Robert R. Twilley. PHOSPHORUS CYCLING IN <u>NUPHAR</u> <u>LUTEUM</u>
COMMUNITIES IN THE LOWER CHOWAN RIVER, NORTH CAROLINA. (Under the
direction of Mark M. Brinson) Department of Biology, July, 1976.

The purpose of this study is to evaluate the role of <u>Nuphar</u> <u>luteum</u>
in the cycling of phosphorus (P) in the lower Chowan River. Measure-
ments were made of (a) seasonal and spatial P concentrations in the plant,
(b) P fractions available to the plant in the substrate and (c) P flux
between plant sediment, and overlying water. The P concentration in the
aboveground biomass (leaves and petioles) peaked during early spring
(241.57 μg-at P/g OW) while the rhizomes declined in P concentrations
during this season. Rhizomes accumulated the greatest amount (66.2%)
of the P bound in the biomass of <u>Nuphar</u> followed by roots (10.6%) with
the remainder (7.1-8.1%) in the three aboveground structures. Total
accumulation of P in the biomass of <u>Nuphar</u> during June in the lower
Chowan River was 0.243 MT. At three sites, the biological available
phosphorus concentration and the P concentration in the roots correlated
better ($r = 0.90$) than did interstitial water and root concentration
($r = 0.74$).

Uptake, translocation, and subsequent secretion of phosphorus by
<u>Nuphar</u> was studied under laboratory and field conditions using radio-
isotopes. In the laboratory studies, roots had the greatest absorption
rate of P, submersed leaves were intermediate, and floating leaves had
the lowest rate. An increase in the concentration of P resulted in
increased absorption rates for submersed leaves and roots. In the Chowan
River, 24-hr experiments tracing P from both water and sediments into
the plant were done simultaneously using a double isotope procedure with

phosphorus-32 and -33 during the winter, spring, and summer. In both laboratory and field experiments translocation of P absorbed by roots occurred more rapidly and extensively than with submersed leaves and floating leaves. Absorption rates for roots were greatest in summer (1.72 µg-at/g dry wt · day) and lowest in winter (1.29 µg-at/g dry wt · day) while the highest absorption rate for submersed leaves was spring (0.55 µg-at/g dry wt · day) and lowest in winter (0.29 µg-at/g dry wt · day). A bidirectional flux of P was measured in Nuphar and the dominant pathway was from belowground to aboveground structures. The flux of P between the aboveground and belowground structures varied between winter, spring, and summer and the translocation rates were affected more by the seasons than the absorption rates. Secretion from submersed leaves and roots were measured only during the summer experiments.

Decomposition studies showed that once the aboveground structures died, the P was quickly regenerated back into the system (half-time ca. 8 days). The flux of P from biomass to detritus was determined by using production rates of Nuphar which equaled 0.032% P/day. During the summer the rate of P movement from aboveground to detritus compartment was 146.22 µg-at/m^2 · day of which 109.67 µg-at/m^2 · day originated from the substrate via root absoprtion and translocation. Adding the daily rate of secretion which also represented regeneration of P from the substrate, the net loss of P from the sediments during the summer via decomposition and secretion was 180.87 µg-at/m^2 · day. The significance of these results is that Nuphar functions as a nutrient pump resulting in a net flux of P from belowground to aboveground structures. This represents a potentially important pathway for the regeneration of P from the substrate to the overlying water.

The radioactive tracer estimates of P inputs by absorption and translocation into aboveground biomass were only 12% of the values calculated by biomass production and P concentrations. It may be that P absorbed by <u>Nuphar</u> is converted to a storage product and later re-distributed via translocation. A 24-hr experiment is too short to measure this proposed redistribution movement of P because of the lag time from absorption to storage to translocation.

PHOSPHORUS CYCLING IN

NUPHAR LUTEUM COMMUNITIES

IN THE LOWER CHOWAN RIVER, NORTH CAROLINA

A Thesis

Presented to

The Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

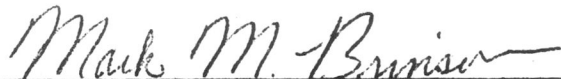Master of Science in Biology

by

Robert Reece Twilley

July 1976

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ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

The dominant pathway of phosphorus (P) movement in the biosphere
is from terrestrial ecosystems to the sediments of aquatic ecosystems.
Because of its importance as one of the major limiting nutrients of
primary productivity (Hutchinson 1957), P has received much attention
in aquatic ecology. The purpose of this research is to evaluate the
role of aquatic macrophytes in cycling of P in the Chowan River. Aquatic
macrophytes may effect P cycling by the following processes: absorption,
accumulation, translocation, secretion, and decomposition. The relation-
ship of these processes is illustrated by a model depicting the possible
pathways of P cycle through macrophyte communites (Fig. 1). There
exists a controversy over which of these pathways of P through rooted
aquatic macrophytes is dominant and therefore the source of P for the
nutrition of the plant (Sculthorpe 1967, Hutchinson 1975, Wetzel 1975).
The controversy centers around three possible dominant pathways: (1) foliar
absorption and subsequent translocation throughout the plant (Sutcliffe
1958, 1962, Den Hortog & Segal 1964); (2) absorption by roots and
translocation to aboveground vegetation (Bieleski 1973, Pearsall 1920,
1921, Denny 1972, Bristow & Whitcombe 1971); (3) a bidirectional pathway
with leaves and roots absorbing nutrients which are distributed through-
out the plant (McRoy & Barsdate 1970). Depending on the dominant pathway,
rooted aquatic macrophytes may act as either a sink or a source of P to
the water.

The pathway of P from sediment to water via root absorption and
subsequent translocation may be important to the P concentration in the
water. The sediment accumulates large quantities of P from sedimentation

Figure 1. A model of the phosphorus cycle through a community of rooted aquatic macrophytes. X_n represents the quantity of P in the compartments and k_{ab} is the flux of P from compartment a to compartment b. The processes that cycle P through the community are: absorption - k_{26} and k_{35}; accumulation - X_n; translocation - k_{65} and k_{56}; secretion - k_{53} and k_{62}; death - k_{61}, k_{54}; decomposition k_{12}, and k_{43}.

The amount of P transferred (k) from one compartment to another is proportional to the quantity (Q) of P in the compartment from which it came. Such a function is described by the following formula: $\frac{dQb}{dt} = \lambda ab \cdot Qa$ where lambda is the rate of transfer.
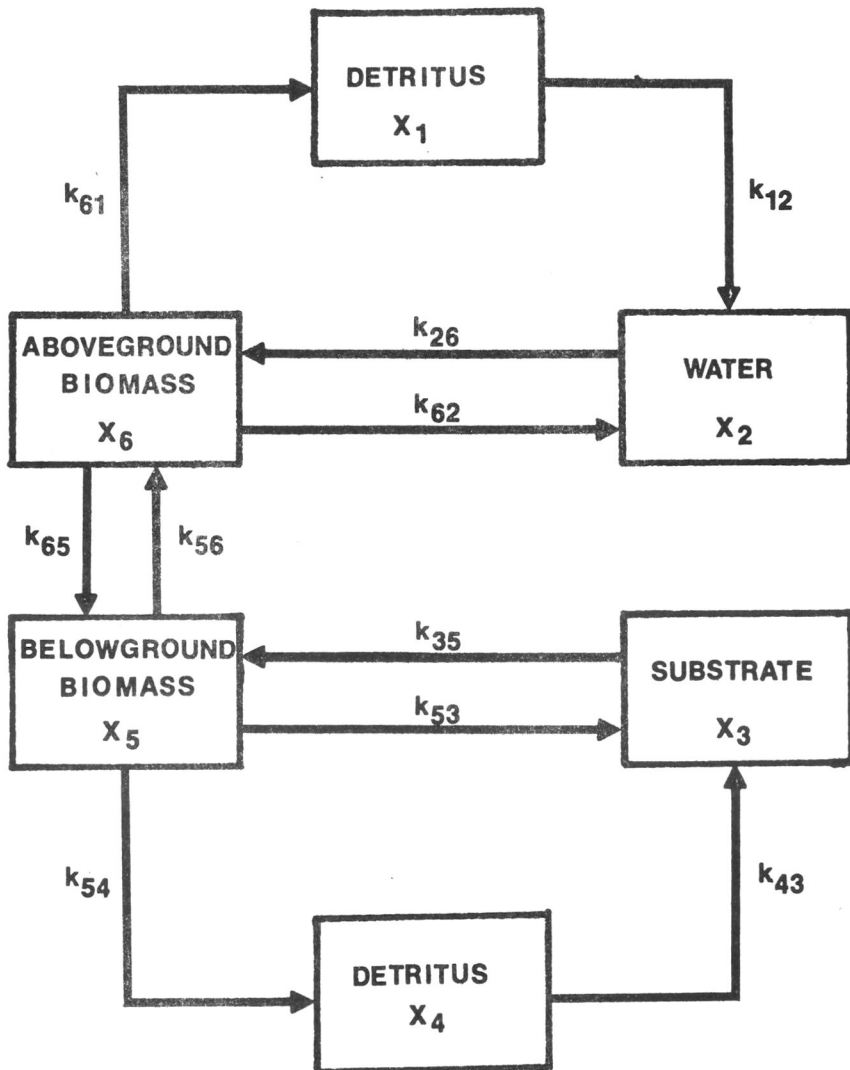
and, under reduced conditions, dissolved P pools develop which have concentrations several orders of magnitude greater than P concentration in the water (Wetzel 1975, Mortimer 1941). Laing (1940) found that Nuphar advena rhizomes were able to respire under anaerobic conditions and Sculthorpe (1967) described a transport pathway of gases from leaves to roots and rhizomes. These mechanisms enable belowground structures to survive reduced conditions in the sediment where these high concentrations of dissolved P exist. By root absorption, subsequent translocation to leaves and decomposition, rooted aquatic macrophytes may be a significant factor in the regeneration of P from the sediment to the overlying water.

The major site of P absorption and dominant translocation pathways vary with species. Studies on Elodea, Lemna, Potomogeton, and Vallisneria demonstrated that aquatic angiosperms absorb salts mainly through the leaves (Sutcliffe 1962). However, Potomogeton thunbergii had a four-fold increase in growth when roots were placed in mud as compared to growth when in a sandy substrate which indicated the importance of roots to the nutrition of this plant (Denny 1972). Recent tracer studies with ^{32}P have shown that even though P is absorbed by both leaves and roots, the roots are the major source of P in Myriophyllum brasilance, M. spicatum, Elodea densa (Bristow & Whitcombe 1971), M. exalbescens (DeMarte & Hartman 1974), and Elodea occidentalis at high P concentrations (Gerloff 1975), and Zostera marina (McRoy & Barsdate 1970). Also, some of the P transported to the leaves from the substrate via root absorption was secreted to the water by the freshwater plant Myriophyllum exalbescens (Demarte & Hartman 1974) and the marine species Zostera marine (McRoy and Barsdate 1970) and

Spartina alterniflora (Reimold 1972). This "pumping activity" of P from substrate by the marine species had pronounced effects on the P concentration in the water. Therefore, there are a variety of ways the aquatic macrophytes could have an effect on the P cycle in the Chowan River.

Nuphar luteum (L.) Sibthorp and Smith, a floating leaved aquatic angiosperm, is the dominant macrophyte on the Chowan River (Blanton, 1976). This perennial herb exists in monospecific stands on a variety of substrates in depths of water from 0.5 to 2 m. Nuphar has two distinct types of leaves--floating and submersed (Fig. 2). Floating leaves occur during a growing season that extends from April to October while the submersed leaves survive the entire year. The laminae of floating leaves of Nuphar are extremely tough and leathery but the cuticle and epidermis of the submersed leaves appears to be reduced (Sculthorpe 1967). This imples that only submersed leaves would be significant in the absorption of P from the water. The biomass of both the floating and submersed leaves has an annual turnover rate of 5.7. The belowground structures (roots and rhizomes) comprise 82% of the Nuphar biomass (Blanton 1976). The roots are very extensive reaching depths of ca. 25 cm. Roots of floating leaved macrophytes are not reduced in structure to the point where they are inadequate for mineral absorption (Sculthorpe 1967). Owing to these structures, Nuphar could absorb P from sediments and transport it to both floating and submersed leaves, and via decomposition and possibly secretion (particularly submersed leaves), released P to the water. Such a mechanism could have important implications for phosphorus cycling where Nuphar is an important part of the community.

Figure 2.   Photographs of the floating leaved aquatic
            macrophyte, <u>Nuphar luteum</u>. Notice the large
            rhizome structure and the two types of
            leaves, floating and submersed.
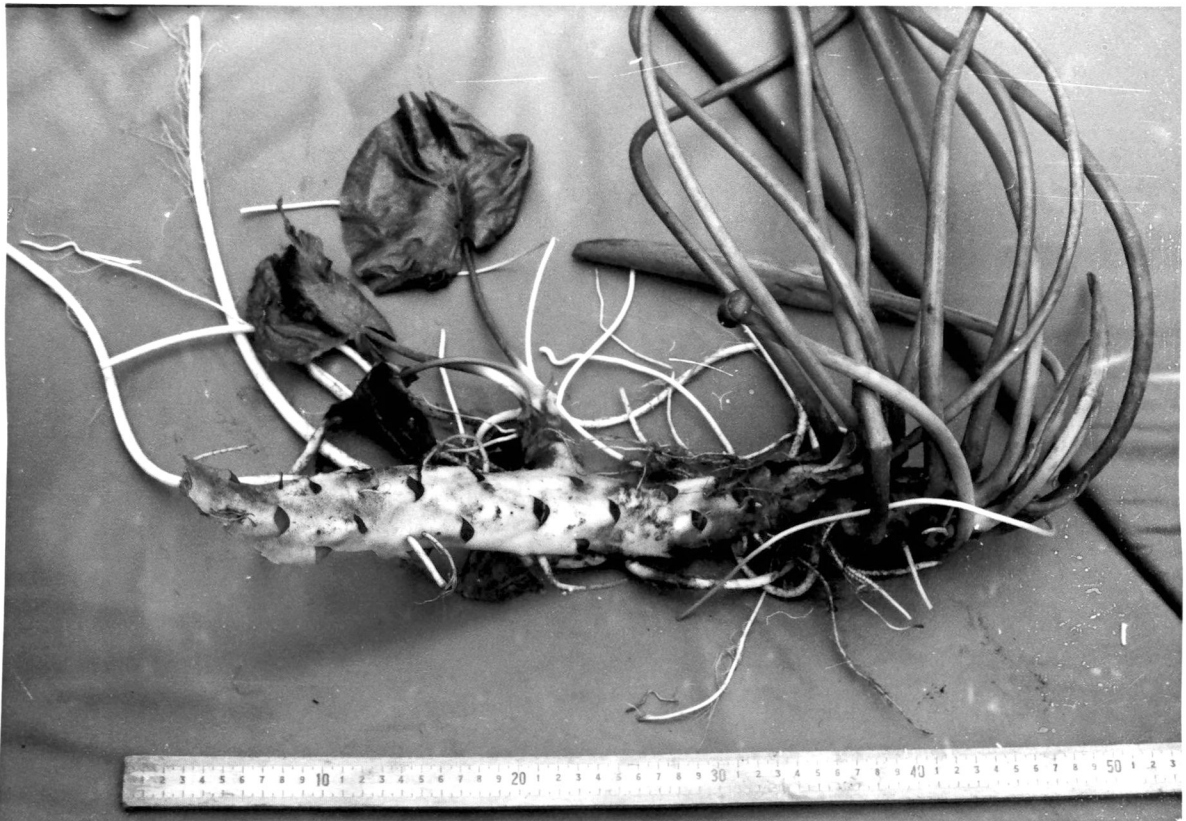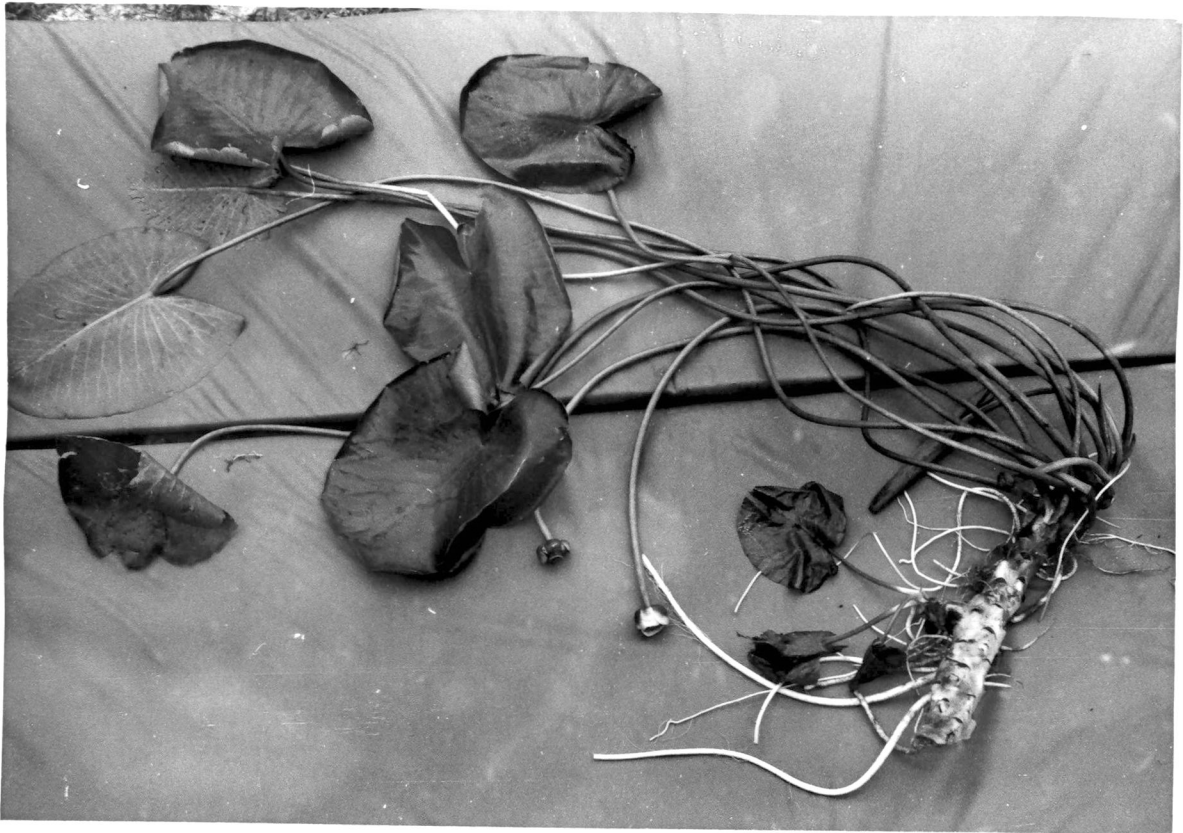
Research Approach

The objectives of this study were (a) to measure the concentration of P in each compartment of the model (Fig. 1), and (b) to measure the rate of flux of P between the compartments. The first objective involved seasonal and spatial P analysis in the plant and measurement of P available to the plant in the sediment. The second objective of measuring flux rates required determination of the absorption, translocation, secretion, and release by decomposition.

Concentration as an Index of Phosphorus Availability

Many studies on nutrient concentration in aquatic plants have been inspired by the potential of this value as an index of the nutrient's availability in aquatic systesm (Anderson et al. 1966, Gerloff & Krombholz 1966, Sculthorpe 1967, Allenby 1968, Gerloff 1969, Fitzgerald 1969, Adams et al. 1971, Gossett & Norris 1971, Seddon 1972, Adams et al. 1973, Gerloff & Fishbeck 1973). The work by Adams et al. (1973) in Pennsylvania is the most extensive tissue analysis survey of aquatic vascular plants. But Wetzel (1975, p. 374) feels that the discovery of bidirectional pathways of nutrients through aquatic macrophytes "negates" any use of plant structures in determining nutrient availability in a medium (water) since the source of the nutrient in the plant may have been elsewhere (sediment). Perhaps, if a nutrient concentration in a plant structure does indicate the nutrient availability in a medium, then this would be reflected in the dominate source or pathway of the nutrient. And if not, then the structure probably relies on some other organ for its nutrient supply.

Aquatic macrophytes have been found to accumulate nutrients in excess of their demand which has been described as luxury uptake (Gerloff & Krombholz 1966). The amount of P bound in the biomass of aquatic macrophytes communities remains relatively constant during the growing season and may represent large quantitites of unavailable P to other organism and pathways. The standing stock of Zostera Marina in the Izembek Lagoon, Alaska, accumulated 3.56 g P/m^2 in the leaves (McRoy et al. 1972). This would be equivalent to a concentration of 57.4 µg-at P/liter in one m^2 of water two m in depth, a very high value indeed. Aquatic macrophytes are also considered to be capable of accumulating nutrients in much higher concentrations than phyto-plankton (Boyd 1971). These concepts concerning nutrient uptake by aquatic plants has led to the proposal that they be used to control nutrient enrichment in waters (Boyd 1970b).

Flux as an Index of Phosphorus Availability

The turnover rate of essential elements that are in low concentra-tion compared to other elements, i.e., phosphorus, indicates the metabolic activity of an aquatic system (Pomeroy 1970). In this respect, a community of aquatic macrophytes contribute to the productivity of a system by regenerating P from the substrate that had been lost from the system. Boyd (1970a) contends that the decomposition of aboveground vegetation releases minerals that originated from the substrate. The objective of measuring decomposition is not only to measure the flux of P into and out of the detritus compartment, but also to determine the degree that this process aids in the exchange of P between water and substrate. To establish this, the amount and direction of P flux though Nuphar had to be determined which involved studies on absorption and

translocation.

Basically, two techniques are available for the study of leaves and roots in inorganic nutrition of aquatic macrophytes. These include the comparison of growth rates of aquatic macrophytes rooted in various substrates and also those that were not rooted at all (Pond 1905, Denny 1972). Another technique involves the isolation of shoots and roots so that isotopes can be selectively introduced to a single compartment (Aldrich & Otto 1959, Funderburk & Lawrence 1963, Frank & Hodgson 1964, Littlefield & Forsberg 1965, McRoy & Barsdate 1970, Bristow & Whitcombe 1971, DeMarte & Hartman 1974). A difference in these tracer techniques was whether the medium surrounding the roots is soil (DeMarte & Hartman, 1974, Reimold, 1972) or water with nutrient concentrations similar to the concentration in the substrate (McRoy & Barsdate 1970, Bristow & Whitcombe 1971). Actually McRoy used both soil and water surrounding roots in studies with _Zostera marina_, but based his conclusions on the experiments with the water medium. He found between a 100 and 1000 fold decrease in ^{32}P radioactivity in 5 cm sections above and to the side of the injection point. A major premise of radioisotope tracer studies is that the isotope is equally distributed in the compartment to which it is injected. This is unlikely when isotopes are injected into sediments to study uptake rates of roots. Also, secretion of P by roots would be difficult to measure in the sediment.

A number of factors interact and affect the absorption of P by plants. The metabolic state of the plant, including photosynthesis, growth, and age may relate to the absorption of P and any treatment which reduces growth may cause a corresponding decrease in the absorption of salts (Sutcliffe 1962). One of the most important factors influencing

P absorption seemed to be the concentration of P in the medium (Jeschke & Simonis 1965, Knauss & Porter 1954). But Olson (1950) concluded that the rate at which individual ions are absorbed was determined by the ratio of their concentration to those of other ions in the medium, rather than by concentration itself. The best reference that I found concerning kinetic studies of nutrient absorption by aquatic plants was Gerloff (1975). By varying the time of incubation of plant structures with isotope and nutrient concentration of the absorbing medium, he was able to determine Vmax and Km for roots and shoots of four aquatic vascular plants.

The approach to study absorption, translocation, and secretion of P was patterned after the work of Gerloff (1975) and McRoy and coworkers (1970, 1972) in an attempt to determine: (1) the rates of P uptake by floating leaves, submersed leaves, and roots; (2) the role of roots in the nutrition of Nuphar; and (3) which of the bidirectional fluxes of P in Nuphar was dominate.

Study Site

The Chowan River begins at the North Carolina-Virginia state line at the confluence of the Nottoway and Blackwater Rivers which arise in Virginia. The river follows a southerly course for 84 km and empties into the west end of the Albermarle Sound (Fig. 3). The river drains a watershed area of 12,766 km^2 located in Virginia and North Carolina. The drainage area lying in the Coastal Plain of North Carolina is 3,269 km^2. The majority of this watershed is forested (60%) (51% of this farm woodlands) while 27% is under agricultural management and 13% is swamp and woodland (Chowan River Interim Report, 1972). Many areas adjacent to the river are low and swampy (elevation of the river is near sea level).

Figure 3. The northern and central coastal regions of North
           Carolina. Inset shows relation of the area to the
           rest of the state. Adopted from Williams et al.
           (1973).

VIRGINIA
NORTH CAROLINA

77 W.   76

CURRITUCK
SOUND

CHOWAN
RIVER

36 N.   ALBEMARLE
         SOUND   36

TAR RIVER

PAMLICO RIVER
ESTUARY

PAMLICO
SOUND

NEUSE
RIVER
ESTUARY

35   35

CORE
SOUND

BOGUE SOUND

0   20   40 KILOMETERS
0   30 MILES

77   76
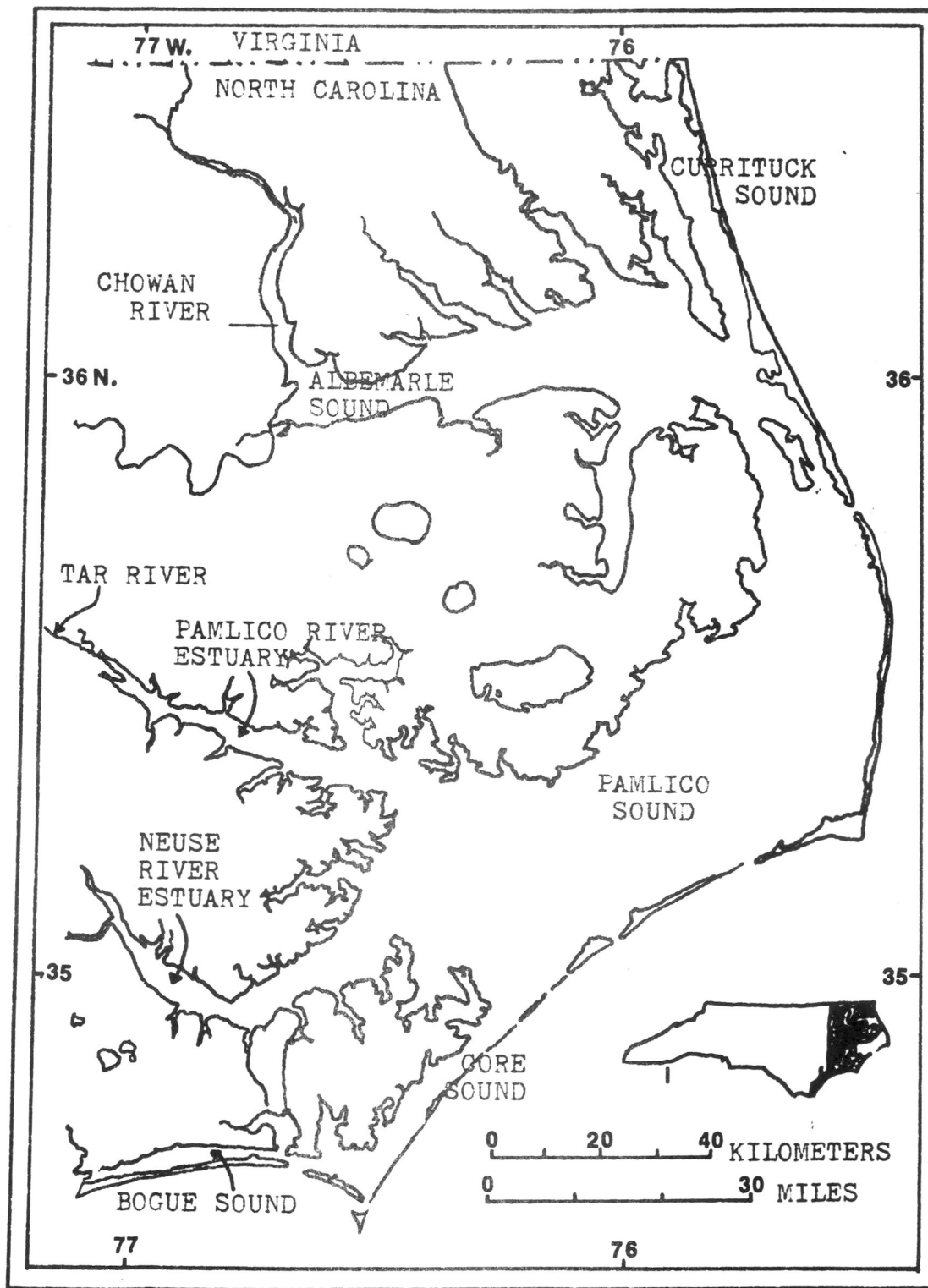
The lower Chowan River broadens below Holiday Island to a width of ca. 3 km and because of its slow current, the river has characteristics of a lake (Fig. 4). The effects of lunar tides are negligible but the system is influenced by wind tides (ca. 0.5 m). As expected, the water temperature varies with the seasons of the year with a maximum in August of 32 C and a low of 4 C in January (Fig. 5). The ortho- phosphate concentration in the river below Holiday Island was ca. 1 μg-at/liter and increases in concentration north of the island (Don Stanley, personal communication).

The Chowan River is characterized as a highly productive river which supports a major commercial fishing industry as well as sports fishing. Beginning in 1970, algal blooms of unusual proportion were reported and by 1972 the blooms had become severe and nuisance problems in the middle and lower sections of the river. Since that year the blooms have been seasonal and less severe. Nutrient inputs to the Chowan River include industrial sources such as a pulp mill in Virginia on the Blackwater River and a fertilizer plant at Tunis, urban and agricultural sources, and natural sources such as rainfall, runoff from forest land, and swamp drainage through tributaries.

Figure 4.   The lower Chowan River. Study sites included Rocky-
           hock Creek, Indian Creek, Keel Creek, and Wiccacon
           Creek. The width of the river at Colerain is ca.
           3 km.

CHOWAN RIVER

US 13

Winton

Wiccacon Cr.

Bennetts Cr.

Catherine Cr.

Holiday Island

Keel Cr.

Indian Cr.

Colerain

N

Rockyhock Cr.

US 17
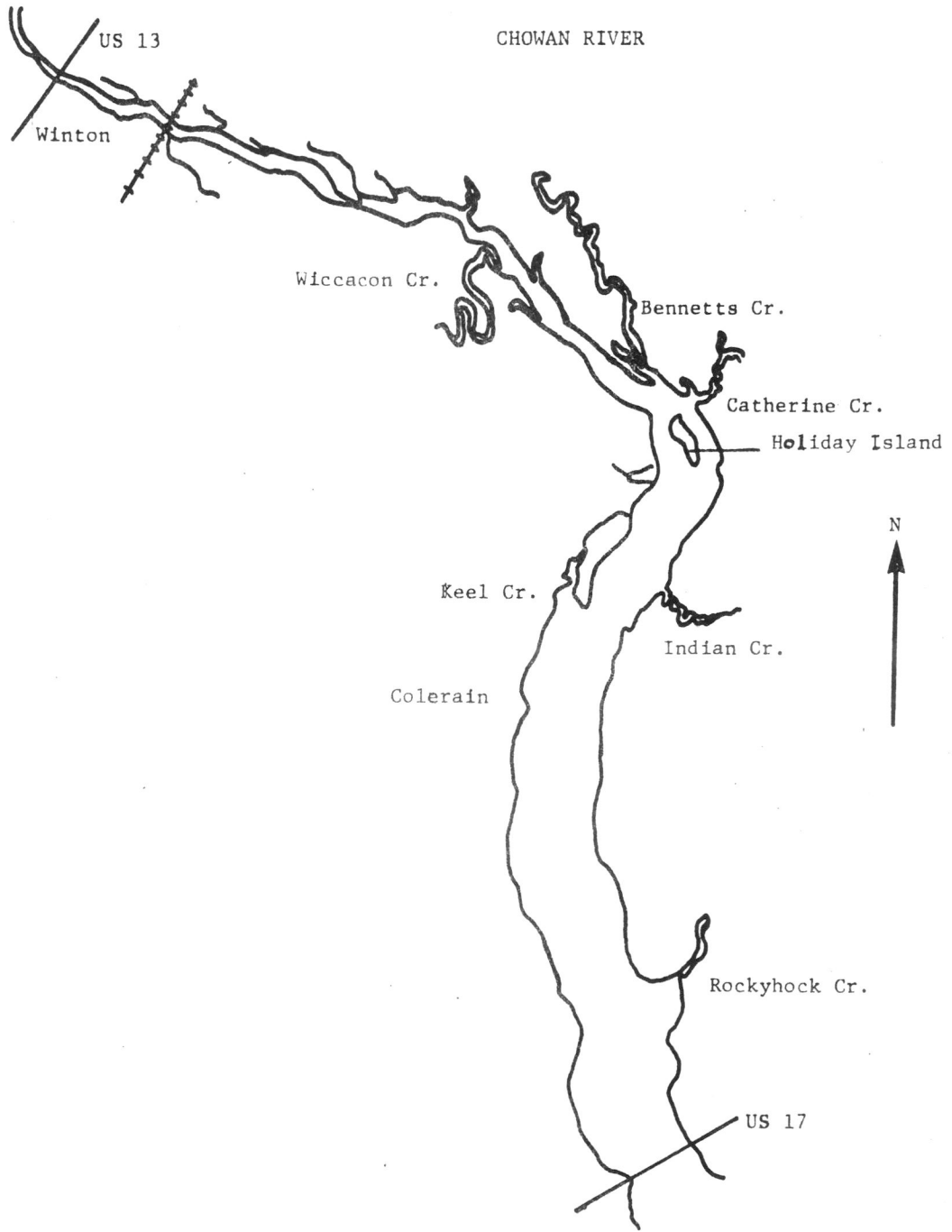
Figure 5. Maximum and minimum water temperatures recorded at
the mouth of Rockyhock Creek during 1974 and 1975.
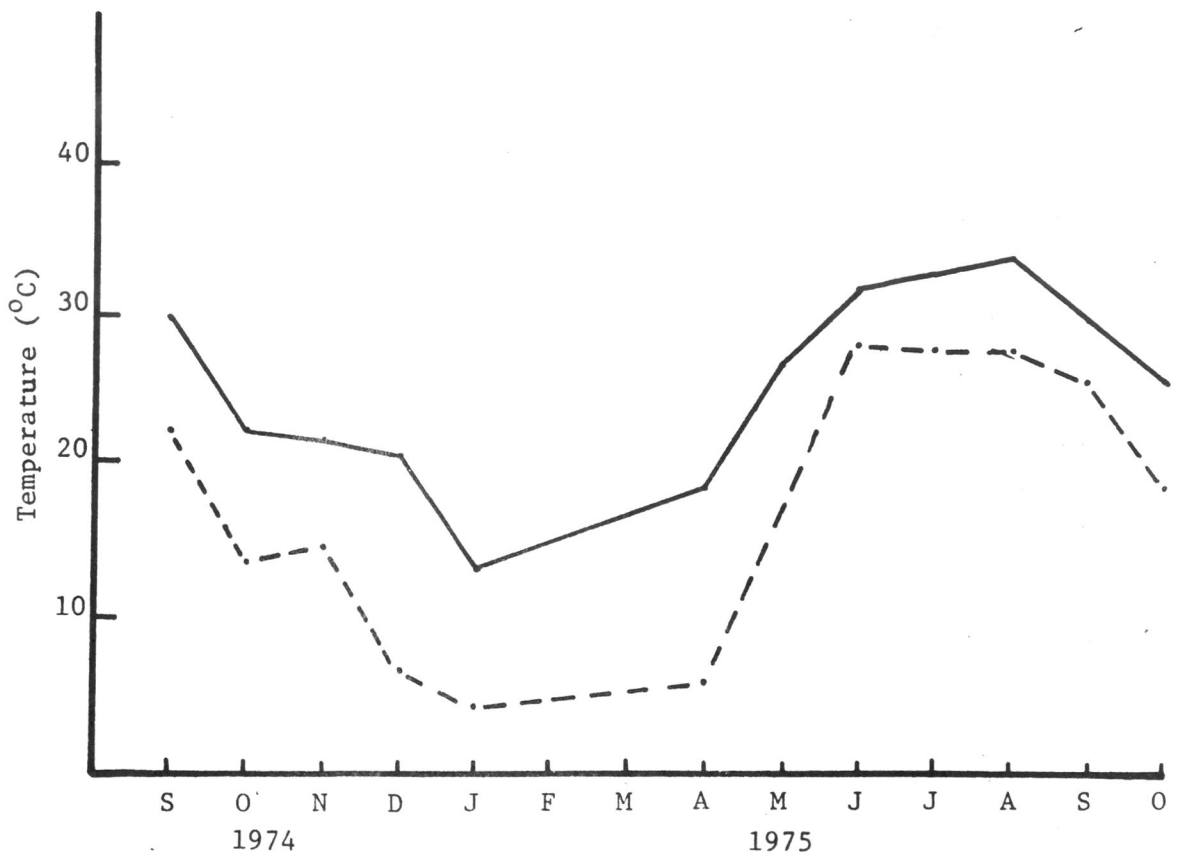
METHODS

Seasonal and Spatial Phosphorus Distribution

Field Sampling

Nuphar luteum was collected at Keel Creek and Indian Creek from
July-November, 1974, and at Keel Creek, Wiccacon Creek, and Rockyhock
Creek from January-September, 1975 (Fig. 4). Each plant community was
sampled at monthly intervals during the growing season and bimonthly
during the winter. During the 1974 sampling period, four 0.35 m^2
quadrat samples along a randomly placed transect perpendicular to the
shore were taken at each location. Three quadrats along two transects
at each site were sampled during the 1975 period. Aboveground biomass
(leaves and petioles) was harvested by hand and belowground vegetation
(roots and rhizomes) with posthole diggers to a depth of 30 cm. The
samples were placed in plastic bags on ice until processed in the
laboratory.

Phosphate Analysis

Nuphar luteum plants were separated into compartments (floating
leaves, submersed leaves, petioles, roots, rhizomes, flowers, and
peduncles) and cleaned by removing the epiphytes with a nylon brush and
rinsing the plants with distilled water. Samples were oven-dried in
paper bags at 85 C for 72 hr and dry weights were recorded. Bulky dried
samples (rhizomes) were chopped in a blender and aliquots of each
structure were ground in a Wiley Mill (40-mesh screen). Ground material
was sealed in small plastic bags and refrigerated (4 C).

Duplicate phosphorus determinations were made on each sample. One
gram aliquots of each ground sample were dry ashed in a muffle furnace

at 480 C for 3 hr. The temperature of the muffle furnace did not exceed 250 C when the samples were placed inside to prevent plant loss from the aluminum pans due to violent combustion which occurs at higher temperatures. After ash weight was recorded, the ash was then placed in 100 ml volumetric flasks to which 10 ml of concentrated hydrochloric acid (HCl) was added. The flask were heated until the ash dissolved. This concentrated acid solution was diluted to approximately 100 ml with distilled water, filtered through Whatman No. 41 filter paper, brought to a final volume of 250 ml and refrigerated (4 C). The weight of the insoluble fraction, presumably silicon, was determined by weighing the filter paper before and after filtering the dissolved samples (air dry at room temperature for 48 hr). This fraction was subtracted from the total ash residue before percent ash values were calculated. Silicon in the aboveground samples was negligible, but 28.9% of the ash weight for roots was attributed to silicon. High silicon concentration were due to the inability to remove these particles during cleaning. Likens and Bormann (1970) found no significant changes in phosphorus concentration in plant material after the insoluble ash was dissolved and added to their samples and no correction was attempted for my samples. Phosphorus was determined by a molybdate blue procedure as described in the E. P. A. Manual (1971) for ortho-phosphate. Significant differences in phosphorus concentration and ash content between structures and study sites were determined by the Newmann-Keuls multiple range test at the 0.05 level of significance (Zar 1974).

All phosphorus data of plant samples was expressed as atom weight per gram of organic weight. The organic weight is assumed to be the ash free dry weight of the plant and is symbolized by OW.

All glassware used during this procedure was originally soaked

overnight with 50% HC1 and at the beginning of each analysis the glassware was rinsed with 25% HC1. After each acid wash, the glassware was rinsed twice with deionized water. The filter paper was rinsed with distilled water before filtering the samples. Ions were removed from plastic containers by soaking them overnight in a 2% nitric acid solution.

To compare the dry and wet ashing procedures eight random plant samples were chosen and ashed using a sulfuric acid-hydrogen peroxide solution similar to that described by Allen et al. (1974). Phosphorus concentrations using this wet ashing technique were not significantly different ($P < 0.05$) from those ashed in the muffle furnace.

Phosphorus Pools in the Substrate

Field Collection

Sediment cores were collected from Rockyhock Creek, Keel Creek, and Indian Creek on 16 August 1975, with a plastic tube (4.7 cm x 50 cm) to a depth of 30 cm and were brought back to the laboratory on dry ice and kept frozen (-10 C) until processed.

Interstitial Water

As suggested by Bray et al. (1973), interstitial water was extracted under anoxic conditions by performing the following procedure in a glove bag purged with nitrogen gas. Cores were separated into 5 cm sections to a depth of 25 cm and each was pressed with 40 lb of pressure in a nylon press for 45 min at room temperature. The press was equipped with a 0.45μ - pore membrane filter that was deareated by soaking it in deionized water purged with N_2 gas to exclude atmospheric oxygen. The interstitial water samples were collected in 15 ml glass graduated centrifuge vials from which 2 ml aliquots were taken in duplicate for

phosphate analysis. These samples were stored in a vacuum desiccator purged with N_2 and frozen (-10 C). Ortho-phosphate was measured as described in the E. P. A. Manual (1971) except 0.32 ml of combined reagent was used since there was only 2 ml of sample. Analysis was with a Bausch and Lomb Sepctrophotometer set at 880 nm.

Biological Available Phosphorus

Each 5 cm core section was analyzed for biological available phosphorus as described by Wentz and Lee (1969a). The sections were dried for 48 hr in an oven (85 C), lightly ground with a mortar and pestle, and stored in acid-washed glass bottles under refrigeration (4 C). Phosphorus was extracted from 200 mg of soil with 25 ml of a dilute $HCl-H_2SO_4$ solution with a pH of 1.1 (Olsen and Dean 1965). These samples were shaken for 30 min on a mechanical shaker and then filtered through a 0.45 μ-pore membrane filter. Twenty ml was used to measure ortho-phosphate by a vanadomolybdate yellow method (Jackson 1958) on a Bausch and Lomb Spectrophotometer set at 420 nm.

Phosphorus Flux Tracer Experiments

General Procedures

Experiments in both the laboratory and field were run to determine the uptake, translocation, and secretion of P by Nuphar. The various plant structures (floating leaves, submersed leaves, and roots and rhizomes) were isolated with plexiglass chambers (Fig. 6) similar to the techniques used by McRoy & Barsdate (1970). The rectangular chambers (22.9 cm x 20.3 cm x 10.2 cm) were made of one-fourth inch plexiglass and had an approximate volume of 4 liters. The chambers used for roots and rhizomes were covered with opaque tape to exclude light. The plant was first placed inside the root and rhizome chamber and then submersed and floating leaves were rolled up and passed through 2.54 cm diameter holes

Figure 6. Plexiglass chambers used to isolate plant struc-
tures of _Nuphar_ _luteum_ and radioactive isotopes.
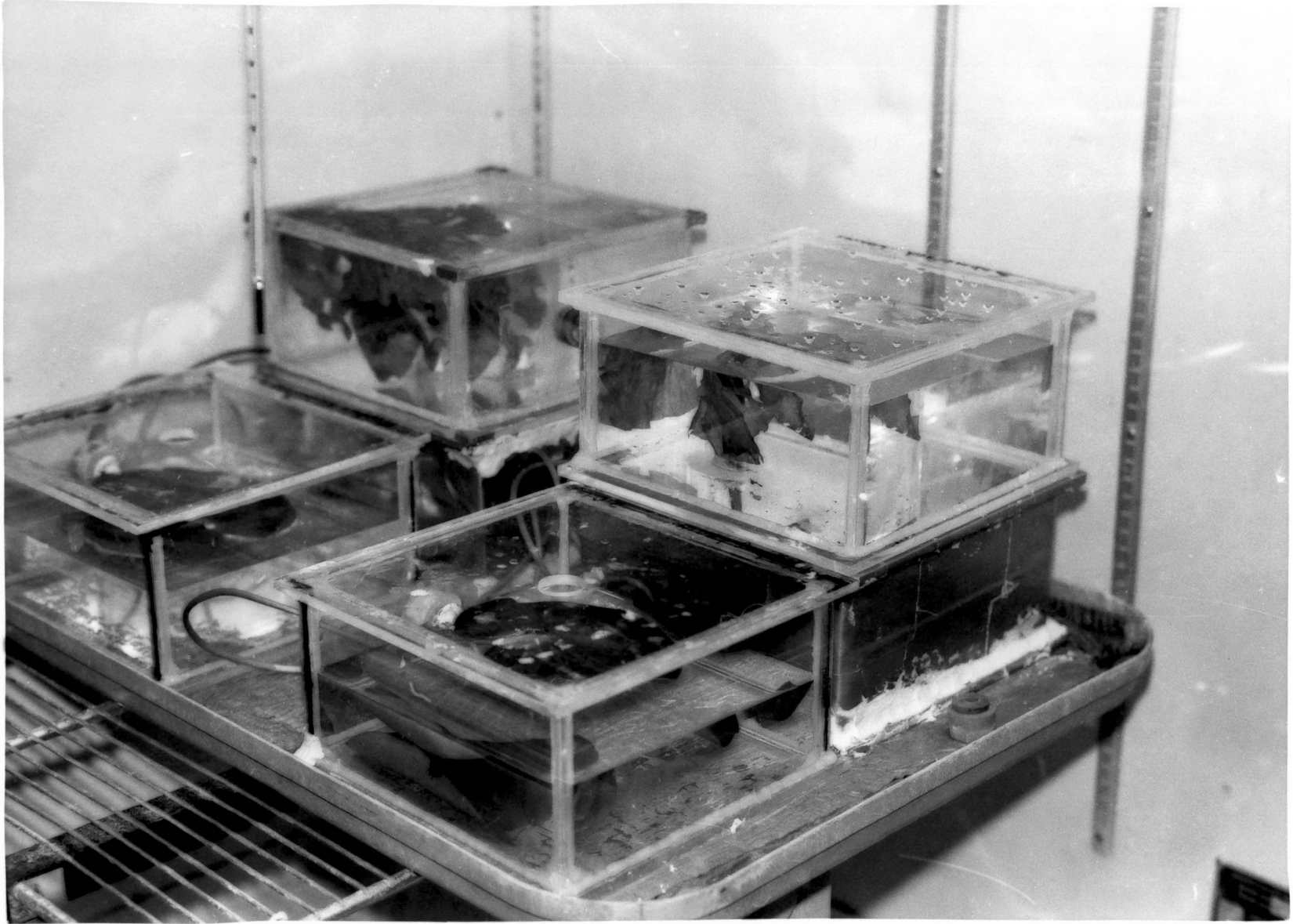
on the top and side of this chamber, respectively. Chewing gum was wrapped around the petioles where they extended from the chamber. A bored-out rubber stopper that had been split on the side was placed around the gum and petioles of floating leaves and pressed inside the corresponding hole. A 5 cm length of suction tubing that had also been cut longitudinally was placed around the gum and petioles of the submersed leaves. When the tubing was fitted into the hole, the submersed leaves petioles extruded through and a seal was formed. The submersed leaves were folded again and put through an identical hole of a second plexiglass chamber. This hole was then fitted around the suction tubing and formed a seal (Fig. 6).

Before each field experiment, plexiglass chambers were scrubbed and rinsed and distilled water was left in them overnight. These samples were assayed on a liquid scintillation counter and no radio-activity was detected above background from any of the chambers.

The surface area of leaves and roots were measured to allow expression of uptake rates based on area in addition to dry weight. The areas of floating and submersed leaves were determined by tracing the perimeter of the leaves on paper and measuring this area with a planimeter. This value was doubled to account for the total area of the submersed leaves.

The surface area of roots was determined by a staining - destaining method (Beasley and Ting 1973) modified by Dr. Prem Sehgal of East Carolina University. The roots were soaked for 5 min in 150 ml of toluidine blue (0.02% in 10% sodium barate) which is a specific stain for cellulose. The roots were rinsed with distilled water to remove the dye and then washed with 100 ml of formalin-acetic-alchol (F.A.A; made

of 90.0 ml of 70% ethyl alcohol, 5.0 ml of glacial acetic acid) for 15 min. Three aliquots of the destaining solution were placed in test tubes and diluted 10 fold. Optical density was read with a Perkin-Elmer Spectrophotometer set at 640 nm. An attempt to convert optical density readings to absolute values failed so relative values were determined by multiplying the optical density readings by 100.

Radioactive plant and water samples from both laboratory and field experiments were assayed with a Packard Tri-Carb liquid scintillation counter (LSC). The LSC has a high counting efficiency for most radioactive isotopes and is able to discriminate between isotopes with different beta energies. A major disadvantage of radioactive plant assay on the LSC is that sample preparation with acids and bleaching agents causes aberrations such as chemical and color quenching and may be immiscible with the organic solvents.

Two methods using H_2SO_4 and H_2O_2 were used to digest the plant material in the tracer experiments. In the first method (used mostly for laboratory experiments), 2 ml of concentrated H_2SO_4 were added to 50 mg of plant material (2 mm diced pieces) and heated on a hot plate for 30 min. The sample was removed from the heat and allowed to cool slightly and then 12-20 drops of H_2O_2 (30%) were added until the sample cleared. To the dissolved sample, one drop of 10N NaOH and the cocktail were added. The other technique involved a dissolving solution containing H_2SO_4, H_2O_2, selenium powder and lithium chloride (Allen et al. 1974). Two ml of this solution were used to dissolve 30 mg of plant tissue in a capped glass scintillation vial. No color remained in any samples except roots, which was cleared by adding a few drops of H_2O_2. Care was taken in both experiments to cool the dissolved samples to room temperature before the cocktail was added to prevent severe chemilumi-

nescence.

Two counting techniques were used for the field and laboratory experiments. Cerenkov counting (Kobayashi and Maudsley 1974) was used exclusively for counting the high energy ^{32}P radiosotope in the laboratory experiments. There was no problem of phase separation with the sample or chemical quenching with this cocktail and the counting efficiency was 46%. The 15 ml of cocktail added to each sample contained 100 mg of 4-methylumbelliferone dissolved in a liter of distilled water.

The field samples were assayed for both ^{32}P and ^{33}P using a double isotope counting technique (Kobayashi and Maudsley 1974). Because the beta energy from ^{33}P was too low for Cerenkov counting, an organic solvent and fluor cocktail was used (RediSolv VI, Beckman). Counting efficiencies were determined by the internal standard method. Standards with just the cocktail had 100% counting efficiencies for each isotope and were used to determine specific activity of the isotopes. Standards for plant and water digestion procedures were made for each isotope to determine the balance point settings and counting efficiencies for each isotope in each channel. The LSC was set for a maximum of 100,000 counts or 50 min of counting for this procedure and Cerenkov counting.

The following formula was used to determine the amount of each isotope in each sample of the double isotope technique:

$$P32 = \frac{N1-N2 \ (ch1/ch2)}{Ch1-Ch2 \ (ch1/ch2)}$$

$$P33 = \frac{N2-N1 \ (Ch2/Ch1)}{ch2-ch1 \ (Ch2/Ch1)}$$

P32 = ^{32}P in the sample (dpm)

P33 = ^{33}P in the sample (dpm)

Ch1 = ^{32}P counting efficiency in channel 1

Ch2 = ^{32}P counting efficiency in channel 2

ch1 = ^{33}P counting efficiency in channel 1

ch2 = ^{33}P counting efficiency in channel 2

N1 = net total observed counts in channel 1

N2 = net total observed counts in channel 2

All quantities of isotopes (dpm) in the samples were corrected for radioactive decay. The specific activity of the isotopes in the chambers after injection was used to determine the absolute amount of phosphorus in a gram dry weight of each plant structure and the amount secreted.

Laboratory Experiments

The absorption and subsequent translocation of P by Nuphar was studied in the laboratory using the radioactive isotope phosphorus-32. Experiments were run on floating leaves, submersed leaves and roots in which the absorption period and external P concentrations were varied. Plexiglass chambers were used as previously described to isolate the plant structures and isotopes. The chambers were placed in an environmental control chamber under constant light (3×10^4 ergs/cm^2) and temperature (29 C). The mean water temperature for all the experiments was 26.5 $\pm$ 3 C. All experiments were run in triplicate on plants collected from Keel Creek on the Chowan River.

Plants collected for the laboratory experiments were kept either in holding tanks in the greenhouse or in a large ice chest filled with water. A black plastic bag was wrapped around the roots and rhizomes of the plants collected from the field to protect them from light. All experiments were run within 48 hr after collection of the plants. Epiphytes and other microorganisms were cleaned off the plant structures before they were enclosed in the chambers. Also, before each experiment, leaf traces were made of those leaves that would be subjected to the isotope. The chambers to which the isotope would be added were filled

with a modified Hoagland's solution (Gerloff & Krombholz 1966) with a
P concentration of 2.0 µg-at/liter in the leaf chambers and 4.0 µg-at/
liter in the root chambers (Table 1). The remaining chambers were
filled with tap water. Incubation periods for the submersed leaves
were 5, 10, 20, 40, and 90 min and for roots were 10, 20, 60, and 240
min. Besides giving information about the absorbing capabilities of
the three organs, the importance of the time studies was to select the
optimal time period for experiments in which P concentrations in
Hoagland's solution was varied. The optimal absorption period when
external P concentration was varied was a time during which linear
absorption of P occurred for submersed leaves and roots which was 10 and
60 min, respectively. About 14 uCi of carrier-free ^{32}P as phosphate was
added to the experimental chamber for each experiment.

After the absorption period the plants were sectioned into leaves,
petioles, roots, and rhizomes and rinsed under a stream of tap water.
The structures were dried for 48 hr at 85 C and dry weights recorded.
Each structure except rhizomes was cut into approximately 2-mm pieces
with scissors which were rinsed with distilled water after cutting each
structure. Rhizomes were ground with a mortar and pestle. Two 30 mg
aliquots of each structure were dissolved and counted as described under
General Procedures.

Field Experiments

Field experiments were run at Rockyhock Creek on 15 and 25 August
1975 for summer rates, on 9 January 1976 for winter rates and 7 May
1976 for spring rates. Plexiglass chambers were used to isolate the
various plant organs and the isotopes as described under General Procedure.
For each experiment 14 uCi of carrier-free radioactive ^{32}P and ^{33}P as
phosphate were injected into specific chambers. All experiments were done

Table 1. Composition and ion concentration of the modified Hoagland solution used in the tracer experiments.

Salt	Molarity	mls of Stock Per Liter Final Sol'n	Concentration (ppm)	
KNO_3	0.1	0.1	P =	0.062
$MgSO_4 \cdot 7H_2O$	0.5	0.8	$N-NH_4$ =	0.05
KH_2PO_4	0.004	0.5	$N-NO_3$ =	0.20
KCl	*	*	K =	0.52
H_3BO_3	*	*	Cl =	1.77
$MnSO_4 \cdot H_2O$	*	*	B =	0.27
$CuSO_4 \cdot 5H_2O$	*	*	S =	13.12
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	*	*	Mg =	9.6
$Fe \cdot EDTA$	*	*	Mn =	0.27
			Mo =	0.009
			Fe =	0.40
			Cu =	0.25

*Trace element stock solutions were prepared at 1,000 X the concentration of the final solution. One ml of each stock solution was added to the final culture medium.

in triplicate for 24 hrs.

The roots and rhizomes of three plants were carefully excavated from the substrate and rinsed in the river to remove soil from the roots and rhizomes. These structures and the submersed leaves were placed in the chambers as previously described under General Procedures. The root and rhizome chamber was filled with a modified Hoagland solution that had a P concentration of 4.0 μg-at/liter and was purged with 200 lb of nitrogen gas (O_2 concentration < 1 ppm). The submersed leaf chambers were filled with ambient Chowan water. The floating leaves were not enclosed. The root and rhizome chamber was injected with ^{32}P and the submersed leaf chamber with ^{33}P. Floating leaves on different plants were enclosed in plexiglass chambers and injected with 10 uCi of ^{33}P during the spring experiments. The technique on 15 August 1975 was different. Both submersed and floating leaves were placed in plexiglass chambers and filled with ambient Chowan water that had a P concentration of 0.32 μg-at/liter. Pipettes were used to inject 14 uCi of ^{32}P into the substrate around the roots of the apical portion of the rhizome at three different locations at depths of 5, 10, and 20 cm. The submersed leaf chamber was injected with ^{33}P and no isotope was added to the chambers with floating leaves which were kept buoyant with inner tubes.

The two field techniques resulted in different amounts of P uptake by the roots. The radioisotope activity in the roots from the 15 August experiment was 8,700 dpm/g dry wt compared to 2,530,640 dpm/g dry wt for the 25 August experiment, and all other structures had less ^{32}P activity in the first field experiment than in the second. Because distribution in the root region was so poor on 15 August, these data are not reported. It appears that by excavating the roots and rhizomes from

the substrate and placing them into a chamber into which the isotope was injected, there was a more equal distribution of the isotope around the absorbing organ which resulted in much higher activities in the plant.

After 24 hrs water samples were taken from each chamber. The plants were removed, rinsed in the river, sectioned into leaves, petioles, roots and rhizomes, and placed in plastic bags. In the laboratory each plant structure was scrubbed with a brush to remove epiphytes and rinsed with tap water. The samples were dried for 48 hrs at 85 C and dry weights recorded. The plant material was assayed similar to the laboratory techniques previously described except the second sulfuric acid method (General Procedures) and organic solvent were used. Water samples collected from the chambers were treated by adding 0.4 ml perchloric acid and 0.2 ml H_2O_2 to one ml of sample in a glass scintillation vial to dissolve particulates. The samples were heated for 30 min on a hot plate, allowed to cool, and 15 ml of RediSolv VI was added.

Decomposition Rates

Phosphorus (P) release rates from Nuphar luteum were determined for senescing plant structures by the net bag technique (Boyd 1970a, Kormandy 1968). Transects of 10-mesh/cm fiberglass net bags containing 35 gm (field weight) each of either aboveground (leaves, petioles) or belowground (roots and rhizomes) vegetation were placed at the mouth of Rockyhock Creek on 4 July 1974 and 30 September 1974 to determine summer and winter rates, respectively. Mature but not senescent plant material of uniform appearance was collected, blotted dry, weighed and placed in the net bags. The percent of the total weight in the bags (35 gm) that

each structure contributed was proportional to the biomass of the structures in the field.

The aboveground bags were allowed to float and belowground bags were buried ca. 10 cm deep in the substrate. Duplicate bags were randomly collected for analysis on a weekly basis for the first month and then monthly for five months. Collected bags were stored on ice until processing. In the laboratory the plant material was picked from the bag, cleaned, rinsed with distilled water and dried for 48 hrs at 85 C and dry weights were recorded. Dried plant material was ground in a Wiley Mill using a 40-mesh screen and then dry ashed (480 C) for three hours in a muffle furnace. Ashed material was dissolved and phosphorus determined by procedures described under Phosphorus Analysis.

So the data could be based on dry weights, the initial dry weights and P concentration were determined by analysing the contents of five bags on the day they were placed in the field. The percent loss of P was determined by comparing the initial concentrations with those of the material undergoing decay.

RESULTS AND DISCUSSION

Seasonal and Spatial Phosphorus Concentrations

Spatial Phosphorus Concentration

The ability of a plant to accumulate mineral nutrients is
reflected in the percent of the dry weight that is ash. In Nuphar
luteum, roots had the highest ash content (17.0%) and floating leaves
the lowest (9.1%) (Table 2). There were significant differences
between ash content of the structures as follows: roots = petioles >
rhizomes = submersed leaves = floating leaves. It is interesting that
petioles were similar in ash content compared to the roots since the
two exist in environments of widely differing nutrient concentration.
There were no significant differences in ash content between sampling
locations nor were there seasonal trends. The average ash content for
all the structures during the two sampling seasons for Nuphar was 12.5%
(Table 2).

The average concentration of P in Nuphar was 153.78 μg-at/ g OW
with no significant difference between structures based on averages of
the five sampling locations (Table 3). There were P concentration
differences between aboveground and belowground structures at two of the
sites. At Keel Creek in 1974, the roots and rhizomes had greater P
concentrations than the aboveground vegetation but the reverse was true
at Indian Creek (Table 3). There were no differences in P concentration
in any of the aboveground structures between any of the sampling loca-
tions.

There were differences between locations for averages of the P
concentration in the whole plant. The 1974 samples from Keel Creek were
the highest (200.79 μg-at P/g OW) and greater than those from Wiccacon

Table 2. Ash content of three types of aquatic macrophytes including
the mean values for the structures of Nuphar luteum.

Type of Plant	%Ash	Reference
Emergent Species	12.0	Hutchinson 1975
Submersed Species	21.0	Hutchinson 1975
Submersed Species with Floating Leaves		
	16.0	Hutchinson 1975
	16.0	Straskraba 1968
Nymphaea odorata	11.2	Sculthorpe 1967
Nuphar advena	8.0	Sculthorpe 1967
Leaves	6.8	Waring 1970
Petioles	10.0	"
Rhizomes	5.9	"
Roots	6.4	"
Flowers	5.4	"
Peduncles	14.7	"
Mean	9.5	"
Nuphar luteum		
Floating Leaves	9.1	This Study
Submersed Leaves	10.1	"
Petioles	15.9	"
Roots	17.0	"
Rhizomes	10.4	"
Flowers & Peduncles	9.6	"
Mean	12.5	"

Table 3. Mean concentration of phosphorus (μg-at/g OW during the sampling season in the anatomical structures of _Nuphar luteum_.

| Structure | Sampling Sites | | | | | Mean | S. E. |
	Keel Cr. 1974	Keel Cr. 1975	Wiccacon Creek	Indian Creek	Rockyhock Creek		
Floating Leaves	161.13 a# B*	168.97 a A	173.06 a A	125.92 a AB	159.94 a A	157.80 A	9.32
Submersed Leaves	172.33 a B	175.89 a A	162.50 a A	145.17 a A	189.90 a A	169.96 A	8.32
Petioles	158.00 a B	164.13 a A	126.70 a A	102.31 a B	170.17 a A	144.26 A	14.41
Rhizomes	237.39 a A	124.16 b A	111.80 b A	74.22 b C	171.59 b A	143.88 A	31.41
Roots	275.09 a A	179.64 b A	101.63 c A	72.12 c C	140.71 c A	153.84 A	39.49
Mean	200.79 a	162.56 ab	135.14 b	103.95 b	166.46 ab	153.78	

\# Small letters denote significant differences between locations at the 5% probability level.

* Capital letters denote significant differences between structures at the 5% probability level.

and Indian Creeks, but not significantly different from those at the other two sites (Table 3). Even though the 1974 and 1975 sampling locations at Keel Creek were similar, the results differed. The roots and rhizomes had a greater P concentration in the 1974 samples than 1975, but the leaves and petioles did not differ between the two sites. Also, there were differences between the aboveground and belowground structures at the Keel Creek sites during 1974, but no differences during 1975. In general, there were fewer differences in P concentration between aboveground structures and locations than for belowground structures which varied considerably between locations.

The expression of P concentration in a square meter of Nuphar community was influenced mainly by the seasonal biomass quantities. Rhizomes were the structures that accumulated the greatest amount of P (66.2%), roots were intermediate (10.6%), and the remainder of the P was distributed among the three aboveground structures (7.1-8.1%) (Table 4). Highest accumulation of P was at Wiccacon and Indian Creek (0.817 and 0.702 g/m^2, respectively) compared to the lowest value from the 1975 samples in Keel Creek (0.367 g/m^2).

The net annual assimilation of P by Nuphar at Rockyhock Creek for 1975 was determined by multiplying its annual production (Blanton 1976) and the P concentration (Table 5). The floating leaves and petioles assimilated the most P and roots the least. Although the belowground biomass contained 78.6% of the P, the aboveground structures assimilated 91.3% of the P per year. The net annual assimilation of P was 19.13 μg-at/m^2.

An extensive biomass survey of Nuphar was made of the lower Chowan River (Blanton 1976) on 16 June 1975. Using this survey and mean P concentration of the Nuphar structures, the biomass of P per m^2 was

Table 4. Mean content of phosphorus (g/m^2) that occurred during the
sampling season in the structures of <u>Nuphar luteum</u>. Included
is the percent of total phosphorus accumulated per m^2 by each
structure.

| Structure | Sampling Sites | | | | | Mean | % of |
	Keel Cr. 1974	Keel Cr. 1975	Wiccacon Creek	Indian Creek	Rockyhock Creek		Total
Floating Leaves	0.017	0.030	0.089	0.054	0.043	0.047	7.9
Submersed Leaves	0.042	0.024	0.060	0.071	0.011	0.042	7.1
Petioles	0.032	0.043	0.084	0.061	0.020	0.048	8.1
Roots	0.054	0.027	0.105	0.070	0.060	0.063	10.6
Rhizomes	0.400	0.243	0.479	0.446	0.392	0.392	66.2
Aboveground	0.091	0.097	0.233	0.186	0.074	0.137	23.1
Belowground	0.454	0.270	0.584	0.516	0.452	0.455	76.9
Total	0.545	0.367	0.817	0.702	0.526	0.592	100.0

Table 5. Phosphorus incorporated during the production of the
structures of Nuphar luteum. Also the amount of P
accumulated in the biomass of Nuphar luteum based on
extensive biomass survey (Blanton 1976). Total amount
of phosphorus accumulated by Nuphar in the Chowan
River by each structure was based on aerial coverage
of 272,267 m^2 (Blanton 1976).

Structure	Net Accumulation* (mg-at m^{-2} yr^{-1})	Biomass mg-at m^{-2}	Total (MT)
Floating Leaves	7.10	2.62	0.022
Submersed Leaves	2.12	1.49	0.013
Petioles	7.56	3.18	0.027
Flowers & Peduncles	0.68	0.16	0.001
Roots	0.27	4.20	0.035
Rhizomes	1.40	17.13	0.145
Aboveground	17.46	7.45	0.063
Belowground	1.67	21.33	0.180
Total	19.13	28.78	0.243

* Phosphorus involved in production of Nuphar luteum.

determined for each structure (Table 5). The total accumulation of P by Nuphar was 28.78 mg-at/m^2, most of which was in the belowground structures. The aerial coverage of these plants (Blanton 1976) times the total accumulation gives an estimate of 0.24 MT of P bound by this aquatic macrophyte in the lower Chowan River (Table 5).

The average P concentration as a percent of the dry weight (including ash) for all the structures of N. luteum was 0.403%. This value is within the range of P concentration found in plants of the Nymphaeaceae family (Table 6). However, the P concentration in the individual structures of N. luteum were higher than those found in the literature. The P concentration in N. luteum during the sampling period was well above 0.13% which is considered the critical content of P for macrophyte production (Gerloff & Krombholz 1966). Using this as the criterion for nutrient limitation, it is unlikely that P was limiting to primary productivity.

The ash values for N. luteum are greater than those recorded for Nuphar advena (Waring 1970) and Nymphaea ordorata (Sculthorpe 1967). Ash content for N. luteum is lower than average values reported for floating leaved species by other investigators (Table 2). The mean ash content of emergent species is similar to N. luteum value while the 21% ash for submersed species is much higher.

Seasonal Phosphorus Concentration

In order to simplify the presentation of P concentrations for the four sites on a seasonal basis, the data were grouped into the six ecological seasonal devised by Allee et al. (1949) as used by Reimold (1972) on his work on P movement through Spartina. The dates for the six seasons were: hibernal-20 November to 4 April; prevernal-5 April to

Table 6. Phosphorus content, as percent P, of species in the Nymphaeaceae family. Values for <u>Nuphar</u> <u>luteum</u> are from this study.

Species	Leaves	Petioles	Roots	Rhizomes	Flowers & Peduncles	Mean
<u>Nuphar</u> <u>advena</u>	0.41 a 0.40 e 0.30 f 0.27 g	0.30 b 0.17 f 0.391 g	0.30 c		0.382 & 0.243 f 0.395 & 0.302 g	0.326 a
<u>Nymphaea</u> <u>tuberosa</u>	0.29 b	0.28 b				0.396 a
<u>Nuphar</u> <u>variegatum</u>						0.410 a
<u>Nymphaea</u> <u>odorata</u>	0.31 b 0.18 e 0.24 f 0.24 g	0.28 b 0.20 f 0.201 g			0.328 & 0.18 f 0.239 & 0.213 g	0.25 b
<u>Nuphar</u> <u>luteum</u> d	0.449	0.361	0.381	0.367	0.334	0.403

a Adams (1973) Pennsylvania

b Reimer & Toth (1970) New Jersey

c Reimer & Toth (1968) New Jersey

d This Study   North Carolina

e Boyd (1970) Par Pond, S. C.

f Cowgill (1973) Linsley Pond, Conn.

g Cowgill (1973) Cedar Lake, Conn.

to 27 May; vernal-28 May to 24 June; aestival-25 June to 29 July; serotinal-30 July to 17 September; autumnal-18 September to 19 November. For example, samples collected on different days during the 25 June to 29 July interval were all plotted on the aestival midpoint of ca. 10 July (Fig. 7). The trends discussed are based on the concentration (μg-at P/g OW) (Table 7) and area (μg-at P/m^2) (Table 8) (See also Appendix).

The P concentration in the aboveground biomass peaked during the prevernal season (241.57 μg-at/g OW), sharply declined to its lowest value during the vernal, aestival, and serotinal seasons, and then gradually increased throughout the autumnal and hibernal seasons (Fig. 7). The P concentration in the belowground biomass also increased in the autumnal and hibernal seasons but in the prevernal season when the aboveground P concentration peaked, the belowground P concentration declined until the aestival season when it had a minor but significant increase. All three aboveground structures (floating leaves, submersed leaves, and petioles) had similar trends in P concentration, but in the belowground biomass, the roots were relatively stable throughout the year, while the rhizomes had a high peak in the hibernal season and another peak in the aestival season (Fig. 8). The greatest decrease in the rhizomes was during the peak in P concentration of the aboveground structures. The changes in the belowground biomass were due primarily to the dynamics of the storage organ, the rhizome.

The amount of P (μg-at) in a m^2 was determined for each sample by multiplying the concentration of P per g OW by the biomass of the structure per m^2 (See Appendix). By averaging these concentrations of P per m^2 for each of the six ecological seasons, seasonal trends in storage by the biomass was determined (Fig. 9). Belowground structures

Figure 7. Seasonal phosphorus concentration (μg-at/g OW) in the aboveground and belowground structures of <u>Nuphar luteum</u> based on mean values for each ecological season.
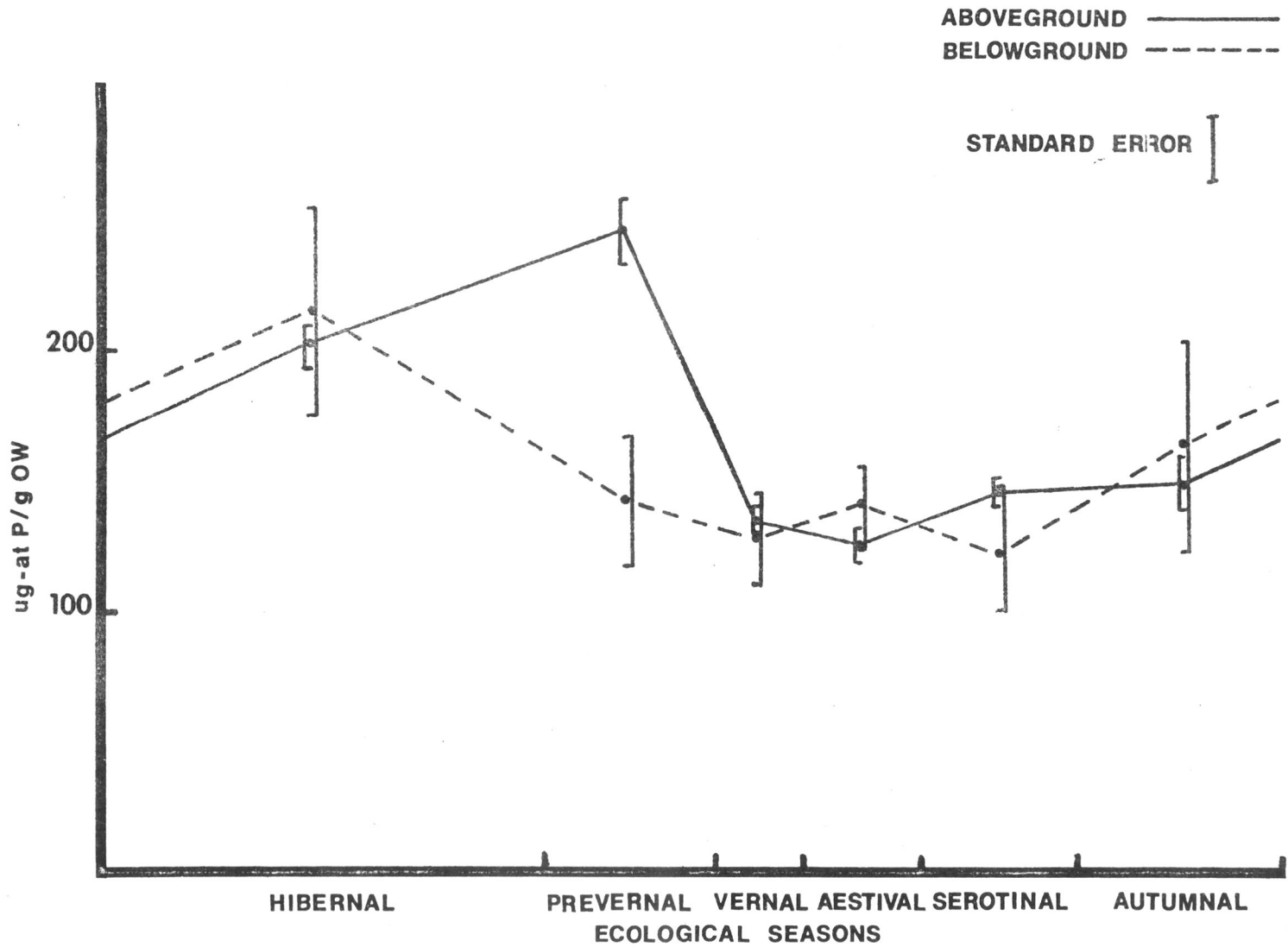
Figure 8. Seasonal trends in phosphorus concentration (μ g-at/g OW) in the structures of <u>Nuphar luteum</u> based on mean values of each ecological season.

FLOATING LEAVES — ·· — ·· —
SUBMERSED LEAVES ———————
PETIOLES — — — —

STANDARD ERROR

200

100

ABOVEGROUND

ug-at P/ g OW

RHIZOMES ———————
ROOTS — — — —

200

100

BELOWGROUND

HIBERNAL   PREVERNAL   VERNAL AESTIVAL SEROTINAL   AUTUMNAL
                        ECOLOGICAL SEASONS
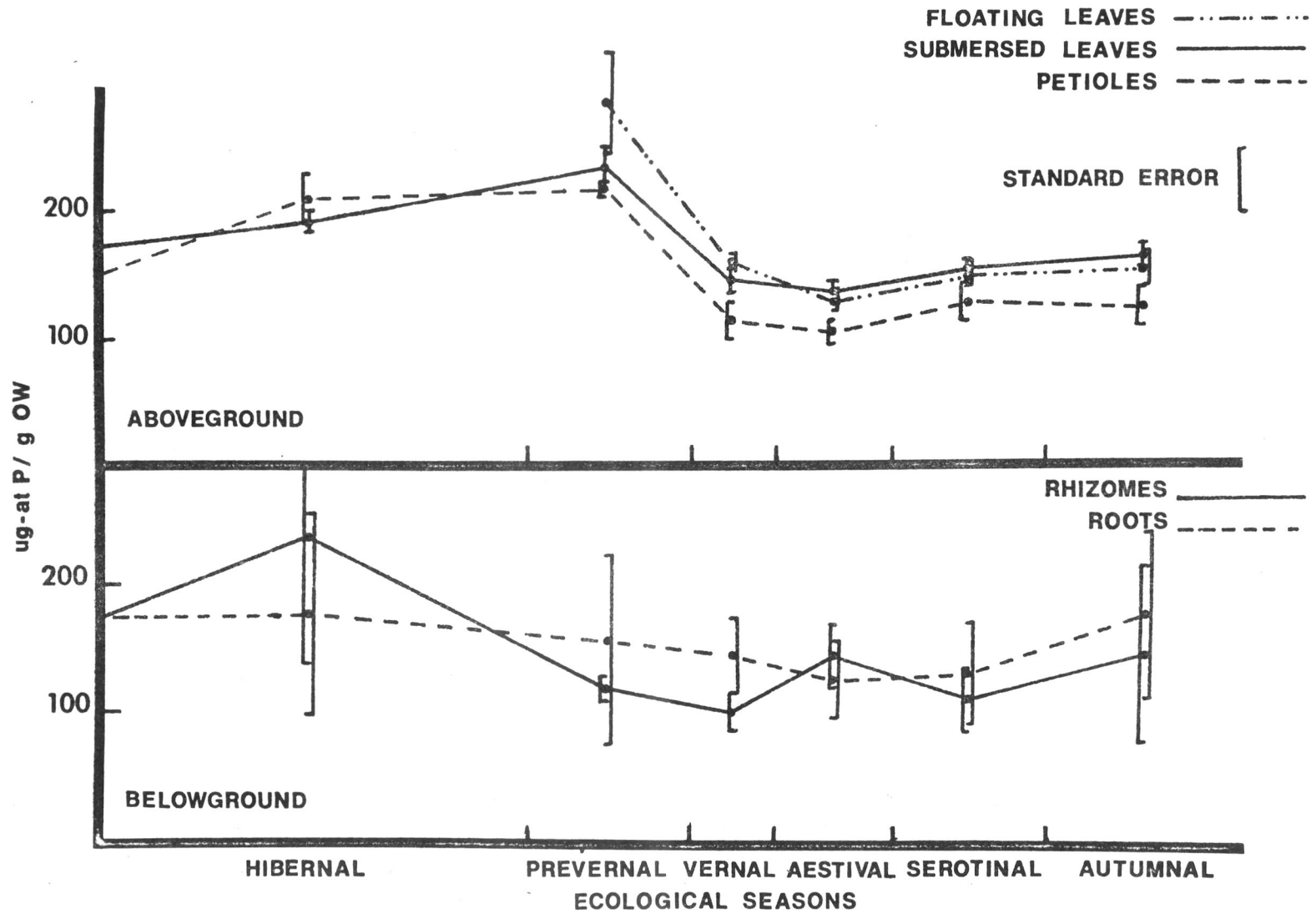
Table 7. Mean seasonal phosphorus concentrations (μg-at/g OW) and standard error in the structures of _Nuphar luteum_.

Structures	Hibernal	Prevernal	Vernal	Aestival	Serotinal	Autumnal
Floating Leaves		283.13	157.34	135.80	149.87	154.36
S.E.		39.49	2.90	7.47	8.30	16.63
Submersed Leaves	192.53	233.43	142.64	137.68	153.96	166.67
S.E.	6.12	13.06	11.38	8.22	6.11	11.44
Petioles	211.70	222.01	115.91	108.36	130.73	125.45
S.E.	21.02	3.55	12.90	8.58	16.96	14.46
Rhizome	244.51	123.99	105.66	149.34	114.37	148.77
S.E.	109.88	6.86	16.23	23.38	26.81	71.89
Roots	186.51	160.84	148.49	132.16	133.09	178.36
S.E.	78.71	67.24	32.76	27.08	37.01	64.75
Aboveground	202.12	241.57	138.63	126.78	140.30	147.72
S.E.	8.40	12.03	7.77	5.44	4.02	9.37
Belowground	215.51	142.42	127.08	140.75	124.45	163.56
S.E.	42.47	28.50	17.99	16.48	23.07	41.89
Mean	208.82	204.68	134.01	132.67	136.40	154.72

showed extremes in fluctuation throughout the year so that no seasonal patterns could be discerned. This was caused by the large standard error in biomass measurements for rhizomes. The accumulation of P by roots was extremely constant at ca. 2,000 μg-at/m^2 throughout the year. The storage of P by aboveground structures did show a pattern. Accumulation of P was low during hibernal and prevernal and then increased sharply during vernal and aestival to a peak in serotinal at 5,507 μg-at/m^2 (Fig. 9). Afterwards, there was a gradual decline in P stored in the biomass until the low value in hibernal was reached. This trend was expected since it resembles the generalized seasonal biomass patterns for aquatic macrophytes (Wetzel 1975, p. 377). An interesting observation of the seasonal patterns of P accumulation by the individual aboveground structures was that the peak in P storage by submersed leaves increased throughout the summer, finally peaking in autumn while the floating leaves and petioles peaked in mid-summer (Fig. 10).

Similar trends have been found by other investigators. Boyd (1969) measured the highest P concentrations in Justicia americana during early spring and the lower concentrations in the summer. He showed that the accumulation of P per m^2 of Justicia increased during the spring and peaked in July as it did for Nuphar. Adams and McCraken (1974) measured P concentration and photosynthetic rates in Myriophyllum spicatum during the growing season. They found that highest P concentrations in late April corresponded with peak photosynthesis and the low P concentration in late April corresponded with peak photosynthesis and the low P concentrations during the summer were measured when low rates of photosynthesis occurred. Photosynthetic rates and P concentrations also peaked again in late August. Another indication of the relationship between plant metabolism and phosphorus uptake is the enhancement of

Figure 9. Seasonal trends in phosphorus concentration (μg-at x $10^3/m^2$) in the aboveground and belowground biomass of <u>Nuphar luteum</u> based on mean values of each ecological season.
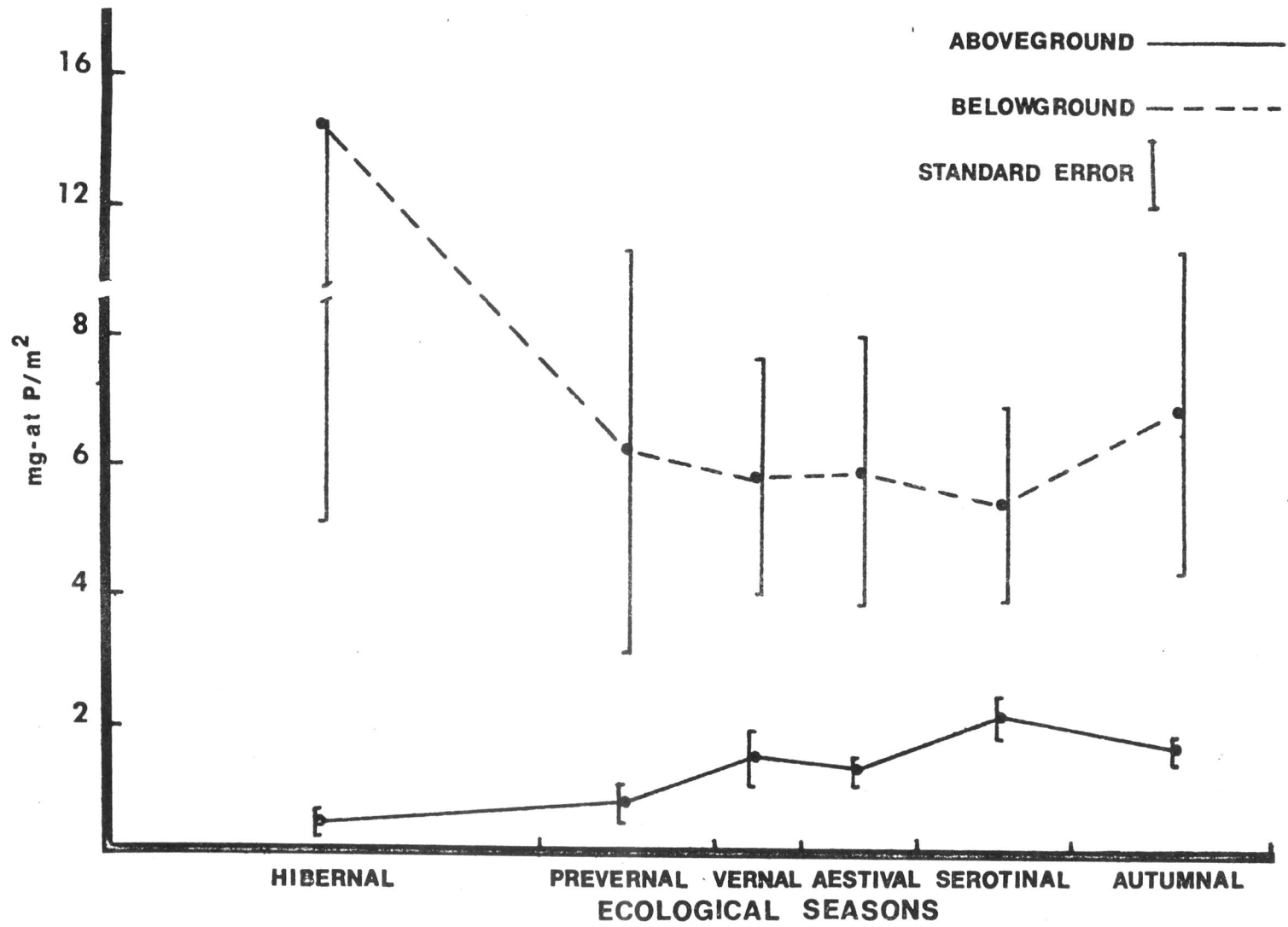
Figure 10. Seasonal trends in phosphorus concentration (μg-at x $10^3/m^2$) in the biomass of each structure of <u>Nuphar luteum</u> based on mean values of each ecological season.

FLOATING LEAVES ——··——··——
SUBMERSED LEAVES ————
PETIOLES —————

STANDARD ERROR

ROOTS —————
RHIZOMES ————

mg-at P/m²

2

1

24
20
16
12
8
4
2

HIBERNAL   PREVERNAL VERNAL AESTIVAL SEROTINAL AUTUMNAL
ECOLOGICAL SEASONS
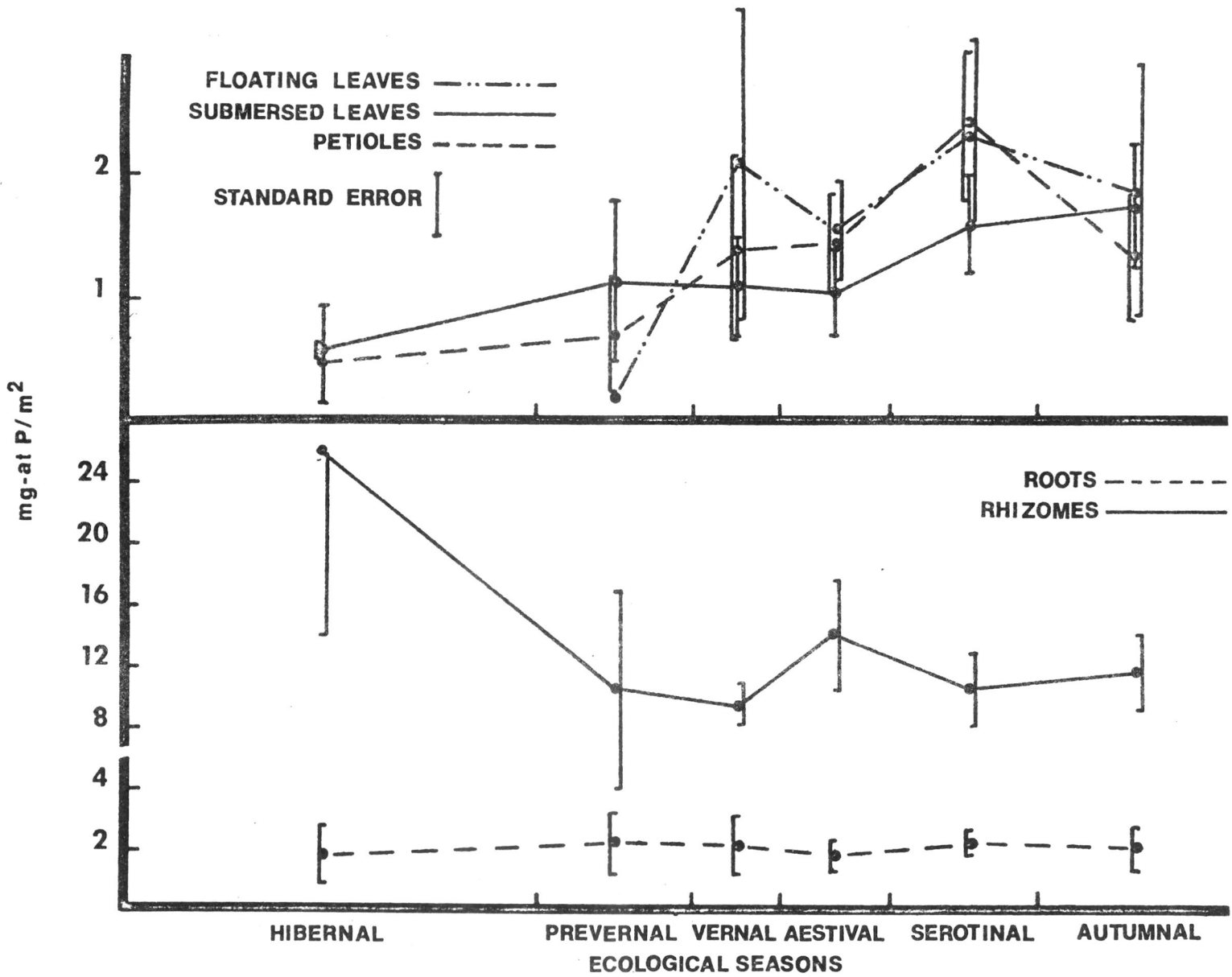
Table 8. Mean seasonal phosphorus biomass (mg-at/m^2) and standard error in each structure of _Nuphar_ _luteum_.

Structures	Hibernal	Pervernal	Vernal	Aestival	Serotinal	Autumnal
Floating Leaves		0.21	2.12	1.57	2.35	1.84
S.E.		----	1.43	0.45	0.78	1.08
Submersed Leaves	0.54	1.16	1.10	1.05	1.62	1.76
S.E.	0.43	0.68	0.44	0.36	0.41	0.49
Petioles	0.52	0.70	1.40	1.47	2.38	1.37
S.E.	0.02	0.46	0.73	0.43	0.60	0.51
Roots	1.98	2.33	2.24	1.80	2.21	2.00
S.E.	0.95	1.07	0.91	0.46	0.41	0.69
Rhizome	26.90	10.74	9.46	14.35	9.35	11.61
S.E.	12.98	6.64	0.63	3.77	2.44	3.44
Aboveground	0.53	0.82	1.54	1.35	2.10	1.62
S.E.	0.18	0.29	0.45	0.22	0.31	0.27
Belowground	14.44	6.53	5.85	5.96	5.46	6.81
S.E.	9.37	3.39	1.82	2.12	1.55	2.45
Total	14.97	7.35	7.39	7.31	7.56	8.43

of uptake in lighted vs. darkened environments (Jeschke & Simonis 1965, McRoy & Barsdate 1970).

Sediment Analysis

Interstitial Water

The average phosphorus concentration in the interstitial water (ortho-phosphate measured as filtered reactive phosphorus--Pi) for Keel Creek, Rockyhock Creek, and Indian Creek was 34.0 µg-at/liter with a range from 2.0 to 73.0 µg-at/liter. There were no significant differences with depth. The average concentration at Keel Creek (60.0 µg-at/liter) was significantly greater than the P concentration at Rockyhock and Indian Creeks (Table 9). The positive relationship between the seasonal average of the P concentration in the roots at these three locations and the interstitial water Pi concentration (Fig. 11) was significant ($r = 0.74$). The interstitial water content of 84.1% in the sediments multiplied by its Pi concentration of 60.0 µg-at/liter results in 11,014 µg-at P/m^2 in interstitial water of substrate at Keel Creek to a depth of 25 cm (Table 9). This was five times the values for Rockyhock and Indian Creeks which had only 22.5 and 24.7% water in sediments, respectively. The average Pi concentration for the three locations was 5,241 µg-at/m^2. The overlying water in Keel, Rockyhock, and Indian Creeks average 1.0 µg-at Pi/liter which was lower than the average for interstitial water by a factor of 34. Considering an average depth of one meter in the _Nuphar_ beds, a m^2 of water contains ca. 1000 µg-at Pi/m^2. This value is one-fifth the amount of Pi in the interstitial water.

The range of Pi concentrations in interstitial water from the Chowan River was similar to the range measured by McRoy et al. (1972)

Table 9. Concentration of phosphorus in the interstitial water, biological available phosphorus, and roots in Nuphar communities at Keel Creek, Rockyhock Creek, and Indian Creek.

Measurement	Keel Cr. $\overline{X}$	Keel Cr. S.E.	Rockyhock Cr. $\overline{X}$	Rockyhock Cr. S.E.	Indian Cr. $\overline{X}$	Indian Cr. S.E.	Mean
Percent Water	84.1		22.5		24.7		
Interstitial Water							
μg-at P/liter	60.0	3.0	20.0	8.0	21.0	6.0	34.0
μg-at P/cm	1.10		0.22		0.25		0.52
Biological Available Phosphorus							
μg-at P/g dry wt	22.68	1.94	9.05	3.12	4.75	0.20	12.16
μg-at P/cm^2	78.70		340.22		174.17		197.68
Sum*							
μg-at/cm^2	79.81		340.44		174.37		198.20
Roots							
μg-at P/g OW	275.10		117.26	72.22	72.12		

* Sum = Phosphorus in the interstitial water + phosphorus in the sediment as biological available phosphorus.

Figure 11. Regression of (A) interstitial phosphorus concern-
tration and the phosphorus concentration in roots,
and (B) biological available phosphorus concentra-
tion and the phosphorus concentration in roots.
Comparisons were made at Keel Creek (□), Rockyhock
Creek (△), and Indian Creek (○).

A. INTERSTITIAL WATER (ug-at P/liter) vs ROOTS (ug-at P/g OW), r=0.74

B. SEDIMENT (ug-at P/g dry wt) vs ROOTS (ug-at P/g OW), r=0.90
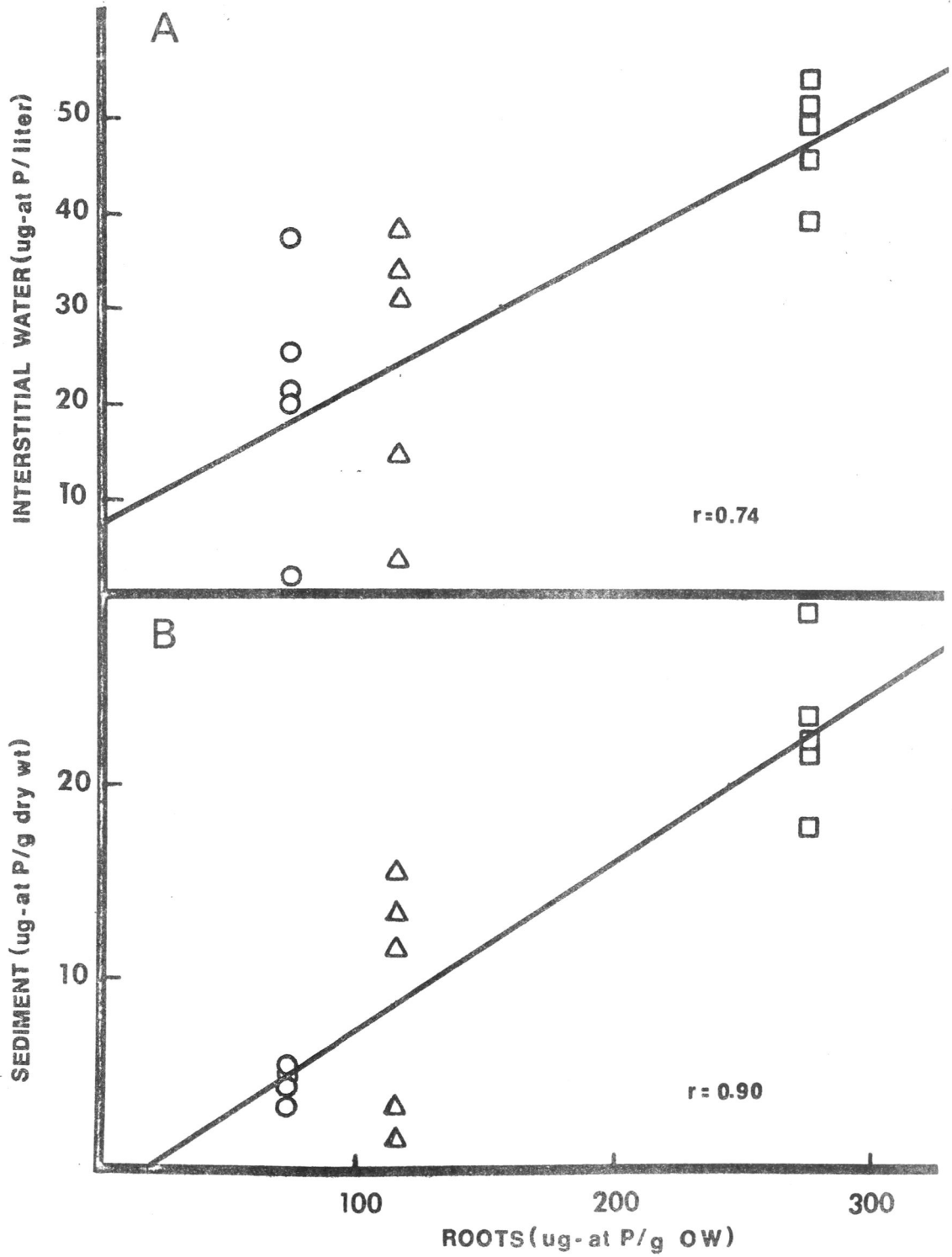
from Izembek Lagoon in Alaska (Table 10). However, the average Pi concentration from this study was well below the mean values for the Chesapeake Bay (Bray et al. 1973) and Lake Innneret (Serruya et al. 1974). Weiler (1973) reported no changes in the concentration of the ions he measured in interstitial water in Lake Ontario from May to August. No seasonal measurements were made in this study.

Biological Available Phosphorus (BAP)

The BAP concentrations at Rockyhock and Indian Creek were not significantly different from each other, but both were significantly lower than the average for Keel Creek (Table 9). These BAP concentrations had a higher positive correlation to the concentration of P in the roots at these sites (r = 0.90) than did interstitial water (r = 0.74) (Fig. 11). The average BAP concentration for the three sites was 12.16 μg-at/g dry wt and no concentration gradient with depth was found in these sediments. Since the water content of the substrate at Keel Creek was so high, the lower BAP/m^2 reported there compared to Indian and Rockyhock Creeks may have been a dilution effect (Table 9). To a depth of 25 cm, the average BAP for the three locations was 1.978 x 10^6 μg-at P/m^2 (Table 9).

As previously mentioned, a m^2 of water in a bed of _Nuphar_ contains ca. 1000 μg-at Pi. Therefore the BAP/m^2 is 2,000 times greater than the Pi in the water. The interstitial water and BAP account for an average of 1.982 x 10^6 μg-at/m^2 in the substrate which is well above the Pi concentration in the medium surrounding a m^2 of aboveground vegetation. Even the movement of a small percentage of P from the substrate to the water via the plant would be significant since the P gradient is so great.

The BAP/g dry wt measurements from this study on the Chowan River

Table 10. Comparison of phosphorus concentrations of interstitial water and biological
available phosphorus from this study to work of other investigators.

Measurement	Location	Phosphorus Concentration	Reference
Interstitial Water	Lake Kinneret, Israel	16.0 to 322.0	Serruya et al. 1974
(μg-at P/liter)	Chesapeake Bay, USA	$\overline{X}$ = 100.0	Bray et al 1973
	Izembek Lagoon, USA	5.0 to 75.0	McRoy et al. 1972
	Chowan River, USA	2.0 to 54.0	This Study
		$\overline{X}$ = 34.0	
Biological Available Phosphorus	Lake Mendota, USA	23.2 to 31.0	Wentz & Lee 1969b
		$\overline{X}$ = 27.4	
(μg-at P/g dry wt)			
	Pamlico River, USA	9.67 to 31.0	Upchurch et al. 1974
	Chowan River, USA	1.50 to 29.06	This Study
		$\overline{X}$ = 12.16	

were below the BAP measurements of Lake Mendota (Wentz & Lee 1969b) and
the Pamlico River (Upchurch et al. 1974) using the same technique
(Table 10). As in this study, Wentz and Lee (1969b) found no concen-
tration gradient with depth in the sediments of Lake Mendota.

In only a few cases has the nutrient level in plant structures
been compared to the nutrient's concentration in the substrate. Misra
(1938) showed the nitrogen content of the rooted aquatic macrophyte,
Potomogeton perfoliatus to correlate with the nitrogen content of the
mud in which it was growing. Chiaudani (1969) indicated that over a
wide range of concentrations, the copper content in the rhizomes
(roots included) depended on the copper content of the sediments in
which the plants were rooted. In this study, the BAP concentrations
and the P concentrations in roots correlated better between sampling
sites than did interstitial water. The roots of Nuphar could be used
to indicate high or low concentrations of P in the sediment. A high
rate of exchange of P between sediment and interstitial water proposed
by Li et al. (1973) could explain the significant correlation (r = 0.78)
in Fig. 12.

Phosphorus Uptake, Translocation, and Secretion

Laboratory results

Absorption - Laboratory experiments were run to observe the response
of submersed leaves, floating leaves, and roots when the time of incuba-
tion with the radioactive isotope and Pi concentration were varied.
Roots exhibited a declining uptake rate after 60 min while after only
20 min there appeared to be no net accumulation in submersed leaves
(Fig. 13a). At 60 min, floating leaves absorbed 0.036 µg-at P/g dry wt
compared to ca. 0.136 µg-at P/g dry wt for submersed leaves and 0.321

Figure 12. Regression of phosphorus concentrations in biologi-
cal available phosphorus and interstitial water with
points from Keel Creek (☐), Rockyhock Creek (△),
and Indian Creek (◯).
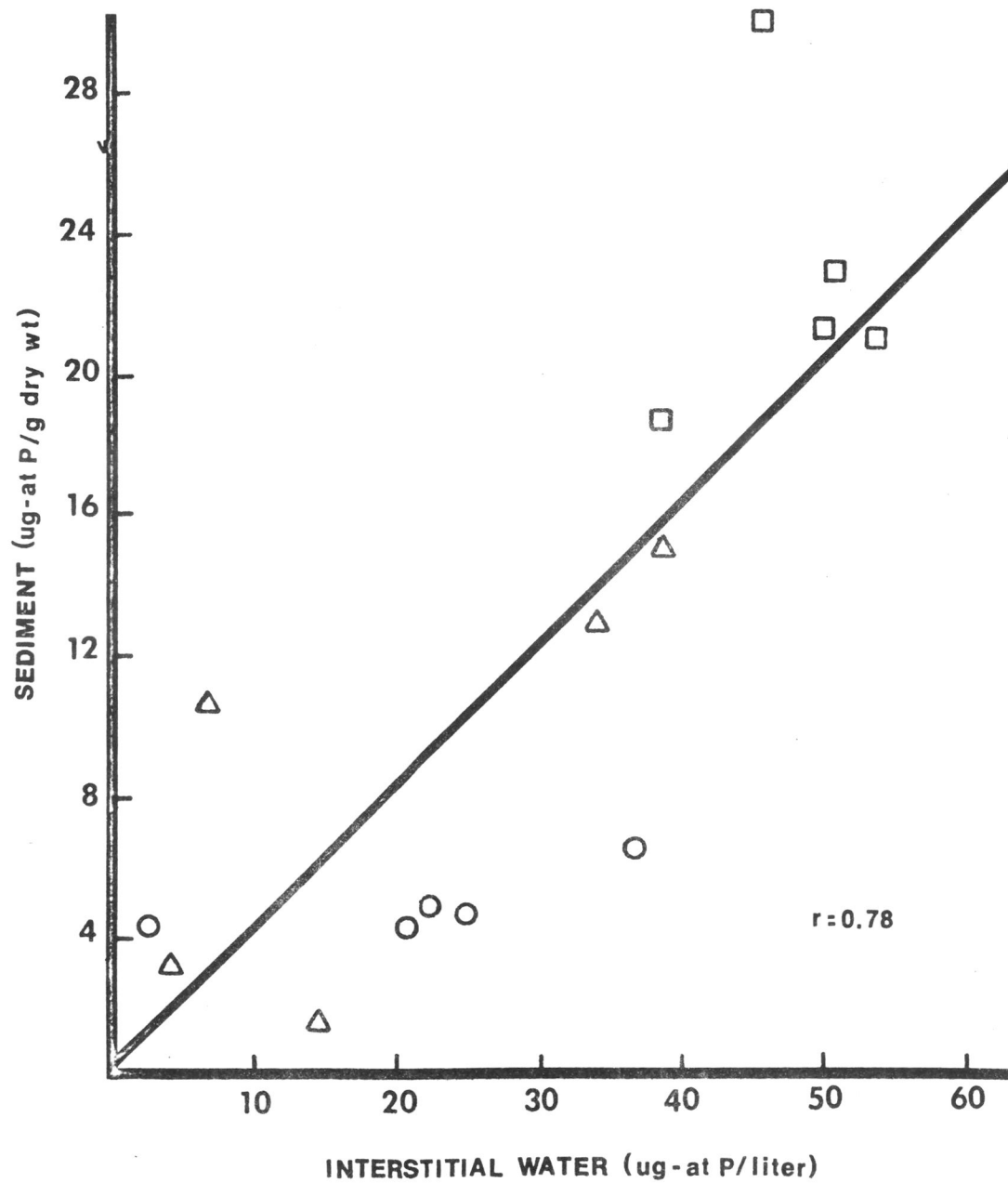
µg-at P/g dry wt for roots (Fig. 13b). Roots had a greater rate of
P uptake than the other two absorbing organs. When the uptake rate of
submersed leaves and roots were expressed per unit area of organ,
similar trends were observed although submersed leaves exhibited
increased net uptake after 20 min of incubation (Fig. 13a). As previously
mentioned in Methods, only the relative area of roots were determined
whereas the exact surface area of submersed leaves was measured.
Therefore the rates graphed in Fig. 13a cannot be compared but represent
only a relative relationship. The coefficient of variation was used to
compare the variability between weight and area as units of expressing
the rates of P uptake by roots and submersed leaves. This statistical
test was an attempt to determine which unit would be best to describe
P uptake rates. For submersed leaves, weight had a lower coefficient
of variation (0.28) than area (0.36), but for roots, area had a similar
coefficient of variation (0.31) with weight (0.34).

An increase in the concentration of P resulted in increased
absorption rates of submersed leaves during 10-min incubation periods
(Fig. 14) and roots during 60-min incubation periods. The increase in
P absorption in the submersed leaves began to level off at about 7 µg-at
P/liter. Results were similar when absorption was based on area (Fig.
14). The ortho-phosphate concentration in the lower Chowan River
averaged ca. 1.0 µg-at/liter (Don Stanley, personal communication) so the
absorption rate of submersed leaves may be limited by low P concentrations
in this system. At a P concentration of 3 µg-at/liter the absorption
rate of P by roots was 0.310 µg-at P/g dry wt · 60 min and a rate of
1.91 µg-at P/g dry wt · 60 min at 35 µg-at/liter--an increase by a
factor of 6.4. The response of roots to various P concentrations were
not plotted in Fig. 14 because only two different concentrations were

Figure 13. Response of roots and submersed leaves of Nuphar
luteum to increase time of incubation with the
radioisotope ^{32}P. Uptake was based on area (A)
and weight (B).
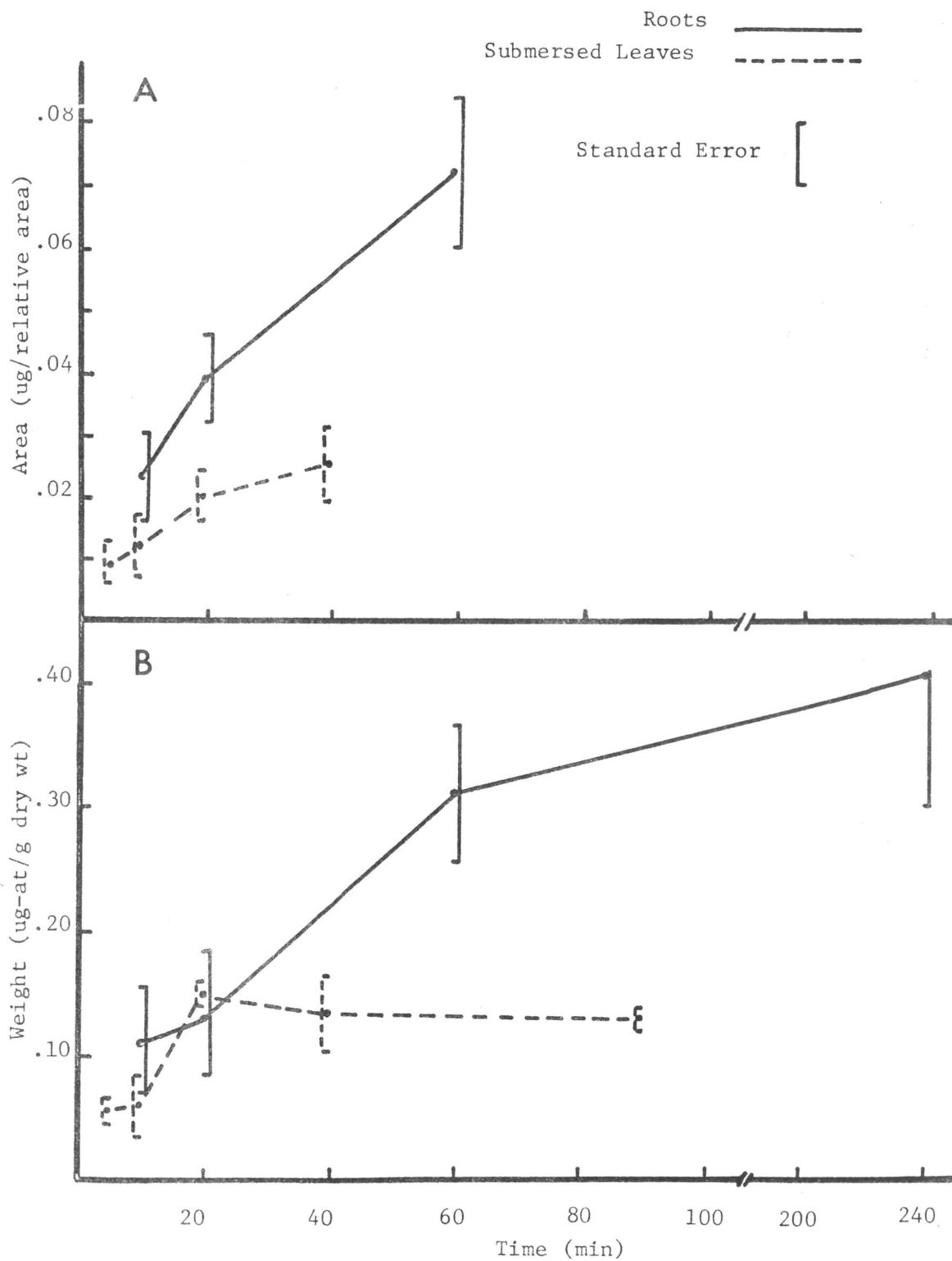
Figure 14.   Response of the phosphorus absorption activity of
            submersed leaves of Nuphar luteum to the increase
            in phosphorus concentration in the absorbing medium
            during a 10-min incubation period. Uptake rates
            are based on weight and area.
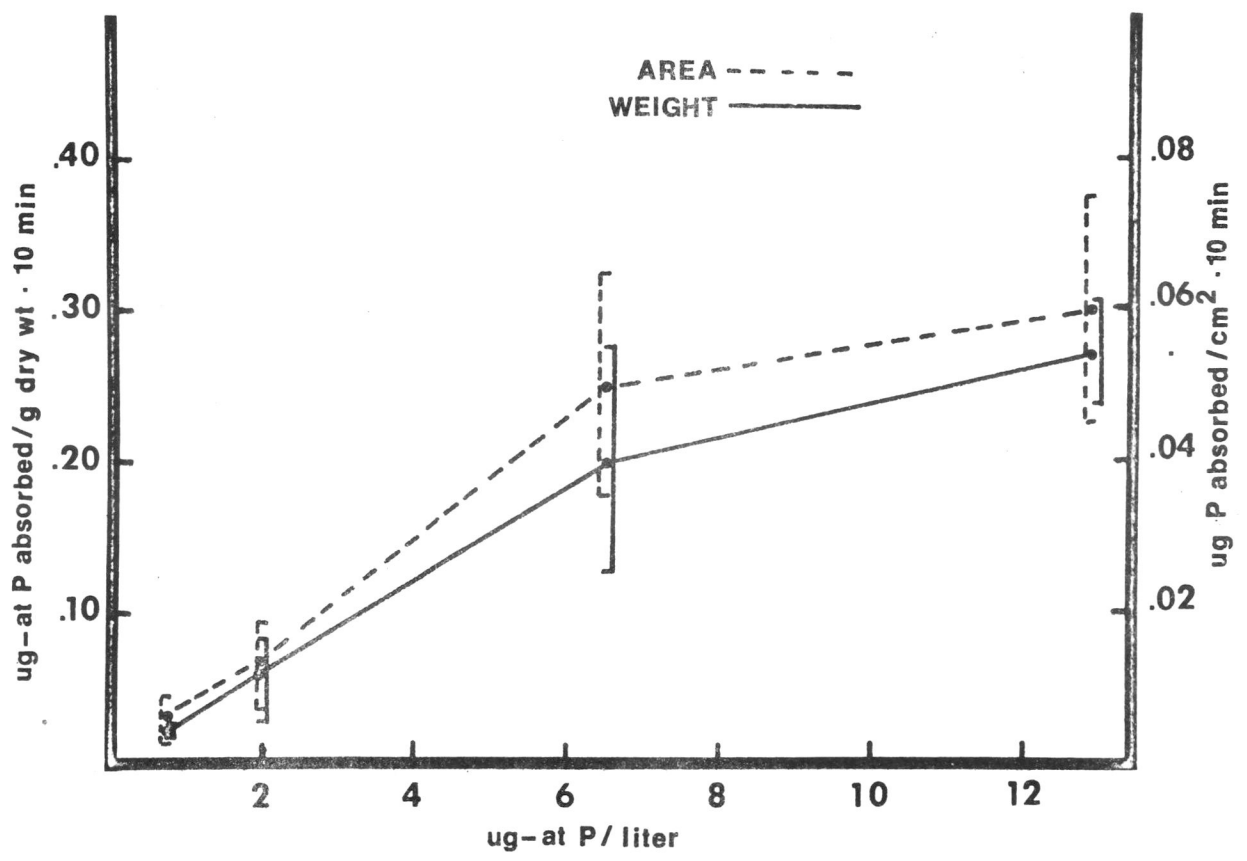
used. These P concentration used for the root experiments equaled the high and low ortho-phosphate concentrations measured in the interstitial water samples described under Sediment Analysis.

The absorption of ions by plants has been related to models that express the kinetics of enzymes and can be expressed by the equations:

$$R + M \xrightarrow[k_2]{k_1} RM \xrightarrow[k_4]{k_3} R + M \qquad (1)$$
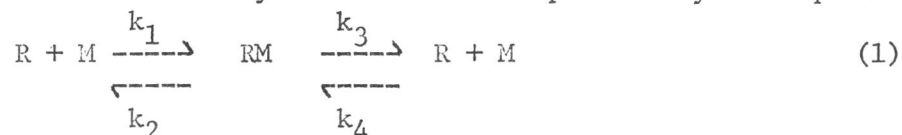
where R is a metabolically produced carrier, M is the ion being absorbed, RM is the carrier-ion complex, and k is the rate constant for each reaction (Epstein & Hagen 1952, Gerloff 1975); and

$$(Vmax + v) \; [M] \; / \; v = Km \qquad (2)$$

where Vmax is the maximum rate of absorption at infinite substrate concentration M, and Km is the Michaelis constant or the ion concentration at (1/2) Vmax (Gerloff 1975). By graphing double reciprocal plots of absorption vs. external concentration Equation 2 results in a Lineweaver-Burke linear form (Lineweaver & Burk 1934). The y-intercept of this linear line and its slope can be used to determine more accurately the values for Vmax and Km. The double-reciprocal plots of absorption vs. external concentration for roots and submersed leaves shows that roots have a greater uptake rate than do submersed leaves (Fig. 15). The two lines for submersed leaves were plotted because the best fit line gave a Vmax of 1.0 which is not compatable with the plot of uptake vs. P concentration in Fig. 14 which appears to be approaching an asymptote of ca. 0.30 µg-at P absorbed/g dry wt · 10 min. But the line through the two points that represent high P concentrations are considered more accurate and gives a Vmax of 0.40 which is similar to the results of Fig. 14. Km from this line is 7.27 µg-at/liter. The Km values for the low and high affinity systems respectively, of other aquatic macrophytes are:

Figure 15. Lineweaver-Burke form of the Michaelis-Menton equation
for both roots and submersed leaves of Nuphar luteum.
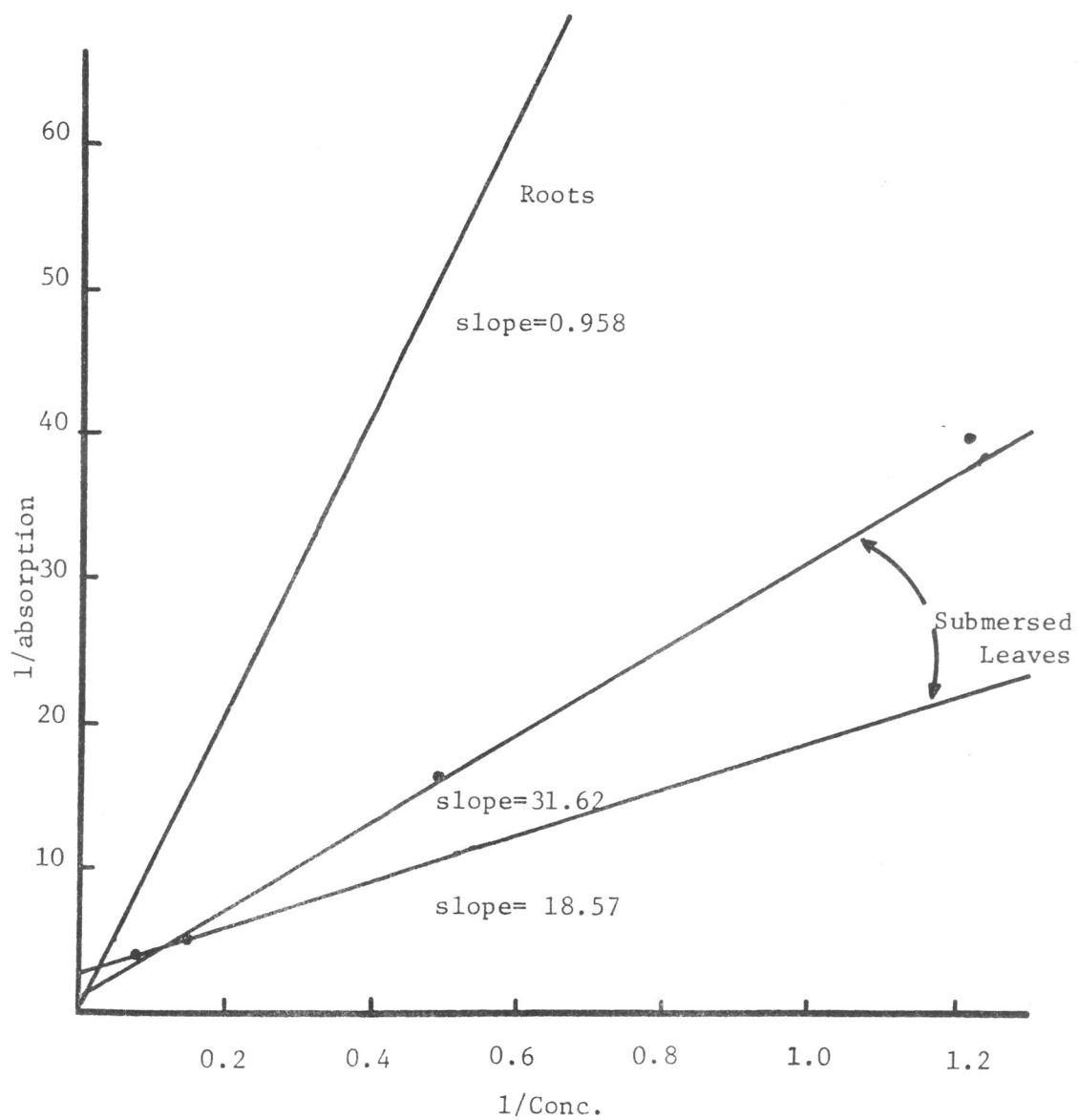
Elodea occidentalis roots - 0.387 and 36.1 µg-at P/liter, Elodea

occidentalis shoots - 0.3555 and 41.6 µg-at P/liter, Ceratophyllum

demersum shoots - 0.074 and 11.9 µg-at P/liter, Myriophyllum spicatum

shoots - 0.015 and 37.1 µg-at P/liter (Gerloff 1975).

The reason no net uptake occurred in submersed leaves after 20 min

may be that rates k_1 and k_2 in Equation (1) were in equilibrium with the

radioisotope ^{32}P. Also, based on principles of plant physiology, ions

released by the carrier-ion complex (M) may be passed to the symplasm or

they remain in the free space where they are transported to main regions

of water loss, i.e. stomata and other epidermal cells. These losses may

be great enough to negate net uptake.

Translocation - Phosphorus absorbed by the submersed leaves was

transported immediately to their petioles and was detected at very low

levels in the rhizomes 20 min after absorption (Fig. 16). After 90 min

of incubation, radioactive P was not detected in any of the other

structures. That activity was recorded in the rhizomes but not in the

roots when ^{32}P was added to the upper chamber containing submersed leaves

is proof that the chamber system was not leaking. Radioactive P was

detected in the roots, rhizomes, and submersed leaf petioles 10 min after

incubation of roots in ^{32}P (Fig. 16). After 60 min, P was also trans-

ported to floating leaves petioles and by 240 min of incubation all the

anatomical structures received P via acropetal translocation. The

amount of radioactivity in the roots and rhizomes varied little between

the 60 and 240 min experiments which indicates that saturation concentra-

tions were reached within an hour. Floating leaves translocated only

5% of the P that they absorbed during 60 min of incubation. Translocation

of P absorbed by roots occurred more rapidly and extensively than with

submersed and floating leaves.

Figure 16. Amount of ^{32}P detected in the various structures of
Nuphar luteum after submersed leaves (A) and roots
(B) had been incubated with ^{32}P at different time
intervals.
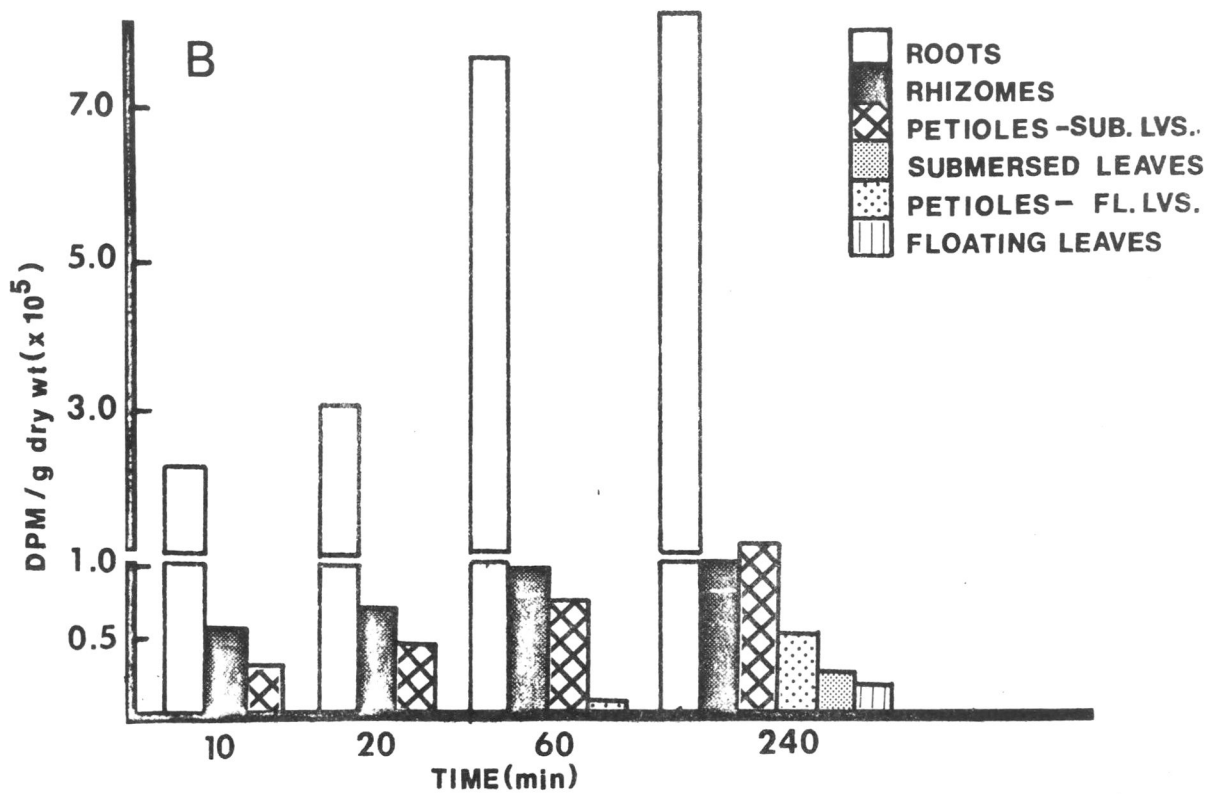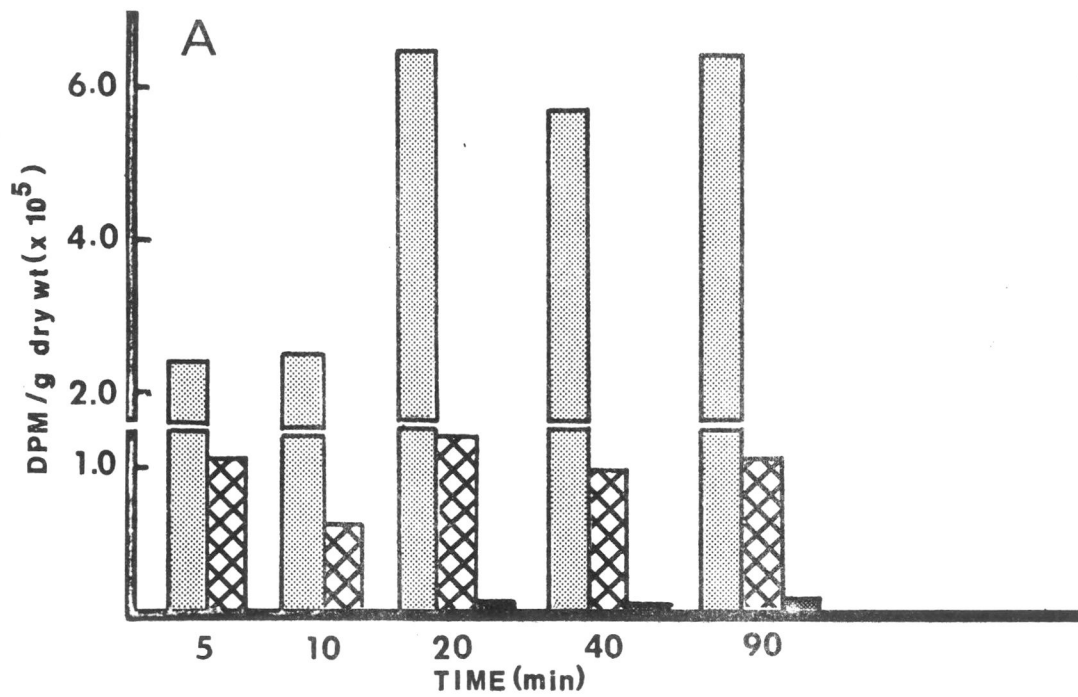
Field results

Absorption - The amount of P transferred (k) from one compartment to another is proportional to the quantity (Q) of P in the compartment from which it came. To calculate the rate coefficient (λ) of P movement between compartment A and B in the field experiments, I used the formula $\lambda = k_A \longrightarrow {}_B / Q_A$ where the values for k and Q were those at the end of the 24-hr experiment (units are %P x 10^3/day). These rates represent exchange of P between water enclosed in plexiglass chambers and plants (Table 11). Movement of P from sediment to the plant was via root absorption and subsequent translocation; the coefficient for movement of P from water to plant represents absorption by the submersed leaves only. There were no significant differences ($P < 0.05$) in the coefficients for absorption by roots (sediment $\longrightarrow$ plant) for the three seasons, but a rank of these rates from highest to lowest was summer > spring > winter. For submersed leaf absorption (water $\longrightarrow$ plant), the summer rate coefficient was significantly greater than spring or winter. Spring has a higher rate coefficient than winter but the difference was not significant. Uptake rates for submersed leaves were affected more by the seasonal change than were roots. During all three seasons, the coefficients for root absorption were higher than submersed leaf absorption.

The concentration of P per gram dry weight in each plant structure that was contributed by submersed leaf and root absorption were determined for the three field experiments by using the specific activity of the isotope in the medium (Fig. 17). By multiplying these values by the seasonal and spatial biomass data on Nuphar luteum (Blanton 1976), the daily flux of P through this plant can be expressed on an area basis (Fig. 18). Trends in absorption resulting from

Table 11. Coefficients and rates of phosphorus exchange between phosphorus in the plexiglass chambers and _Nuphar luteum_: sediment---> plant is via root absorption; water ---> plant is via submersed leaf absorption; plant ---> sediment is via root secretion and plant ---> water is via submersed leaf secretion.

Process	Summer $\overline{X}$	S.E.	Winter $\overline{X}$	S.E.	Spring $\overline{X}$	S.E.
Coefficients (% P x 10^{-3} · day)						
Absorption						
Sediment ---> Plant	287.0	40.0	157.0	80.0	237.0	60.0
Water ---> Plant	108.0	7.0	19.6	2.0	28.1	3.5
Secretion						
Plant ---> Sediment	0.19	0.03	*		*	
Plant ---> Water	0.31	0.03	*		*	
Rates (µg-at P/g dry wt · day)						
Absorption						
Sediment ---> Plant	1.72	0.19	1.29	0.54	1.42	0.29
Water ---> Plant	0.46	0.14	0.29	0.09	0.55	0.07
Secretion						
Plant ---> Sediment	0.10	0.04	*		*	
Plant ---> Water	1.05	0.35	*		*	

expression of data in this manner are similar to the previous discussion (Table 11). The roots absorb 1.72 μg-at P/g dry wt · day in the summer, 1.42 μg-at P/g dry wt · day in the spring and 1.29 μg-at P/g dry wt · day in the winter (Table 11). These values are greater than absorption rates for submersed leaves in the spring, summer, or winter (0.55, 0.46, and 0.29 μg-at P/g dry wt · day, respectively). The spring rate for submersed leaves was higher than the other two seasons. Absorption rate for floating leaves during the spring was lower than the rate for submersed leaves (0.19 μg-at P/g dry wt · day). These absorption rates are based on the dry weight of the absorbing organ and not on the total dry weight of the plant. In the summer, a square meter of roots would absorb 46.63 μg-at P/day compared to 5.26 μg-at P/day absorbed by submersed leaves in the same area. Roots absorbed 33.01 μg-at P/m^2 · day and submersed leaves 3.32 μg-at P/m^2 · day during the spring. Winter rates were lower for both roots and submersed leaves (20.58 and 0.92 μg-atP/m^2 · day, respectively). The rank of absorption rates from the field and laboratory experiments are the same: roots > submersed leaves > floating leaves.

Excavating the roots from the substrate may have caused a loss in the more active absorbing regions of the roots. Lauchli (1972) states that the terminal 10 cm of the root is where the main transfer of ions into the xylem occurs. For the majority of roots, it was not possible to remove this terminal section from the substrate and therefore the absorption rates may be underestimates.

Translocation - In the summer, all the anatomical structures except submersed leaves received more P from acropetal translocation than by submersed leaf absorption and subsequent translocation (Fig. 17). Similar results occurred in the spring except submersed leaf petioles

Figure 17. The amount of phosphorus (μg-at/g dry wt) trans-
located to each structure via submersed leaf
absorption (----) or root absorption (——) during
a 24-hr period in winter, spring, and summer.

WINTER

WATER
0.29

→0.271
SUBMERSED
LEAVES
0.003←

→0.010
PETIOLES
SUB. LVS.
0.001←

→0.0002
RHIZOMES
0.011←

→0.0004
ROOTS
1.233←

1.29
SUBSTRATE

SPRING

WATER
0.55

0.189←
→0.0001
FLOATING
LEAVES
0.002←

→0.515
SUBMERSED
LEAVES
0.003←

→0.0002
PETIOLES
FL. LVS.
0.029←

→0.036
PETIOLES
SUB. LVS.
0.003←

FLOWERS
PEDUNCLES
0.008←

→0.0003
RHIZOMES
0.024←

→0.0009
ROOTS
1.352←

1.42
SUBSTRATE

SUMMER

WATER
0.46   1.05

→0.0002
FLOATING
LEAVES
0.006←

→0.082
SUBMERSED
LEAVES
0.003←

→0.005
PETIOLES
FL. LVS.
0.072←

→0.005
PETIOLES
SUB. LVS.
0.081←

→0.0003
FLOWERS
PEDUNCLES
0.028←

→0.002
RHIZOMES
0.038←

→0.046
ROOTS
1.063←

0 103   1.72
SUBSTRATE

Figure 18. The daily flux of phosphorus (μg-at per m^2 of <u>Nuphar</u>
<u>luteum</u> community) during the winter, spring, and
summer. The sources of phosphorus for the bidirec-
tional flux are via submersed leaf absorption (----)
and root absorption (———).

WINTER

WATER
0.92

0.85
SUBMERSED
LEAVES
0.01

0.03
PETIOLES
SUB. LVS.
0.002

0.03
RHIZOMES
1.69

0.01
ROOTS
18.88

20.58
SUBSTRATE

SPRING

WATER
3.32

0.48
0.0003
FLOATING
LEAVES
0.004

3.21
SUBMERSED
LEAVES
0.02

0.001
PETIOLES
FL. LVS.
0.11

0.05
PETIOLES
SUB. LVS.
0.02

FLOWERS
PEDUNCLES
0.01

0.03
RHIZOMES
2.33

0.02
ROOTS
30.52

33.01
SUBSTRATE

SUMMER

WATER
5.26   12.42

0.003
FLOATING
LEAVES
0.10

0.97
SUBMERSED
LEAVES
0.03

0.01
PETIOLES
FL. LVS.
1.43

0.03
PETIOLES
SUB. LVS.
0.40

0.001
FLOWERS
PEDUNCLES
0.10

0.26
RHIZOMES
4.21

1.20
ROOTS
27.94

2.71   46.63
SUBSTRATE

80

received more P from submersed leaves than from rhizome and roots
(Fig. 17). Flowers and peduncles received P via root and submersed leaf
absorption during the summer but via only root absorption during the
spring (Fig. 17). The contribution of P to the aboveground vegetation
from root absorption was much higher in the summer than the spring
per gram dry weight of the structure (Fig. 17). Phosphorus absorbed by
floating leaves was detected in only the petioles of the floating leaf
and this amount was small. During the winter, the belowground struc-
tures (roots and rhizomes) received most of its P via root absorption
while the source of P for the aboveground structures (submersed leaves
and petioles) was absorption by submersed leaves. A bidirectional flux
occurred in Nuphar for all three seasons measured.

About four times more P moved acropetally than basipetally in
Nuphar during the summer. In the spring and winter the differences were
greater in magnitude ($\simeq$ 25 times greater for both seasons) but the
absolute values were less (Table 11). Of the P absorbed by submersed
leaves, 82% was translocated while only 40.1% of the P absorbed by the
roots was translocated during the summer. Therefore, the difference in
bidirectional movement was due to the much lower absorption rate of the
submersed leaves (5.26 μg-at/m^2 $\cdot$ day) compared to the roots (46.63 μg-
at P/m^2 $\cdot$ day).

The rate of translocation varied between the three seasons
measured. This change was indicated by the difference between seasons
in the concentration of P in the structures of Nuphar contributed by
translocation from either roots or submersed leaves (Fig. 17). Floating
leaves, floating leaf petioles, flowers, and peduncles received much
more P via submersed leaf and root absorption during the summer than
spring. Rhizomes received more P from both acropetal and basipetal trans-

location during the summer than during spring with the least amount
received in winter. Petioles of submersed leaves received more P/g
dry weight from acropetal translocation during the summer, while in
winter most of its P was from submersed leaves. This also implies
unequal seasonal effects on the bidirectional pathways.

The translocation rates were affected more by the seasons than the
absorption rates. The winter absorption rate for roots was one-half
the summer rate but the amount of translocation of P from the roots
during the winter was one-tenth the summer value (Table 12). There was
only a 30% difference in absorption values for roots between summer and
spring but a 85% difference in the amount of P translocated acropetally.
The same was true when absorption by submersed leaves and basipetal
translocation are compared (Table 12). Also, the concentration of
absorbed P in the absorbing organs (roots and submersed leaves) was
highest during spring followed by winter which was greater than summer
(Fig. 17).

Cutting the rhizomes to place the belowground structures inside a
chamber might have caused a decrease in the rate of acropetal transloca-
tion of P. Observations have been made that the osmotic pressure in the
root of some submersed plants may be primarily responsible for upward
movement of water and therefore ions through the plant (Sculthrope 1967).
Frank and Hodgson (1964) showed that the removal of tubers resulted in
decreased translocation of labelled fenac, a herbicide, in Potomogeton
pectinatus. The removal of the tubers decreased the root presure which
caused less upward movement of ions. To place Nuphar luteum in the
plexiglass chambers in this study, the rhizomes had to be cut which
probably caused a decrease in the exudation pressure. In a preliminary
laboratory experiment, gas bubbles were observed escaping through a film

Table 12. Daily rates of absorption, translocation, and secretion of phosphorus through a m^2 of _Nuphar_ _luteum_ community during winter, spring, and summer.

		Season					
		Winter		Spring		Summer	
Process	Direction	$g\text{-at}/m^2$	$\%^a$	$g\text{-at}/m^2$	$\%^a$	$g\text{-at}/m^2$	$\%^a$
Absorption	Roots	20.579		33.012		46.627	
	Submersed Leaves	0.917		3.317		5.259	
Translocation	Acropetally	1.697	8.0	2.493	7.6	18.691	40.1
	Basipetally	0.066	7.0	0.098	2.9	4.291	82.0
	BG ---> AG[b]	0.011	0.05	0.159	0.5	14.485	31.0
	AG ---> BG	0.037	4.0	0.049	1.5	4.166	79.0
Secretion	Submersed Leaves	c		c		12.422	26.6
	Roots	c		c		2.708	51.5

a - Percent of the phosphorus absorbed that was translocated.

b - BG = Belowground biomass (roots and rhizomes)

   AG = Aboveground biomass (leaves, petioles, and flowers)

c - Below the level of detection ($< 10^{-4}$)

of stopcock grease that had been applied to the cut surface of a rhizome. This was possibly the release of pressure in the belowground organs.

Secretion - No secretion of P originating from submersed leaves or roots was detected in the floating leaves for the summer field experiment. Secretion was detected in both the submersed leaves and roots but only during the summer. Secretion by the submersed leaves was greater (12.42 μg-at P/m^2 · day) than secretion by roots (2.71 μg-at P/m^2 · day) (Fig. 18). This trend was also observed by comparing the rate coefficients for secretion (Table 11). The submersed leaves secreted 86% of the P translocated to them from the belowground structures compared to 33% secreted by the leaves of Zostera marina (McRoy & Barsdate 1970). This represents a source of P to the water column whose origin was the substrate. Secretion during the winter and spring was below the limits of detection ($<10^{-4}$ μg-at P/g dry wt · day). During these seasons the percent of P absorbed by the roots that was translocated to aboveground structures was 0.05 and 0.50%, respectively, compared to 31% translocated during the summer (Table 12). It may be that a threshold value of P must be translocated to the submersed leaves and roots before secretion will occur and that this value was excreeded only in the summer. Therefore, secretion as a process of regenerating P from the substrate assumed importance only during the summer which was the season submersed leaves appeared to be vigorously growing. During other seasons, submersed leaves were torn and badly damaged.

A major assumption used in determining the flux of P by translocation and secretion was that the ratio in the absorbing medium between radioactive atoms of phosphorus (^{32}P and ^{33}P) and the stabile phosphorus atom (^{31}P) remains constant as both atoms moved through the plant. Other

investigators have used the initial specific activity of the absorbing mediums to compute P translocated and secreted (McRoy et al. 1972, Reimold 1972). This could be a problem since it is possible the specific activity of the isotope changes once inside the plant and the measurements of translocation and secretion would be an over- or under-estimate. This expected change is based on the phenomenon of solute concentration gradients that occur in plants that enable the movement of ions to the xylem. Phosphorus concentration of the xylem sap were not measured in this study. But from other studies some idea of the relationship between the concentration of P in exudates (water moving through vascular tissue) and absorbing mediums can be obtained. Epstein (1972) found that it was common for exudate concentration of salts in roots to be in excess of that of the absorbing medium. Based on this observation, the secretion and translocation rates from this study would be underestimates. But work by Anderson & Collins (1969) on SO_4^{2-} in maize roots and Baker & Weatherly (1969) on salts in roots of <u>Ricinus communis</u> have shown that the relationship between the exudate concentration and the absorbing medium concentration is hyperbolic. For sulfate, the exudation concentration was higher than the absorbing medium until the sulphate concentration of the absorbing medium reached between 4 and 4.5 mM. Above this concentration the exudate concentration was lower than the concentration of SO_4^{2-} in the absorbing medium. For more accurate measurements of translocation and secretion, the ratio should be determined between the specific activity of the radioisotope to the phosphorus concentration being translocated.

Decomposition

Dry Weight Loss

The equation used to determine the decompsition and phosphorus (P) releases rates was $X/X_o = e^{-\lambda t}$ where X = weight of plant or P at time t, X_o = original weight, e is the base of the natural logarithm, and λ is the decomposition or P release constant (Olson 1963, Gosz et al. 1973). To solve for λ based on a day, the following equation was used: $\lambda = lnX_o - lnX_t/t$ (day). A useful index for comparing decomposition and P release rates between species and structures is the half time which was calculated by dividing 0.693 by the rate, λ.

The aboveground vegetation of Nuphar luteum lost ca. 95% of its dry weight in one month and there were no seasonal differences (Fig. 19). The half time of the decomposing plant material was about a week with a λ value of 0.080 in the summer and 0.089 in the winter. The aboveground structures of Justicia americana, an emergent macrophyte found in the Chowan River, decomposed much slower and had seasonal differences in the rates of decomposition. The half time during the summer was 27 days compared to 63 days in the winter.

The belowground vegetation of Nuphar decomposed slower than aboveground vegetation for both seasons; during the summer the loss of plant material was faster ($\lambda = 0.032$) than during the winter ($\lambda = 0.008$) (Fig. 19). During the summer, half the weight of the decomposing plant material was released in 22 days compared to 83 days during the winter. The half time for the dry weight loss of the belowground structures was similar in the summer (23 days) but twice as fast in the winter (34 days) compared to Nuphar belowground biomass.

A number of factors interact in decomposition including fragmentation,

Table 13. The constants and half-times (days) for decomposition and phosphorus release by the aboveground and belowground structures of <u>Nuphar</u> <u>luteum</u> and <u>Justicia</u> <u>americana</u> during summer and winter.

Plant	Compartment	Season	Decomposition		Phosphorus Release	
			λ [1]	half-life [2] (days)	λ	half-life (days)
<u>Nuphar</u>	Aboveground	Summer	0.080	8.7	0.076	9.2
		Winter	0.089	7.8	0.110	6.3
	Belowground	Summer	0.032	21.5	0.046	15.0
		Winter	0.008	82.5	0.014	50.2
<u>Justicia</u>	Aboveground	Summer	0.026	26.5	0.055	12.6
		Winter	0.015	63.0	0.009	72.2
	Belowground	Summer	0.030	23.4	0.041	17.1
		Winter	0.020	34.0	0.025	27.6

1 $\lambda = \ln X_o - \ln X_t / t$ (days)

2 half-life $= 0.693/\lambda$

Figure 19. Percentage of the original dry weight remaining
during a period of decomposition of the aboveground
and belowground structures of <u>Nuphar luteum</u> and
<u>Justicia americana</u> during summer and winter.

DECOMPOSITION
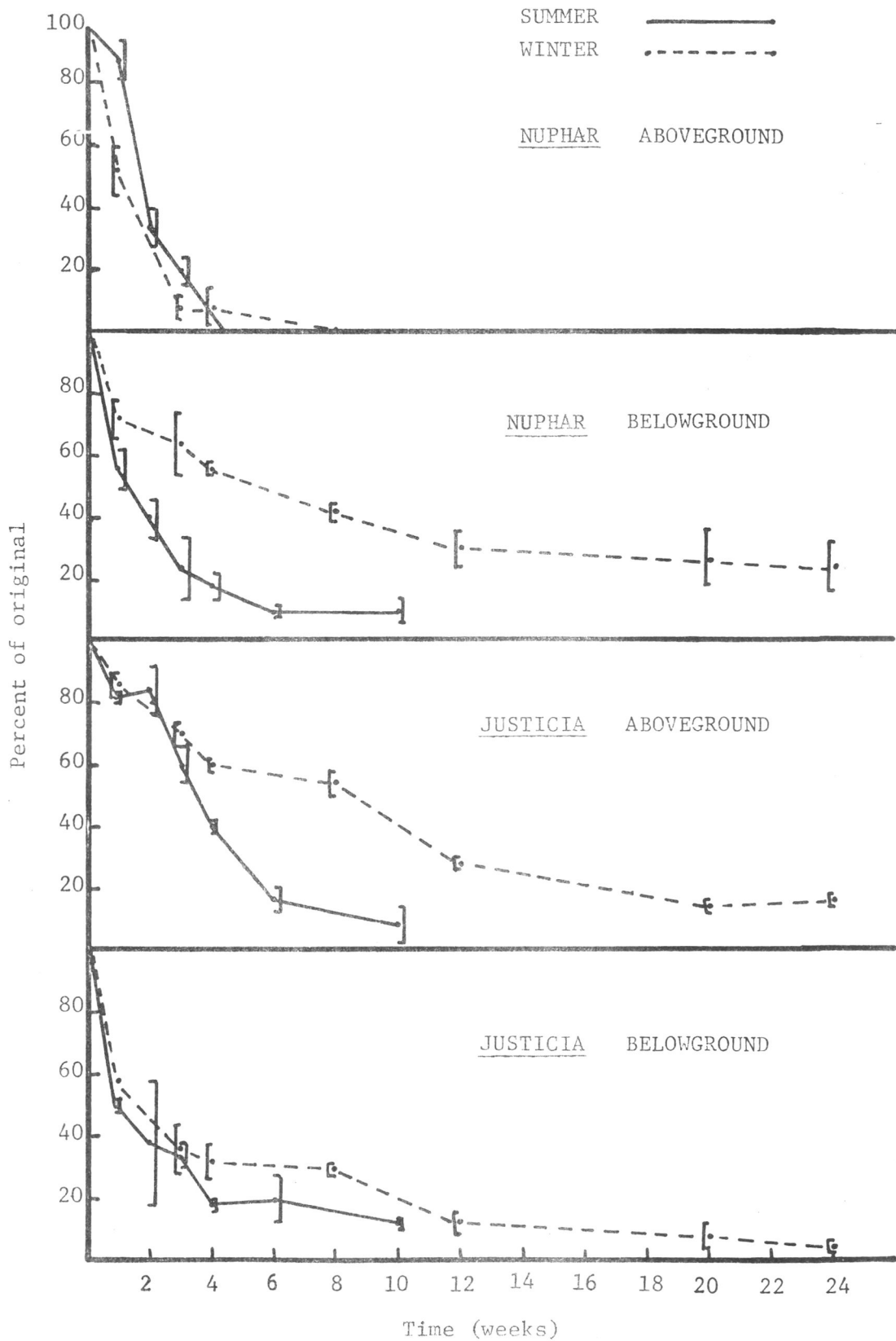
mechanical breakdown, autolysis, leaching, and microbial decay. Rapid
decay of the aboveground structures of Nuphar indicates that the processes
of autolysis and leaching were of major importance. Leaching was
established as the major process in the decomposition of eelgrass leaves
(Harrison & Mann 1975). Regardless of the process, aboveground detritus
was quickly regenerated back into the system. The time for half the loss
of litter in terrestial studies is between one and two years (Gosz et al.
1973) compared to one week for leaves of Nuphar.

The dry weight loss of the aboveground biomass via decomposition
was greater in Nuphar luteum than in a similar species Nymphaea odorata.
Kormondy (1968) experimented with N. odorata and measured 50% and 25%
dry weight loss at the end of one month from leaves lying on the sedi-
ment and suspended, respectively. A major factor for the difference may
have been that Kormondy used bags with a smaller mesh (25 mesh/cm).
Results of Nuphar luteum decomposition of aboveground biomass was similar
to studies on submersed aquatic macrophytes. Watermilfoil lost 65% of
its original dry weight in a month (Nichols & Keeney 1973) and
Potomogeton decayed from 6 to 95% of its original dry weight from 7 to
14 days. The range of percent decay for belowground structures of
Nuphar after one month was from 35 to 85% compared to Golley's (1960)
range of 2 to 70% decay of cellulose during April placed in different
types of terrestial soil. He hypothesized that the range in soils of
old fields was due to the density of fungal populations and the absence
of other cellulose in the soil ("buffer system"). The belowground
decomposition rate of Nuphar during winter (0.008) is at the more rapid
end of the range of decomposition rates (0.01 to 0.001) for organic
matter within mineral soils as established by Olson (1963).

Phosphorus Release

The rate constants for phosphorus release from Nuphar luteum were greater than rate constants for dry weight loss except in the above-ground structures during summer which were very similar (Table 13). Phosphorus release was also greater than dry weight release in Justicia americana except in aboveground structures during winter. The rates of P release for aboveground biomass of Nuphar was greater in the winter (λ = 0.11) than summer (λ = 0.076) and these rates were greater than those for the belowground structures in which summer rate (λ = 0.046) was greater than in winter (λ = 0.014) (Table 13). The time for half the P to be released from the aboveground detritus was 8 days. This is lower than the half-time for belowground structures during the summer (15 days) and winter (50.2 days). This short half-time for aboveground structures of Nuphar are lower than those for aboveground and belowground biomass of Justicia (Table 13). This quick rate demonstrates that once Nuphar aboveground detritus is produced by death, the P is quickly regenerated back into the system. But because of the wave activity, this P is flushed out into the river and probably not available for recycling by the same plants.

Boyd (1970a) and Nichols & Keeney (1973) measured a 50% less of P from submersed bags of Typha and Myriophyllum, respectively, after one month compared to 95% loss of P from aboveground structures and a range of 20 to 90% loss from belowground structures of Nuphar. In Typha, the percent of P in the dry weight decreased rapidly during the first week and then increased to 85% the original value after 64 days had elasped (Boyd 1970a).

SYNTHESIS

This section will combine the results of the concentration and
flux experiments previously discussed and the role of <u>Nuphar</u> <u>lutuem</u>
in the exchange of P between sediment and water during winter, spring,
and summer will now be considered. The discussion will deal mainly with
the net fluxes of P through a m^2 of <u>Nuphar</u> communities, water, and
substrate (Fig. 1). The concentrations of P in the water and substrate
compartment are considered to be constant. Based on an average depth
of one m and an ortho-phosphate concentration of 1 μg-at/liter
(Don Stanley, personal communication), the Pi content in the water is
1000 μg-at/m^2. The P content in the substrate compartment is the sum
of the interstitial water and biological available phosphorus (BAP)
fractions measured and was 1.98×10^6 μg-at/m^2 assuming a depth of 25 cm.

The daily flux of P from aboveground structures to the detritus
compartment during the prevernal, vernal, aestival, serotinal, and autumnal
seasons was based on the turnover rate of the biomass assuming that the
P content of detritus is in steady state. The turnover rates for biomass
were determined during a growing season which extended from April to
October which is similar to the period represented by these five ecologi-
cal seasons. The average turnover time for the aboveground structures
(floating leaves, submersed leaves and petioles) was 31 days assuming a
constant rate of turnover during the growing season (Blanton 1976).
Based on this turnover time the daily turnover rate would be 0.032%. To
calculate the daily flux of P from the aboveground compartment to the
detritus for the five previously mentioned ecological seasons, the mean
P biomass (μg-at/m^2) in the aboveground compartment (Table 8) during each

ecological season was multiplied by the turnover rate (0.032%/day).

The aboveground structures contained a mean of 3939.40 µg-at P/m^2 for

the entire growing season (prevernal, vernal, aestival, serotinal, and

autumnal seasons) and, at a daily turnover rate of 0.032%, the average

rate of flux of P from the aboveground structures to the detritus was

125.77 µg-at/m^2 • day during the growing season. The daily turnover

rate for belowground biomass was 8×10^{-4}% based on a turnover time of

1257 days (Blanton 1976).

Absorption rates for roots were measured in the laboratory at two

different P concentrations which resulted in the lower concentration

having an absorption rate 6.4 times less than that of the high concen-

tration (page 63). The lower P concentration used in these laboratory

experiments was equal to that in the medium used in the field experiments

and the high P concentration was similar to the high range of P concen-

tration in the interstitial water (Table 9). Since only the low

concentration was used in the field experiments, rates of P movement

were multiplied by 6.4 to correct for this underestimate. Other sources

of error such as damage to the roots during field procedure and the

assumption of constant ratio between labelled and unlabelled P are not

accounted for and therefore these values may still be underestimates.

The significance of these seasonal models (Fig. 20) is the comparison with

each other.

<u>Seasonal Flux of Phosphorus</u>

Winter

Because of the low rates for absorption, translocation, and decompo-

sition and the undetectable rate of secretion, Nuphar luteum does not

contribute to an upward flux of P from sediments to water (Fig. 20).

Figure 20. The daily flux of phosphorus (μg-at) through a m^2 of <u>Nuphar</u> <u>luteum</u> community during the winter, spring, and summer. Values in the compartments are P biomass.
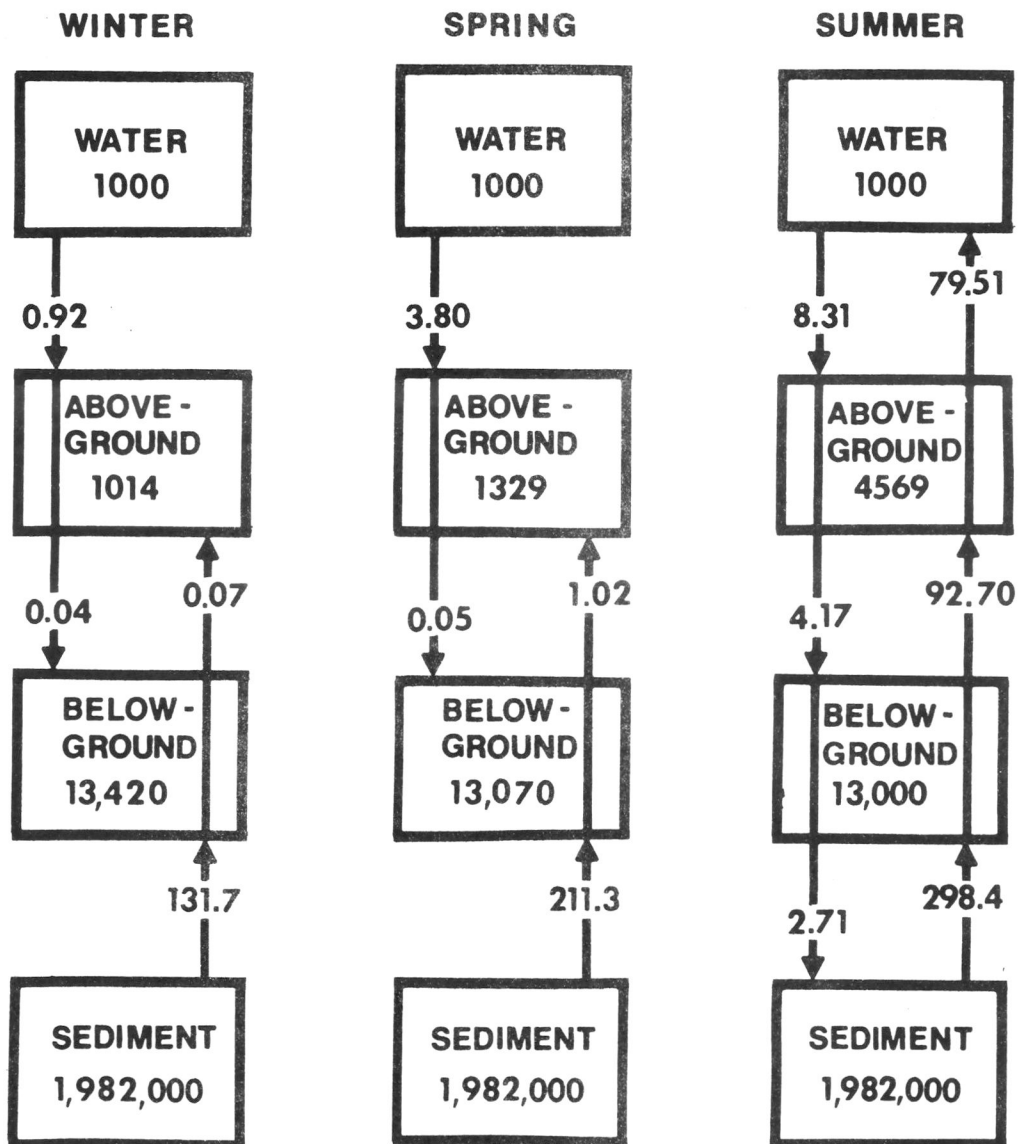
Phosphorus accumulated in the plant from both the water and substrate. This coincides with the observation of increased P concentration in the aboveground and belowground structures during the winter (Fig. 7). The major source of P for the belowground compartment was the substrate while the aboveground biomass received most of its P from the water. Although the dominant pathway was from belowground to aboveground biomass, the flux was small (0.033 μg-at/m^2 $\cdot$ day) and insignificant when compared to the P in the biomass compartments (Fig. 20). Of the total inputs (absorption and translocation) to the aboveground biomass compartment, only 7% originated from the substrate which implies that roots are not important in the mineral nutrition of these structures during the winter. This low translocation rate is probably related to the low demand for P by the aboveground structures since their production is presumably arrested. The high daily absorption rate of roots compared to submersed leaves is only 7 x 10^{-3}% of the P content in the substrate, whereas the rate of submersed leaves is 9 x 10^{-2}% of the P content in the water compartment.

Floating leaves and petioles were not present during winter but submersed leaves and petioles survived. Since biomass turnover rates were not measured during this time it will be assumed that the flux of P from the aboveground compartments to the detritus equals the mean biomass content of P which was 1014 μg-at/m^2 (Table 8). This release most likely occurred during the later part of the season. The turnover rate of the belowground biomass gives a daily flux of 10.67 μg-at/m^2 from this compartment to the detritus compartment which is only 8% of the daily absorption rate.

The mean accumulation of 1014 μg-at P/m^2 in the aboveground compartment is similar to the ortho-phosphate content of the water. But the P concen-

tration in the belowground biomass was only 0.6% of the available P in the substrate compartment. Since the fluxes of P are small, this accumulated P in the aboveground biomass represents a slowly cycling pool of P that is largely unavailable to the aquatic system.

Spring

The influence of <u>Nuphar</u> on the regeneration of P from the substrate increased slightly due to the increase in absorption, translocation, and decomposition rates (Fig. 20). Secretion of P from neither submersed leaves nor roots was detected. The aboveground and belowground biomass still received most of their P via absorption from their own surfaces, but the percent of the inputs to the aboveground biomass from the substrate did increased to 21% from 7% during the winter. The net inputs into the aboveground compartment increased to 4.77 μg-at P/m^2 · day from 0.95 μg-at P/m^2 · day in the winter. The net accumulation of P in the belowground biomass increased to 210.8 μg-at P/m^2 · day from a low winter rate of 131.7 μg-at P/m^2 · day. This increase indicates that the translocation rate did not rise in proportion to increased absorption. This is interesting since demand was high in the aboveground vegetation during spring time growth. An explanation of the difference will be discussed later (page 104).

The prevernal and vernal biomass measurements will be discussed with the spring tracer results since both represent plant activity at the beginning of the growing season. The mean aboveground biomass data and the daily rate for detritus flux (0.032%/day) equals a flux of 42.54 μg-at P/m^2 · day in prevernal and 147.7 μg-at P/m^2 · day in the vernal season. Since 21% of the aboveground P biomass originated from the substrate, the net loss from the substrate would be 8.93 and 31.01 μg-at P/m^2 · day during the prevernal and vernal season, respectively. These

values are much higher than the amount measured from the tracer data and this discrepancy will be discussed later. The detritus flux rate in the vernal season is 3% the Pi concentration in the water compartment. Nuphar luteum during the spring served as a sink for P that was significant proportion of the P in the water but only a minor fraction of P in the substrate. The increased flux of P from the belowground to the aboveground biomass compartments and the eventual decomposition of the aboveground compartment has enhanced the exchange of P from the substrate to the water compartment.

Summer

During the summer secretion from both submersed leaves and roots was significant and all the other rates of P movement increased (Fig. 20). The greatest increase in P cycling through Nuphar was P absorbed from the substrate by the roots and translocation to the aboveground biomass. The belowground structures provided 75% of the total inputs to the aboveground compartment which was nearly a 100-fold increase (0.969 μg-at P/m^2 · day in spring and 88.54 μg-at P/m^2 · day in summer). The amount of P absorbed by the roots that remained in the belowground biomass compartment was similar to the value for spring. The roots became very important to the nutrition of the total plant during the summer.

The net release from the substrate via acropetal translocation of 88.54 μg-at P/m^2 · day was only 4 x 10^{-3}% of the two fractions of P available to the plant in the substrate. The effect of Nuphar on P in the substrate compartment was small since this compartment is much more concentration in P than the water compartment. This value is 9% of the P storage quantity in the water.

During the summer, Nuphar was a source of P to the water compartment

via secretion at a daily rate of 71.20 μg-at P/m^2 · day. This was 80% of the net transport of P to the aboveground structures from the belowground compartment via translocation. This pumping activity represented 7.1% of the P content in the water compartment and at a constant rate would release the equivalent of the P content in water in 14 days. This P source would cause an increase in the water column of 0.07 μg-at P/liter per day in the vicinity of the plant bed. To the epiphyte communities associated with Nuphar beds, this could be an important source of P. Considering an aerial coverage of ca. 272,267 m^2 of Nuphar in the lower Chowan River, secretion releases 0.60 kg P/day in the river. Nearly half the aerial coverage of Nuphar is below Holiday Island (Fig. 4) and in this region of the river the secretion by plants would increase the P concentration in the water by only 1.5 x 10^{-5} μg-at/liter · day. This pathway would have an insiginificant effect on the P cycling in the Chowan River. But in low stress aquatic systems such as ponds, canals, and other impoundments where the density of Nuphar could be high, the secreting activity of this plant would have a definite effect on the P in the water during the summer.

The amount of P transported from aboveground biomass to the detritus from leaf turnover was 4662 μg-at P/m^2 in aestival, 6278 μg-at/m^2 in serotional, and 4687 μg-at/m^2 in autumnal for a total input during the summer and autumn of 15,630 μg-at P/m^2. This is 70% of the total detrial flux during the growing season (Table 14). Based on the daily biomass turnover rate of 0.032%/day, the rate of P from aboveground to detritus compartment was 146.2 μg-at P/m^2 · day. Considering 75% of the inputs to the aboveground biomass was from the substrate, the total release of P from the substrate during the aestival, serotinal, and autumnal seasons was 11,720 μg-at P/m^2 or an average of 109.7 μg-at P/m^2

Table 14. The total (μg-at/m^2) and daily flux (μg-at/m$^2 \cdot$ day) of phosphorus to the detritus compartment during each ecological season based on turnover rate of the biomass.

Season	Total Released	Daily Rate
Hibernal	1041.05	10.67
Prevernal	1515.31	42.54
Vernal	5260.97	147.68
Aestival	4661.84	130.86
Serotinal	6278.29	176.23
Autumnal	4686.86	131.56

˙ day. Adding the daily rate of secretion which also represents regeneration of P from the substrate, the net loss of P from the sediments during the summer via decomposition and secretion was 180.9 μg-at P/m^2 · day. Based on the aerial coverage of Nuphar in the lower Chowan River, this represents an input of 1.53 kg P/day to the river.

Nuphar luteum was very active during the summer and was a source of P to the water compartment whose origin was the substrate. Therefore it does play a mjaor role during the summer by regenerating P from the substrate to the overlying water via both decomposition and secretion. In most areas of the Chowan River, the P released from the aboveground structures by these processes was probably exported from the plant beds by wave activity and thus was unavailable for reuse by the plant community. In small ponds or in areas such as Keel Creek that are protected from the wind, more recycling may occur.

Comparison to Other Studies

Roots have also been determined the major absorbing organ in the submersed aquatic macrophytes Zostera marina (McRoy & Barsdate 1970), Myriophyllum brasiliense and M. spicatum (Bristow & Whitcombe 1971). Bristow and Whitcombe also studied translocation in two species of Myriophyllum and Elodea densa and found that most of the P in the shoots derived from acropetal translocation (over 95% in M. brasiliense, 57% in M. spicatum, and 74% in Elodea). The amount of P translocated to leaves and stems of Zostera marina from the substrate via root absorption was calculated from McRoy's data (1972) to be 70%. In this study, 75% of the P inputs (absorption and translocation) to the aboveground vegetation originated from the belowground structures via translocation during summer which decreased to 21% and 7% in spring and winter, respectively.

Nuphar luteum exhibited an active pumping system during the summer similar to that described in Zostera marina (McRoy et al 1972) and Spartina alterniflora (Reimold 1972). Zostera, a submersed marine macrophyte, released 2,012 µg-at P/m^2 · day into the Izembek Lagoon in Alaska, and Spartina, an emergent marine macrophyte, released 19,355 µg-at P/m^2 · day during the summer on the coast of Georgia. These rates are much higher than the 71.20 µg-at P/m^2 · day secreted by Nuphar during the summer. A better comparison of the secreting activity of these aquatic plants is by dry weight since secretion per m^2 is influenced by biomass which can vary greatly. Spartina secretion is still extremely high at 59 µg-at P/g dry wt · day (based on 79% water content), but Zostera secretes 2.85 µg-at P/g dry wt · day which is similar to but lower than the value for Nuphar (6.72 µg-at P/g dry wt · day using the 6.4 factor due to low P concentration in the field medium). These differences in secretion can be attributed to a number of factors including light and turbidity, water chemistry, temperature, water velocity and the physioligical characteristics of the plant. These same factors also influence productivity and distribution of aquatic plants (Westlake 1973) and it is interesting that the magnitude of difference in the productivity of marine emergent and submersed macrophyte communities and freshwater macrophyte communities is similar to their difference in secretion (P/m^2 · day). Also, secretion occurred only in the summer for both Spartina and Nuphar.

Seasonal trends in the absorption of P have also been shown for Justicia americana (Boyd 1969) and Spartina alterniflora (Reimold 1972). It is evident from this work on Nuphar that absorption, translocation, and secretion are affected by the seasonal metabolism of the plant. Nuphar and Spartina were most active in the summer months when productivity and biomass was at a maximum and the lowest activity was reported during the

hibernal season. However, Boyd measured the highest absorption rate of P and nitrogen by _Justicia_ during the early part of the growing season (early spring) before the period of rapid growth. He cites that maximum absorption of mineral nutrients before active growth is an advantage for aquatic plants in their competition with phytoplankton for nutrients in the water during the summer. Boyd's data on P dynamics in _Justicia_ was calculated by changes in biomass P between sampling periods. Since the absorption rates for _Nuphar_ and _Spartina_ were measured with radioactive tracers, the differences in conclusion may be in the method of measuring the rates. Biomass sampling does not yield detailed information on pathways of absorption, subsequent translocation and loss via secretion.

Evaluation of Tracer Studies

A large discrepancy results when comparing phosphorus accumulation data from tracer studies with data calculated from seasonal productivity rates. Differences between the inputs of P to the aboveground biomass compartment based on production (determined by multiplying turnover rates of biomass and mean P concentrations in the biomass for an ecological season) and the inputs of P into the aboveground compartment based on the tracer studies are difficult to resolve. The mean daily input into the aboveground compartment during growing season based on average P biomass of 3939 μg-at P/m^2 and a turnover rate of 0.032%/day was 125.8 μg-at P/m^2 $\cdot$ day. This ranged from a low during early spring at 42.54 μg-at P/m^2 $\cdot$ day to a high in late summer of 176.2 μg-at P/m^2 $\cdot$ day (Table 14). The highest daily inputs into the aboveground compartment from both translocation and absorption based on the tracer studies was during the summer at 17.34 μg-at P/m^2 $\cdot$ day. This is only 12% of the mean daily input into the aboveground compartment for the aestival, serotinal, and autumnal

seasons. McRoy et al. (1972) also found his tracer data to be under-estimates compared to the daily flux of P through a m^2 of Zostera marina based on productivity but by a smaller margin (tracer data was 43% of productivity data).

The values for P cycling through Nuphar based on tracer studies compared to the values based on the productivity of this plant indicates the tracer values are a gross underestimate. Possible sources of error are damage to the active absorption areas of the root by excavation, change in internal pressure by cutting the rhizome, and change in the specific activity of the isotope as it moved through the plant. These factors suggest underestimates in acropetal movement of P. However, the possible underestimates of P secreted from submersed leaves have little to do with this discrepancy. Errors in the opposite direction, i.e., basipetal translocation, are believed to be minimal since leaves and petioles were not noticeably damaged during the experiments. Since the tracer studies resulted in much lower estimates of P cycling in Nuphar than a very similar technique on Zostera the reason may be in some inherent difference between structure and/or physiology.

The Mobility of Phosphorus

One of the critical assumptions underlying the design of the laboratory and field tracer experiments, and in interpretation of the results, is that the phosphorus largely remains in a highly mobile form after absorption. An alternate hypothesis is that the absorbed phosphorus is first converted to a storage product and redistributed (translocated) throughout the plant at some later time. This proposed "lag", if longer than 24 hr, could result in a misinterpretation of the experiments reported here.

There is some evidence for P storage and later redistribution throughout the plants. The observation during the prevernal season that the P concentration in the rhizome decreased when the demand in the aboveground structures was high indicates a flux of stored P to the aboveground structures from the rhizome (Fig. 8). Smith et al. (1976) found that 36% of the P inputs to the aboveground structures of Typha during the increased demand in spring was supplied from P in the rhizome which was stored during the winter. The forms that make up stored P in the plant include lipid-P and ester-P. Bieleski (1973) describes phytic acid, a phosphate ester of myo-inositol, as a major form of P found in storage pools of plants. It has been isolated in tubers such as potato (Samotus & Schwinner 1962) and in a few aquatic plants (Roberts & Loewas 1968).

The 24-hr tracer studies may have measured only the daily flux of mobile P through the Nupher transport system that had been absorbed from the external environment. Therefore, the redistribution mechanism via hydrolysis of P storage products such as phytic acid stored in the rhizome may be the reason for the discrepancy between the tracer and productivity measurements of P input to aboveground biomass. This could explain the difference in Zostera and Nuphar in the ability of the tracer study to measure P cycling in the plant since Zostera does not have as extensive a rhizome (storage) as Nuphar and therefore this mechanism may not be as significant.

Conclusion

This research has demonstrated that phosphorus simultaneously moves acropetally and basipetally in <u>Nuphar</u> <u>luteum</u>. The dominant pathway of P is from the belowground to the aboveground structures and the quantity of this net flux varies with the seasons of the year (Fig. 20). During winter the acropetal flux of P was insignificant compared to the P content of the aboveground structures. This pathway increased 30-fold (to 0.97 μg-at/m^2 · day) in the spring. But in the summer the flux of P from belowground to aboveground structures was 88.54 μg-at/m^2 · day which was much higher than values for winter or spring. During the winter, spring, and summer, the P transported from sediment to aboveground structure via root absorption and translocation was later released to the water by decomposition. But during summer, P from the substrates via the same pathway was also released by secretion from submersed leaves at a rate of 71.20 μg-at P/m^2 · day. The significance of these results is that <u>Nuphar</u> functions as a nutrient pump resulting in a net flux of P from belowground to aboveground structures which represents a pathway for the regeneration of P from the substrate to the overlying water.

A major question developed from this research resulted from the discrepancy between measurement of P movement utilizing radioactive tracers and the values obtained from field studies of leaf production. The problem is that radioactive tracer estimates of P inputs by absorption and translocation into the aboveground biomass are only about 12% of the values calculated by biomass production and P concentrations. A possible explanation is that the bulk of the P absorbed by the roots may

be converted to some storage produce of P which later becomes the source of P via redistribution to developing sinks such as production of floating and submersed leaves during the growing season. A 24-hr tracer experiment of the design used in this study will measure the movement of very mobile P in the transport system (xylem and phloem), but the time is too short to measure the redistribution movement of P because of the lag time from absorption to storage to translocation. In some aquatic plants where storage of absorbed P and subsequent release upon the formation of sinks is significant, this type of experimental design is inadequate in determining the absolute flux of P through the plant. Such was the case for Nuphar luteum.

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APPENDIX

Table A. Phosphorus concentration in each structure of <u>Nuphar</u>
<u>luteum</u> at Keel Creek during the 1974 sampling season.

			Structures		
Dates	Floating Leaves	Submersed Leaves	Petioles	Roots	Rhizomes
12 Jul '74					
μg-at/g OW	149.72	162.73	128.11	241.07	202.07
μg-at/m^2	464.25	1768.19	1551.46	2265.29	25,404.93
13 Aug '74					
μg-at/g OW	162.86	153.34	150.33	342.18	222.63
μg-at/m^2	497.13	1479.12	1109.92	2956.95	6,070.45
18 Sep '74					
μg-at/g OW		189.88	143.66	270.81	334.21
μg-at/m^2		1861.62	698.66	1001.74	10,685.95
16 Oct '74					
μg-at/g OW	170.82	167.50	141.37	279.39	105.82
μg-at/m^2	1077.29	822.19	1177.03	1111.75	4,405.96
20 Nov '74					
μg-at/g OW		188.20	226.57	242.03	322.21
μg-at/m^2		907.81	534.89	1306.44	17,719.92

Table B. Phosphorus concentration in each structure of Nuphar luteum at Keel Creek during the 1975 sampling season.

Dates	Structures				
	Floating Leaves	Submersed Leaves	Petioles	Roots	Rhizomes
3 May '75					
μg-at/g$_2$ OW	255.20	250.17	216.22	269.21	118.90
μg-at/m^2		906.87	372.38	1203.82	6,213.30
4 Jun '75					
μg-at/g$_2$ OW	155.64	161.11	136.61	200.65	123.76
μg-at/m^2	620.97	802.40	719.89	862.20	8,440.21
2 Jul '75					
μg-at/g$_2$ OW	132.85	135.23	118.23	124.01	188.36
μg-at/m^2	1331.79	652.25	1695.26	663.56	12,582.95
30 Jul '75					
μg-at/g$_2$ OW	169.39	157.58	126.67	138.57	103.48
μg-at/m^2	1844.87	776.90	2682.18	787.79	4,126.02
27 Aug '75					
μg-at/g$_2$ OW	131.76	175.35	222.92	165.84	86.28
μg-at/m^2					

Table C. Phosphorus concentration in each structure of <u>Nuphar</u>
<u>luteum</u> at Indian Creek.

Dates	Structures				
	Floating Leaves	Submersed Leaves	Petioles	Roots	Rhizomes
17 Jul '74					
μg-at/g$_2$OW	113.94	129.03	78.52	64.36	70.11
μg-at/m^2	2653.00	1997.84	2678.36	1141.03	11,629.73
22 Aug '74					
μg-at/g$_2$OW		142.34	113.97	60.87	71.72
μg-at/m^2		2837.43	1650.13	1995.37	14,548.95
25 Sep '74					
μg-at/g$_2$OW	137.89	141.45	89.50	93.03	85.50
μg-at/m^2	2608.73	·1490.92	2660.65	2347.29	12,481.96
23 Oct '74					
μg-at/g$_2$OW		167.84	127.25	70.20	69.55
μg-at/m^2		2868.54	948.08	3552.22	18,878.99

Table D. Phosphorus concentration in each structure of <u>Nuphar</u>
<u>luteum</u> at Wiccacon Creek.

Dates	Structures				
	Floating Leaves	Submersed Leaves	Petioles	Roots	Rhizomes
10 May '75					
μg-at/g OW	311.05	213.62	224.69	121.88	135.18
μg-at/m^2	206.01	2216.73	1448.10	4039.16	21,539.35
10 Jun '75					
μg-at/g OW	154.37	135.18	102.21	112.15	79.85
μg-at/m^2	4424.36	1819.23	2582.46	3392.34	9,861.21
9 Jul '75					
μg-at/g OW	132.50	141.11	110.27	92.44	176.90
μg-at/m^2	2333.56	1493.39	2151.64	3608.79	23,859.90
6 Aug '75					
μg-at/g OW	134.38	154.03	90.16	90.05	59.54
μg-at/m^2	2836.67	2431.77	2593.48	2647.55	7,501.65
3 Sep '75					
μg-at/g OW	133.02	168.54	106.17	91.63	107.53
μg-at/m^2	4717.38	1675.51	4801.30	3224.76	14,515.47

Table E. Phosphorus concentration in each structure of <u>Nuphar</u> <u>luteum</u> at Rockyhock Creek.

		Structures			
Dates	Floating Leaves	Submersed Leaves	Petioles	Roots	Rhizomes
26 Jan '75					
μg-at/g OW		196.85	196.85	130.72	166.81
μg-at/m^2		162.83	509.29	2647.53	36,071.43
5 Apr '75					
μg-at/g OW		236.49	225.11	91.44	147.90
μg-at/m^2		343.82	270.35	1735.53	4,464.34
28 May '75					
μg-at/g OW	162.01	131.63	108.91	132.68	113.36
μg-at/m^2	1300.64	672.22	897.42	2461.92	10,075.50
25 Jun '75					
μg-at/g OW	150.01	108.75	92.38	125.43	156.88
μg-at/m^2	1065.05	176.64	697.09	1424.56	7,410.45
23 Jul '75					
μg-at/g OW		149.22	122.66	145.66	101.73
μg-at/g^2		185.64	52.74	1693.55	5,197.43
20 Aug '75					
μg-at/g OW	167.81	125.56	104.92	77.61	
μg-at/m^2	1833.34	509.53	1445.44	1640.90	

EPILOGUE

It is the purpose of this epilogue to discuss suggestions for developing nutrient budgets on aquatic plant communities that have evolved from my work on phosphorus and _Nuphar luteum_. Extreme caution should be used when measuring the rate of nutrient flux through plant communities with isotopic tracers. Incubation periods should be long enough for the isotope to reach equilibrium in the plant's organs which in structures such as rhizomes in _Nuphar_ may take an entire growing season, especially if the pathway hypothesized in this thesis occurs. Incubation periods with intervals of weeks instead of days should be considered.

I still feel the technique in which isotopes are injected into sediments to measure uptake rates of roots are inadequate because of the poor dispersion of the isotope. A proposed method is that the roots and rhizomes are excavated, placed into chambers as described in this thesis and afterwards this chamber is filled with sediment that has been mixed with the isotope. This would alleviate some of the problems discussed in this thesis with the excavation of belowground organs.

Tracer studies along cannot adequately measure the cycling of an element in a plant community. In attempts to determine the above, there should be a control based on biomass productivity measurements.

The lack of detailed information on the fractions of phosphorus in _Nuphar_ emphasized the importance of such measurements of plant nutrients for a better understanding of their relationship to the nutrition of the plant. As in chemical analysis of water, sediment, etc., the seasonal measurement of the individual fractions is more descriptive than just the total amount. Concerning phosphorus, a most important consideration should be the quantity and location of phytic acid accumulation.