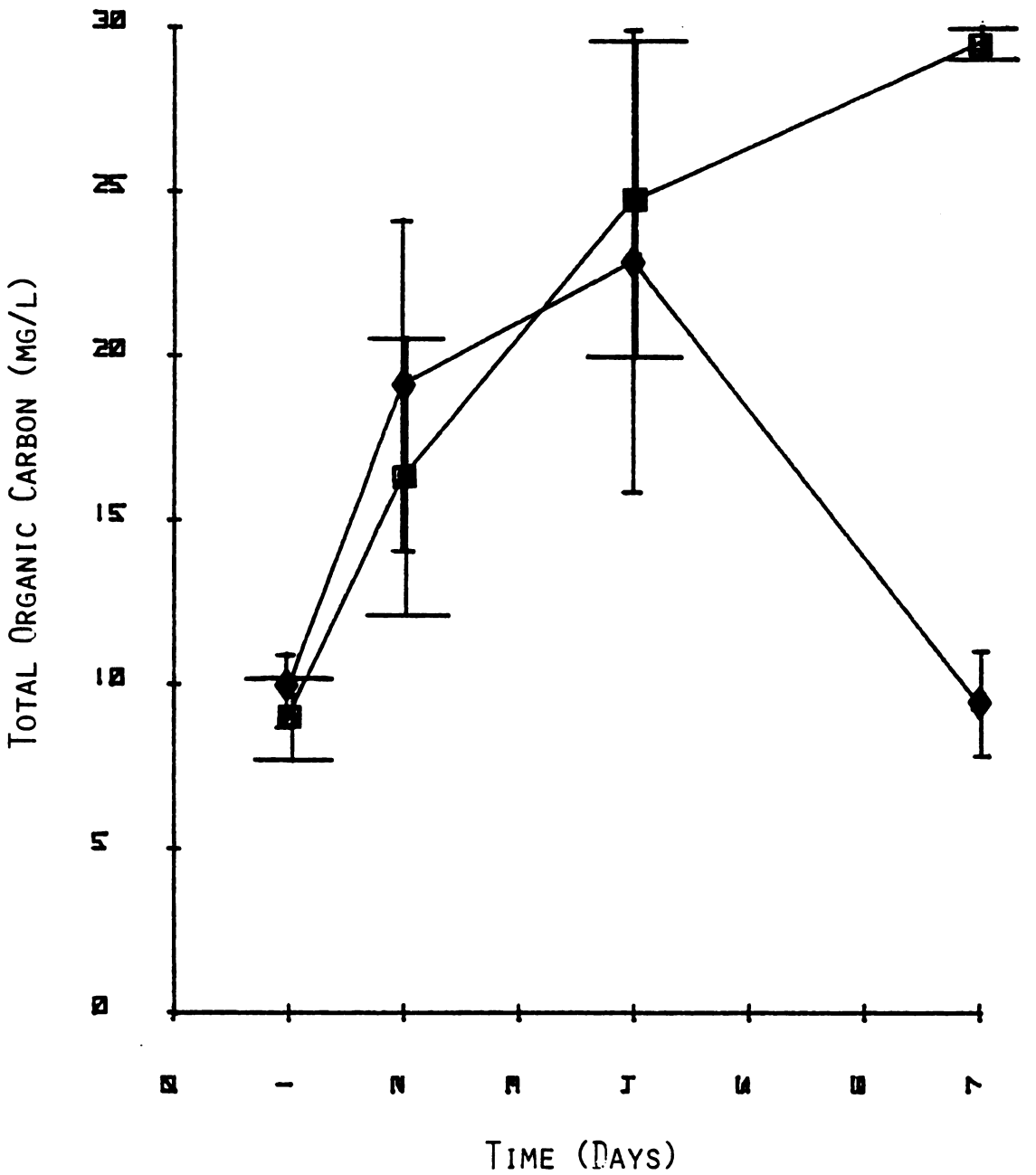

FIGURE 9. NET PRODUCTIVITY OF SCENEDESMUS QUADRICAUDA AFTER ADDITION OF FOLIAGE MEDIUM FROM P ENRICHED AND P STARVED VALLISNERIA. (■— - ENRICHED; ◆— - STARVED). VERTICAL BAR REPRESENTS ± ONE STANDARD ERROR OF MEAN

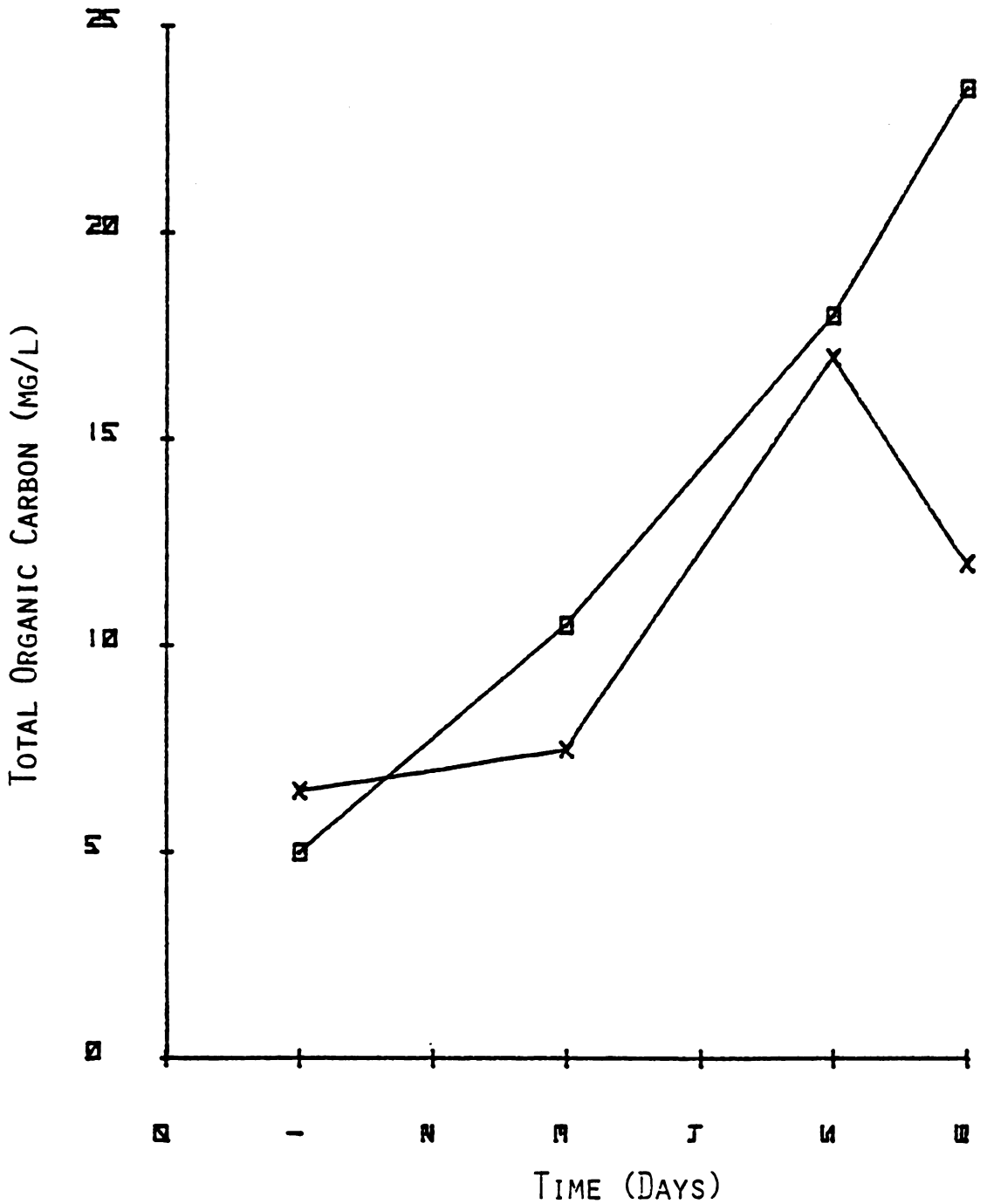

FIGURE 10. NET PRODUCTIVITY OF P STARVED AND P ENRICHED SCENEDESMUS GROWN WITH CONSTANT SHAKING. (⊡ - ENRICHED; × - STARVED)

increased net productivity. No differences in pH were noted between the two cultures with and without phosphate. The pH increased from 7.5 to 11 before algae became stressed in both cultures.

DISCUSSION

Phosphorus uptake, translocation and release

The amount of labelled phosphorus accumulated in roots or foliage of Vallisneria, and the amount released by the foliage were dependent upon the concentration of PO_4-P supplied to root or foliage compartments. Only when Vallisneria was supplied with a high concentration of PO_4-P did ^{33}P release to the water increase significantly. Experiments of Barko and Smart (in press) have suggested that nutrients that are available from the sediment in surplus of the plant's requirement should be released in significant concentrations from rooted aquatic plants. The amount of total phosphorus present in the foliage for individual plants differed greatly, which may explain why PO_4-P concentration did not show significant differences in ^{33}P release relative to the concentration over tenfold increases in some cases. It was assumed, in the analysis of data, that the ratio between ^{33}P and unlabelled phosphorus remained constant as phosphorus was absorbed, translocated or released from the plant (Whittaker 1961).

Similar amounts of ^{33}P release into the foliage medium resulted whether PO_4-P was supplied to the root or shoot medium. Both of these sites have been found to be active in nutrient uptake in many aquatic plants (Denny 1972). Bristow (1975) found that the level of phosphate in the foliage compartment medium was unrelated to rate of translocation of ^{32}P. He concluded that the uptake of ^{32}P by one compartment was

independent of the supply of ^{32}P in the other compartment. In the Vallisneria studies, phosphate levels in the foliage medium significantly affected ^{33}P release from the plant. Autoradiographs further confirmed that phosphorus uptake could occur through the foliage or through the roots. Acropetal and basipetal translocation occurred within the plants as seen from the autoradiography. Bidirectional movement of phosphorus has been found in other macrophytes including Zostera marina (McRoy and Barsdate 1970), Myriophyllum exalbescens (DeMarte and Hartman 1974), and Nuphar luteum (Twilley et al. 1977). Nichols and Keeney (1976) found some bidirectional flux of nitrogen in Myriophyllum spicatum. In Vallisneria, basipetal movement did occur; however, plants exposed to ^{33}P for one hour appeared to incorporate as much ^{33}P in their roots as did plant foliage exposed for 10 hours to ^{33}P (Figure 4). Rate of translocation from leaves to roots was not measured. Schults and Malueg (1971) working with Vallisneria americana found translocation of phosphorus occurring in both directions with more translocation from roots to the foliage than downward from foliage to roots.

Autoradiographs supported findings that suggested initial rapid uptake and translocation from the roots (Figure 2). That 51.7% of ^{33}P injected into the rooting medium was found in the foliage with 0 mg/l PO_4-P added and only 5.8% ^{33}P was found in the foliage with medium at 0.01 mg/l PO_4-P suggested that total phosphorus was limiting in the tissue. This data could explain why release of ^{33}P into the foliage medium relative to the amount taken up by roots was relatively low (Table 2). Only when the plants were given an excess of nutrients, as was the case with the Lake Lansing sediment (200 mg total P/l) or the addition of the 10 mg PO_4-P/l treatment, was the percent release of ^{33}P much greater. Gerloff and

Krombholz (1966) found the critical phosphorus tissue content of Vallisneria americana, a closely related species of V. spiralis, to be 0.13 percent by weight. Above this level, addition of phosphorus would result in luxury consumption. However, the biomass of the roots and foliage may also have influenced phosphorus uptake. The dry weight of roots of plants given 0.01 mg/l PO_4-P was 18.4 mg and 51.5 mg for those plants absorbing 55.5 percent and 66.5 percent ^{33}P, respectively. The dry weight of the root given 0 mg/l PO_4-P was only 2.1 mg for that plant absorbing 1.8 percent labelled phosphate. Plants with greater root biomass may be able to absorb and translocate more phosphorus then those plants with less root biomass.

Those plants given 0.1 mg/l PO_4-P to the root compartment did not absorb as much ^{33}P as those plants given less inorganic phosphate (Table 3). Dry weights indicated that on the average, plants that had the least root biomass took up less ^{33}P. Plants given 0.1 mg/l PO_4-P had an average root weight of 6.68 mg, while they absorbed an average of 17.1 percent ^{33}P. Plants given 0.01 mg/l had roots weighing 16.7 mg, and they absorbed 40 percent ^{33}P. The latter set of plant roots weighed 2.4 times more than the former and absorbed 2.8 times more ^{33}P. It is likely that phosphorus absorption is a function of root biomass and ion concentration. Bristow et al. (1971) found a correlation between root mass and the proportion of phosphate taken up by roots in Myriophyllum brasiliense.

Macrophyte-algal interactions

A direct transfer of phosphorus from Vallisneria to the epiphytes colonizing the plant and to algae in the foliage compartment was found. Epiphytic and planktonic algal populations absorbed up to 31.6 percent

of the ^{33}P released to the medium from the plant. Since epiphytes could not be completely scraped from the containers or plant, this is likely an underestimate of the amount of ^{33}P in algal cells.

Allen (1971) studied the release of dissolved organic carbon from Najas flexilis to its epiphytic community. His data suggested that an epiphyte-macrophyte interaction occurred in relation to carbon release. A similar interaction might have occurred with phosphorus release in Vallisneria. Although uptake and translocation occurred immediately upon injection of ^{33}P, release of phosphorus fluctuated, and usually significant differences between PO_4-P concentration and ^{33}P release were not seen for several days. Where plants began to senesce before epiphytic colonization occurred on the glass or plant, only 2.02 percent of ^{33}P translocated was released for the treatment using 0.01 PO_4-P (Table 3). Where colonization did occur, 37.7 percent of the ^{33}P translocated was released for the same concentration. However, for PO_4-P concentrations of 0 mg/l, percent release for colonized and uncolonized containers was similar. Hutchinson (1975) suggests that the excretion of dissolved organic compounds leading to the increase in epiphytic colonization may be an adaptive mechanism for plants since invertebrates of freshwaters generally feed on epiphytic communities rather than on the higher plants. This same adaptive mechanism may be true for phosphorus release from plants.

Increased net productivity of Scenedesmus cultures indicated that the algae could utilize the phosphorus released from the macrophyte. All algal cultures showed an increase in net productivity through the first four days after being given aliquot samples from phosphorus-enriched and phosphorus starved Vallisneria. Since algae have been found

capable of luxury consumption of phosphate, an increase in net production over a short time could occur for both phosphorus-starved and phosphorus-enriched cultures. Allen (1971) suggested that organic materials released from Najas first underwent microbial degradation before being absorbed by algae. The form of phosphorus released from Vallisneria was not determined. Since axenic cultures were not used, bacterial degradation prior to algal uptake may have occurred. Lean and Nalewajko (1976) discussed the relationship between algal uptake of phosphorus and phytoplankton productivity. Algal cell volume and phosphorus uptake were not directly correlated. In their cultures of Scenedesmus, almost all ^{32}P was absorbed by the algae before cell volume increased. In the present study, it was not analyzed if phosphorus uptake and net productivity were directly correlated; however, an increase in net productivity was attributed to the addition of phosphorus to the cultures, derived from Vallisneria foliage.

The contribution of released phosphorus from aquatic macrophytes to surrounding algal and epiphytic populations could be significant, especially in phosphorus-limited waters. In an estuary, McRoy and Goering (1974) studied the transfer of nitrogen from sediments through macrophytes to an epiphytic community and offered this as an explanation for high stocks of epiphytic algae in low nutrient waters. In phosphorus-limited waters, in the littoral area of lakes, rooted macrophytes may contribute to an increase in algal productivity. Phosphorus lost to the sediments could be absorbed by rooted aquatic plants and released from their leaves into the water making available a previously unobtainable source of phosphorus for epiphytic and planktonic algal populations. Phosphate release from Elodea is currently being studied in situ (G. Lie, in preparation).

Further studies should examine the rates of phosphorus release from other rooted aquatic plants and the impact on biota in natural waters. A knowledge of the form of phosphorus released is needed as is the extent to which phosphorus becomes available to biota. This may be important in predicting the trophic status of freshwater lakes.

CONCLUSIONS

Phosphorus released from the foliage of Vallisneria was partially incorporated into epiphytic and algal populations. Net productivity of algae was enhanced when supplied with the foliage medium in which the phosphorus was released from the macrophyte. The total phosphorus released from the foliage was relatively low and may be attributed to several factors. The enclosed root compartment could have created a very different environment than that in which the plant had been previously grown. Acclimation to the nutrient medium and partitioned environment may not have been successful within the three days prior to treatment. It was also indicated that plants were phosphorus limited in many cases. Phosphorus limitation could be a major cause of a small percentage of released phosphorus into the foliage medium.

APPENDIX

APPENDIX

A1. Nutrient composition of modified Gaudet's medium (Gaudet 1963)

Compound	Concentration (mg/l)
Calcium nitrate ($Ca(NO_3)_2.4H_2O$)	30
Potassium nitrate (KNO_3)	8
Potassium chloride (KCl)	6.5
Magnesium sulfate ($MgSO_4.7H_2O$)	75
Sodium sulfate (Na_2SO_4)	20
Manganese sulfate ($MnSO_4.H_2O$)	0.34
Zinc sulfate ($ZnSO_4.7H_2O$)	0.30
Boric acid (H_3BO_3)	0.15
Potassium iodide (KI)	0.075
Copper sulfate ($CuSO_4.5H_2O$)	0.004
Ammonium molybdate ($(NH_4)_6Mo_7O_{24}$)	0.008
Sodium-Iron EDTA	0.5
Sodium bicarbonate (Na_2HCO_3)	30

A2. Nutrient composition of modified Bold's Basic Medium (James 1974)

Compound	Concentration (mg/l)
Sodium nitrate ($NaNO_3$)	250
Magnesium sulfate ($MgSO_4.7H_2O$)	50
Dipotassium phosphate (K_2HPO_4)	100
Potassium monophosphate (KH_2PO_4)	150
Calcium chloride ($CaCl_2$)	25
Sodium chloride (NaCl)	25
Sodium bicarbonate (Na_2HCO_3)	100
Zinc sulfate ($ZnSO_4$)	8.8
Molybdenum tetraoxide (MoO_4)	0.71
Cobalt nitrate ($Co(NO_3)_2.6H_2O$)	0.49
Manganese chloride ($MnCl_2$)	1.44
Copper sulfate ($CuSO_4.5H_2O$)	1.57
Boric acid (H_3BO_3)	11
EDTA stock	50
H-Fe stock	4.98

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