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Distribution, Growth, and Phosphorus Relationships of Water Milfoil in Lake Winnipesaukee, New Hampshire

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COMPLETION REPORT

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ACKNOWLEDGEMENTS

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ABSTRACT

Myriophyllum heterophyllum Michx. has been described as the
dominant aquatic vascular plant in the littoral of Lake Winnipesaukee,
N.H. Its distribution, throughout the lake, was mapped and its
habitat preferences were noted. The plant is present in areas with
a silt-sand substrate and low wave action at depths of 0.5 to 3 m.
The density of the stands was determined at three sites. Rapid
colonization and stand expansion occurred through vegetative re-
production. Tissue phosphorus levels of M. heterophyllum varied
seasonally during 1975-1976 and reached a maximum of 0.75% dry
weight during late May. A correlation between tissue phosphorus
and sediment phosphorus was observed for the three study sites.
In situ phosphorus enrichment of the water produced a marked increase
in tissue phosphorus of M. heterophyllum. Growth of the plants
occurred from April through October with the maximum growth rate
(June through August) at more than 1.5 cm/day. In batch culture
tissue phosphorus levels of shoot apices are proportional to phos-
phorus levels in the medium. Luxury consumption (Gerloff, 1975)
of phosphorus was evident. Two uptake mechanisms appeared to
function in phosphorus uptake. In culture both uptake and growth
were affected by temperature, while growth rate was not affected
by the phosphorus concentration in the medium. Phosphorus was
released from decaying plants.

INTRODUCTION

Aquatic vascular plants have been shown to be important

components of the nutrient cycles of shallow lakes and ponds or

the littoral zone of deeper lakes when they are abundant. Rooted

aquatic vascular plants such as Myriophyllum or Zostera are capable

of removing nutrients from the water and sediments and of releasing

them back into the water while living or decaying (McRoy and Barsdate,

1970; Bristow and Whitcombe, 1971; Jewell, 1971; DeMarte and Hartman,

1974; and Nichols and Keeney, 1976). The plants thus act as

nutrient "pumps" in the manner described for Myriophyllum exalbescens

by DeMarte and Hartman (1974):

> Aquatic plants can play an important role in
> recycling nutrient elements in aquatic systems, not only
> when they die and decompose, but also during periods
> of active growth. Available evidence indicates that
> Myriophyllum exalbescens returns nutrients to the water
> along a substrate-root-stem-leaf pathway. The substratum
> of fresh-water ecosystems can be a major source of
> inorganic nutrients to submersed vascular hydrophytes.
> These plants not only have a passive role as a potential
> pool of nutrient elements available to the water phase
> upon decomposition but they can also serve an active
> role in moving elements from the substratum to the
> surrounding water.

The rampant growth and rapid spread of Myriophyllum heterophyllum

(water milfoil) throughout Lake Winnipesaukee have made the plant

dominant in the littoral zone of many areas of the lake. The abundance

of water milfoil probably exerts a strong influence upon the nutrient

cycles in the areas where it grows. Phosphorus, considered a limiting

nutrient in many freshwater systems (Wetzel, 1976), may be cycled by

M. heterophyllum in Lake Winnipesaukee in much the same manner as

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described for M. exalbescens.

M. heterophyllum (broadleaf water milfoil) is a species native to North America. It is found in ponds, quiet streams, and ditches of many states from the plains to the Atlantic coast as well as in Mexico and Ontario and Quebec, Canada (Muenscher, 1944). In New England M. heterophyllum has been reported in three states: Rhode Island, Massachusetts, and New Hampshire. Within these states its distribution appears to be limited. It is possible that the species is outside of its natural range in New England. In New Hampshire no stations other than Lake Winnipesaukee have been reported for the plant. This apparently limited distribution may be due to the plant's recent introduction into the state, a lack of thorough collecting of aquatic vascular plants in New Hampshire, or to a scarcity of appropriate environments.

Heavy growth of M. heterophyllum in a pond in Massachusetts has been reported by Colt and Hellquist (1974). The plant occupies about one third of the western sector of the pond where it is dominant. No other vascular plants grow in or near the stand. According to Barre Hellquist M. heterophyllum is present in other Massachusetts ponds in such great abundance.

M. heterophyllum behaves as a weed in Lake Winnipesaukee. Curtis (1959) states that the term "weed" has many definitions, but continues to describe the characteristics of weedy plants:

> Plants which can invade ruderal or cultivated areas have certain characteristics in common. They are very vigorous, in the sense that they are of rapid growth; they have the ability to withstand and surmount high intraspecific competition; they show great tolerance of soil disturbance, partial defoliation, or other regressive influences; and generally they possess a high reproductive potential. Accompanying this vigor is a

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great genetic variability of both morphological and
ecological characters. Many of the species have efficient
means of spread and may invade in large numbers . . .

The behavior of M. heterophyllum in Lake Winnipesaukee conforms
to this description in many ways.

The rapid spread and growth of M. heterophyllum throughout
the lake is similar to the behavior of the European species
Myriophyllum spicatum that was introduced into Chesapeake Bay during
the early 1900's and became a nuisance species by the 1950's
(Coffey and Mc Nabb, 1974). The plant reached Michigan by the
early 1960's. M. spicatum reached Budd Lake, Clare County, Michigan
at that time and during the next decade increased in density
until in 1972 the population had "exploded" to cover one quarter
of the surface of the 70 ha lake. Problems with M. spicatum in
Currituck Sound, North Carolina began in 1965 when about 40 ha
were infested with the plant and another 200 to 400 ha were colonized.
By 1966 M. spicatum had infested 3200 ha and become established
in an additional 2680 ha (Crowel et al., 1967).

It is difficult to draw a distinction between a natural and
an introduced population of aquatic vascular plants and, in some
cases, it may be impossible. Many aquatic plants can be introduced
by man, or "naturally", through forces such as high winds, floods,
or animals. M. heterophyllum may have been introduced into Lake
Winnipesaukee by waterfowl or man.

M. heterophyllum seeds or plant fragments may have reached
Lake Winnipesaukee from more southern areas. Colonization may have
been unsuccessful (not occurring at all or only on a very limited

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scale) for many years until lake conditions changed sufficiently to
support growth of the species. Therefore, M. heterophyllum could
have been absent from the lake or present in only very small numbers
until, over the years, natural and/or manmade changes produced an
environment (including light, temperature, nutrients, substrate,
lack of competition, etc.) that suited the plant. The proper environ-
ment allowed for rapid growth and colonization of the plant and
produced the "weed problem" that exists today. Manmade changes in
the environment such as dredging have been suggested as factors
promoting the growth of M. spicatum in Lake Wingra through the creation
of an environment suitable to the plant (Nichols and Mori, 1971).
It is possible that such disturbances in Lake Winnipesaukee have
produced conditions favoring the growth of water milfoil.

The spatial relationships between M. heterophyllum and other
aquatic macrophytes may indicate its ability to compete with other
plants. M. heterophyllum is most often present in Lake Winnipesaukee
as dense, nearly unispecific stands. In areas where there is an
abundance of other aquatic vascular plants M. heterophyllum grows
mixed with these plants. It does not grow as vigorously in mixed
stands as it does in pure stands. The fact that M. heterophyllum
grows alone in areas of the lake that would support growth of other
species as well might indicate that water milfoil is able to out-compete
other species. M. spicatum has caused the disappearance of other plant
species such as Valisneria and Potamogeton in Lake Wingra, Wisconsin
(Nichols and Mori, 1971) and has been described as a very aggressive
plant able to restrict other species (Nichols and Keeney, 1976). Coffey
and McNabb (1974) found that M. spicatum was able to overgrow submersed

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native species because it was able to reach the surface (and therefore more light) when it was rooted at 3 to 5 m. The same explanation probably holds for M. heterophyllum in Lake Winnipesaukee. As it becomes established with other species such as Sagittaria graminea, a short rosette plant, it begins to shade them. The shorter plants would then die from lack of light, leaving a unispecific stand of water milfoil.

M. heterophyllum is a submerged, rooted hydrophyte of the family Haloragaceae. The species possesses finely divided (pinnatisect) leaves arranged alternately or in whorls along a seldom-branched stem. Its perfect or imperfect flowers are in the axils of small, serrate leaves along a thickened section of stem that rises above the surface of the water.

Many of the 13 species of Myriophyllum in North America are difficult to distinguish from one another vegetatively. Many keys to aquatic vascular plants provide excellent means of separating the species sexually, but almost no information on how to separate the species vegetatively. One species easily confused with M. heterophyllum is Myriophyllum verticillatum, a species common in New England. The production of winter buds by M. verticillatum, as well as its smaller size and finer stem, are characters that can sometimes be used to distinguish the two species vegetatively. Sexual material is necessary for accurate identification of the two species. M. spicatum var. spicatum and M. spicatum var. exalbescens are two varieties of a species that is a noxious weed in many areas of the country. The species (both varieties) is readily distinguished from M. heterophyllum by its distinctly whorled leaves (usually in fours), its leaf shape, and its

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long internodes. M. spicatum is uncommon in New England.

This study was designed to assess the influence of M. heterophyllum on the phosphorus cycle in the littoral zone of Lake Winnipesaukee and to determine the extent of the plant's growth in the lake. Batch cultures were used to study phosphorus uptake by M. heterophyllum shoots under a variety of conditions. Levels of phosphorus in plant tissue and sediments were monitored seasonally to observe changes in amounts present and to determine whether any correlation between the two exists. The density and growth rates of the plants were determined to estimate biomass changes and rates of stand expansion. Locations of plant stands throughout the lake were mapped. Environment preference of the plants was determined to examine colonization rates and to note areas with a potential for water milfoil growth.

DESCRIPTION OF STUDY SITE

Lake Winnipesaukee is the largest lake in the state of New Hampshire. Its area, 18044.1 ha (44586.3 A), covers sections of Belknap and Carroll counties. Lake Winnipesaukee is oriented in a northwest and southeast direction and is 27.2 km (16.9 mi) long by 12.1 km (7.5 mi) wide. Its shoreline extends nearly 386 km (240 mi). The maximum depth of the lake is 51.2 m (168 ft) and its mean depth is 18.3 m (60 ft). In past years Winnipesaukee has been considered a clean, relatively unpolluted lake in spite of extensive development along the shoreline (72%).

Lake Winnipesaukee is an important recreational area in New Hampshire. Its shoreline varies from wooded lots to summer cottages with lawns extending to the lake shore. The primary industry of the many towns in the region is tourism. The lake is used for fishing, swimming, and boating during the summer and for ice fishing, snowmobiling, and skating during the winter as well as for a water supply for the city of Laconia and some of the summer homes. The combination of heavy use by many people and a growing environmental awareness in some has created concern for the future of this lake. The "crystal" clear water and sandy shorelines have begun to disappear and large stands of water milfoil have begun to carpet the shoreline, making recreational use of the affected areas difficult and almost impossible in some cases.

Circumnavigation of the lake by boat and observation of the stands from the lake surface were used to locate and map the Myriophyllum heterophyllum populations of Lake Winnipesaukee. Myriophyllum species

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are easily seen and recognized from the surface by an experienced observer since they grow in shallow water (usually less than 4 m deep) and are bright green in color. Substrate type (e.g. mud, sand, silt, rock, or cobble) was characterized for each location of M. heterophyllum by sight and dredging. The exposure of the area to wind and wave action was also noted along with the relative degree of shoreline disturbance (e.g. presence of roads, cottages, marinas, etc.).

Three primary study sites were chosen from the plant observations in Lake Winnipesaukee. Choice was based on population density, practicality for use of SCUBA, location, and accessibility. The sites were in Alton Bay, Smith Cove, and Green's Basin.

The Alton Bay site was chosen for its dense stand of M. heterophyllum along the Alton Town beach. Alton Bay proper lies within the town of Alton, at the southernmost portion of Lake Winnipesaukee. The section of the bay studied was the southeastern shore of the bay along the Alton Town beach from Harmony Park to Downing's boat ramp. This section is approximately 0.45 ha in area. The extensive stand of M. heterophyllum covered the southern end of the bay as well as all of Parker's Marina, a total area of approximately three times that studied. The maximum depth in this area is about 4 m. The substrate type is normally silt and sand, but is mixed with sawdust in the deeper areas. The sawdust washed into the bay from sawmills once located along the Merrymeeting River.

Alton Bay water is tea-colored, especially during February through May, from the input of the Merrymeeting River. The river enters the bay at the southern tip. It passes through Parker's Marina before entering the beach area. The bay is very long and narrow and receives

northerly winds. Wave action is low to moderate and moves in a north-south direction.

Alton Bay is the first part of the lake reached by tourists driving up from the south. Lake use is heavy, particularly from recreational boating in summer and snowmobiling in winter. The shoreline of this study area is nearly 100% developed with one marina, a public beach, commercial buildings, and summer homes. Major roads flank both shores.

The Smith Cove study site was on the west side of the lake in the town of Gilford. The cove is connected to the main lake by a shallow channel. Because of its protected nature, the cove is exposed to little wave action. Its maximum depth is approximately 6 m. The substrate of the cove is a sand and silt mixture in shallow regions that grades into organic mud (gyttja) in deeper areas. The water is somewhat turbid, the degree dependent on boat traffic (Baker, 1973). An inlet, Lazy Meadow Brook, enters the cove on its north shore.

M. heterophyllum stands are distributed throughout the cove at Ostrand's Marina, Lazy Meadow Brook, Glendale Dock, and Fay's Marina. The stand chosen for the quadrat study was along Ostrand's Marina. The M. heterophyllum population was sparse the first year and was chosen since its expansion could be easily monitored. Maximum depth at this site is approximately 3.5 m. The substrate is sand and silt.

The shoreline of Smith Cove is very heavily developed. Three marinas and many cottages line the shore of the cove. Concern has been expressed by Smith Cove residents about the heavy use of the cove and subsequent decline in water quality.

The Green's Basin study site was at the northernmost end of Lake Winnipesaukee. The Basin is in a wooded, relatively unpopulated area

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of the watershed. It covers an area of 43.4 ha. $\underline{M}$. heterophyllum
is present in varying density throughout much of this area. Two $\underline{M}$.
heterophyllum stands were chosen for study within Green's Basin. One
stand is along a beach belonging to the Cruner family on the western
shore of the basin and is called Green's Basin Beach in this study.
The site has a sand and silt substrate in shallow areas and a gyttja
bottom in deeper areas. The section of the $\underline{M}$. heterophyllum stand
studied was 0.24 ha in area. Lake use in this area is light. The
shoreline is disturbed only slightly with fewer than eight summer
cottages set back from the shore.

The other stand, called Green's Basin Marsh, is at the north end
of the basin. The area is filled with dense stands of $\underline{M}$. heterophyllum
and other aquatic macrophytes. The substrate is organic mud and sand.
Maximum depth is approximately 2 m. Wave action is negligible. The
shoreline consists of woods and Typha latifolia marsh. There are no
summer homes in this area.

During the course of the study the $\underline{M}$. heterophyllum populations
at two ot the primary sites, Green's Basin Beach and Alton Bay Beach,
were killed with application of the herbicide Silvex (2,4,5-trichloro-
phenoxypropionic acid or 2,4,5-TP) during June 1975 and July 1976,
respectively, by private environmental consulting firms. Dieback,
regrowth, and phosphorus levels of the plants were monitored following
herbicide application.

MATERIALS AND METHODS

Phosphorus Analysis

The procedure for phosphorus analysis of Myriophyllum heterophyllum tissue was used both for the samples collected in the field and for the samples taken from the batch cultures. The upper 5 cm of a M. heterophyllum shoot, including the apical section (apical meristem), was chosen for analysis as the indicator of the tissue phosphorus level of the plant. This section of the plant has been found to be the most metabolically active and to exhibit the most uniform nutrient levels from plant to plant because the apices are quite similar in age (Gerloff, 1973). The 5 cm sections (apices) were rinsed under a stream of tap water to remove detritus and loosely attached fauna and algae. Microscopic examination of the apices showed a very small population of attached epiphytes, so a thorough scrubbing of the apices was unnecessary and in fact may have injured the apices. Rough treatment of the apices can cause leaf breakage, loss of tissue, and possible loss of phosphorus from the injured portions of the plant. After rinsing, the apices were shaken to remove excess water. Five apices constituted a single observation. The observations were analyzed in triplicate (one sample).

The apices were placed in porcelain evaporating dishes or aluminum foil pans, dried for a minimum of 24 hours at 105 C in a forced-draft oven, and ashed in porcelain crucibles in a muffle furnace for one-half hour at 250 C and then for a minimum of seven hours at 550 C.

Phosphorus was extracted by boiling the ash in 5% hydrochloric acid.

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An aliquot of the digest was analyzed for total phosphorus with the ammonium molybdate-ascorbic acid method modified from Golterman (1969). The absorbance of the solution was read at 650 nm on a Fisher Electro-photometer II. Absorbance was converted to parts per billion phosphorus with a standard curve. From the phosphorus concentration in the sample the total phosphorus in the tissue as percent dry weight was calculated. Sediment phosphorus was analyzed in a similar manner.

Plant and Sediment Collection

Plant apices were collected on a weekly basis during the summer of 1975 and monthly thereafter (through October 1976) from each of the primary study sites and at other locations throughout the lake. Collection of apices was random at each site, unless it was being done along a transect line. Apices were harvested at a depth of 1 to 2 m with the use of SCUBA or a potato hook (garden cultivator) from the surface. The apices were placed in plastic bags along with some lake water for transportation back to the lab. The samples were refrigerated for a maximum of 24 hours until they could be prepared for analysis.

Plant and sediment samples were collected at intervals along the transect lines and within the quadrats for phosphorus analysis. Apices were collected by the methods described previously. In 1975, surficial sediment samples (0 to 3 cm) were scooped into 100 ml glass jars. Sediment traps were used during 1976 for collection of contemporaneous sediment. The traps consisted of bottomless plastic jugs inverted and attached to a stake 0.5 m above the sediment surface.

Density and Growth Measurements

Plant density and distribution were determined with the use of line transects or quadrats at the three primary sites. At Alton Bay Beach and Green's Basin Beach line transect studies similar to those of Schmid (1965) were implemented in sections of the Myriophyllum heterophyllum stands. Thirty-meter plastic-coated lines marked at 1 m intervals were stretched out at right angles from the shore into the plant stand. Each transect line was anchored at both ends with wire stakes. Transects were placed 10 m apart along the shore. Plants intersecting the line were counted by a diver and totaled for each meter segment.

A 10 X 10 m quadrat was constructed along the lake bottom at Ostrand's Marina. Nylon cord and short wire stakes were used to mark the quadrat which was divided into nine 3.3 X 3.3 m sections. Plants were counted and mapped within each quadrat. This method was chosen over line transects for Ostrand's Marina because plant density was sufficiently low to enable counting and mapping of individual plants.

Growth rates were measured with a tagging procedure. During the summer of 1975 plants along the transect lines at Alton Bay Beach and Green's Basin Beach were tagged at the base of a single shoot with colored plastic tape. Length of the shoot was measured from the base of the plant to its apex. Many of the tagged plants were lost during the growing season because the tags seemed to cause the shoots to break off. Another tagging method was implemented during the 1976 growing season in Alton Bay Beach and Ostrand's Marina. Single shoots of ind-ividual plants were marked with nylon monofilament tied 5 cm from the shoot apex. The filament was tagged with a styrofoam disk 2 cm in

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diameter. The disks floated making the marked shoots easily visible under water. The shoot was measured from the point of filament attachment to its apex. This method of tagging did not cause shoot breakage.

In Situ Phosphorus Enrichment

A phosphorus enrichment experiment (Fig. 1) was performed to test the effects of in situ phosphorus loading (into the water) on Myriophyllum heterophyllum tissue phosphorus levels. The study site chosen was in Green's Basin marsh because of its high density of M. heterophyllum, shallow water, and its protected and isolated nature. Plants were "enriched" with a high concentration of phosphorus (1 g P/l) as KH_2PO_4. The phosphorus solution was placed in three 19 l (5 gal) carboys on shore. Tygon tubing (0.79 mm i.d.) was run from each carboy through aluminum conduit (for protection) to a stake placed among the plants. The stakes were placed about 3 m apart. The phosphorus solutions flowed from the carboys into the water surrounding the plants at a rate that varied from 0.22 to 0.58 l of solution per day (0.22 to 0.58 g phosphorus per day) at each stake. Problems arose with bacterial blooms that clogged the tubing and slowed down or stopped the flow periodically. Flow was restarted on each collection date if necessary. Apices were collected within a 0.5 m radius of each stake prior to enrichment and at regular intervals thereafter for phosphorus analysis.

Batch Cultures

Apices grown in batch culture were placed in a volume of nutrient medium at the start of the culture. The nutrient supply was not renewed during the course of the culture period. This type of culture method differs from the chemostat culture in which the nutrient medium is constantly renewed.

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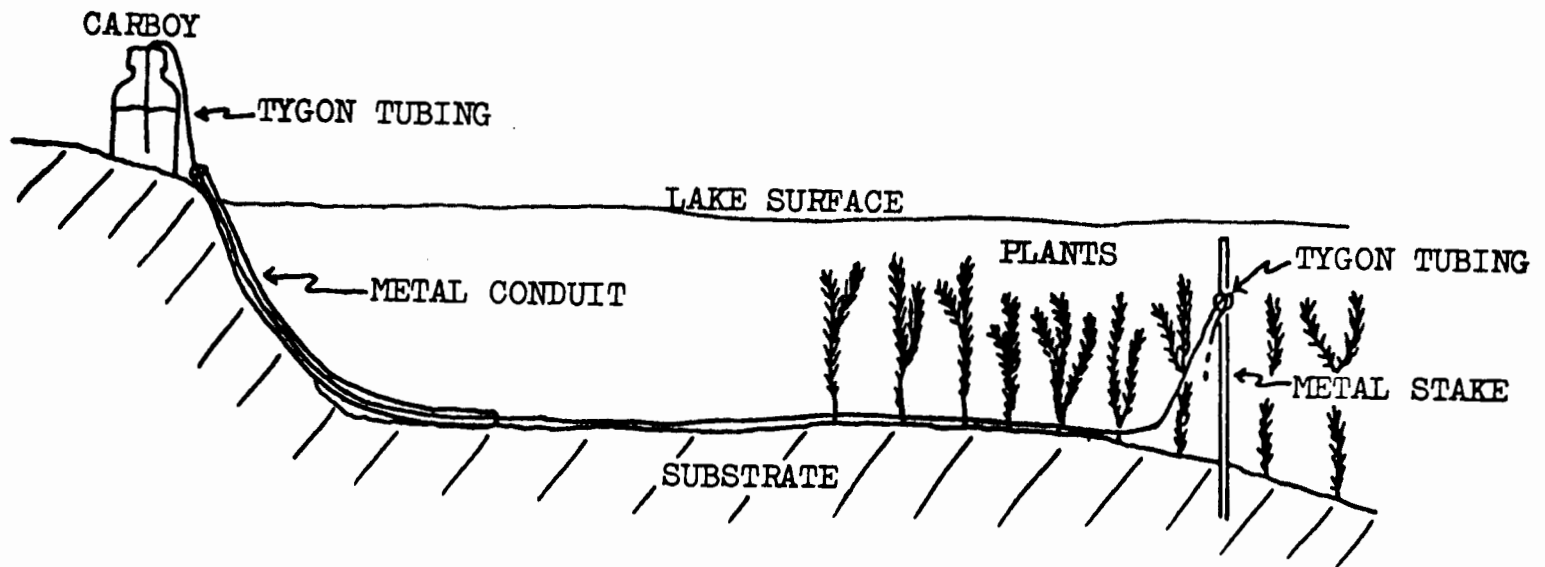
Figure 1.   Diagram of the in situ phosphorus enrichment setup in Green's Basin Marsh.

Apices used in batch culture were collected from Lake Winnipesaukee. They were grown for one week or less at room temperature in aquaria with tap water and modified Hoagland's solution minus phosphorus. Continuous light was supplied by 40 Watt cool-white fluorescent bulbs 15 to 20 cm away from the side of the aquaria. Gerloff (1973) observed no differences in growth or nutrient uptake rates in plants subject to constant illumination or a light-dark cycle. Axenic cultures were not maintained due to the difficulty and complexity of preparation (Wilson, 1972) and the need for large amounts of plant material. Any study of the natural system or an extension of that system into the laboratory would necessarily include attached algae and bacteria. As mentioned previously, the rinsed 5 cm apices usually hosted only a very small population of epiphytes.

When prepared for culture, the apices were cut from the shoots into 5 cm lengths and their apical (meristematic) portion was pinched off, unless growth was to be studied. The removal of the meristem prevented new growth of any significance for the duration of the cultures. Five apices were placed in a 500 ml Erlenmeyer flask filled with 500 ml of modified Hoagland's solution with varying levels of phosphorus. The flasks were capped with foam stoppers to retard evaporation and placed in a culture chamber. Temperature in the chamber was held at 15 C unless otherwise noted. The flasks were not aerated. All batch cultures were run in groups of three or five.

After culture the apices were removed from the flasks, rinsed, and prepared for phosphorus analysis. In Batch 1 phosphorus levels in the medium were measured using 100 ml of medium in place of the 100 ml of distilled water and the tissue digest aliquot.

Batch cultures were used for these short-term studies because they are easy to set up and maintain. Chemostat cultures (similar to those used with algae), involving constant input of fresh nutrient medium and outflow of old medium, were attempted because they are more analogous to the natural system, but algal blooms occurred throughout the systems and were difficult to control. Successful chemostat cultures would necessarily involve the use of an axenic system because any bacteria or algae could create bloom conditions and alter nutrient uptake results or outcompete the macrophyte being cultured. Because of time constraint batch cultures were used instead of chemostat cultures.

RESULTS

Myriophyllum Stand Locations

Many Myriophyllum heterophyllum populations were observed during
the shoreline survey of the lake. The entire lake has not been surveyed
to date and it is expected that more stands of M. heterophyllum exist
than have been described. M. heterophyllum was the only Myriophyllum
species observed during the study period.

M. heterophyllum is present in areas of the lake characterized
by silt and sand substrates, shallow depths (less than 4 m), and
protection from heavy wind and wave action. Areas with rocky, sandy,
or steeply sloping bottoms are usually devoid of M. heterophyllum as
are areas of great turbulence. The degree of development along the
shoreline seems to have little effect on the presence or absence of water
milfoil. Streams entering the lake frequently contain M. heterophyllum
stands. Many of the marinas on the lake are filled with dense stands
of the plant.

M. heterophyllum was also found in a pond (Blackadar Pond) that
had been formed by a dammed stream in Alton, New Hampshire off Route 28.
A report was also received of its presence in a farm pond in Alton.

Many residents of Lake Winnipesaukee claim that water milfoil
has spread rapidly throughout the lake during the past five years.
They state that prior to this time the plant was not present in the
lake or present only in small amounts. The ability of the plant to
migrate and colonize new areas was confirmed by our observations. M.
heterophyllum populations in a small stream at Lee's Mills, Robert's
Cove Marina, and Ostrand's Marina were nonexistent or very sparse during

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the summer of 1975. By the end of the summer of 1976 all three of these populations became much greater in density. The increase in density at Ostrand's Marina was quantified by quadrat mapping of individual plants. Plant cover increased from less than 25% cover in 1975 to 75 to 90% cover in 1976.

Aquatic Macrophytes and Algal Epiphytes

Myriophyllum heterophyllum is not always present in areas having populations of other aquatic macrophytes, but it is frequently associated with other genera. In Lake Winnipesaukee M. heterophyllum is usually present in dense, almost unispecific stands with scattered occurrences of Chara spp., Nitella spp., Potamogeton amplifolius, Sagittaria graminea, or Utricularia purpurea for example. These other species occupy patches within the M. heterophyllum stand that are not colonized by water milfoil. In other areas, such as parts of Green's Basin Marsh, M. heterophyllum grows mixed with such species as Nymphaea odorata or Nuphar lutea. In these mixed stands growth of M. heterophyllum is sparse and less vigorous than that in the unispecific stands.

The finely dissected leaves of water milfoil offer an excellent substrate for algal epiphytes. M. heterophyllum plants are covered with epiphytes in varying densities throughout the year. Such algae are predominantly diatoms, green, or blue-greens. Younger sections of M. heterophyllum shoots are usually free of epiphytes as compared to older shoot sections.

Heaviest epiphyte cover was observed during late summer and occasionally during winter or early spring, when growth of the plants was slowest. Plants in Green's Basin Marsh and Lees Mills were covered with heavy epiphyte growth (mainly gelatinous blue-greens such as Nostoc sp.)

during late July and August of 1975. During this time the apical portions of the plants died and no shoot elongation occurred. During late August and September the epiphyte cover died back and some growth of new apices began. In both of these areas water milfoil growth was dense and in water 1.0 to 1.5 m deep.

A similar increase in epiphyte cover occurred again in Green's Basin Marsh during June 1976. Epiphyte cover was extremely heavy. Apical sections of the plants again died. Following the death of epiphytes in July, new apical growth occurred and continued to the end of October. Heavy epiphyte cover, mainly diatoms such as <u>Synedra</u> spp. and <u>Eunotia</u> spp., was also noted in Alton Bay Beach during April 1976 before the start of rapid growth and in Ostrand's Marina, where epiphytes were mainly filamentous Zygnematales, at the end of the growing season in October 1976.

Phenology of Myriophyllum heterophyllum

Myriophyllum heterophyllum is a perennial plant in Lake Winnipesaukee. Flowering begins during late June and continues through August. Certain apices of the plant produce small, serrate leaves instead of the usual pinnate leaves and thickened, stiffer stems. The apices extend above the water surface to a height of 5 to 10 cm. Small flowers, either staminate or perfect, are produced in the axils of the aerial leaves. Fruit is set during late July and August after which the aerial shoot portions sink back into the water and begin to decompose, releasing the fruit. Flowering did not occur in all M. heterophyllum stands. Flowering plants were usually found in the more shallow areas (1.0 to 1.5 m) such as Lees Mills Stream, Green's Basin Marsh, Twentymile Brook, Merrymeeting River, and Parker's Marina.

At the approach of winter the large, older shoots settle to the bottom and partially or completely die back, sloughing off leaves and becoming dark in color and brittle or stiff. New shoots that were formed in the autumn remain compact and green throughout the winter. During the spring the shoots and new apices from some of the older shoots begin elongating. The remainder of the old tissue decomposes. The shoots elongate rapidly during May through August and may reach 2.0 to 2.5 m in length.

At the end of the growing season elongation of the shoots nearly ceases and new compact shoots are produced at the base of the plant for overwintering.

At any time of the year segments of shoots may be broken off from the plant. The segments float and drift temporarily, eventually sinking to the bottom where they produce adventitious roots and new apices.

Vegetative colonization is an important means of reproduction of water milfoil in Lake Winnipesaukee. In Alton Bay large areas of the rooting fragments were found on substrate that had not yet been colonized.

Established plants increase in size by sending out rhizomes that produce new shoots. This method of reproduction was important in producing the stand expansion described for Ostrand's Marina.

Line Transect and Quadrat Studies

Four transects were laid 10 m apart perpendicular to the shore at the west end of Green's Basin. The transects extended along the lake bottom for 28 m. Depths ranged from 0 to 3.4 m along transect 4. Few plants were counted at depths of less than 0.5 m (Fig. 2). The maximum plant density occurred between 1.0 and 2.5 m. At depths of less than 0.5 m the substrate was mostly coarse sand while in deeper water, to approximately 3.0 m, it was a silt, sand, and organic mud mixture. Plant density decreased at depths greater than 2.5 to 3.0 m, where the substrate was primarily composed of soft, organic mud. Distribution of Myriophyllum heterophyllum plants was patchy along the transects. Often large areas of substrate were completely bare, while other sections had densities of 21 plants/m^2 (Fig. 3). The plants along these transects were small, having from one to 10 shoots/plant as compared to the larger plants observed at Alton Bay that often had more than 10 shoots/plant.

Counts of plants along the eight transects in Alton Bay Beach (Fig. 4) indicated a patchy distribution. Cover was nearly continuous along many of the transects except for transects 6, 7, and 8, that passed through the edge of the stand where density decreased. Transects 1, 2, and 3 were within a swimming area, where plants were subject to
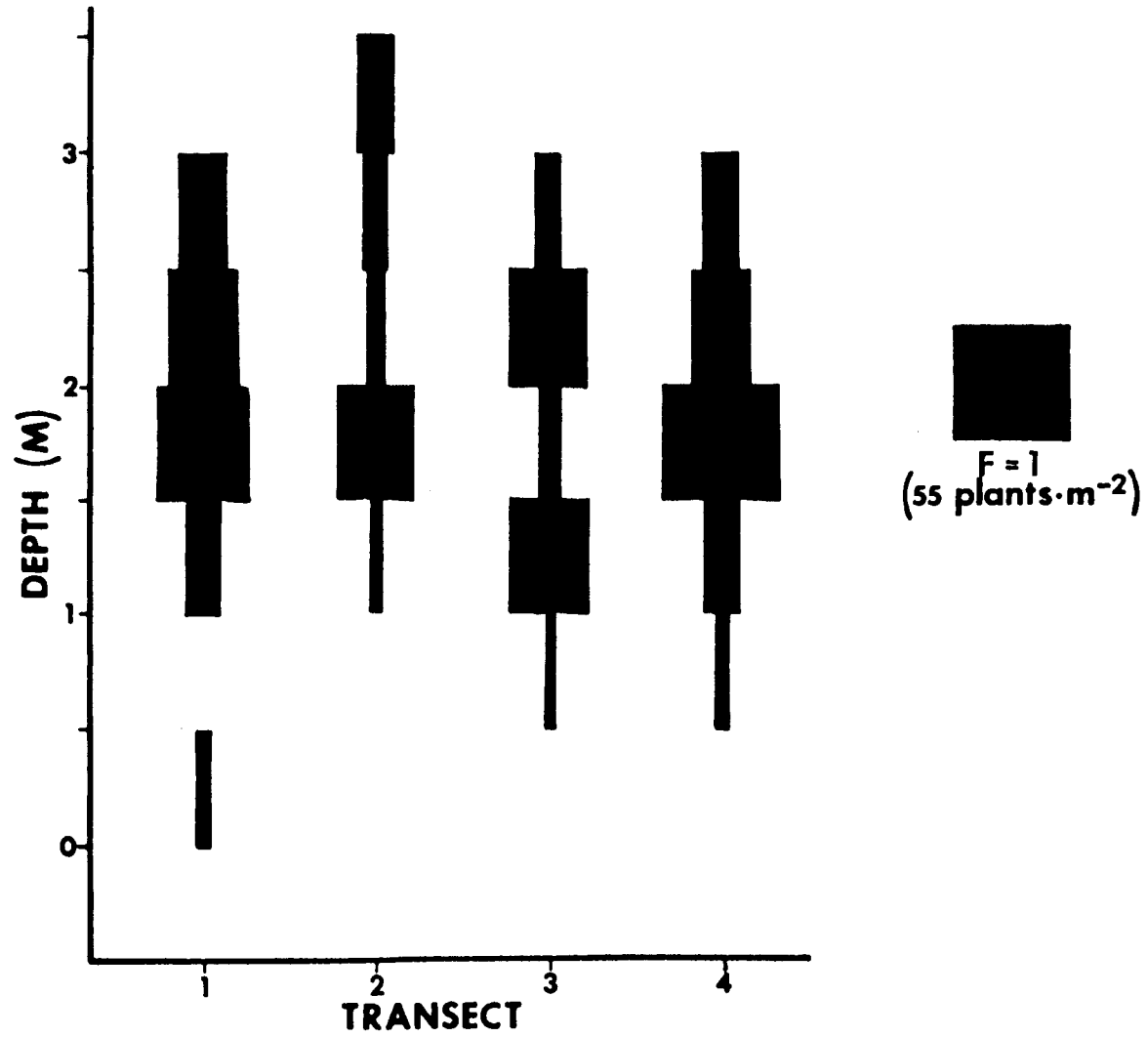
Figure 2. Frequency of plant occurrence with depth at Green's Basin Beach, summer 1975.
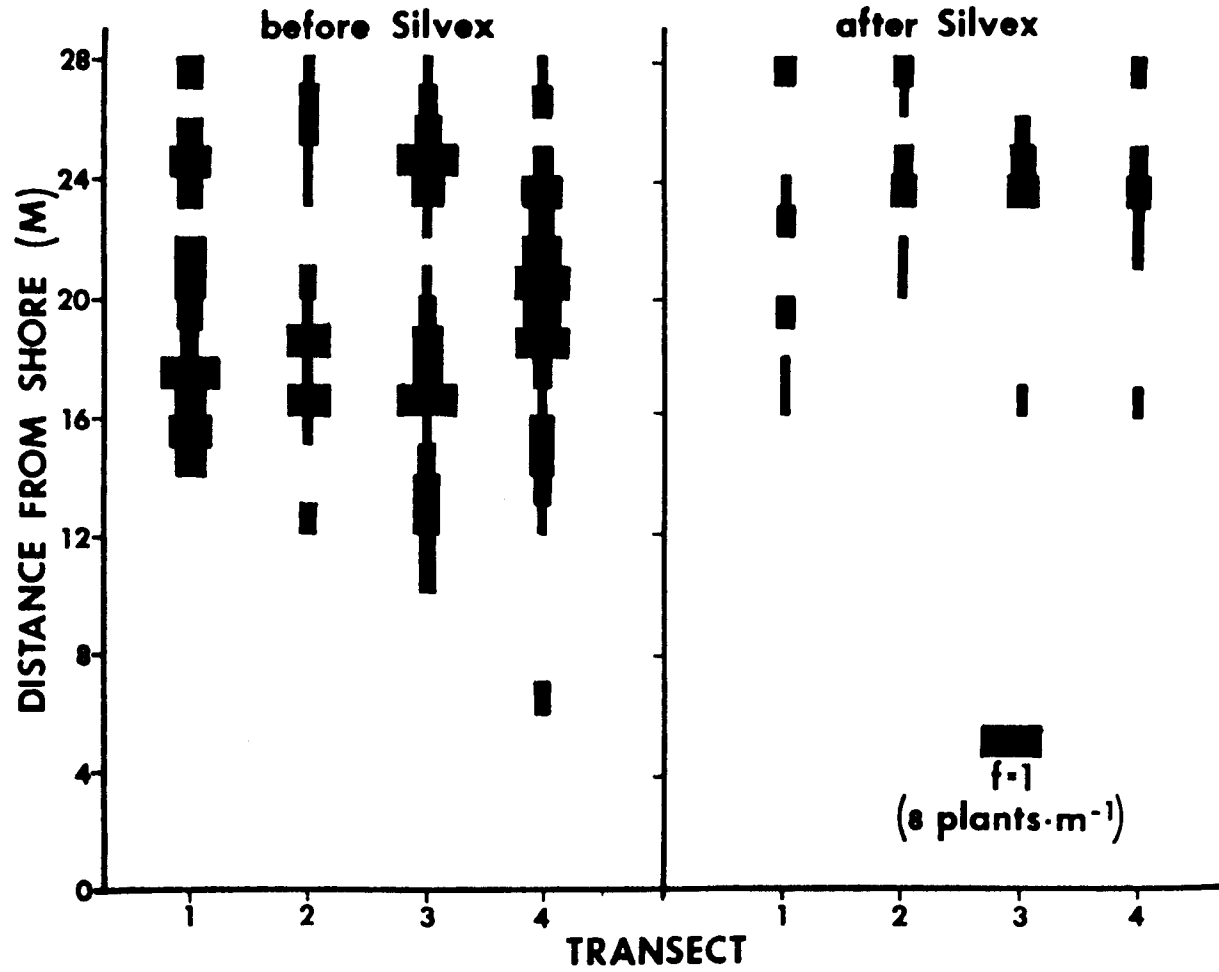
Figure 3. Frequency of plants along transect lines at Green's Basin Beach before and after application of Silvex, summer 1975.
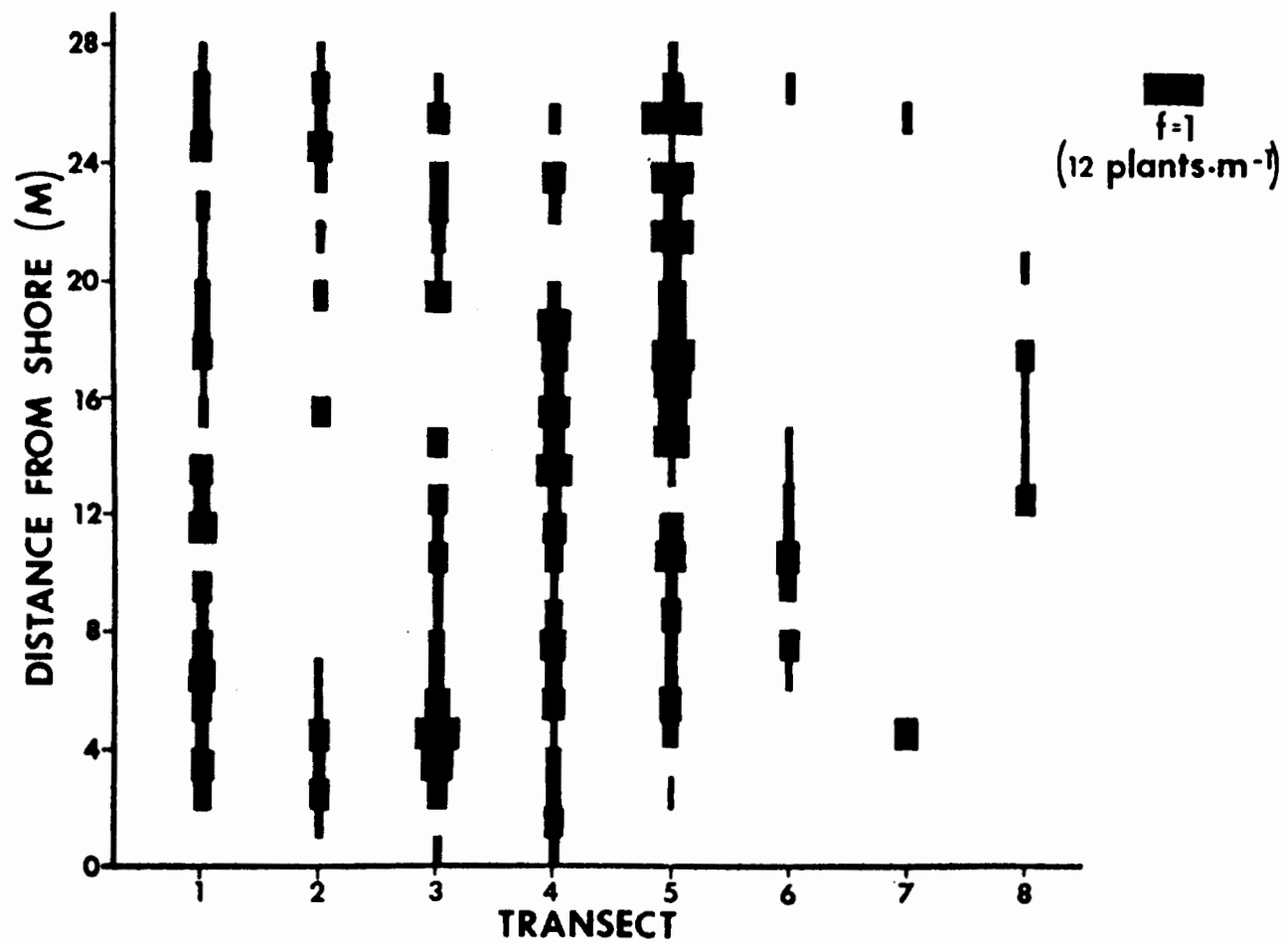
Figure 4.   Frequency of plants along transect lines at Alton Bay Beach, summer 1975.

breakage.

The "shore effect", with no or few plants present in water less than 0.5 m deep was readily observable at Green's Basin Beach. The effect was not evident for the Alton Bay Beach transects, all of which were at depths of more than 0.5 m. Transects 5 through 8 began at the retaining wall of Harmony Park where no shore is present. Transects 1 through 4 began off shore at points parallel to the edge of the wall. A few plants were observed along the beach at depths of less than 0.5 m (Fig. 5).

Maximum plant density occurred at depths between 1.0 and 2.5 m on most transects (except transect 5). At these depths, the sediment is silt, sand, and mud with some wood chips. The substrate in deeper areas is thickly covered with wood chips. Plants reached densities of up to 40 plants/m^2.

At Moultonboro Town Beach a row of "terrestrial" plants had become established from drift of plant segments onto the beach. The plants were anchored in the sand with roots up to 10 cm long and had aerial shoots up to 5 cm in length.

Biomass (g dry plant/m^2) was estimated for each of the primary study sites from plant density and dry weight. Estimates are expressed as a range of values because the plant density values used in the calculations equal the mean plant density plus and minus one standard error. An average of 10 shoots/plant, a height of 100 cm/shoot, and a dry weight of 0.006 g/cm of shoot was assumed. Only above-ground portions of the plants were included in the biomass estimates. Using the equation:

(shoots/plant)(cm/shoot)(g dry wt/cm) = g dry wt/plant
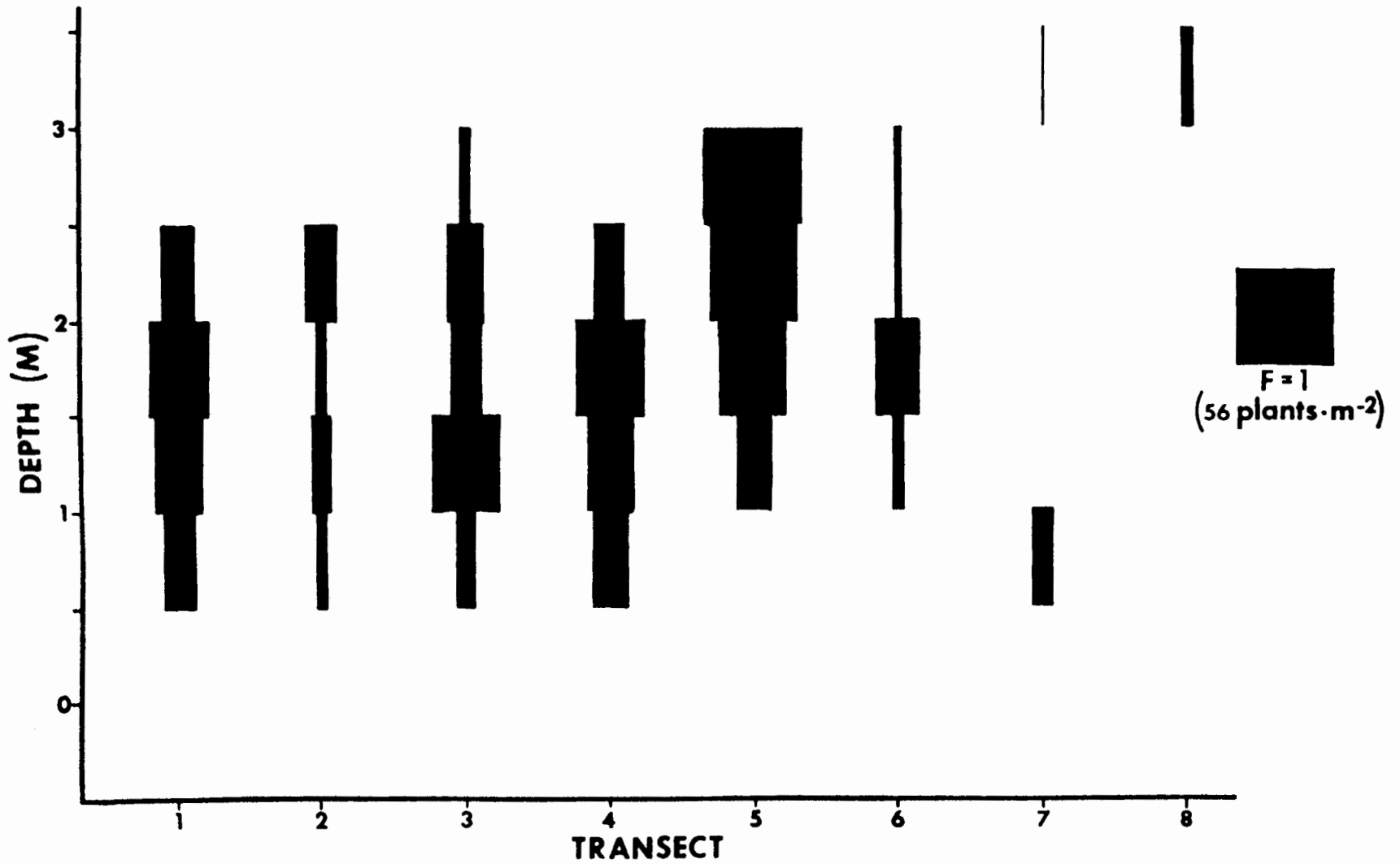
Figure 5.   Frequency of plant occurrence with depth at Alton Bay Beach, summer 1975.

the total dry weight/plant can be calculated, e.g.:

(10 shoots/plant)(100 cm/shoot)(0.006 g dry wt/cm)

= 6 g dry wt/plant

Within a stand of M. heterophyllum dry weight of individual plants depends upon the height and number of shoots and the age of individual shoots. Older shoots have a greater dry weight:length ratio.

Biomass estimates for Alton Bay Beach and Green's Basin Beach range from 72 to 132 g dry weight/m^2 and 84 to 120 g dry weight/m^2 respectively. Mean plant density for both sites (17 plants/m^2) is the same, but the ranges of the biomass estimates differ due to the differences in standard error of mean density at each site. Green's Basin Beach plants appeared smaller (both in number of shoots and height) than did the Alton Bay Beach plants which sometimes reached a height of 2 m. Therefore, biomass may be overestimated for the Green's Basin Beach stand and underestimated for the Alton Bay Beach stand.

The method of density measurement made in the quadrats at Ostrand's Marina can also be used to estimate biomass. The mapping of the location of each plant in the quadrats during 1975 revealed a sparse cover of plants: 218 plants on 100 m^2 or approximately 2 plants/m^2. With the previously estimated value of 6 g dry weight/plant, a biomass estimate of 12 g dry weight/m^2 can be made. By 1976 plant cover had increased from 25% of the total area to more than 75% so that plants could not be counted individually. With the assumption that the increase in percent cover was due to an increase in plant numbers, biomass would increase to three times the 1975 value: 36 g dry weight/m^2. In spite of the dramatic increase, plant biomass at Ostrand's Marina during 1976 was less than half that of either Alton Bay Beach or Green's Basin Beach.

Increase in plant cover at the Ostrand's Marina quadrats appeared
to be due primarily to an increase in the size of the existing plants.
Colonization by new plants accounted for a small part of the increase.
The observations confirm the ability of the plants to colonize new areas
through production of new shoots from rhizomes as well as by rooting
of vegetative fragments.

Observations of increase in plant cover at Ostrand's Marina indicate
the probability of a similar occurrence at Alton Bay from 1975 to 1976.
No significant increase occurred at Green's Basin Beach in 1976 due to
effective control with Silvex in 1975.

Growth Rates

Growth rates (elongation) of Myriophyllum heterophyllum shoots
were measured at the three primary study sites from June until August
in 1975 and from June to October in 1976. Measurements from April to
June would have been desirable for the determination of initial growth
after ice out. Measurements of shoot elongation at Green's Basin Beach
and Alton Bay Beach were interrupted with the application of Silvex
in June 1975 and July 1976 respectively. Sufficient data are available
to provide an approximation of growth rates on a seasonal and depth
basis.

Growth rates during June and July 1976 (Fig. 6) were unaffected
by depth. Shoots elongated at about the same rate at depths of 1.5
to 3.4 m. Location of the plants within the stand at Green's Basin Beach
did not affect growth rates. All plants along each transect elongated
between 1.7 and 1.9 cm/day during June 1975 with no significant differences
in growth rate.

A seasonal variation in shoot elongation was apparent (Fig. 7).
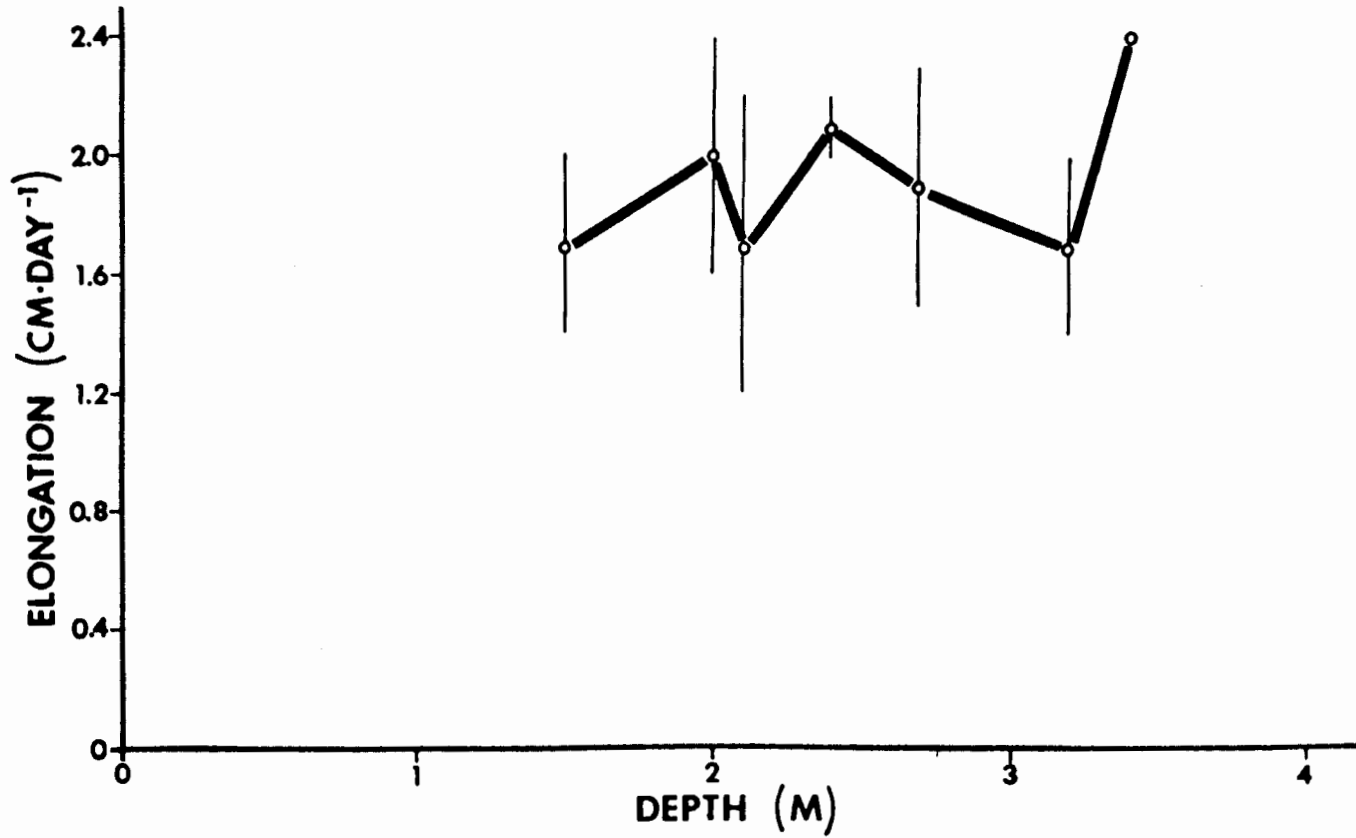
Figure 6. Elongation rates of <u>Myriophyllum</u> <u>heterophyllum</u> shoots with depth at Alton Bay Beach, 24 June 59 to July 1975. (+ one standard error)
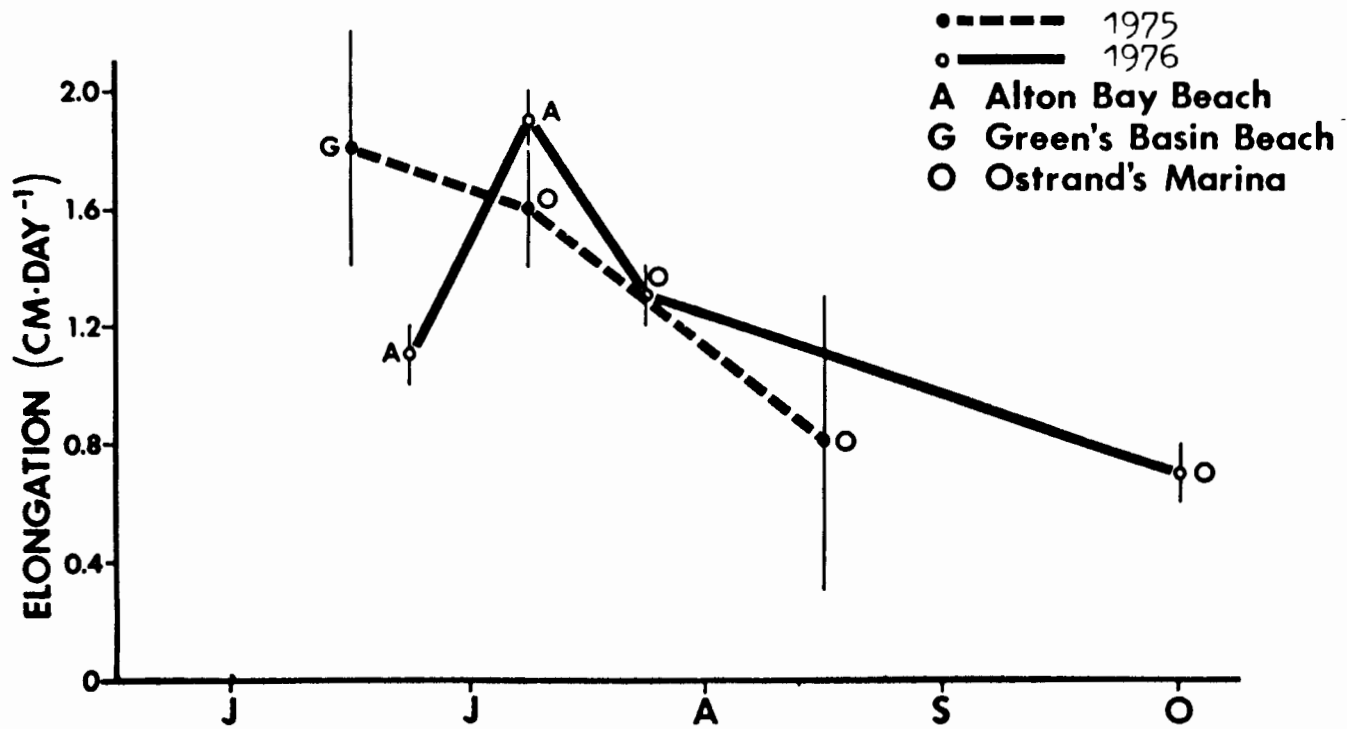
Figure 7. Elongation rates of <u>Myriophyllum</u> <u>heterophyllum</u> shoots with time at all study sites. (<u>+</u> one standard error)

Shoots began to elongate after ice-out in April and continued to grow throughout the summer. The period of most rapid growth was in late June and July after which it tapered off but continued until late August. During September to November elongation of existing shoots seemed to stop and senescence begin. However, during this time new shoots were produced for overwintering.

Maximum summer growth rates occurred between June and July. The most rapid elongation rate, 1.9 cm/day, was measured in Alton Bay in early July 1976. Growth rates of 1.8 cm/day were observed in Green's Basin Beach in early to mid June 1975. It is possible that growth rates prior to June, during April and May, may have been equal to or greater than those recorded for June and July. From July onward growth rates decreased rapidly. Late July and August growth rates were as low as 0.8 cm/day and 0.7 cm/day in Ostrand's Marina in 1975 and 1976 respectively.

Tissue and Sediment Phosphorus

Tissue phosphorus levels in Myriophyllum heterophyllum during the same month were not uniform throughout Lake Winnipesaukee. Tissue phosphorus levels varied as much as 0.42% dry weight between Green's Basin Beach and Parker's Marina in October, 1975, and 0.50% between Lazy Meadow Brook and Lees Mills Cove in October, 1976. June 1976 tissue phosphorus values present a great range of levels among the twelve populations studied. Lazy Meadow Brook, Alton Bay Beach, and Green's Basin Beach apices were all high in phosphorus (greater than 0.7% dry weight) while Green's Basin Marsh and Lee Stream apices were much lower in phosphorus (less than 0.4% dry weight).

Seasonal changes in tissue phosphorus levels were evident for a number of M. heterophyllum populations: Alton Bay Beach, Lazy Meadow

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Brook, and Twentymile Brook (Fig. 8). The seasonal patterns of tissue

phosphorus levels were different for each population. Maximum phosphorus

levels in Alton Bay Beach plants were reached during June of 1975 and

1976. Minimum values occurred during December to March. In Lazy

Meadow Brook peak tissue phosphorus levels occurred in December 1976,

June 1976, and October 1976. The October level of 0.87% dry weight

was among the highest levels measured for any population. During the

winter months of January to April 1976 tissue phosphorus levels were low.

Maximum tissue phosphorus levels of 0.83% were reached during

June 1976 in Twentymile Brook. During the remainder of the year the

phosphorus levels were considerably lower. Two other high values

occurred in February and April. Tissue phosphorus was minimum during

May and July.

The study of tissue phosphorus levels in the Green's Basin Marsh

stand did not reveal any seasonal fluctuation (Fig. 8). Phosphorus

levels were relatively constant throughout the year. A maximum was

reached in September 1976 (0.53% dry weight) after nearly four months

of phosphorus enrichment in the area. Plant samples were collected

at a distance of at least 5 m from the phosphorus enrichment setup,

but the long term enrichment could have affected the phosphorus levels

of the plants sampled.

Analysis of apices from different depths revealed changes in tissue

phosphorus with depth. Phosphorus levels in Green's Basin Beach plants

were greater at 1.1 and 1.7 m than they were at 2.7 m during June 1975

(Fig. 9). During July the converse appeared true: plants at 3.0 m

contained less phosphorus than did those at 3.7 m. In Alton Bay the

relationship of tissue phosphorus to depth changed seasonally (Fig. 9).

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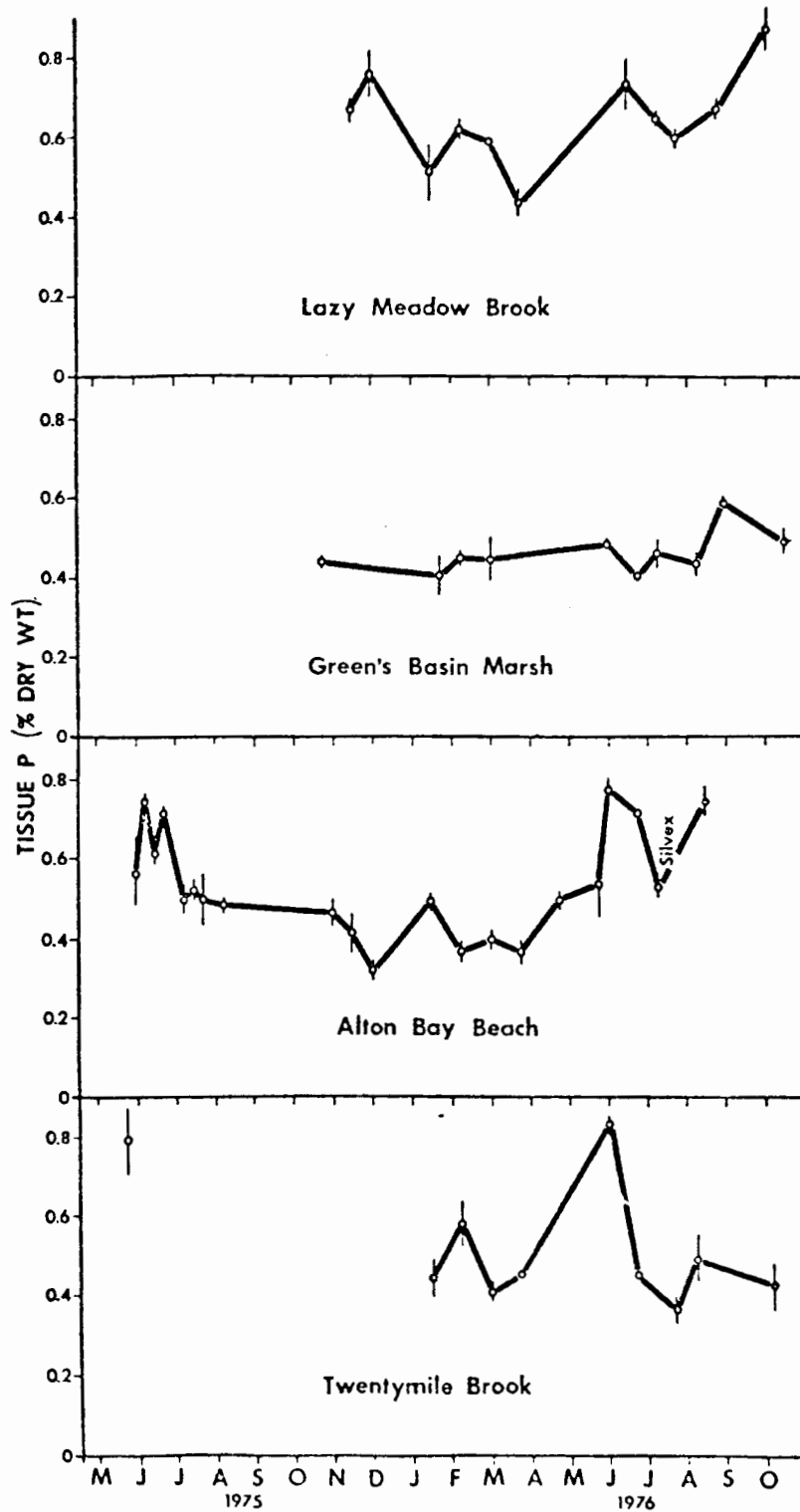
Figure 8.   Tissue phosphorus levels of <u>Myriophyllum heterophyllum</u>
            plants in Lazy Meadow Brook, Green's Basin Marsh, Alton
            Bay Beach, and Twentymile Brook; June 1975 through
            October 1976. (+ one standard error)

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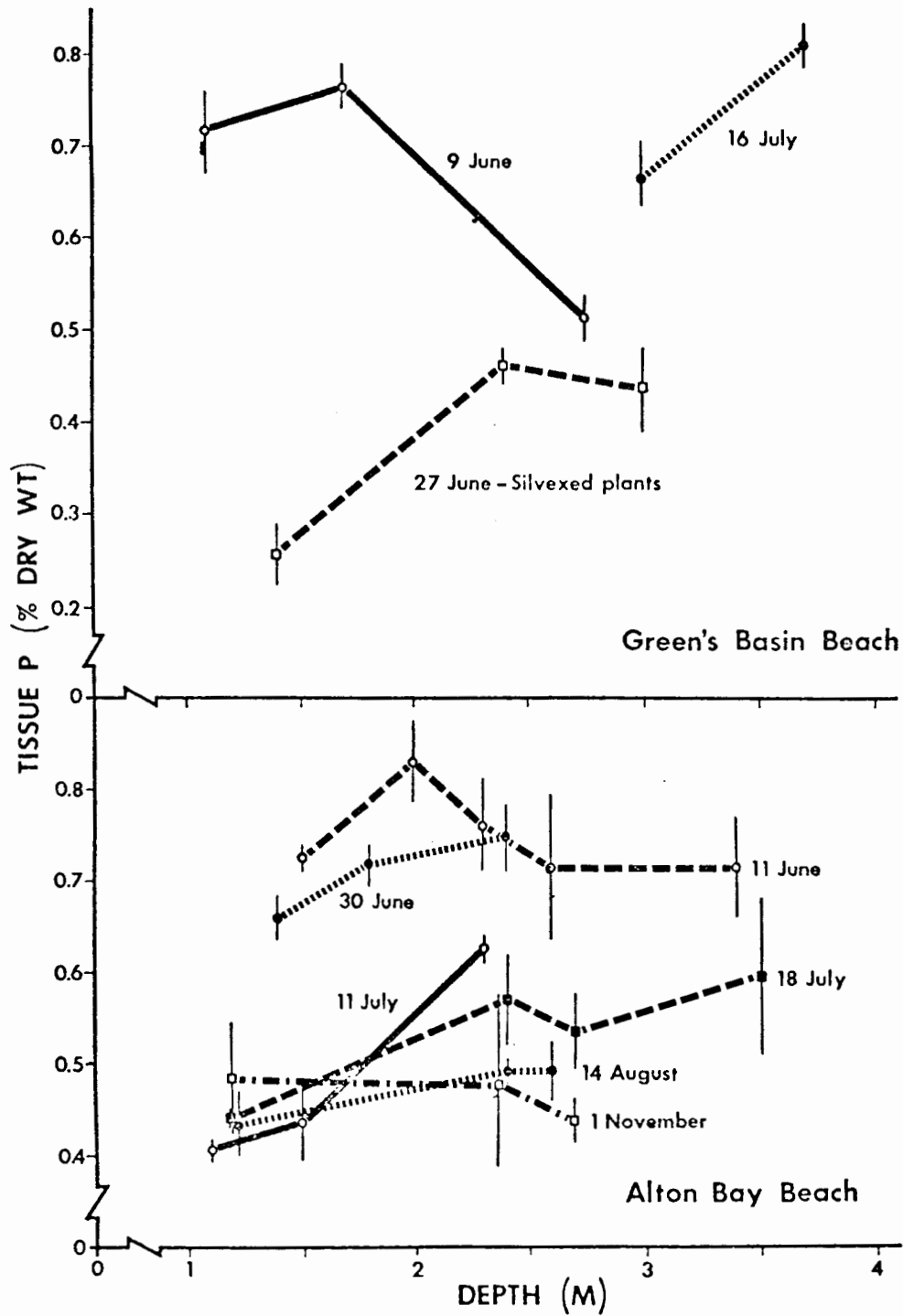
Figure 9.   Tissue phosphorus levels in response to depth at Green's Basin Beach and Alton Bay Beach, summer 1975.   (+ one standard error)

During early June tissue phosphorus versus depth followed a similar
pattern to that in Green's Basin Beach. Tissue phosphorus was greatest
at 2.0 m. July and August tissue phosphorus levels produced a pattern
of increasing tissue phosphorus with increasing depth. At all depth
intervals tissue phosphorus was maximum during June and decreased
thereafter (Fig. 10). Tissue phosphorus of plants at depths of 2.0
to 2.5 m was greater than that at other depths from June until August,
when phosphorus levels for plants at all depths became uniform.

Sediment phosphorus levels measured during the summer of 1975
remained nearly constant in Green's Basin Beach and Ostrand's
Marina. Ostrand's Marina sediment, which was predominantly sand and silt,
was the lowest in phosphorus (0.018 to 0.39% dry weight) of the three
areas. Phosphorus levels were maximum in late June (0.039%), minimum
in early July (0.018%), and were only slightly higher during late July
(0.028%) and August. Green's Basin Beach sediment phosphorus levels
were 0.037% to 0.043% throughout the summer with a minimum in early June
and a maximum in early July. Phosphorus levels of Alton Bay sediment
fluctuated during the summer with maxima during late June and early
August and minima during early June and late July. Variation
of sediment phosphorus may have been due to sampling technique. Sediment
phosphorus values ranged from 0.050 to 0.085%. These phosphorus values
are higher than those at the Ostrand's Marina and Green's Basin Beach
sites.

The large standard error of the mean phosphorus values for many
of the collection dates indicate that many of the fluctuations in
sediment phosphorus levels may not be significant. Mean values for all
sediment phosphorus analyses during 1975 at all three sites are: Ostrand's
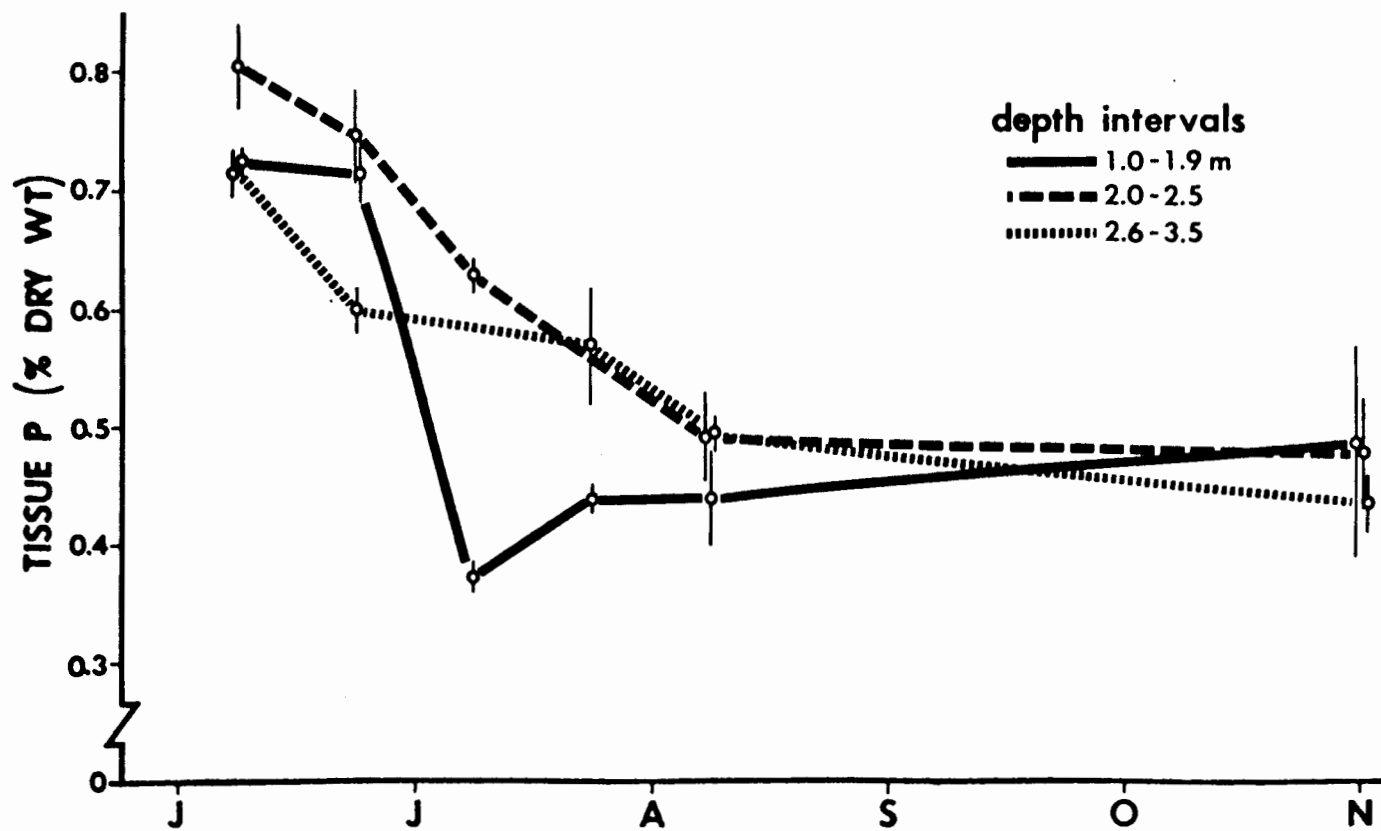
Figure 10.   Tissue phosphorus changes with time for three depth intervals at Alton Bay Beach, summer 1975.   (+ one standard error)

Marina - 0.25%, Green's Basin Beach - 0.040%, and Alton Bay Beach -
0.072%. The differences in sediment phosphorus between sites are
significant and may affect the tissue phosphorus levels at each site
if M. heterophyllum obtains its phosphorus primarily from the sediments.

Green's Basin Beach sediments increased in phosphorus content very
slightly with depth (Fig. 11). Sediment phosphorus in Alton Bay Beach
did not change with depth (Fig. 11).

Percent phosphorus in "contemporaneous" sediment from traps was
greater than in sediment scooped from the bottom: 0.060% greater in
Alton Bay, 0.087% greater in Green's Basin Beach, and 0.134% greater
in Ostrand's Marina. The trapped sediment contained small amounts of
sand and other matter that had been resuspended by wave action and settled
into the traps. The percent organic matter in the traps (range = 9 to 26%)
was higher on the average than that of the substrate (range = 1 to 38%)
because it usually contained smaller amounts of sand.

When tissue phosphorus values of all three sites are plotted against
corresponding sediment phosphorus values (Fig. 12) three clusters of
points are produced, one for each of the locations. The Alton Bay
Beach cluster is distinct from the other two, Ostrand's Marina and Green's
Basin Beach, which overlap slightly. A positive correlation (R = 0.844)
exists between mean tissue phosphorus of the apices at each site and
the mean phosphorus value of the sediment in which the apices were growing
(Fig. 13). Alton Bay Beach apices were highest in tissue phosphorus
and were growing in sediment with the highest phosphorus levels. Con-
versely, Ostrand's Marina sediment was lowest in phosphorus and the plants
rooted in it were lower in phosphorus than the plants at the other two
sites. However, the standard errors of the tissue phosphorus means
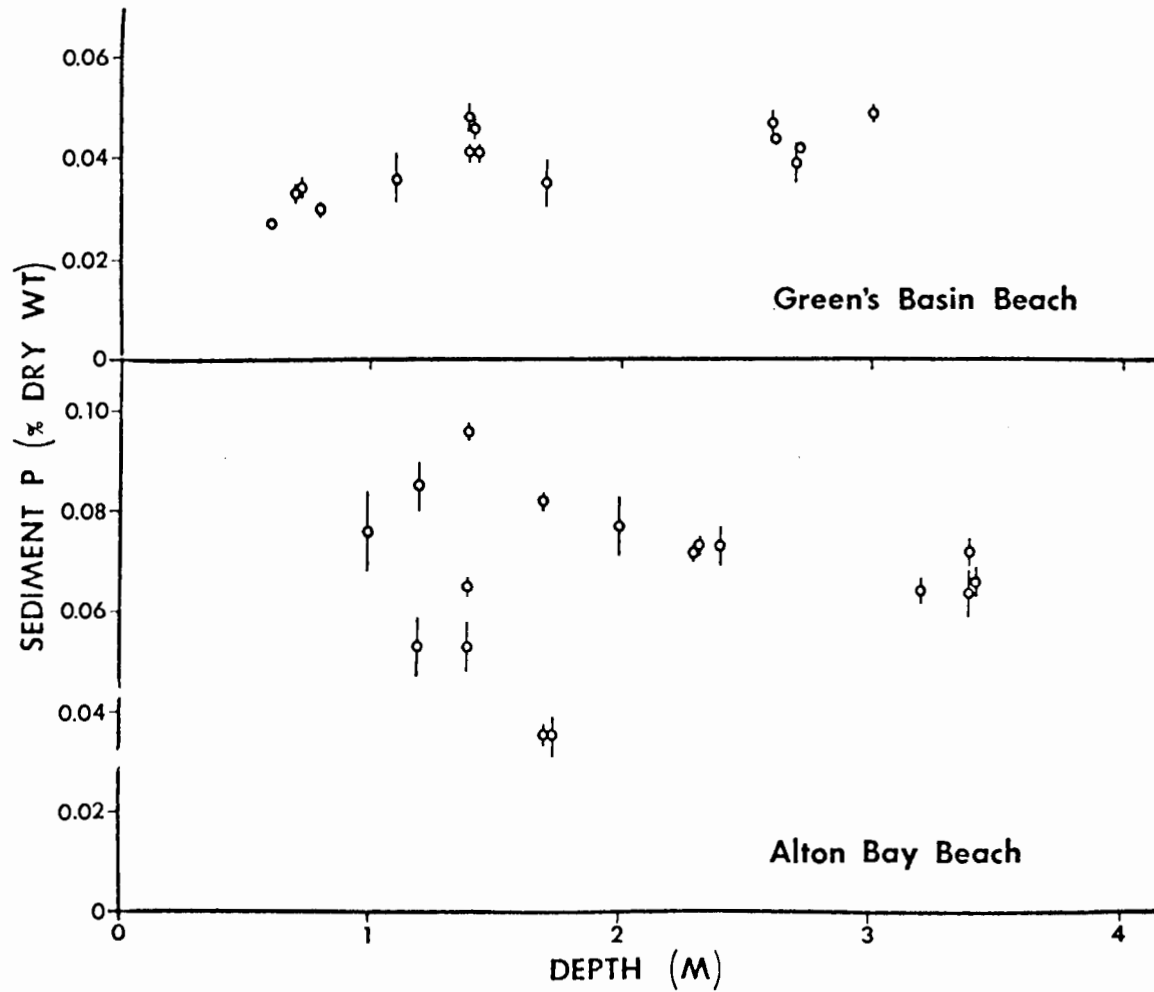
Figure 11. Green's Basin Beach and Alton Bay Beach sediment phosphorus levels in response to depth, summer 1975. (+ one standard error)
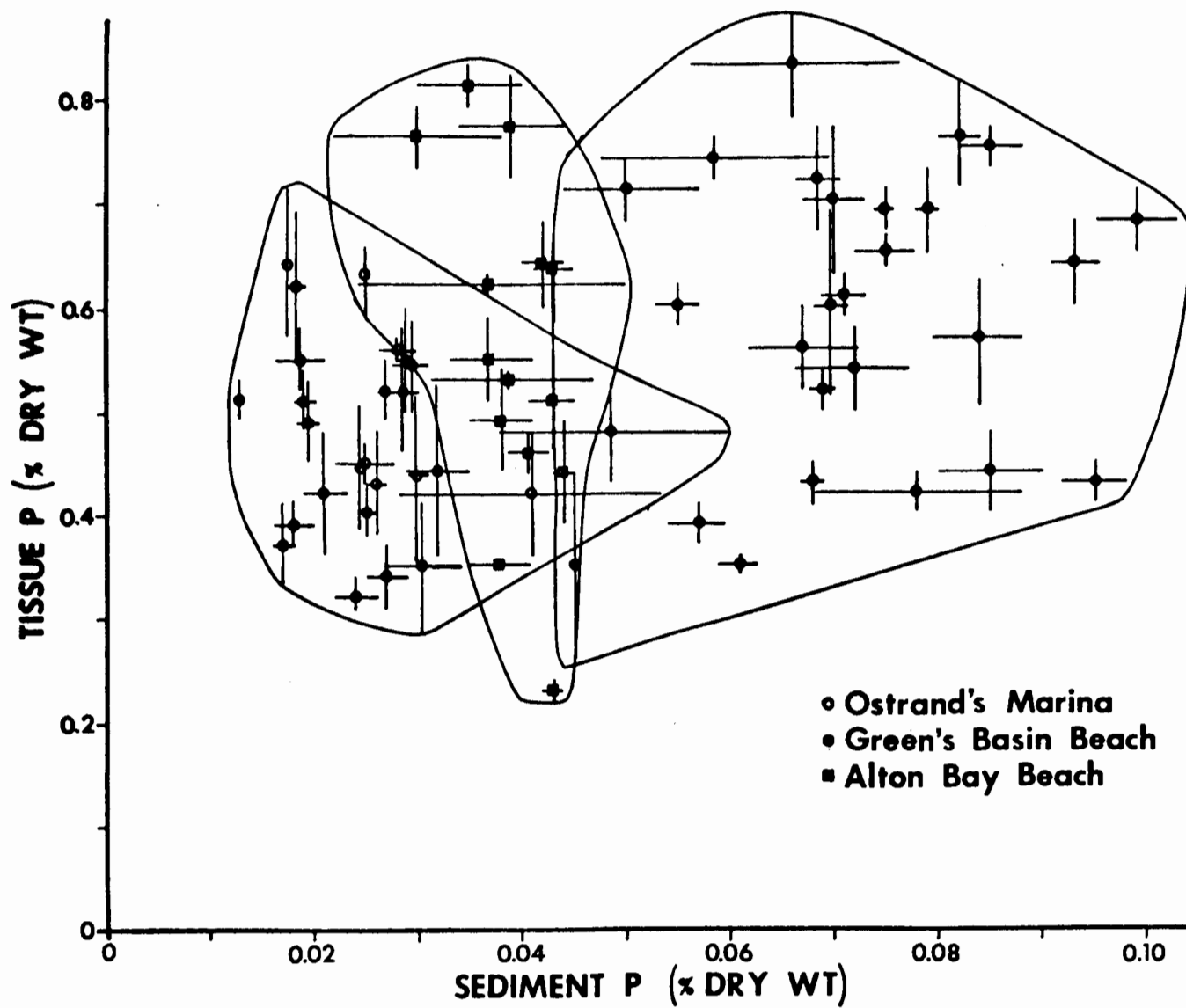
Figure 12. Tissue phosphorus as related to sediment phosphorus at three study sites, summer 1975. ($\pm$ one standard error)
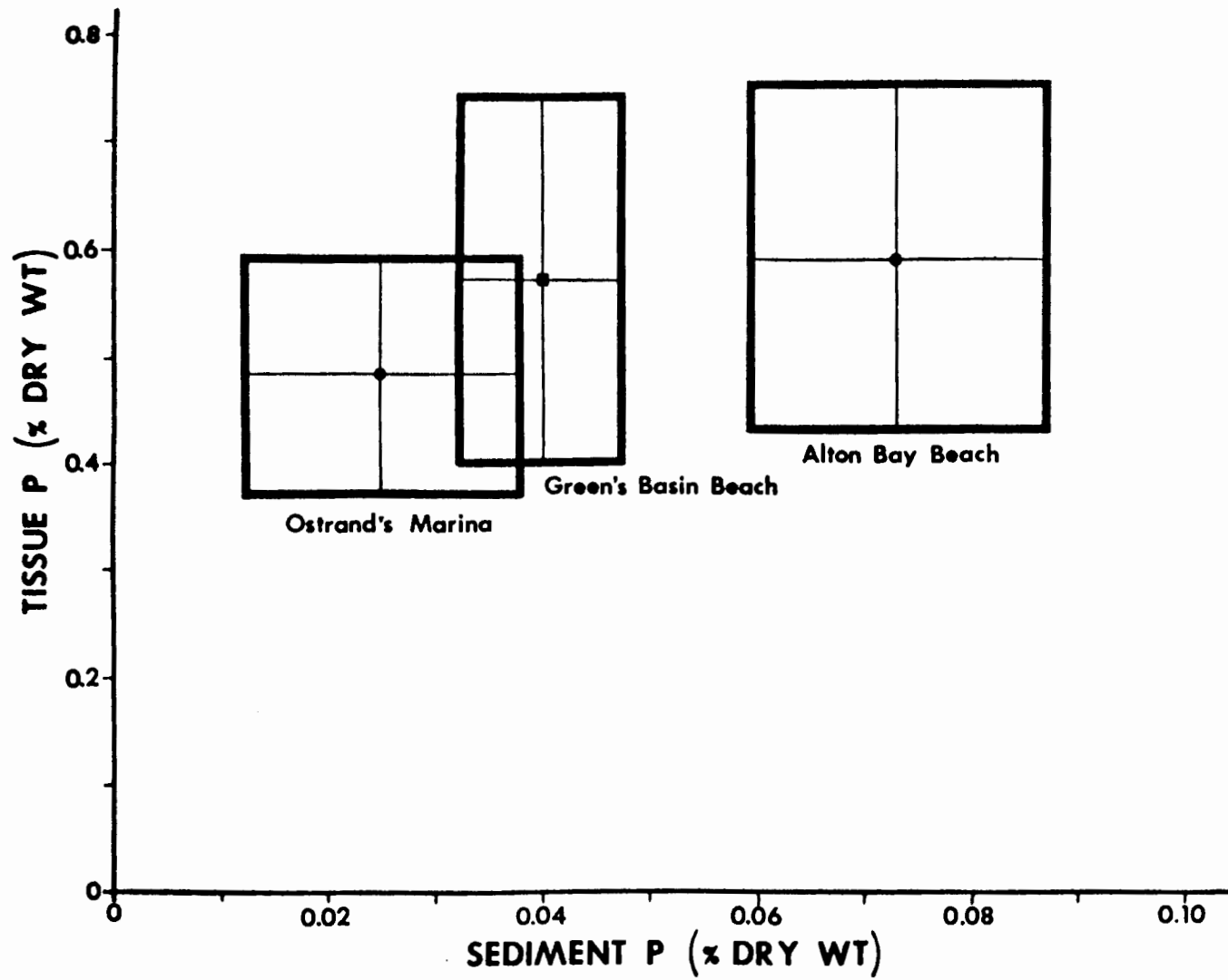
Figure 13. Mean tissue phosphorus as related to mean sediment phosphorus at three study sites, summer 1975. (+ one standard deviation)

overlap graatly for Green's Basin Beach and Alton Bay Beach.

In Situ Phosphorus Enrichment

In situ enrichment with phosphorus of Myriophyllum heterophyllum
plants in Green's Basin Marsh produced significant increases in the
tissue phosphorus (Fig. 14). After 14 days of enrichment plants at
stakes 1 and 2 had phosphorus concentrations of 0.72% and 0.62% while
that in control plants was 0.45%. Continued enrichment from day 14
to day 40 produced continued high levels of tissue phosphorus (0.65 to
0.77%). Plants at stake 3 were not enriched until day 28. After 16
days of enrichment tissue phosphorus levels increased from 0.35% to
0.67%. Input of the phosphorus solution (1 g/1) at each stake supplied
between 0.22 and 0.58 g of phosphorus/day to the plants.

At some time between days 44 and 71 phosphorus flow from the
reservoirs slowed and finally stopped due to clogging of the tygon
tubing by bacterial growth. During this time, in the absence of
additional phosphorus, the tissue phosphorus levels decreased to values
slightly above (stake 1) or slightly below (stake 2) tissue phosphorus
levels in the control plants. Flow of the phosphorus medium was not
restarted on day 71. Without enrichment the tissue phosphorus levels
on day 110 were between 0.47 and 0.60%, and were not significantly
different in the control and experimental plants.

Prior to the start of phosphorus enrichment the apices of most
plants in the area of the stakes had died, presumably due to the heavy
epiphyte cover. During the course of the enrichment new apices were
formed. By day 28 the apices reached lengths of approximately 5 cm
and by day 110 they were 30 cm long. Growth of the apices did not
appear to be affected by the addition of phosphorus (tagging studies

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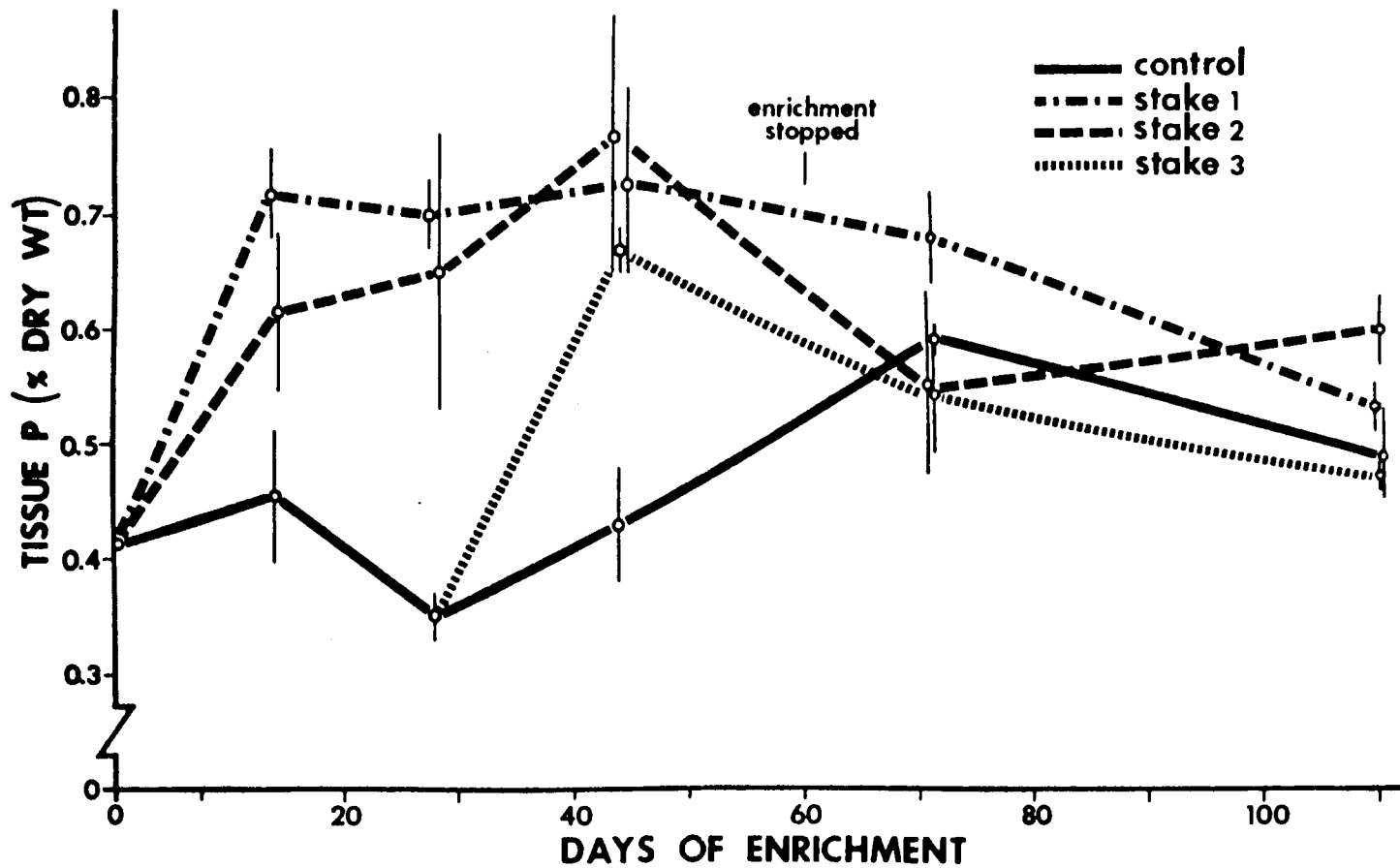
Figure 14. Effects of in situ phosphorus enrichment on tissue phosphorus levels of Myriophyllum
heterophyllum in Green's Basin Marsh, summer 1976. (+ one standard error)

were not conducted). The growth rate between day 28 (26 July) and day 110 (16 October) was slow compared to that at other study sites.

Effects of Herbicide Application

The herbicide Silvex (2,4,5-trichlorophenoxypropionic acid) was applied to several Myriophyllum heterophyllum stands by two commercial firms. Application of Silvex causes death of water milfoil within three days. Five populations in Lake Winnipesaukee were treated with Silvex: Salmon Meadow Cove - summer 1974, Smith's Cove - summer 1974, Green's Basin Beach - 16 June 1975, Moultonboro Town Beach - June 1976, and Alton Bay Beach and Parker's Marina - 12 July 1976.

The effects of Silvex on M. heterophyllum plants were studied at two primary sites, Green's Basin Beach and Alton Bay Beach. Within four days after herbicide application shoots became chlorotic and fell to the lake bottom. Plants in water deeper than 2 m either remained healthy or were only slightly affected. Monocotyledonous plants and algae in the same area were unaffected by the Silvex. In Green's Basin Beach Sagittaria graminea, Eleocharis acicularis, Valisneria americana, and Nitella and Chara spp. appeared unharmed. M. heterophyllum tissue began sloughing off and decomposing within two weeks after spraying. The water in the area of the affected stands became very turbid with decomposing plant tissue.

Only remnants of basal stems and roots of the plants remained after three weeks. Plants at depths greater than 2 m that had been affected by the herbicide began to produce new apices at the stem nodes. Phosphorus analyses of some apices of the remaining living plants showed a marked increase in tissue phosphorus levels (Fig. 9).

An examination of the Green's Basin Beach area one year after

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herbicide application revealed small numbers of M. heterophyllum plants growing in deep water and a few plants colonizing more shallow areas. Water in the area was more turbid than it had been when the water milfoil population was dense. Plant species unaffected by Silvex did not rapidly take over the area that M. heterophyllum had occupied.

Eradication of the M. heterophyllum stands at all sites was essentially complete in that few plants remained during the season the herbicide was applied and few plants were present in the following season.

Attempts to control M. heterophyllum growth through mechanized cutting and harvesting were made in conjunction with the Town of Alton at the Town Beach. Various cutting methods, such as dragging a chain or chain-link fencing through the plants by boat or a power winch on shore, produced unsatisfactory results. The flexible plants were bending down instead of snapping off as the "cutter" passed through them. As a result only a small fraction of plants was harvested and further attempts were abandoned.

Batch Cultures

Batch cultures were designed for the study of phosphorus uptake and release by Myriophyllum heterophyllum apices and the growth of the apices in culture. The responses were monitored under varying conditions of temperature, phosphorus, and other nutrient concentrations in the medium, and for varying lengths of time.

In Batch 1 apical sections and nonapical sections (the second 5 cm of the shoot) were used in a ratio of one to two because insufficient apical sections were available. Tissue phosphorus increased in each culture as external phosphorus (phosphorus in the medium) increased (Fig. 15). The uptake of phosphorus by apices did not saturate at external phosphorus

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Figure 15. Tissue phosphorus in response to external phosphorus
concentration, Batch 1. ($\pm$ one standard error)

concentrations of 0.5 ppm, a phosphorus concentration in excess of natural

levels. The daily change of tissue phosphorus levels versus external

phosphorus concentration produced a straight line relationship at each

temperature (correlation coefficients all 0.89 or greater). The slopes

of the regression lines for 18, 30, and 4 C were 0.048, 0.043, and

0.030, respectively. The intercepts of the lines differ slightly. The

differences of the equations for uptake rate at different temperatures

indicate that the uptake of phosphorus by the apices is affected by

temperature since all other environmental factors were identical.

Although uptake rates were not studied for intermediate temperatures,

the results indicate that the optimal temperature for phosphorus uptake

was approximately 18 C.

At all temperatures and external phosphorus concentrations, phosphorus

in the medium decreased with time as a result of phosphorus uptake by

the M. heterophyllum (Fig. 16). Phosphorus levels in the medium decreased

most rapidly during the first one to two days of culture, but the decrease

(and therefore uptake by the plants) continued to the end of the culture

at day 12. At the low external phosphorus concentrations (0.270 ppm

and lower) phosphorus was undetectable at the end of the culture period,

particularly in the 30 C and 18 C cultures. Uptake, or decrease in

external phosphorus, was more rapid at higher temperatures than at 4 C

(Fig. 17).

The rates of elongation of apical sections and apex initiation

by subapical sections were used as growth indicators in culture. Although

the indicators do not provide an accurate measure of growth (i.e. increase

in weight) they do provide an approximation (Wilson, 1972).

Length increase was independent of external phosphorus concentrations.

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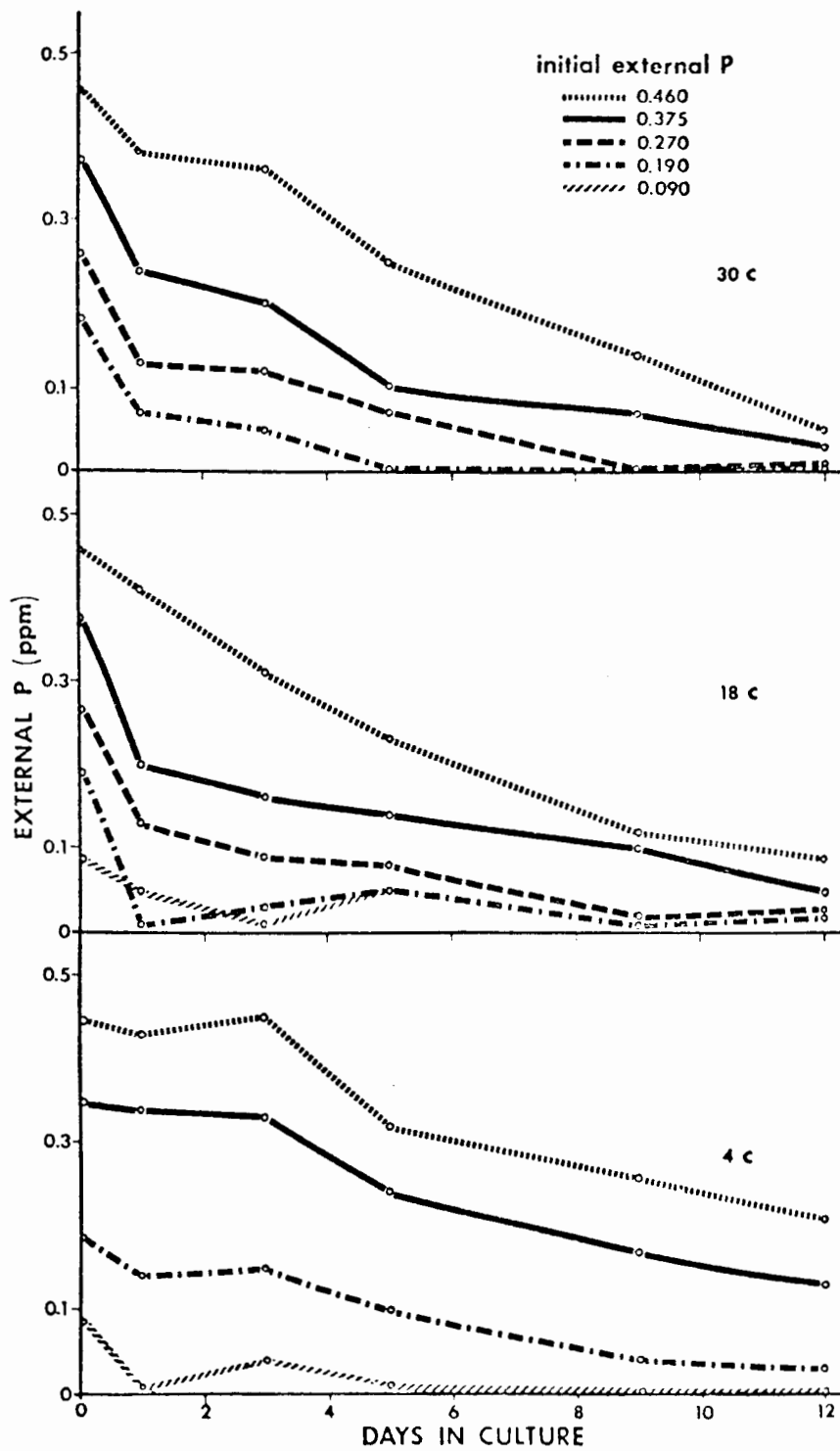
Figure 16. Change in external phosphorus concentration with time, Batch 1.

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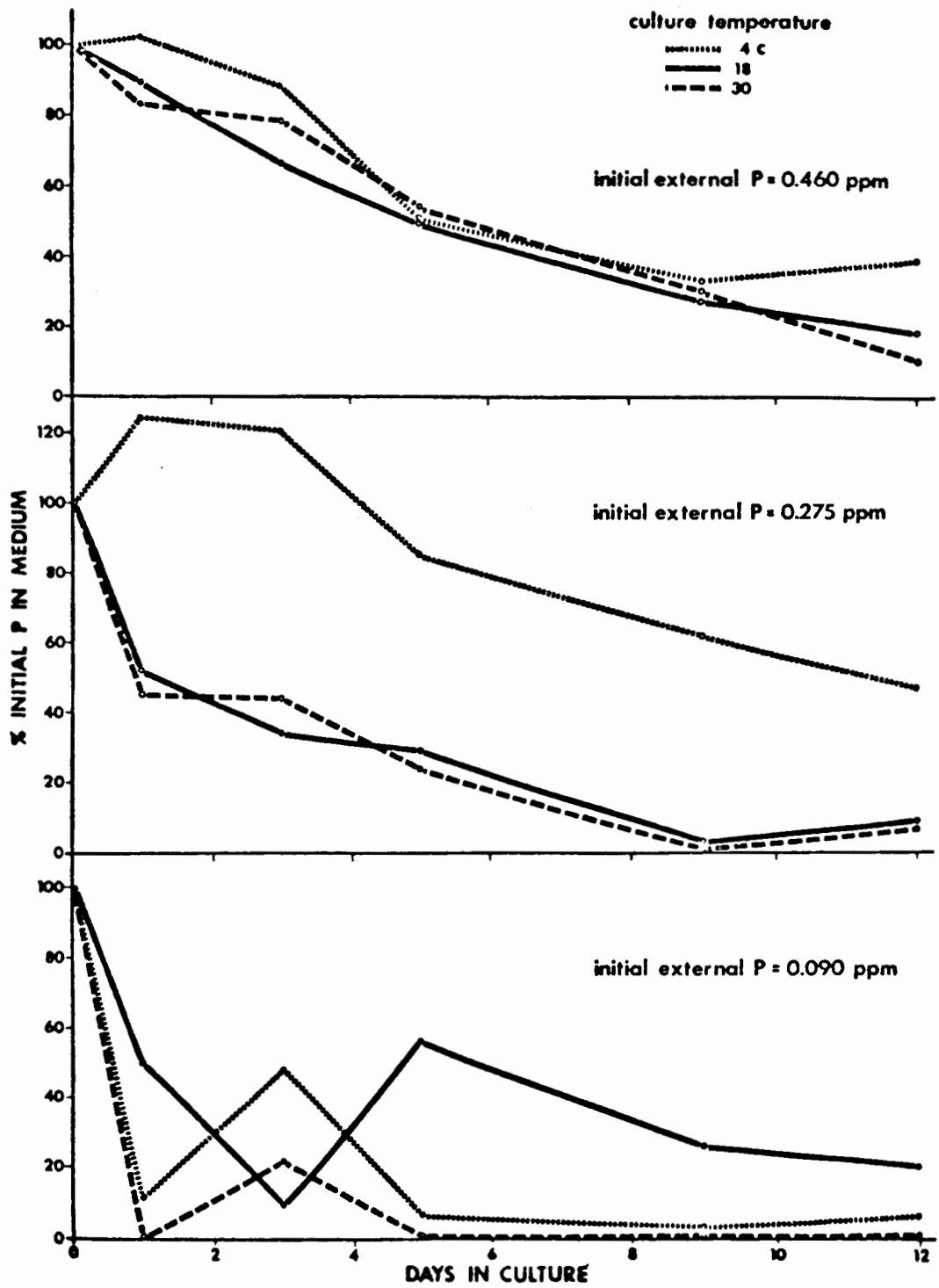
Figure 17. Change in percent of original external phosphorus concentration
with time, Batch 1.

The means and standard deviations of length increase for each medium phosphorus level overlapped at each temperature. The initiation and length of new apices was also unaffected by external phosphorus concentrations.

Temperature affected both the rate of elongation of apical sections as well as the initiation and length of new apices. Sections grown at 4 C had the smallest mean length increase and almost no initiation of new apices. Length increase and apex initiation were greatest at 18 C and 30 C. Length of new apices initiated was maximum at 30 C.

During culture at 4 C the culture chamber was accidentally shut off for a number of hours. The temperature rose to about 15 C before the problem was discovered, and may have affected phosphorus uptake by the apices. However, any effects of the event were not evident in the data.

Tissue phosphorus increased until day 5 in all external phosphorus concentrations in batch 2 (Fig. 18). No increase in tissue phosphorus occurred after day 5. Lack of external phosphorus was an unlikely explanation for the cessation of uptake because the high initial phosphorus levels could not have been depleted in only five days. Standard deviations of most tissue phosphorus values were large and may have indicated some unusual occurrence or an insufficient sample size.

There was no correlation between tissue phosphorus and external phosphorus concentration (Fig. 19). An uptake saturation may have been reached at phosphorus levels greater than 1 ppm, but the data are not reliable enough for any specific conclusions.

Phosphorus uptake rates in response to external phosphorus concentration (Fig. 20) confirm the erratic results. The culture was repeated
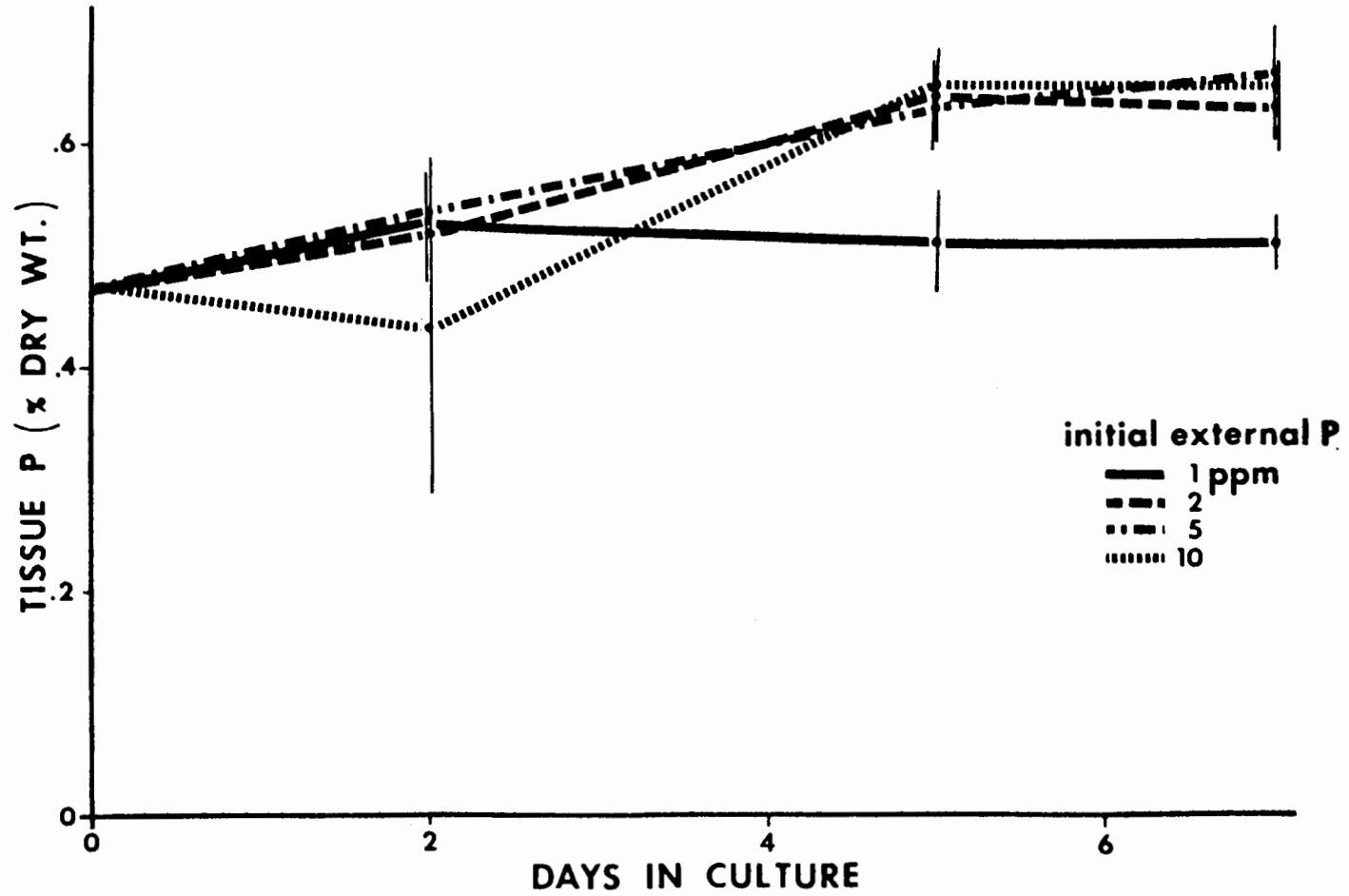
Figure 18.   Tissue phosphorus changes with time, Batch 2.   (+ one standard error)
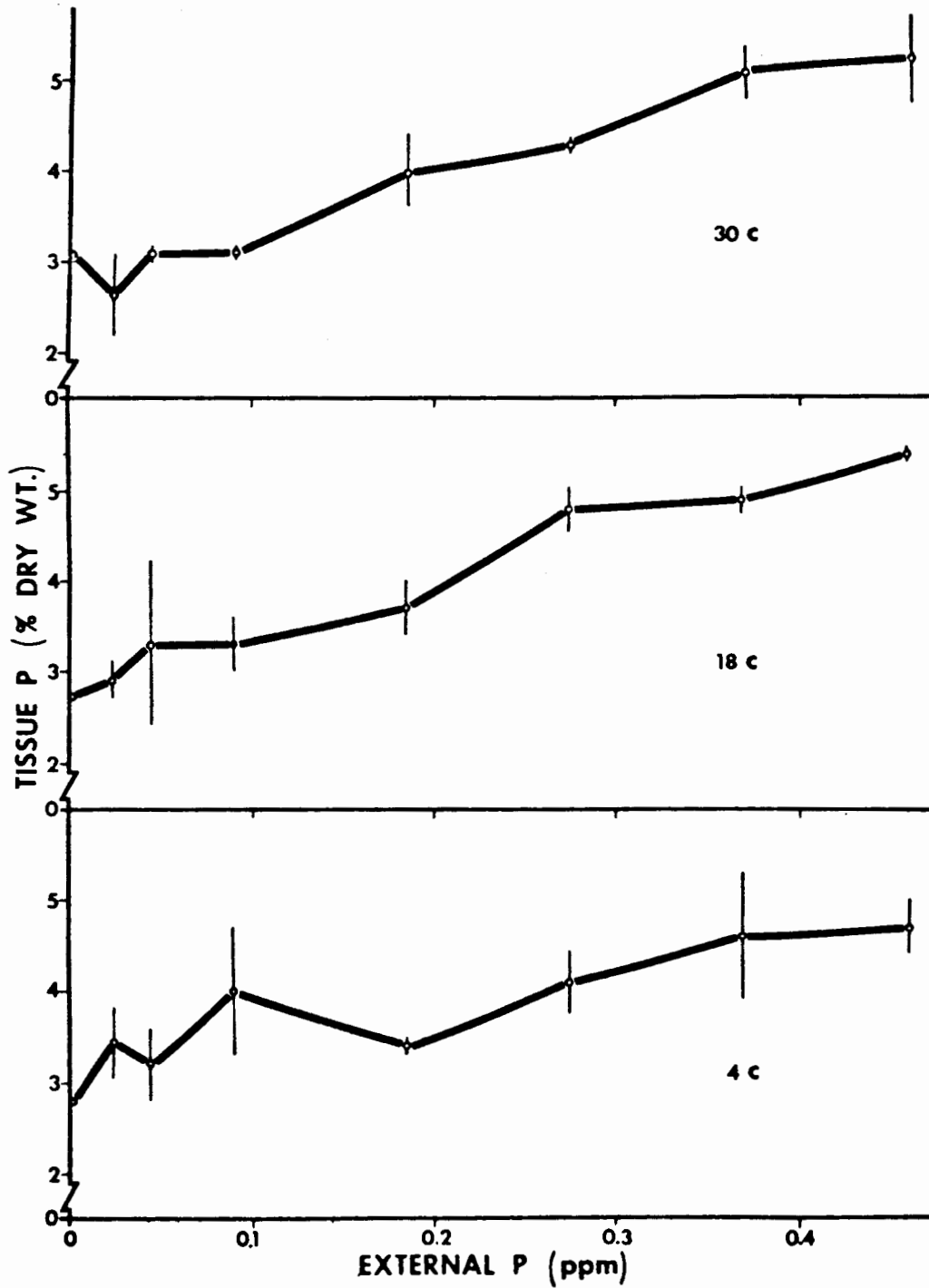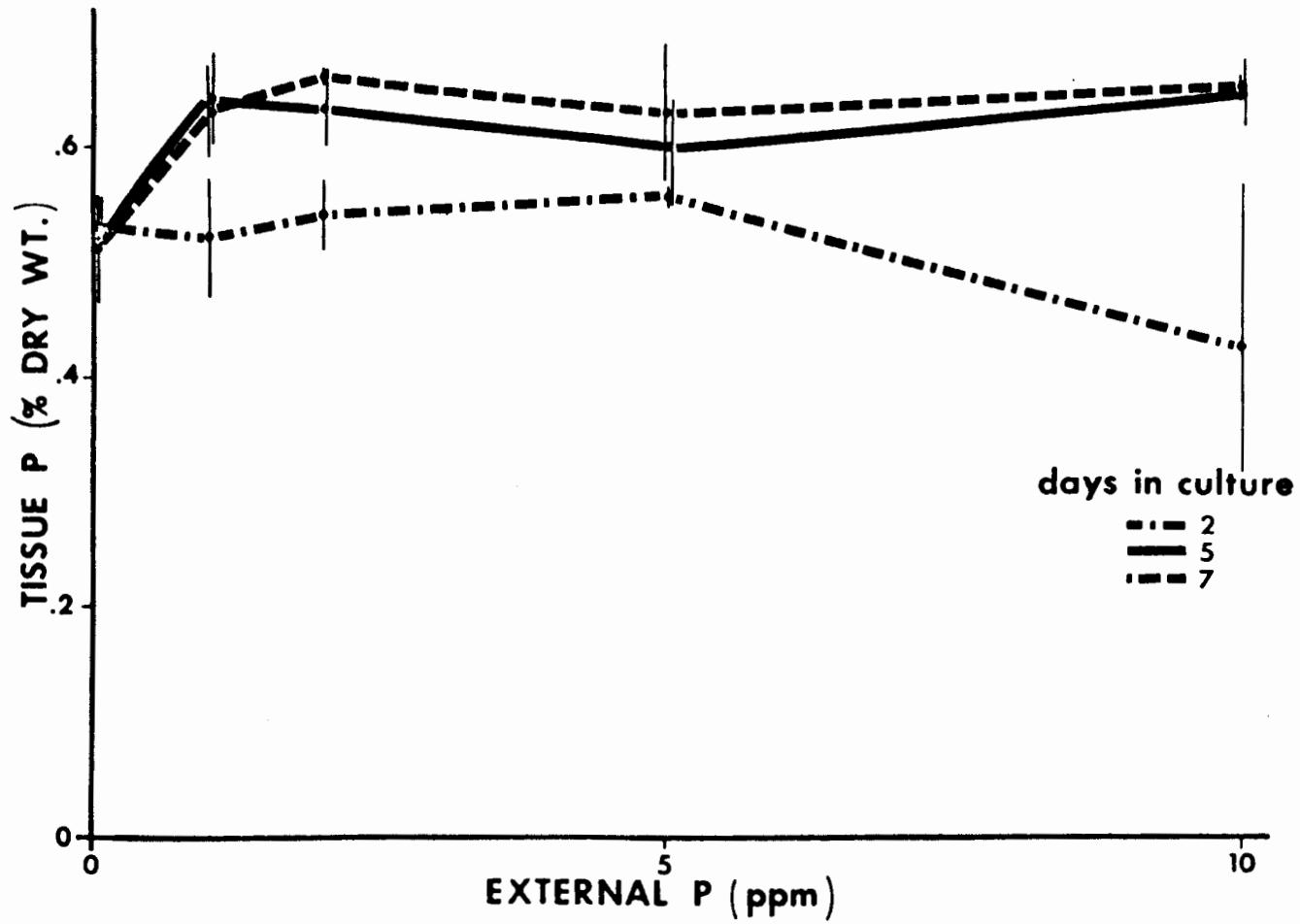
Figure 19. Tissue phosphorus in response to external phosphorus concentration, Batch 2. (+ one standard error)
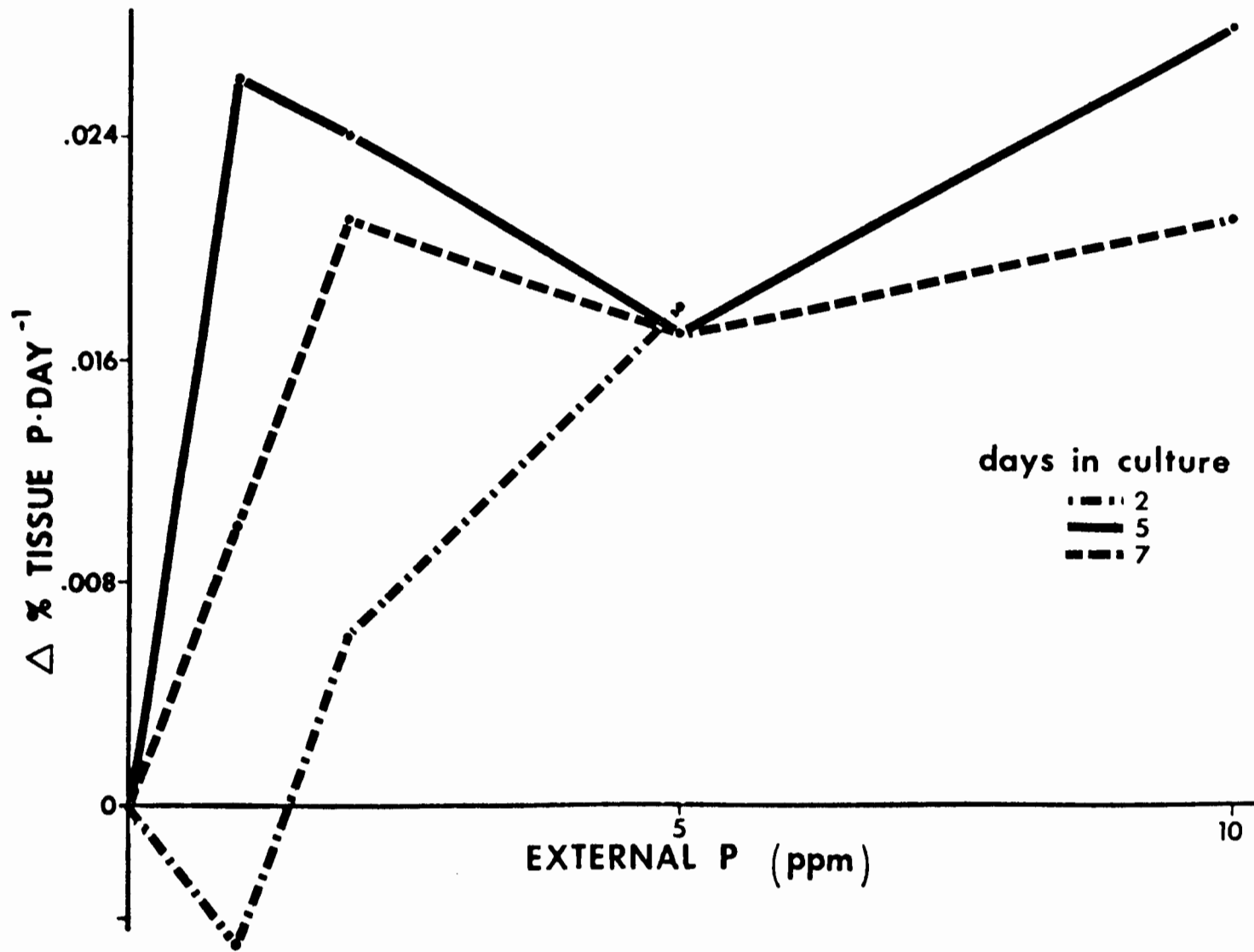
Figure 20. Phosphorus uptake rate in response to external phosphorus concentration, Batch 2.

in Batch 3 with five relicates rather than three.

Tissue phosphorus levels in Batch 3 increased with time at every external phosphorus concentration (Fig. 21). The increase was most rapid during the first five days of culture, after which lower uptake rates occurred (Fig. 22). As in Batch 1, tissue phosphorus (Fig. 23) and uptake rates (Fig. 24) were directly related to phosphorus concentration in the medium. The results for phosphorus levels of 1 to 10 ppm support, or are a continuation of, the results obtained in Batch 1 for phosphorus levels of 0 to 0.5 ppm.

As phosphorus is removed from the medium by the plants the levels of available phosphorus decrease. The decrease would account for the decreasing uptake rates with time. Uptake rates decrease more rapidly at low external phosphorus concentrations than at high ones because greater percentages of available phosphorus are removed at lower concentrations (Fig. 25). Nearly 100% of available phosphorus at the initial level of 1 ppm was removed after 15 days, while less than 50% was removed from the initial 10 ppm level during the same time. Therefore, although uptake rates of phosphorus are dependent upon external phosphorus concentration, the relationship is not a direct one (Fig. 25).

The section of Batch 1 cultured at 18 C was combined with Batch 3 to provide data for phosphorus uptake by M. heterophyllum at external phosphorus concentrations of 0 to 10 ppm. Uptake rates by the apices in response to external phosphorus concentration did not produce a straight line when graphed, but instead produced two distinct sets of points: those at 0 to 0.500 ppm and those above 0.500 ppm (Fig. 26). Uptake rates in the first set of points "saturate" at phosphorus levels of approximately 0.400 ppm, while those above 0.500 ppm increase with

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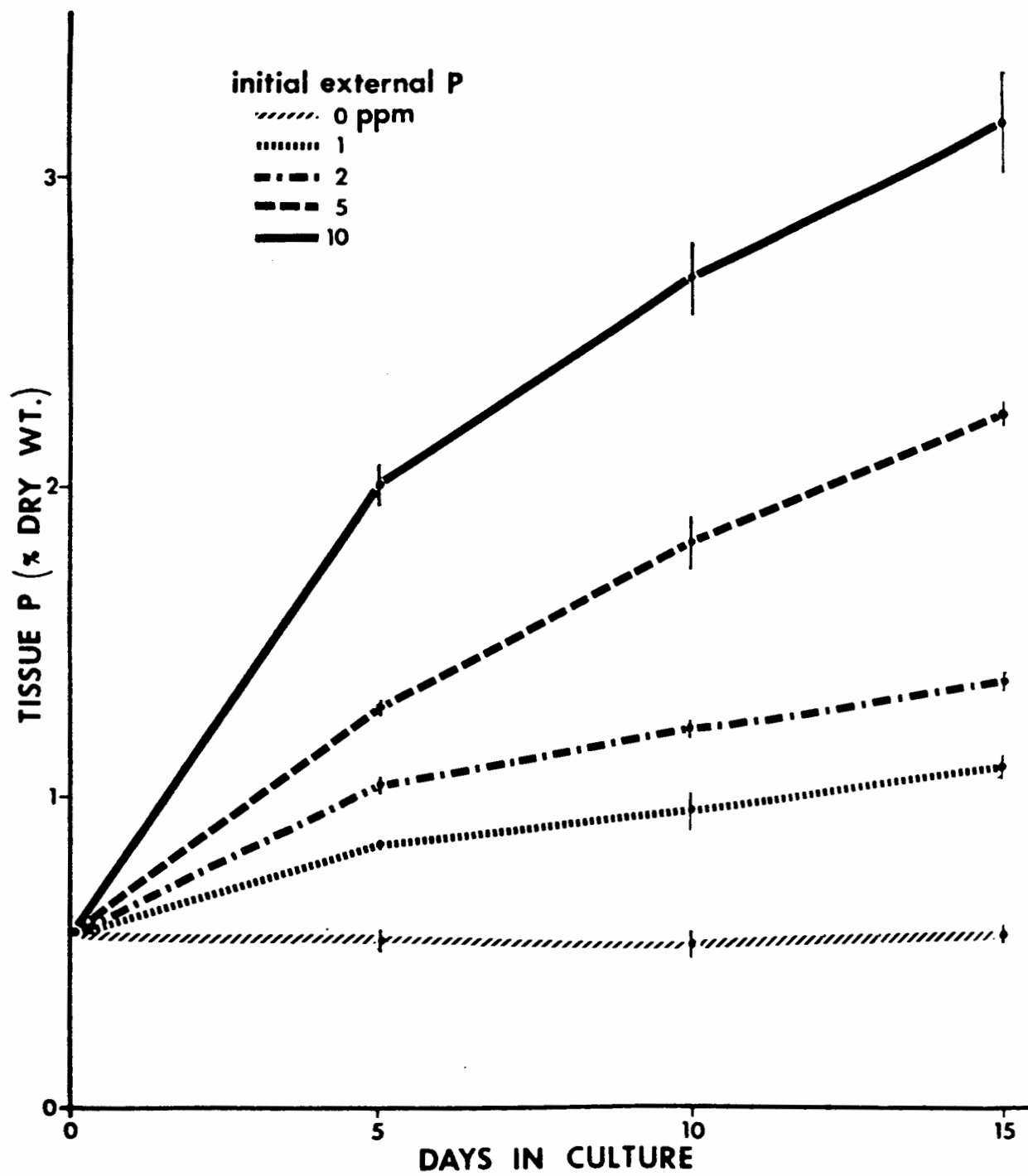
Figure 21. Tissue phosphorus changes with time, Batch 3. (± one standard error)
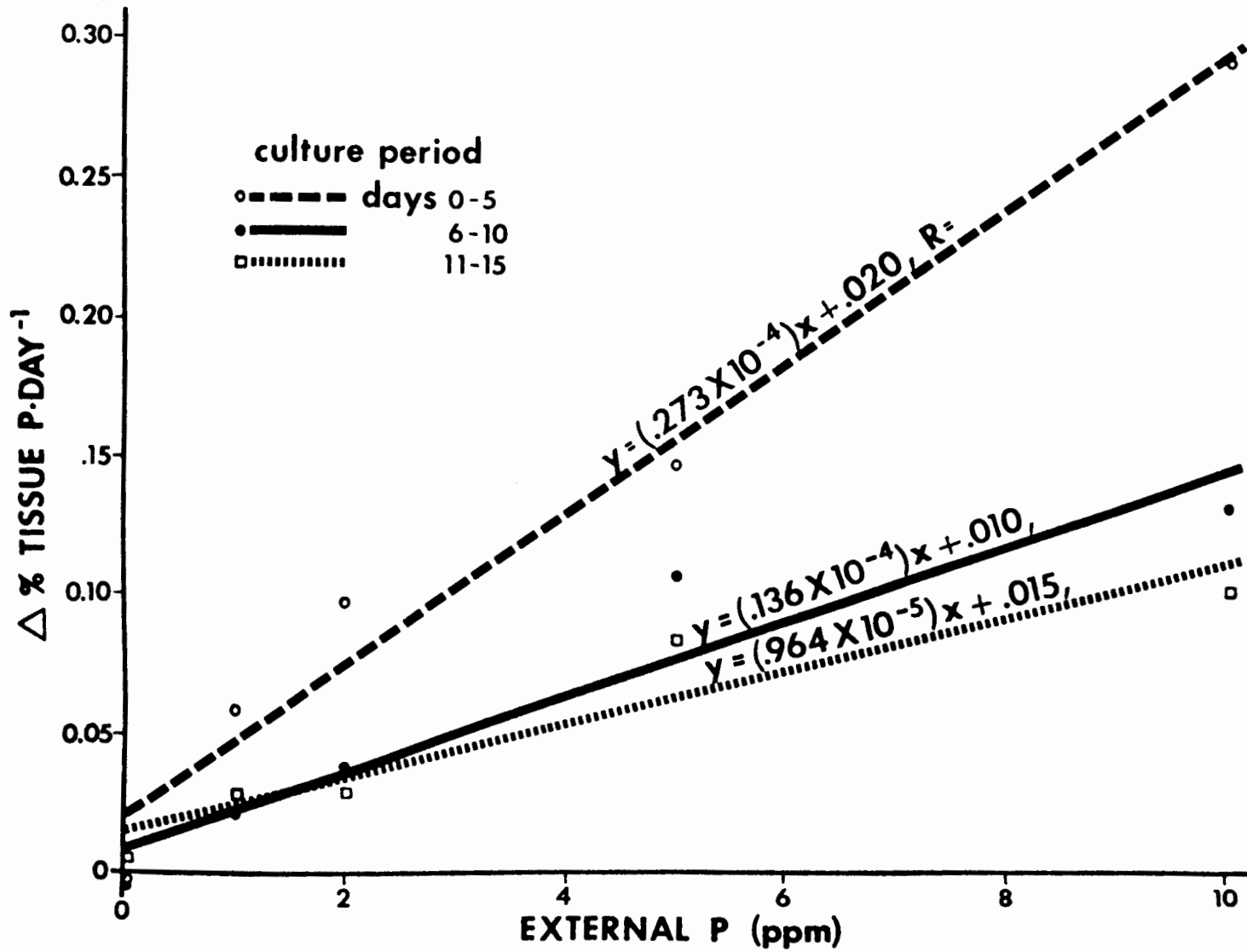
Figure 22. Phosphorus uptake rate in response to external phsophorus concentration, Batch 3.
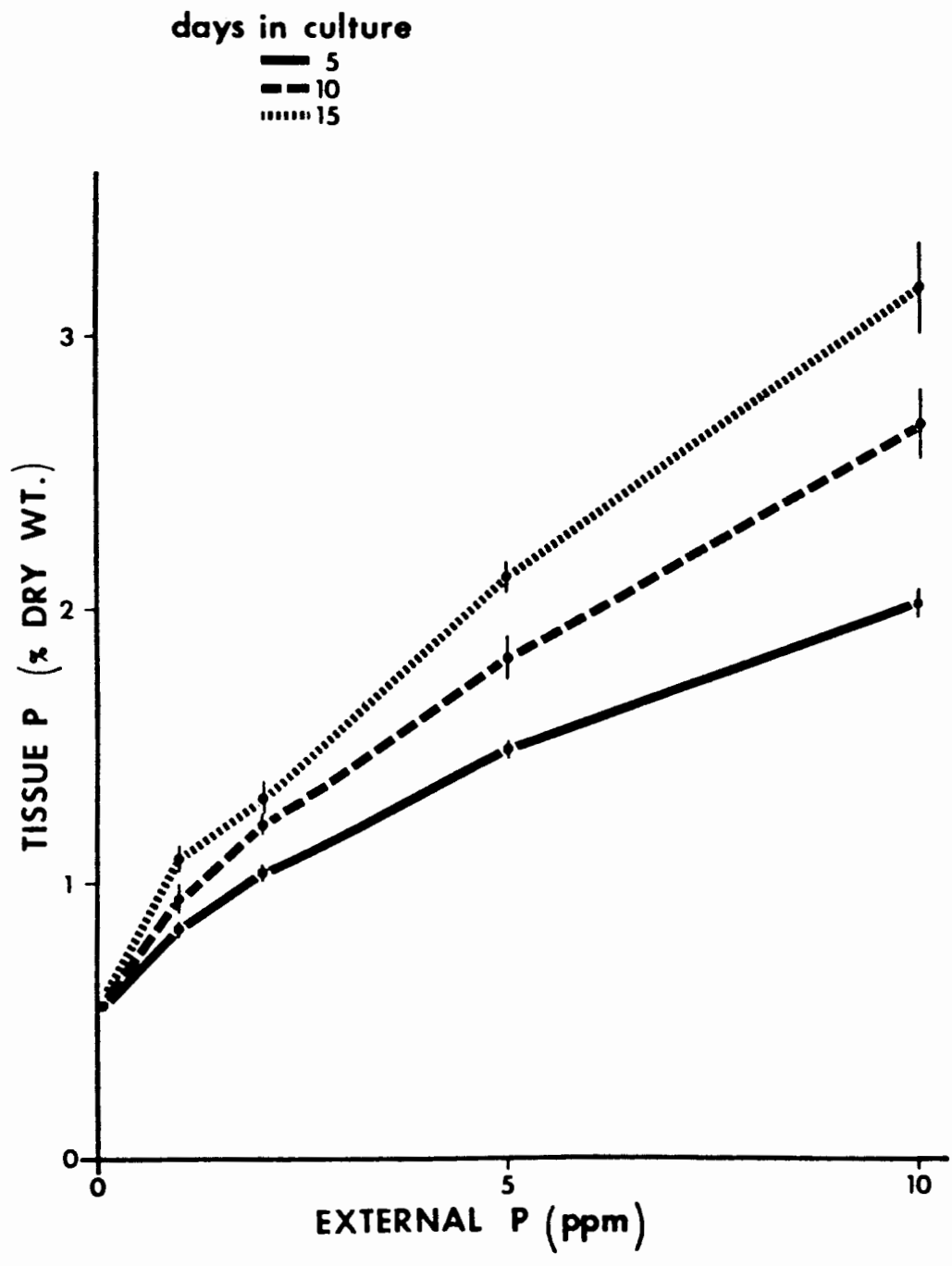
Figure 23.   Tissue phopshorus in response to external phosphorus
concentration, Batch 3.   (+ one standard error)

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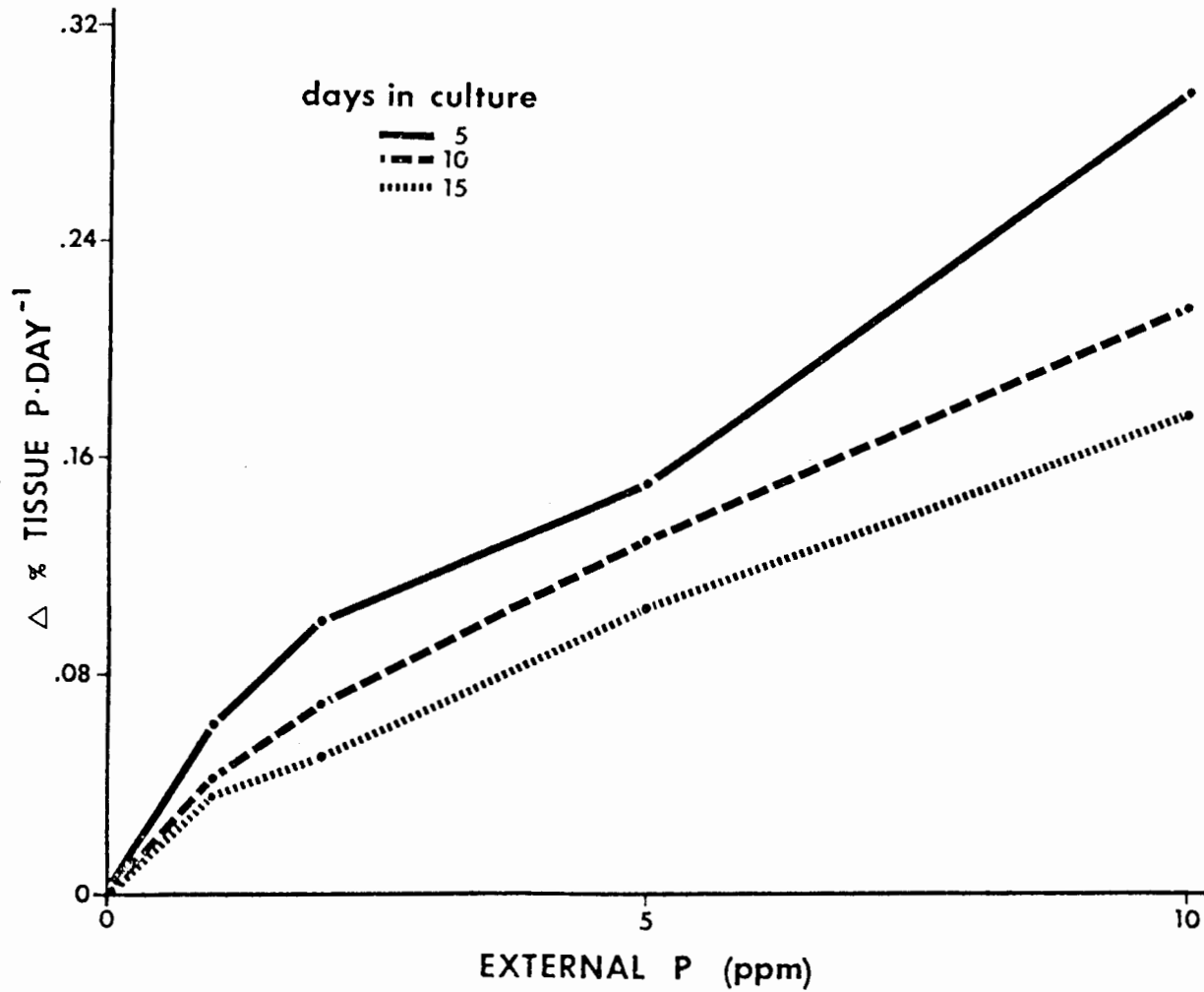
Figure 24. Phosphorus uptake rate in response to external phosphorus concentration after 5, 10, and 15 days in culture, Batch 3.
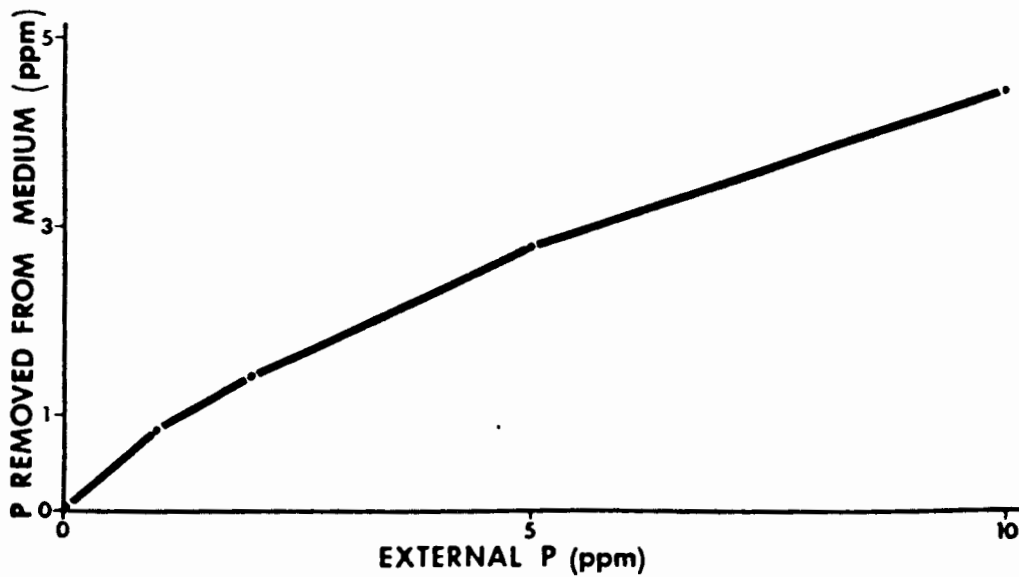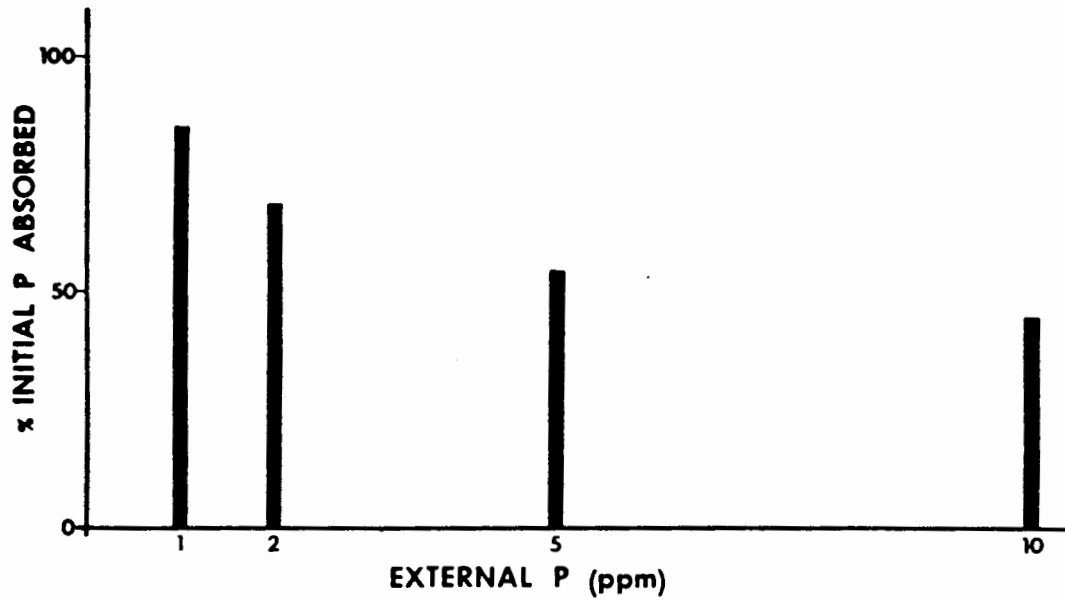
Figure 25. Percent of initial external phosphorus absorbed during culture and amount of phosphorus removed from the medium in response to external phosphorus concentration, Batch 3
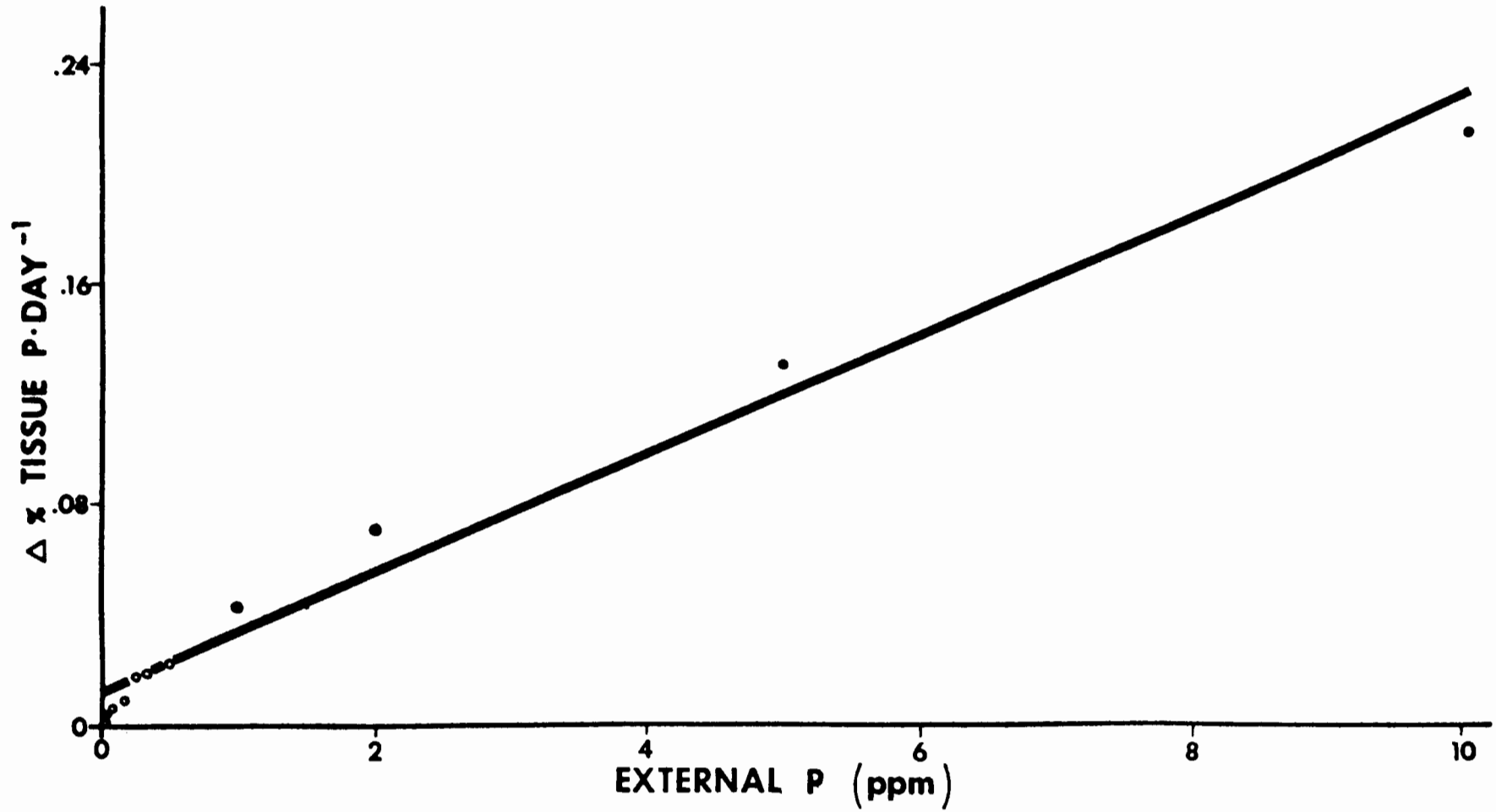
Figure 26. Phosphorus uptake rate in response to external phosphorus concentration, Batch 1 and 3.

increasing external phosphorus concentrations. The results suggest that two types of "uptake mechanisms" may be functioning simultaneously, one at high concentrations and another at low concentrations.

Uptake by mechanism A is directly related to the high phosphorus concentrations at which it operates. Mechanism B, which operates at low phosphorus concentrations, becomes saturated at approximately 0.400 ppm. By subtracting the saturation value of mechanism B from the values of mechanism A the two curves are separated and can be seen to operate together at all phosphorus concentrations (Fig. 27). A Hofstee plot of the data in figure 24, in which uptake rates and phosphorus concentrations are converted to moles/g/day and moles, respectively, produces a hyperbola (Fig. 28). The shape of the curve is indicative of two first-order reactions governing uptake at different phosphorus ranges (Hagen and Hopkins, 1955).

It seemed possible that the M. heterophyllum was not absorbing all its phosphorus through active uptake but instead some phosphorus was being adsorbed onto the plant surface in the form of insoluble phosphate salts produced by combination of phosphate ions with some of the divalent cations in the medium. Apices were cultured in modified Hoagland's medium without divalent cations, such as Ca, Fe, Mg, Mn, etc. in Batch 4. After 10 days of culture the tissue phosphorus levels had not increased at any external phosphorus concentration. The apices, which were healthy prior to the start of the culture, were becoming chlorotic and necrotic at the end of the culture. The Na^+ concentrations in the medium were probably damaging the plant, to the extent that they interfered with the physiological processes of uptake and metabolism. No attempts were made to further alter the growth medium.
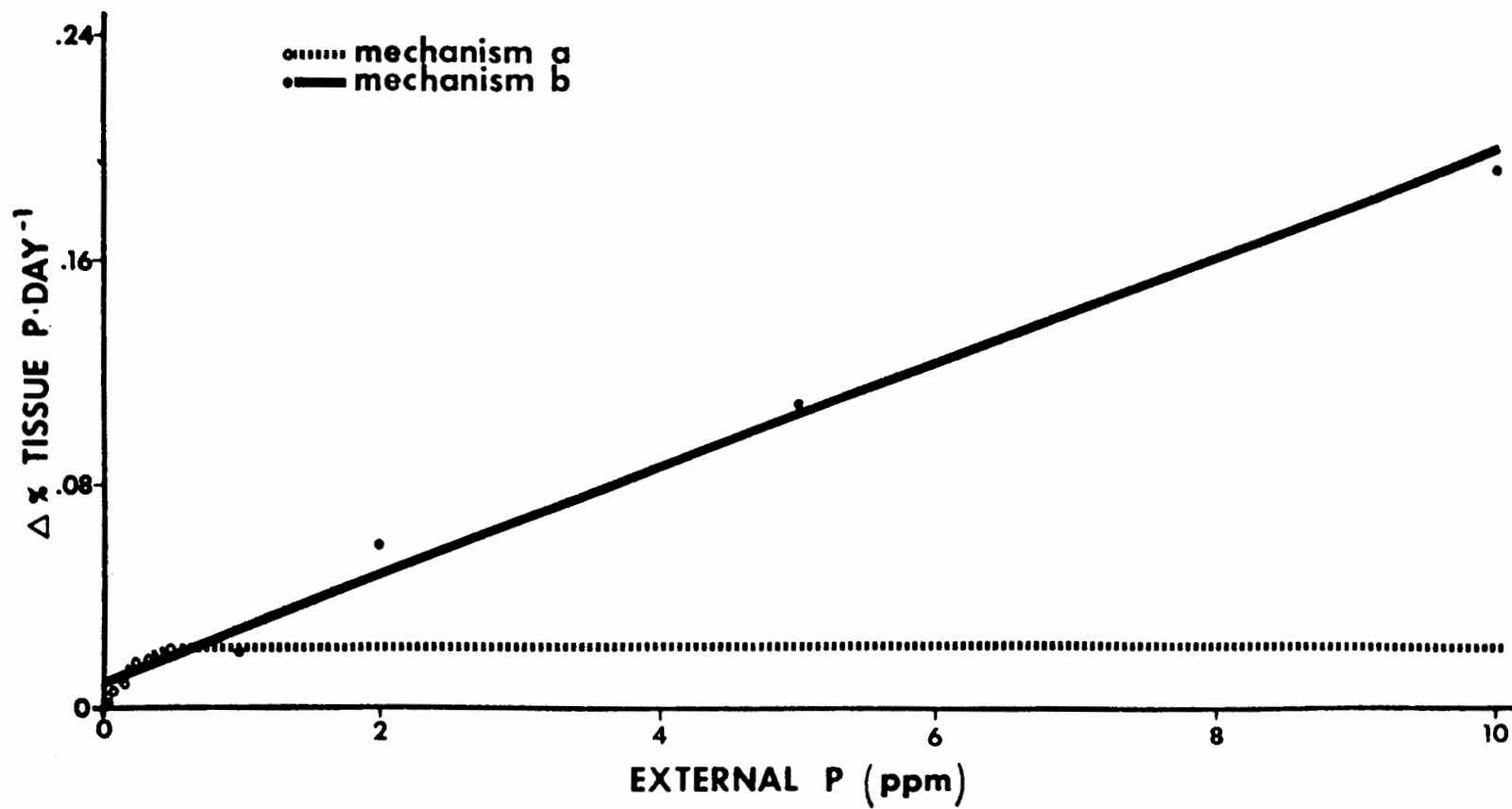
Figure 27. Separation of two uptake curves for uptake rate in response to external phosphorus concentration, Batch 1 and 3.
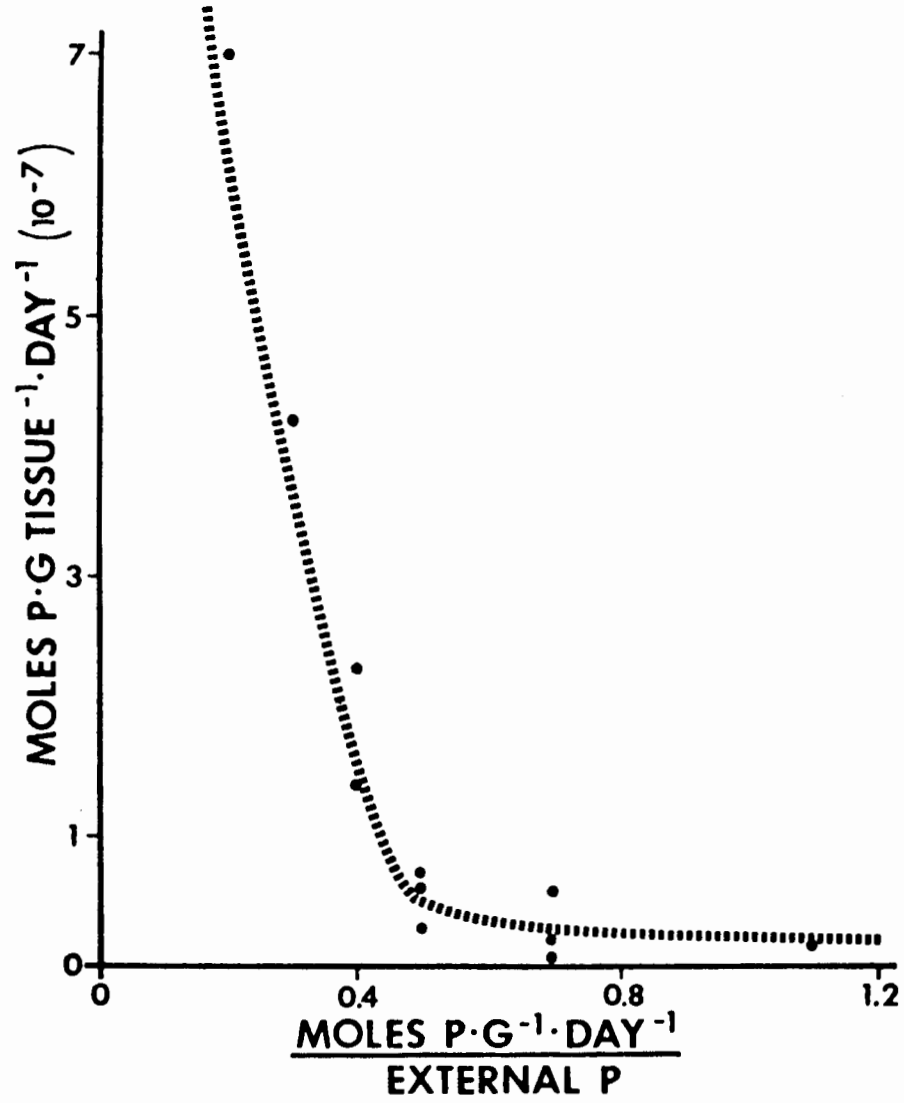
Figure 28. Hofstee plot (uptake rate vs. uptake rate/substrate concentration) of Batch 1 and Batch 3 data.

Another approach for assessing the possible influence of phosphate

compounds that may have precipitated onto the surfaces of M̲. heterophyllum

cultured at high external phosphorus levels was undertaken in Batch 5.

Apices were placed in an acid bath of 1 N hydrochloric acid for varying

lengths of time after culture to facilitate the dissolving of any phosphate

compounds on the plant surface, thus separating external from internal

phosphorus. The acid bath did not damage the plant tissue except when the

plants were held in the bath for 15 minutes, the longest time used. After

this length of time the leaf tips were slightly damaged by the acid.

Tissue phosphorus levels were unaffected by the acid bath, even after

15 minutes. The results indicate that the levels of externally precipitated

phosphate compounds are minimal or they are insoluble even under acidic

conditions.

The pH of the medium was monitored during culture of Batch 5. It

rose from about 4.5 to 6.0, at the end of the culture period. The pH

was similar for all external phosphorus concentrations throughout the culture

period.

Significant amounts of phosphorus were released into the medium by

decomposing apices both in distilled water and in a Silvex solution in

Batch 6. The apices in distilled water released phosphorus slowly because

they were not killed at day 0 but were allowed to die in the dark (Fig. 29).

The apices in Silvex were killed rapidly by the herbicide and quickly

released their phosphorus. After 66 days in distilled water the dry weight

of the decomposing apices was less than half of the initial dry weight.

26% of the total phosphorus in the system remained in the tissue. Although

final dry weight was slightly higher for the apices in the Silvex treatment,

the percent of the total phosphorus retained in the tissue after 66 days
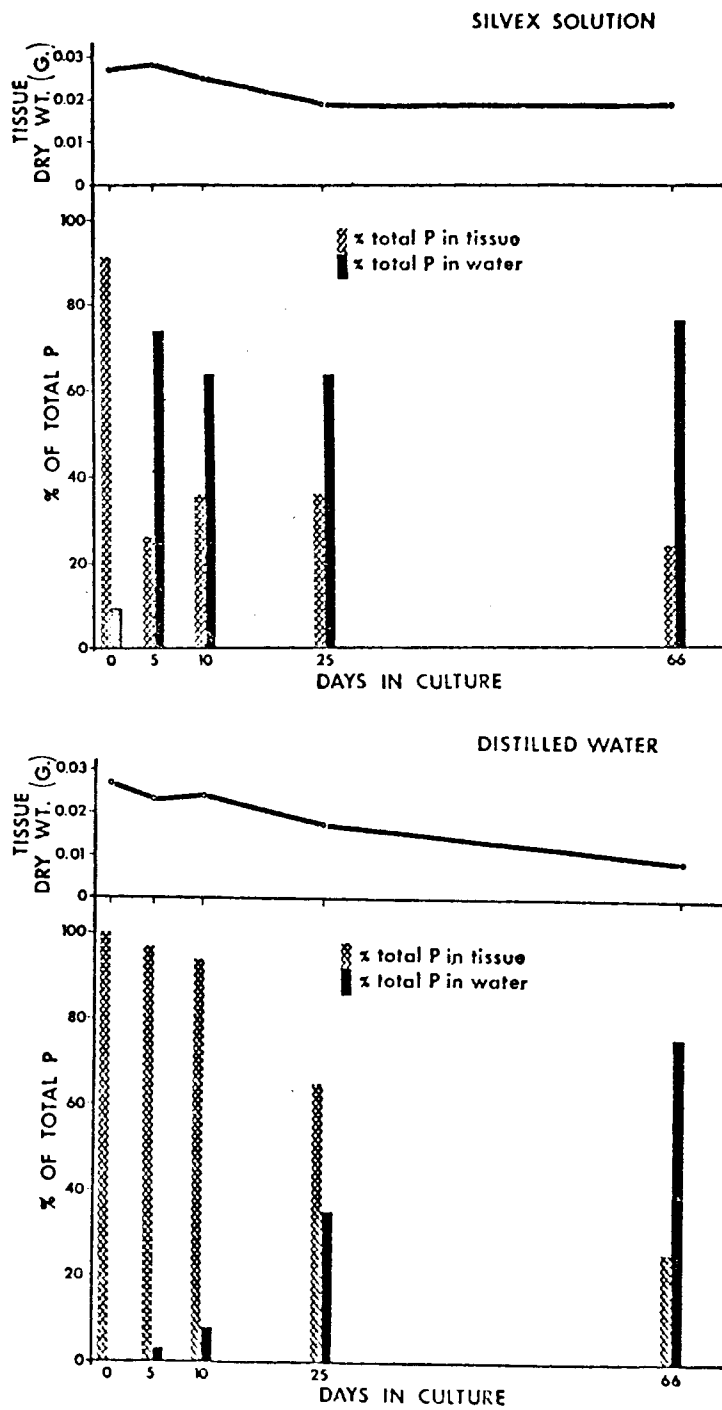
Figure 29. Phosphorus release from plants decaying in distilled water and Silvex solution, Batch 6.

was similar (24%) to that in the distilled water treatment.

To separate active uptake of phosphorus by the apices from any passive uptake or adsorption that might occur simultaneously, the uptake of phosphorus by live apices, dead apices, and cheesecloth (nearly all cellulose) was studied in Batch 7. Lake water and modified Hoagland's solution were used as growth media to demonstrate the effects, if any, of other nutrients present in large amounts in the modified Hoagland's solution (relative to lake water).

Cheesecloth sections absorbed no measurable amount of phosphorus from either lake water or modified Hoagland's solution. Dead apices, which initially had higher phosphorus levels than cheesecloth but lower levels than live apices, took up small amounts of phosphorus in response to external phosphorus concentrations. The dead apices took up slightly more phosphorus from the modified Hoagland's solution than from the lake water. Live apices had the greatest increase in phosphorus in both modified Hoagland's solution and lake water (Fig. 30). Live apices absorbed approximately the same amounts of phosphorus from lake water and modified Hoagland's solution alike (Fig. 31), indicating that the high nutrient levels of modified Hoagland's solution had no effect on phsophorus uptake after five days. Had the length of culture been longer, concentrations of nutrients other than phosphorus may have become important as they decreased. Phosphorus levels in the tissue (and uptake rates) increased with increasing external phosphorus concentrations up to 1 ppm, where they became constant (Fig.32). The cessation of uptake may be due to the short culture length or to other factors. Standard deviations from the mean were large and indicated the need for a larger sample size.

The results indicate that most of the phosphorus absorbed by live
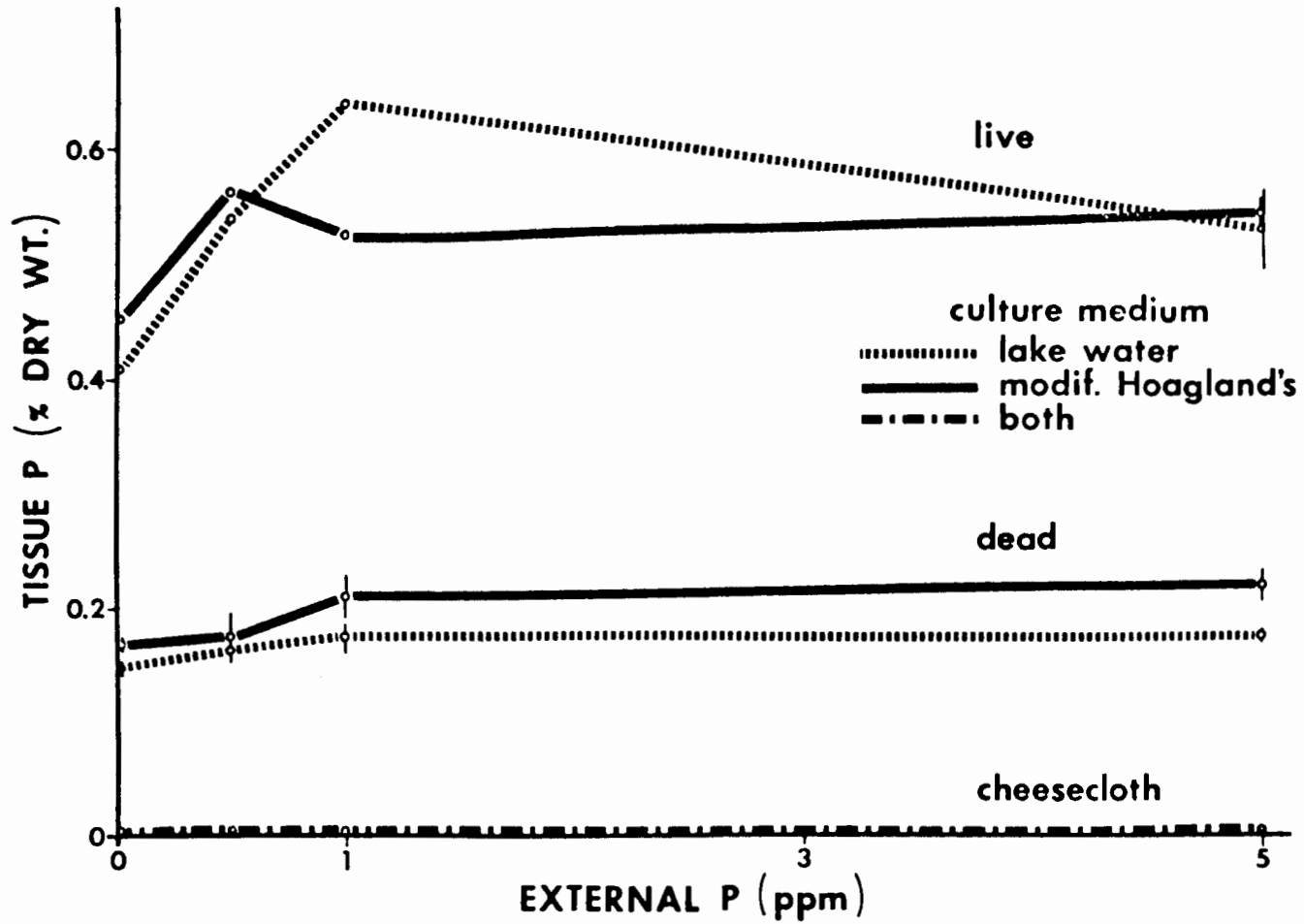
Figure 30.   Phosphorus levels in live and dead apices and cheesecloth in response to external phosphorus concentration, Batch 7.
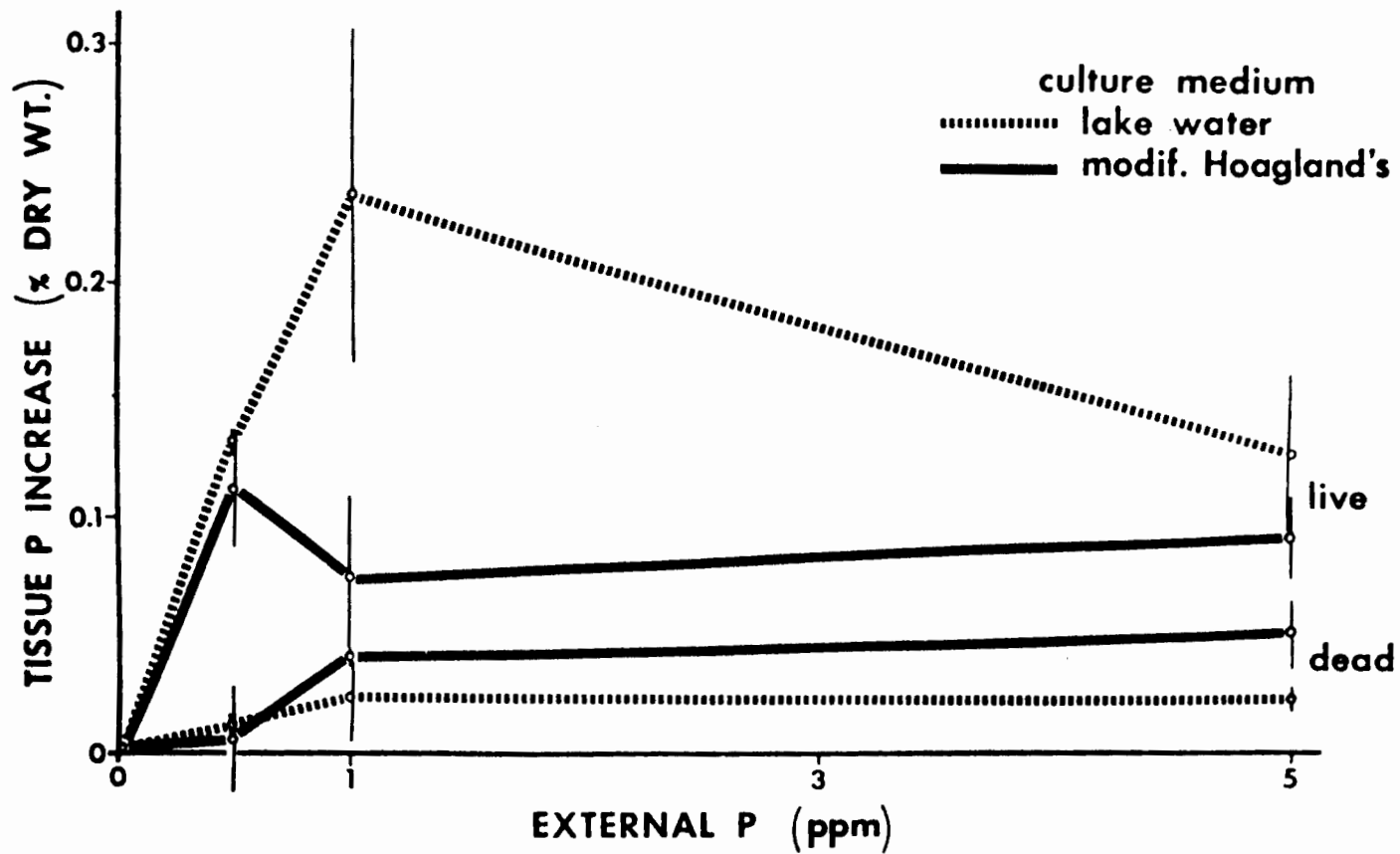
Figure 31. Tissue phosphorus of live and dead apices in response to external phosphorus concentration, Batch 7. (+ one standard error)
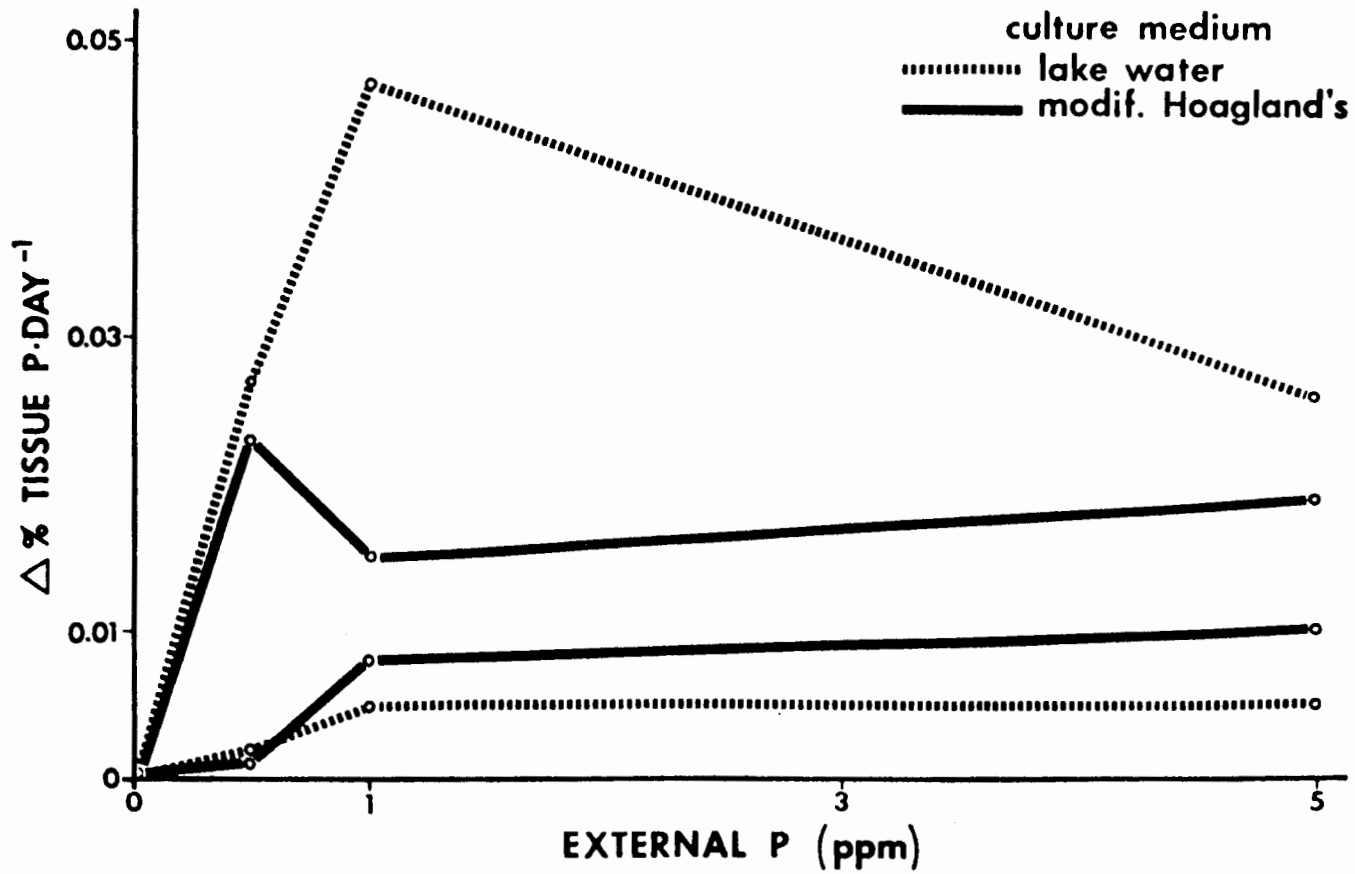
Figure 32. Phosphorus uptake rate of live and dead apices in response to external phosphorus concentration, Batch 7.

apices resulted from active processes, although small amounts are absorbed by passive means. Adsorption of phosphorus onto the leaf surfaces as a phosphate complex did not appear significant.

External phosphorus concentrations greater than 0.500 ppm (greater than those used in Batch 1) produced no effect on elongation of apices in Batch 8 (Fig. 33). Length increase was nearly the same for apices grown at 0, 0.500, and 1 ppm phosphorus for 15 days. Growth during the first six days of culture was much more rapid than during the last nine days, possibly due to the decrease of nutrients in the culture medium as the culture progressed.
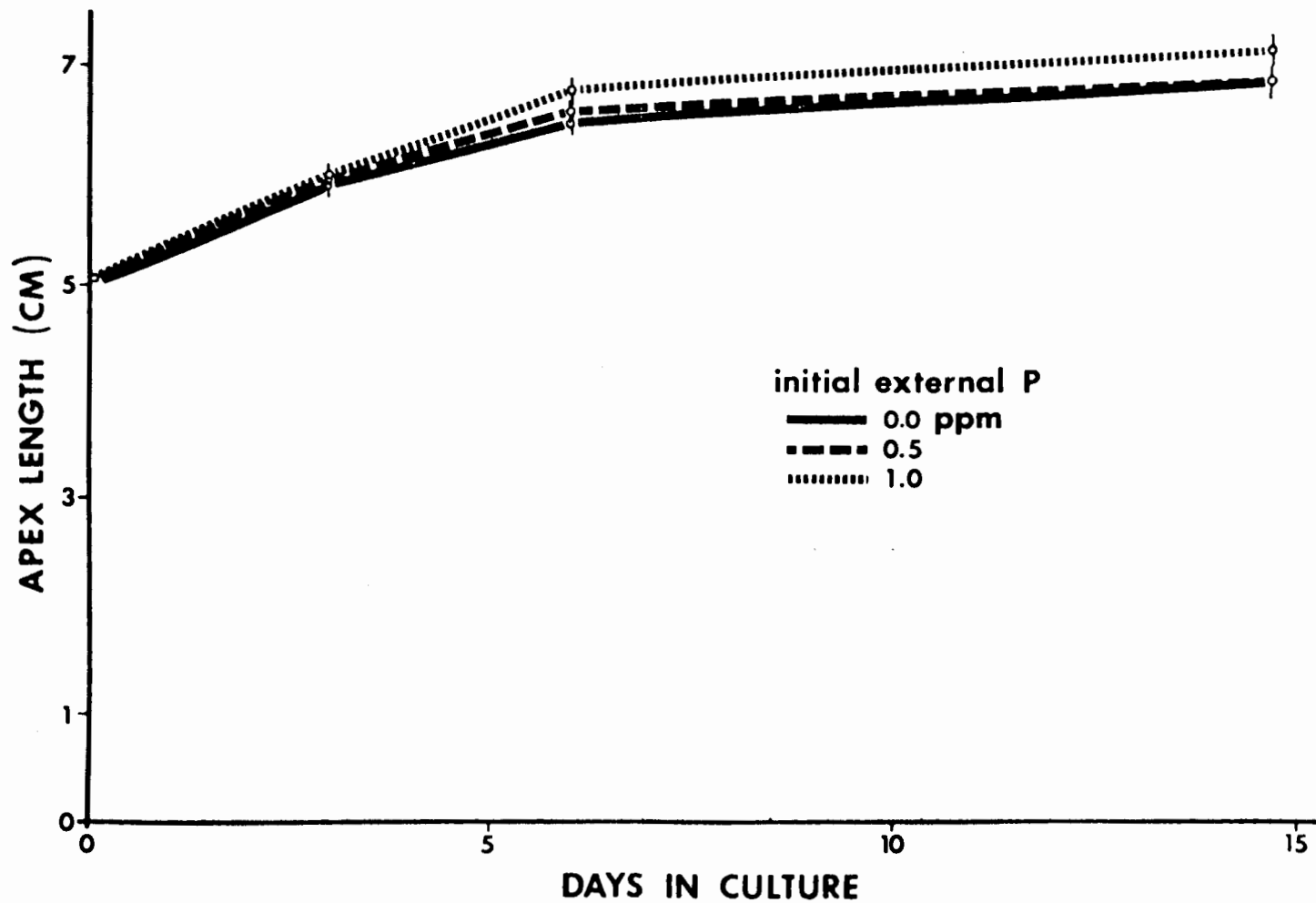
Figure 33. Shoot elongation with time in response to external phosphorus concentration, Batch 8. (± one standard error)

DISCUSSION

Distribution of Myriophyllum heterophyllum

Factors such as water quality, sediment quality and type, and
light can influence the distribution of aquatic vascular plants within
a lake. Myriophyllum heterophyllum populations are distributed in isolated
stands throughout Lake Winnipesaukee. Substrate texture and wave action
may be the controlling factors of its distribution. Populations of
water milfoil are present in areas with silt and sand substrates and low
wave action. Sand or highly organic mud bottoms may inhibit water milfoil
growth because it is not present in shallow waters where the substrate
is mostly sand or in the deep water where the bottom is organic mud.
It is also possible that at shallow depths wave action is inhibiting
and at greater depths light is limiting. However, M. heterophyllum is
not found in sandy or muddy areas even at its preferred depths of 1 to
2.5 m. M. heterophyllum is not present in a few area in the lake where
depth, substrate type, and wave action seemed to be suited for its
colonization and growth. Other factors such as water or sediment quality
may inhibit growth of the plant or the area may not yet be colonized.

Schmid (1965) studied aquatic vascular plant distribution with
SCUBA. He found that substrate texture influences vegetation distribution.
Lower plant density was observed on bark, cobble, and boulder substrates
than on other substrates. The distribution of six species was correlated
with substrate texture. Distribution of Myriophyllum spicatum was affected
by wave action and sediment type in the Neuseidlersee of Austria. The
action of the wind inhibited M. spicatum growth toward the center of the

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shallow lake, while sedimentation of extremely fine silt along the shore, due to the presence of a reed band, inhibited plant growth (Schiemer and Prosser, 1976). The inhibition of M. spicatum colonization is similar to that found in Lake Winnipesaukee except that in Winnipesaukee wave action is limiting along the shore and the fine sediments are limiting in the deeper areas away from shore. Pearsall (1920) and Spence (1967) described turbulence and substrate type as the major factors affecting plant distribution in the English lakes and Scottish lochs respectively.

Depth, as related to wave action, sediment type, and light availability, is also an important controlling factor of aquatic plant distribution. Schmid (1965) found that the distributions of eight aquatic vascular plant species were correlated with depth. As depth increases wave action decreases and sediment particle size usually decreases. Such changes with depth would affect plant distribution as described above. Light availability decreases with depth due to the absorption of light by the water and suspended matter. M. heterophyllum is not present at depths greater than 5 m possibly because sufficient light is not available for photosynthesis. Blackburn et al. (1968) describe light as the most variable and most important condition for aquatic weed growth. The relationship between M. heterophyllum distribution and light was not studied. Such an investigation might be profitable.

Sediment quality (e.g. pH, redox potential, and nutrient levels) affects the distribution of rooted vascular hydrophytes in addition to substrate particle size (Misra, 1938). Few other studies on this subject have been conducted. Misra found that different sediment types, classified according to their organic matter content, varied in nutrient content, pH, and particle size. The physical and chemical characters were closely

correlated with benthic vegetation. Sculthorpe (1967) attributes the effects of substrate on plant distribution to texture rather than chemical properties.

Water quality has been studied as a possible factor affecting vascular hydrophyte distribution. Results have been inconclusive in most cases. Hellquist (1975) attempted to correlate concentrations of some nutrients (nitrates, total phosphates, chlorides, and free carbon dioxide) and alkalinity with the distribution of Potamogeton species. He was unable to show a correlation between species distribution and concentration of any nutrient. A relationship between alkalinity and distribution of certain Potamogeton species was observed. Hellquist concluded that phosphorus levels in the water would not affect distribution of rooted aquatic plants because they probably can obtain phosphorus from the substrate.

McCombie and Wile (1974) described a correlation between conductance of water (as a result of nutrients) and the distribution of some species, while Denton (1966) was unable to correlate water quality with plant distribution. Most rooted aquatic plants may not be entirely dependent upon nutrient levels in the water for nutrition because they obtain some of their requirements from the sediment. Since M. heterophyllum is strongly rooted it is probably dependent upon sediment and not water for many nutrients. Therefore, water quality, except in the case of toxicity problems from pollutants, would not affect its distribution.

Water milfoil was found in at least six marinas in Lake Winnipesaukee. Local residents often ask whether the marinas are supplying "pollution" or nutrients necessary for the growth of M. heterophyllum. Nutrient input

is probably not the cause of the water milfoil problem because the plant is present in other areas in addition to marinas. The apparent "preference" of the plant for marinas may be a coincidence. Both the plant and the marina builder choose protected sites in shallow water where wave action is low and a silt and sand substrate is present. Marinas are also disturbed areas and often have been dredged. Such dredging (and filling) has been cited as a cause for the growth of M. spicatum in Lake Wingra (Nichols and Mori, 1971). Dredging may remove the thick layer of organic mud that overlies the sand in Lake Winnipesaukee to produce a substrate conducive for growth of the plant. Certainly the blame for the weed problem in Lake Winnipesaukee cannot be laid on marina owners.

Density, Biomass, and Stand Expansion

The growth of Myriophyllum heterophyllum was very dense at Alton Bay Beach (71-132 g dry weight/m^2) and Green's Basin Beach (84-120 g dry weight/m^2). Boyd (1975) reported a biomass of 81.9 dry g/m^2 (731 dry lb/acre) of M. heterophyllum in an Alabama fish pond, a value close to the minimum biomass estimates of Alton Bay Beach and Green's Basin Beach.

Although Alton Bay Beach biomass figures for 1976 are not available, growth seemed more lush in 1976 than during the previous year. The increase in numbers of plants observed in Ostrand's Marina between 1975 and 1976 indicates that biomass can increase significantly from one year to the next. It is not known at what density the plants cease spreading as a result of crowding or competition for light and nutrients.

Observations of plants by SCUBA showed that increased biomass within an established stand of plants is a result of two reproductive processes: 1) increase in size of existing plants through production of new shoots

from the rhizomes and 2) rooting of plant fragments with subsequent production of new shoots. Both processes were evident in Ostrand's Marina and Alton Bay Beach. The size (number of shoots) of a M. heterophyllum plant appears to be proportional to its age. Plants at both sites increased in size between 1975 and 1976.

Rooting plant fragments were observed at Alton Bay Beach in previously uncolonized areas and in a few instances in the Ostrand's Marina quadrats. Rooting of plant fragments is clearly an effective means of reproduction because numbers of plants increased in the quadrats. Smith (1971) calculated that one shoot fragment of Myriophyllum spicatum was capable of producing 250×10^6 new fragments within one year.

Because water milfoil is a perennial plant, its growing season lasts from ice-out in mid April until October or November. Growth rates were measured only from June through October. During the summer months growth was most rapid, approaching nearly 2 cm/day. Myriophyllum exalbescens in University Bay of Lake Mendota, Wisconsin exhibited its most rapid weight increase from June to August (Lind and Cottam, 1969). Growth of M. heterophyllum in Lake Winnipesaukee followed this pattern. The weight of M. exalbescens decreased rapidly from August through September and then increased slightly in October. Growth of M. heterophyllum continued, but more slowly, through October. The apparent continuation of growth may be due to the averaging of growth rates from August to October, as no measurements were made between those dates. Growth may have stopped by September.

Seasonal biomass measurements were not made, but total standing crop of M. heterophyllum would have been greatest during July or August when growth rate was maximal and before autumn dieback had begun. After

August the decrease in growth rate and the sloughing off of tissue produced an apparent decrease in biomass. Maximum standing crop for M. spicatum in Lake Wingra (Adams and McCracken, 1974) and Myriophyllum verticillatum in Ösbysjön (Forsberg, 1959) occurred during late August.

Growth of M. heterophyllum is temperature dependent, and is most rapid during June through August when water temperatures are highest. The field observation is confirmed by Batch Culture 1 where growth was most rapid at temperatures between 18 and 30 C. The metabolic rates of Myriophyllum alterniflorum and Myriophyllum tenellum were more rapid at 23 C than at 2 C, regardless of whether the plants were collected in midwinter or midsummer (Boylen and Sheldon, 1976). Biomass (and therefore growth rate) of M. spicatum was greater in warm water stations than in cool water stations at a nuclear reactor site (Grace and Tilley, 1976).

The rapid stand expansion of M. heterophyllum from plant fragments and rhizomes and its rapid growth rate make it an effective colonizer in Lake Winnipesaukee. It is present in many areas of the lake, but many areas suitable for its growth are yet uncolonized. It is probable that the plant will establish stands in many more areas of the lake in the future and that the size of present populations will increase.

Phosphorus Cycling by Myriophyllum heterophyllum

Tissue phosphorus levels of Myriophyllum heterophyllum varied seasonally at each site. The patterns of the seasonal changes differed spatially. Caines (1965) described a seasonal pattern of tissue phosphorus levels for Myriophyllum alterniflorum similar to that of M. heterophyllum at Alton Bay Beach. Both species reached maximum tissue phosphorus levels during late spring and minimum levels during the winter.

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Myriophyllum exalbescens exhibited a maximum in tissue phosphorus during
May, low tissue phosphorus throughout the summer, and a slight increase
in the fall (Wile, 1974). Winter values were not reported.

Temperature and growth rate of the plants probably produce changes
in tissue phosphorus levels on a seasonal basis. In batch culture, phos-
phorus uptake was more rapid at high than at low temperatures. During
the winter months water temperatures of approximately 4 C would slow
phosphorus uptake by slowing metabolism of the plants. As water temperature
rises in the spring after ice-out, the phosphorus uptake rate of the
plants would increase allowing more rapid accumulation in the tissue.

Temperature affects the growth rate of the plants as well as their
phosphorus uptake rates. During the spring as phosphorus uptake rates
increase, growth rates also increase. Until the May or June maximum
of tissue phosphorus, uptake rates (supply to the tissue) of phosphorus
are greater than usage rates for growth. At a certain point during June,
temperatures may reach a level at which phosphorus uptake remains constant
and growth rate reaches a maximum. The combination of the two factors
produces a phosphorus usage rate greater than phosphorus uptake rate,
thereby "diluting" the phosphorus in the plant. Tissue phosphorus would
decrease rapidly at this point, exhibiting a period of maximum growth
and minimum tissue phosphorus. A hypothetical graph of phosphorus uptake,
temperature, and growth rate shows the possible relationship of these
factors.

Caines (1965) attributed the tissue phosphorus decrease in M.
alterniflorum during early summer to the initiation of flowering. He
theorized that phosphorus is moved into the flowering parts of the plants
producing a decrease in phosphorus in other parts of the plant. The lack

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of flowering plants in Alton Bay Beach, Green's Basin Beach, and the scarcity of flowering plants in Green's Basin Marsh would not allow the use of Caines' explanation. Flowering did occur in Twentymile Brook, Parker's Marina, and Lees Mills Stream. Phosphorus levels in flowering apices were usually equal to or slightly lower than those in the vegetative apices.

Seasonal changes in the availability of phosphorus to the plants might be another explanation of changes in tissue phosphorus. However, it is thought that most of the phosphorus obtained by water milfoil is from the sediment rather than from the water. Seasonal changes in sediment phosphorus were minimal. Phosphorus levels of the water were not monitored at each site and they may have influenced tissue phosphorus levels. No seasonal variation of the low phosphorus values of Smith Cove water was evident.

M. heterophyllum plants growing in streams (Lazy Meadow Brook and Twentymile Brook) and those in Green's Basin Marsh did not exhibit the same seasonal pattern of tissue phosphorus as Alton Bay Beach plants. Tissue phosphorus of Green's Basin Marsh plants remained nearly constant throughout the year, possibly due to the heavy epiphyte cover that frequently affected the plants, causing reduced growth rate or reduced photosynthesis due to shading. The actual effects of heavy epiphyte growth on aquatic macrophytes are not known.

Tissue phosphorus levels of the Twentymile Brook and Lazy Meadow Brook plants may have been affected by the constant flow of water over the plants. Although phosphorus levels in the water are usually low, a constant flow of water by the plants may make nutrients more available ofr uptake. Hellquist (1975) reported greater total phosphorus levels

in streams and rivers than in lakes and ponds. Total phosphorus in Lazy Meadow Brook water was often higher than at Smith Cove during 1975. The greater amount of total phosphorus may indicate a greater availability of phosphorus to the plants, either from the water or from the substrate. Seasonal variations in the flow rate of the streams could also account for the seasonal tissue phosphorus variations. A study of the water and sediment phosphorus levels along with tissue phosphorus levels in these two streams would be profitable.

Sources of nutrients for aquatic vascular plants have been under debate for a long time (Sculthorpe, 1967). Many researchers feel that the water is more important than the sediment as a nutrient source and vice versa. It has been shown that Myriophyllum species are capable of absorbing phosphorus through both shoots and roots (DeMarte, 1969; Bristow and Whitcombe, 1971; and DeMarte and Hartman, 1974). Uptake of phosphorus by M. heterophyllum shoots was evident in batch culture and in the in situ enrichment study where the shoots readily took up phosphorus supplied to the water. Caines (1965) produced an increase in the tissue phosphorus of M. alterniflorum and Myriophyllum spicatum through direct fertilization of the lake water with calcium superphosphate. However, it is difficult to believe that the water can be the main source of phosphorus for M. heterophyllum when phosphorus levels are low. Phosphorus values for the water at each study site are unavailable, so it cannot be proven that tissue phosphorus is not related to phosphorus availability in the water of Lake Winnipesaukee.

Phosphorus concentrations in the water milfoil plants are many times greater than those of the water in Smith Cove. Boyd (1970) reported that the ratios of nutrients contained in the water of Par Pond were

dissimilar to those found in M. heterophyllum plants growing there. He did not discuss the ability of the plants to take up nutrients from the water and to concentrate them in ratios varying from those found in the water. Myriophyllum spicatum plants exhibited differences in tissue nitrogen according to their location in Lake Wingra (Nichols and Keeney, 1976) but no differences in nitrogen in the water were observed. The distribution of M. exalbescens was not related to phosphorus concentrations in the water (Jones and Cullimore, 1973), but the authors stated that aquatic plants depend upon phosphorus in the water and not on the less readily available phosphorus in the sediments. Their discussion mostly concerned weakly rooted or nonrooted plants such as Potamogeton and Ceratophyllum species. Their observations on Myriophyllum did not fit this generalization. Hellquist (1975) was unable to correlate distribution of Potamogeton species with phosphorus concentration in the water. From these findings it seems evident that Myriophyllum species are not dependent upon the water as a phosphorus source, although other species might be. The only situation in which Myriophyllum might be dependent upon the water as its sole phosphorus source is when fragments are released from the plant and they can no longer obtain nutrients through the plant roots (Wilson, 1972). Even in this case the plant could utilize stored phosphorus until it was able to root in the substrate. As was shown in the batch cultures, M. heterophyllum apices store large amounts of phosphorus.

Sediments probably provide a major portion of necessary phosphorus to rooted M. heterophyllum plants. Lake Winnipesaukee sediments contained up to 0.1% total phosphorus. Not all of this phosphorus is available for uptake by the plant roots, because some of it is strongly bound with

other elements. However, Harter (1968) reported the capacity of lake sediments to loosely bind phosphorus and later to release it, making it available to aquatic plants.

Ample evidence for uptake of phosphorus from sediments by species of rooted hydrophytes is available. Myriophyllum species are capable of taking up both phosphorus (DeMarte, 1969; Bristow and Whitcombe, 1971; and DeMarte and Hartmen, 1974) and nitrogen (Toetz, 1974) from sand, mud, and water in both the light and dark and translocating the nutrients to the shoots. McRoy and Barsdate (1970) reported that Zostera marina takes up phosphorus from the sediments and moves it into the leaves. Although Waisel and Shapira (1971) have found that M. spicatum roots are capable of taking up phosphorus, they contend that the primary function of roots is not for nutrient uptake but for the production of growth hormones. The roots of M. heterophyllum possess an endodermis which, according to Sculthorpe (1967), contrasts with the supposed reduction of vascular tissue in aquatic plants and would indicate the function of roots in nutrient absorption.

The dependence of different species upon roots for nutrient uptake depends upon how strongly rooted the plants are (Denny, 1972). Plants dependent upon their root systems for nutrient uptake grew better in mud, which was higher in nutrients, than they did in sand. M. heterophyllum possesses an extensive system of fibrous roots that penetrate 20 to 30 cm into the substrate. It would appear that these roots function in nutrient uptake as well as anchoring the plant. Analysis of phosphorus levels in the plants and the substrate in which they were growing showed a correlation between tissue phosphorus levels and sediment phosphorus levels. The mean tissue phosphorus of all plants at each site was dependent

upon the mean sediment phosphorus levels of that site, indicating that, although M. heterophyllum shoots are capable of taking up phosphorus, the sediment may function as the major phosphorus source for the plants in Lake Winnipesaukee.

The rapid uptake and storage capacity of M. heterophyllum for phosphorus was demonstrated in the batch cultures. Most of these cultures were at phosphorus concentrations thousands of times greater than those found in the water or sediments at Lake Winnipesaukee. However, phosphorus uptake by water milfoil shoots was rapid, regardless of the external phosphorus concentration. The ability of M. heterophyllum to store large amounts of phosphorus as determined by the batch culture studies was reflected in the tissue phosphorus levels observed for plants in situ. During some months plants reached tissue phosphorus concentrations greater than 0.7% dry weight. The high phosphorus levels are a function of luxury consumption (Gerloff, 1975) and are not necessary for growth and metabolism of the plant.

Although M. heterophyllum stores large amounts of phosphorus, all phosphorus absorbed may not remain in the plant, but may instead be released through its foliage. DeMarte and Hartman (1974) reported that healthy as well as injured M. spicatum plants release phosphorus-32 to the surrounding water, making it available for uptake by other plants or algae. Release of phosphorus from the plants can also occur upon death and decay, as observed in Batch 6. Plants surviving Silvex application in Green's Basin Beach were high in phosphorus, indicating they may have absorbed phosphorus released from decaying plants. M. heterophyllum may function as a phosphorus pump by removing phosphorus from the sediments through its roots, into its shoots, and out into the surrounding water in

the manner described for M. spicatum (DeMarte and Hartman, 1974).

Much of the phosphorus released from the foliage of live Myriophyllum could be taken up by the large epiphyte population covering the older portions of the shoots. McRoy and Goering (1974) determined that such is the case for Zostera marina and its epiphytes. Carbon-14 and nitrogen-15 obtained from the sediments by Z. marina roots were excreted through the leaves to the epiphytic algae. It is probable that the same nutrient release to epiphytes occurs for M. heterophyllum.

If M. heterophyllum cycles phosphorus from the sediments in the manner described for Z. marina, by virtue of its great biomass, it probably dominates the phosphorus cycling in the littoral zone it nhabits in Lake Winnipesaukee.

Value of Myriophyllum heterophyllum

To discuss the importance of Myriophyllum heterophyllum in Lake Winnipesaukee would seem presumptious to many because it is considered nothing more than a noxious weed by many people living around the lake. Although it may "pump" phosphorus out of the sediment and into the water, M. heterophyllum probably serves as a phosphorus trap by removing most of the available dissolved phosphorus as well as particulate phosphorus (by filtration of the water) from the water in the areas in which it grows. Such trapping has been observed directly in a laboratory aquarium, and indirectly by noting the increase of water turbidity after the removal of the M. heterophyllum stand at Alton Bay Beach. The trapping of phos-phorus could prevent formation of algal blooms that are also considered problems because they interfere with swimming, use of the water for drinking, and may cause fish kills. In some southern Ontario impoundments aquatic macrophytes contain quantities of minerals in their tissues that, when

compared to mineral levels in the water, comprise a large portion of the nutrients available in the environment (Wile and McCombie, 1972). The plants remove the nutrients from circulation for extended periods of time compared to the generally more rapid nutrient turnover by algae. The same may be true of water milfoil for the areas in which it grows.

M. heterophyllum provides food and shelter for many forms of animal life: fish fry, insects, invertebrates, crayfish, etc., all of which are important parts of the food chain in Lake Winnipesaukee.

Understandably, the rank growth of water milfoil in Lake Winnipesaukee is a nuisance. It creates problems for swimmers and boaters with its luxuriant growth. Control measures for some M. heterophyllum stands may be necessary for safety and economic reasons.

Widespread use of herbicides (Silvex being the most common in Lake Winnipesaukee) may create another set of problems in the future. The herbicide almost completely kills the stand on which it is sprayed. Destruction of the plant populations eliminates a valuable nutrient trap, and a food and "shelter organism" of the lake. Such habitat modification may upset the delicate ecological balance within the lake. In addition, the long-range effects of Silvex (and other herbicides) on the ecology of a lake are largely unknown. Silvex degradation and the effects of its degradation products upon aquatic organisms have not been studied extensively. It is found in significant quantities in water for as long as five weeks after application (Mulligan, 1968). Until the long-range effects of Silvex are known it should not be used as a widespread control measure for water milfoil.

The trapping ability for nutrients by water milfoil could effectively be put to use in combination with a control program for the plant.

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Harvesting as a means of water milfoil control would remove considerable quantities of nutrients from the environment. Plants in Alton Bay Beach and Green's Basin Beach would contain between 0.36 and 0.66 g phosphorus/m^2 during the period of maximum biomass in July or August, assuming that tissue phosphorus levels are approximately 0.5%. When areas of plants as large as a hectare are harvested, removal of phosphorus from the environment becomes significant.

Further regrowth of the plants and subsequent harvesting would remove still more nutrients. Control of growth with an herbicide would only return nutrients to the environment making them available for uptake by remaining M. heterophyllum plants as well as algae. Wile (1974) lists three advantages of harvesting as a means of aquatic plant control: 1) removal of biomass (decreasing sedimentation), 2) less drastic effects on the ecology than chemicals would create, and 3) nutrient removal.

Batch Cultures

Batch cultures were used for the study of phosphorus uptake by Myriophyllum heterophyllum shoots for their ease in establishment and maintenance. Because the nutrient media used in the cultures were not changed during the culture period, concentrations of many nutrients would have decreased significatly during that time. However, concentrations of nutrients in the modified Hoagland's solution used (Table 3) were probably sufficient initially so that nutrient limitation after 15 days (the longest culture period used) was unlikely. Ideally, a chemostat culture providing a constant exchange of nutrient medium should be used. Use of this type of culture system was attempted, but it proved to be impractical, as discussed previously.

Temperature is an important influence on uptake rates, as seen in

Batch 1. At low temperatures (4 C) uptake was slower than it was at higher temperatures (18 and 30 C). Increased temperature affects both diffusion of ions and active uptake of ions by increasing the rate of both. Higher temperature produces more rapid movement of the ions in the solution and therefore a more rapid rate of diffusion. Ions moving into a cell by a passive mechanism, diffusion, would move more rapidly at higher temperatures. Ions being actively absorbed by a cell, therefore involving an enzyme system of the cell, would also be taken up more rapidly at higher temperatures because the rate of most enzyme reactions doubles for each 10 C rise in temperature (Lehninger, 1975). Therefore, whether uptake of ions (phosphate ions in this case) is passive or active, the rate of uptake is affected by temperature. Temperature of the water and sediments in the natural system probably affects phosphorus uptake rates there, as well.

The pH of the culture medium has been described by a number of authors as an important factor influencing the uptake of ions by plants. Hagen and Hopkins (1955) and Olsen (1953) found that uptake of phosphate ions by excised roots of Hordeum vulgare (barley) and Secale cereale (rye) was maximum at pH 4.0 to 5.0. Higher pH decreased phosphate uptake rates. Batch cultures of M. heterophyllum had an initial pH of 4.5 that increased to approximately 6.0 to 6.5 after nine days (Fig. 60). The gradual pH increase could have produced a decrease in phosphorus uptake as the culture progressed, similar to that seen in Batch 3. Conversely, chemical adsorption and/or precipitation would have been enhanced because of the increasing insolubility of phosphate salts at higher pH levels. It would have been desirable to buffer the culture medium to prevent or minimize pH changes.

Light was a constant factor in all of the batch cultures and could

not account for any of the differences found for phosphorus uptake.
Gerloff (1973) found no indication that a light-dark cycle was superior
to continuous light in culturing aquatic plants. He did not investigate
the differences in uptake between plants in the dark and in the light.
McRoy and Barsdate (1970) studied the differences in phosphorus-32 uptake
by shoots of Zostera marina and found that uptake was greatest in the light
but that it continued in the dark as well. Changes in light levels on
a seasonal and diel basis in the natural system may affect phosphorus
uptake by M. heterophyllum, but because light levels in culture were
constant they can offer no explanation of the results obtained. Changes
in phosphorus uptake by M. heterophyllum as a result of changing light
intensity, quality, and varying light-dark cycles would constitute an
interesting and valuable study.

Batch cultures were constructed for the study of phosphorus uptake
kinetics of M. heterophyllum. Special questions were: 1) How much phosphorus
could the plant take up?, 2) How rapidly could phosphorus be taken up?,
3) At what phosphorus levels would the uptake mechanism(s) be saturated?
At both low (0 to 0.5 ppm) and high phosphorus levels (1 to 10 ppm)
uptake rates were proportional to external concentrations, that is,
uptake rates were greatest at highest external phosphorus concentrations.
No apparent saturation point of uptake was reached, regardless of the
external phosphorus levels. The maximum external phosphorus concentration
used in culture was 10 ppm, a value far greater than any found in Lake
Winnipesaukee, and yet the phosphorus uptake mechanism was not saturated.

The plot of Batch 1 and Batch 3 together, in addition to showing no
saturation level of phosphorus, produced a curve indicative of two types
of uptake mechanisms: one operating at low phosphorus concentrations

and the other one operating at high phosphorus concentrations. The
existence of two mechanisms was supported with the Hofstee plot of the
same data. The Hofstee plot of a single first-order reaction is a straight
line. A curvilinear plot suggests that more than one reaction is taking
place simultaneously. Similar results were found by Gerloff (1975)
for both rubidium and phosphorus uptake in a number of aquatic vascular
plants. Excised roots of many agricultural crop plants, such as corn,
rye, and barley, have dual mechanisms of ion uptake (Hagen and Hopkins,
1955; Noggle and Fried, 1960; Andrew, 1966; Torii and Laties, 1966;
Carter and Lathwell, 1967; Gerson and Poole, 1971; and Nissen, 1973).

The existence of a dual uptake mechanism is widely recognized. One
mechanism (mechanism A) operates or is dominant at low ion concentrations,
and the other (mechanism B) operates or is dominant at high ion concen-
trations. Mechanism A exhibits a saturation curve typical of active uptake
kinetics where uptake rates increase with increasing ion concentration
until the uptake mechanism becomes saturated at a relatively low ion
concentration. Mechanism B takes up ions at rates proportional to ion
concentration and does not become saturated. Whether mechanism B operates
simultaneously with mechanism A or comes into operation at a threshold
ion concentration is unknown.

The actual mechanisms of ion uptake, whether they are active, passive,
or a combination of both, are not fully understood. Hiatt (1968) attributes
both mechanisms to passive, diffusive uptake involving electrostatic
association and Donnan phenomena. Electrostatic association is the
binding of certain inorganic ions within the cell by organic or amino
acids that provide nondiffusible charges while Donnan phenomena are the
attraction of electrolytes by the large, nonmobile, charged surfaces of

such compounds as proteins (Salisbury and Ross, 1969). Gerson and Poole (1971) hypothesize that the dual uptake mechanisms can be attributed to a single, uncharged carrier species at the plasma membrane (of root epidermal cells). Evidence of inhibitor interference of uptake and accumulation of ions against a concentration gradient suggest that uptake may be active (Bielski, 1966).

The question of whether active or passive uptake of phosphate ions by M. heterophyllum occurs is an interesting one. Although the method of uptake was not studied in detail, results of Batch 7 suggest that uptake might be active. Very low levels of phosphorus were taken up by either the autoclaved tissue or the gauze (mostly cellulose), while much greater amounts were accumulated by the live tissue. Although the character of the M. heterophyllum tissue could have been altered by the autoclaving process used to kill it, the difference between the uptake of live and dead plants could be attributed to active uptake requiring metabolic activity of living plants. The small amount of phosphorus taken up by the autoclaved apices may have been through diffusion into the tissue or adsorption of the phosphate ions onto its outer surface.

The question of whether the phosphorus was actually entering the plant tissue or whether it was simply adsorbed onto the surface of the plant arose. Phosphate ions are highly reactive and they complex easily with many cations such as iron. Iron II phosphate precipitates onto the roots of plants grown hydroponically in nutrient solutions (Biddulph, 1952). The precipitate causes removal of phosphorus and iron from solution and forms an effective barrier on absorbing surfaces, reducing the amounts of either element that can be taken up by the plant. Precipitation of phosphate salts of Ca, Mg, Fe, etc. was considered a possible factor in

the apparent uptake of phosphorus by M. heterophyllum.

The use of an acid bath to remove any precipitates adhering to the epidermis (Batch 5) produced no change in tissue phosphorus levels of the plants after culture. It is possible that low pH did not dissolve the salts that were present or that there were none present. Culture of apices in both modified Hoagland's solution with added phosphorus (high in cations necessary for phosphate precipitation) and lake water (comparatively low in cations) produced little difference in tissue phosphorus levels. The results suggest that precipitation of phosphate salts, which would have been more possible in modified Hoagland's solution than in lake water, is not a significant factor in phosphorus uptake.

M. heterophyllum shoots absorb very great amounts of phosphorus. Because growth of the plants was not affected by the concentration of phosphorus in the medium or the tissue phosphorus levels observed, it can be concluded that phosphorus was not limiting to growth in any of the cultures where growth was measured. Uptake of phosphorus by the plants was then at rates greater than necessary for growth and metabolism of the plants. Gerloff (1975) referred to this excess phosphorus (or any other nutrient) uptake as luxury consumption, which is characteristic of microscopic algae as well as many aquatic vascular plants. The minimum tissue phosphorus level at which maximum growth occurs is the critical minimum. At tissue phosphorus levels above the critical minimum no increase in growth rate is observed.

The critical minimum of phosphorus for M. heterophyllum has not been established. Critical minimum of phosphorus for Myriophyllum spicatum is only 0.07% dry weight (Gerloff, 1975). Although critical minima vary among species, the critical minimum of phosphorus for M. heterophyllum

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is probably close to that of M. spicatum. Tissue phosphorus of all plants studied in the field or used in batch culture was five to ten times the critical concentration of M. spicatum.

While phosphorus levels in the medium did not affect growth rate of the apices, temperature did. Culture at 4 C slowed shoot elongation and initiation of new apices significantly. The effects of increasing temperature between 4 and 18 C were not studied so the temperature necessary for maximum growth rate was not determined. Because there was no significant difference in growth rate or apex initiation between 18 and 30 C, it can be assumed that the temperature at which maximum growth rates will occur (or the minimum temperature necessary for maximum growth rate) is between 4 and 18 C and is probably within a smaller range between the two temperatures. Elodea sp. growth was not sensitive to temperature changes between 20 and 30 C while its growth rates increased with increasing temperature below 20 C. (Gerloff, 1973).

Death and subsequent decay of the M. heterophyllum apices in Batch 6 released significant amounts of phosphorus regardless of whether they were killed with Silvex or whether they died from lack of light. Probably because of the rapid action of Silvex on the apices those in the Silvex solution released phosphorus rapidly. The released phosphorus remained in the medium surrounding the decaying apices and possibly was bound to the organic Silvex molecules (or their degradation products) or the petroleum based carrier of the herbicide. Phosphorus was slowly released from the plant tissue in distilled water because of the gradual death of the plants in the dark.

After 66 days 24% of the total phosphorus in the system remained in the Silvex-killed tissue and 26% in the dark-killed tissue. The

remaining phosphorus levels were much lower than those reported by Foree (1971) for aerobically decomposing algal cells. After one-half to one year as much as 50% of the original phosphorus remained in the undecomposed algal cells. Foree determined two types of nutrient release dependent upon initial phosphorus concentrations in the cells: 1) a regeneration of the nutrient proportional to the degree of decomposition when that nutrient is below the critical minimum and 2) regeneration of an excess proportion of the nutrient when it is loosely bound because it is at levels above the critical minimum for the plant. The rapid release of phosphorus by decaying M. heterophyllum tissue may have been a result of tissue phosphorus levels above critical minimum.

After 60 days of decay of herbicide-killed M. spicatum in an aerated water-only system, approximately 35% of original phosphorus remained in the tissue (Nichols and Keeney, 1973), similar to the 24% and 26% phosphorus remaining in M. heterophyllum. Phosphorus levels found in the water of the herbicide-killed M. heterophyllum followed the same pattern described by Nichols and Keeney: initial increase of phosphorus in the water was rapid, reaching a maximum at about 10 days and leveling off thereafter. Phosphorus release by decaying Callitriche sp. (Jewell, 1971) followed much the same pattern.

Decrease in dry weight for both the dark-killed and herbicide-killed plants was slow. Dark-killed plants decomposed more rapidly than did the herbicide-killed plants (30% of original dry weight remaining and 74% remaining, respectively). After 60 days Jewell (1971) reported 20% of the original mass of Callitriche sp. remaining, and 22% of the original dry weight of Myriophyllum exalbescens remained after 60 days of decay (Nichols and Keeney, 1973). The apparent large proportions of remaining

dry weight of M. heterophyllum may have been due to the differences in initial dry weight placed in each flask. Five apices of uniform length were placed in each flask, but initial dry weight was not measured.

As is evident from the graphs for Batch 8, calculations of phosphorus loss from the tissue and phosphorus levels in the medium were calculated on a percent-of-total basis. Calculations were made in this manner to account for the differences in the total phosphorus pool in each flask because initial dry weights (and therefore phosphorus weights) were not uniform for each flask. Total phosphorus was greater in those systems that were allowed to run the longest and least in those systems that were run for the shortest periods. It then appears that systems somehow gained phosphorus the longer they were allowed to stand. No explanation can be offered for this phenomenon except that it is possible that the differing amounts of tissue added initially happened to be greatest in those flasks chosen for the longer culture periods.

Suggestions for Further Study

Further study of growth rates and biomass of water milfoil is necessary to more accurately determine the amount of the plant present in Lake Winnipesaukee. More plant tissue and sediment should be analyzed for phosphorus to determine whether the sediment phosphorus and tissue phosphorus relationship exists in other locations in addition to the three described. As mentioned previously, phosphorus-32 studies of phosphorus cycling are necessary to determine the exact relationships among sediments, plant roots and shoots, epiphytes, and water. The role of Myriophyllum heterophyllum in the ecology of Lake Winnipesaukee should be more clearly defined before drastic and permanent control measures are applied.

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SUMMARY

1.   Myriophyllum heterophyllum is present in Lake Winnipesaukee
in dense, isolated stands. The stands are located in areas characterized
by silt and sand substrates and low wave action. The greatest plant
density occurs at depths of 0.5 to 3 m.

2.   The density of M. heterophyllum plants was 17 plants/m^2 in
1975 in Alton Bay and Green's Basin Beach. Biomass estimates were 72
to 132 g dry weight/m^2 in Alton Bay and 84 to 120 g dry weight/m^2 in
Green's Basin Beach. Density and biomass were much smaller in Ostrand's
Marina.

3.   Stand expansion and colonization of M. heterophyllum into new
areas were very rapid. The Ostrand's Marina stand increased from 25%
cover in 1975 to over 75% in 1976.

4.   Shoot elongation of M. heterophyllum was most rapid during June
through August, at more than 1.5 cm/day.

5.   Tissue phosphorus levels of M. heterophyllum shoots correlate
with sediment phosphorus levels for the three major study sites, indicating
that 1) sediment is probably the major phosphorus source for the plant
and/or 2) the supply of phosphorus to each site affects the tissue phosphorus
levels.

6.   Phosphorus cycling in the littoral may be greatly influenced
by the presence of M. heterophyllum, which removes phosphorus from both
the water and sediment. The plant stores large amounts of phosphorus
in its tissues, as much as 0.75% dry weight was observed in untreated

samples. Physical removal of phosphorus-rich particulate matter by the finely-dissected leaves also occurs.

7. In situ phosphorus enrichment of the water surrounding M. heterophyllum plants produced an increase in tissue phosphorus.

8. M. heterophyllum is potentially useful as an indicator organism for available phosphorus in Lake Winnipesaukee. Further research on its phosphorus uptake behavior, under a variety of conditions, is necessary before its use as an indicator organism for phosphorus becomes practical.

9. M. heterophyllum apices take up large amounts of phosphorus (luxury consumption). The amount of phosphorus taken up is proportional to the phosphorus concentration in the medium and is influenced by temperature.

10. Two uptake mechanisms appear to function in phosphorus uptake. Whether these mechanisms involve active or passive uptake or both is unknown.

11. Growth of apices was unaffected by phosphorus concentrations employed. Growth rates were influenced by temperature, being slower at lower temperatures. Initiation of new apices was greatest at high temperature.

12. Decay of M. heterophyllum tissue released significant amounts of phosphorus into the surrounding water in batch culture.

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