UPTAKE AND TRANSLOCATION OF ^{15}N BY *POTAMOGETON NODOSUS*

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PREFACE

This study is an investigation of ammonia uptake and translocation by Potamogeton nodosus, a rooted aquatic plant. The major objectives are to determine if ammonia is absorbed by the root and rhizome systems and, subsequently, translocated to stems and leaves under laboratory conditions and in situ. The isotope ^{15}N is used as a tracer to detect uptake and translocation. Measurements of ^{15}N are accomplished by mass spectroscopy.

I wish to express my sincere gratitude to my thesis advisor, Dr. Dale W. Toetz, for his advice, constructive criticism and financial assistance provided through his research funds. My appreciation is also expressed to the chairman of my committee, Dr. Kenneth Wiggins, for his continuous support and guidance. I am also indebted to Dr. Herbert Bruneau and Dr. Glenn Todd for their assistance in the preparation of this manuscript.

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CHAPTER I

INTRODUCTION

The study of aquatic ecosystems is a field of intensive research because of widespread concern for water conservation and pollution control. Both abiotic and biotic components of aquatic ecosystems are interwoven and interrelated, producing a highly complex self-sufficient unit.

One aspect in the analysis of ecosystems is nutrient cycling, i.e., the movement of elements between living and non-living parts of the ecosystem. The element nitrogen has received a large share of the attention of ecologists, since the most characteristic components of living material are nitrogenous (Fogg, 1953).

Keeney (1972) defined and summarized the primary processes involved in the aquatic nitrogen cycle as: nitrogen fixation, denitrification, nitrification, ammonification, and assimilation of different forms of inorganic nitrogen by components of the flora.

This investigation was directed towards an understanding of ammonia assimilation by aquatic macrophytes. The symbol NH_4 is used to represent ammonia, both as an ion and an undissociated molecule. Mineralization (conversion of organic N to inorganic N) occurs in sediments of lakes and ponds that serve as a substrate for rooted, aquatic plants (macrophytes). Thus, ammonia and other nutrients may become available within the sediments (Misra, 1938). However, it is not known whether

NH_4 in the sediments is used by macrophytes. The purpose of this research was to resolve this issue for Potamogeton nodosus, an emergent plant with floating leaves.

A glossary of terms appears in the Appendix.

Significance

Macrophytes and attached periphyton represent important components of aquatic ecosystems. Aquatic weeds, often nuisances, provide breeding grounds for mosquitos (Peltier and Welch, 1969), infest drainage and irrigation canals (Blackburn et al., 1968), modify physical conditions of river beds, and impede water flow (Butcher, 1933), contribute sizeable amounts of BOD (thus causing serious dissolved oxygen problems), clog skimmer walls of water intakes of hydro-electric plants (Peltier and Welch, 1970), and contribute significantly to the primary production of aquatic habitats (Rich et al., 1971).

A complete understanding of the nitrogen cycle is necessary if achievement of effective control and management of ecosystems is to be accomplished. Specifically, data are needed on the role of rooted aquatics in this cycle.

Nature of the Problem

A number of studies have been undertaken to establish the capabilities of selected species of aquatic plants in absorption and translocation of elements and compounds. Absorption and movements of herbicides in aquatic weeds have been investigated by Earl et al., (1951), Aldrich and Otto (1959) and Funderburk and Lawrence (1962, 1963). More recently, uptake of phosphate, carbon dioxide and nitrogen has been

studied (Bristow, 1969; Peltier and Welch, 1969; McRoy and Barsdate, 1970; Bristow and Whitcombe, 1971; and Toetz, 1971).

Controversy exists on the role of roots in the nutrition of hydrophytes. Sculthrope (1967) stated "there is no doubt that for emergent hydrophytes the substrate is the prime source, and the root system the major site of absorption, of water and dissolved nutrients". He also indicated that there is an upward translocation of these nutrients to the foliage. However, other workers hold that absorption of nutrients by the roots of submerged, rooted aquatics is negligible (Waisel and Shapira, 1971).

The importance of lake sediments as a source of nutrients for aquatic plants is demonstrated to some extent by the work of Butcher (1933), Misra (1938), Moyle (1945) and Peltier and Welch (1969). Frink's (1967) data supports the position that lake water alone is an insufficient source of nitrogen for aquatic weeds and algae.

Since the interstitial water of sediments is rich in ammonia (Keeney et al., 1970 and Konrad et al., 1970) and some evidence suggests this NH_4 is available for absorption by roots of macrophytes, it seems plausible that nitrogen moves from sediments via roots and rhizomes through the stems to the leaves, where it may be used in biosynthesis or excreted.

The following are three null hypotheses, which were to be tested:

Null Hypotheses

H_o1: There will be no significant translocation of ^{15}N labeled ammonia from roots and rhizomes of Potamogeton nodosus to the stem-leaf tissues

from aqueous solution under controlled conditions.

H_o2: There will be no significant uptake by root-rhizome systems of Potamogeton nodosus exposed to ^{15}N labeled ammonia under field conditions.

H_o3: There will be no significant acropetal translocation of ^{15}N labeled nitrogen by Potamogeton nodosus under field conditions.

CHAPTER II

REVIEW OF SELECTED LITERATURE

Introduction

In Chapter I the problem was defined and brief attention was given to previous work in this field. An expanded survey of the literature is presented to emphasize the scope of the problem.

Each of the subjects listed below lend insight into the broad concept of nutrient cycling in aquatic ecosystems and specifically to the role of rooted macrophytes in the nitrogen cycle: 1) factors that affect the growth and distribution of macrophytes, 2) nutrient absorption and translocation in higher aquatic plants, 3) lake sediments as a source of nutrients, and 4) excretion of nutrients by macrophytes.

Factors Affecting Growth and Distribution of Macrophytes

Butcher (1933) made an extensive study of the factors governing the distribution of higher plants in the rivers of Britian. He concluded that the current was a prime ecological factor, since it produces mechanical strain on plants and also determines the nature of the river bed. The latter, in turn, greatly influences the type of vegetation. Butcher suggested that macrophytes have fundamental importance in the general biological character of a stream in the following ways: 1) provide a place for growth of epiphytic algae, 2) provide oxygen,

shelter and habitation for the fauna and 3) act as cementing agents of river bed components.

The abundance, competitive ability and rate of growth of aquatic macrophytes were considered in relation to water quality and light penetration by Blackburn et al., (1968). Light penetration was the most important limiting factor in canals where nutrients were abundant.

Peltier and Welch (1969) investigated factors affecting growth of aquatic weeds: Nutrient concentation, light penetration and temperature. Laboratory experiments were conducted to determine the source of nutrients for plant growth. Physical factors influenced the variability of plant growth, but the importance of enriched sediment was noted. In one experiment where roots and foliage were partitioned, Potamogeton pectinatus L. grown in sediments showed 3.5 times greater growth than those in sand.

Moyle (1945) conducted surveys of 225 lakes and streams in Minnesota. Plants inhabiting different bodies of water were identified, and chemical analyses of water samples taken were made at the same time. The effect of water chemistry upon growth and survival of these hydrophytes was observed experimentally. Moyle separated the flora into three major groups (soft-water flora, hard-water flora and the alkali- or sulphate-water flora) on the basis of plant preference for water quality, concluding that water chemistry was the most important single factor influencing aquatic plant distribution. However, other factors, such as fertility and type of bottom sediment and the physical nature of the habitat, greatly influenced distribution of aquatic plants, even in such free-floating plants as Lemna minor.

Misra (1938) furnished strong evidence that edaphic factors con-

trol the distribution of aquatic plants. In situ experiments on the growth of Potamogeton perfoliatus and laboratory studies on P. perfoliatus, P. alpinus and Sparganium minimum growing in three different types of sediment showed the importance of substratum in influencing development of vegetation. Misra concluded: 1) physical and chemical characteristics of lake sediments are closely correlated with the type of vegetation supported, 2) nitrogen is available in sediments in the form of ammonia and 3) sediments of moderate organic content contain the most available ammonia.

During a three-year study period, nitrogen and phosphorus were found not to be related to the year-to-year growth of Najas in Pickwick Reservoir (Peltier and Welch, 1970). Physical and climatic data indicated that heavy infestations were correlated positively with irradiance.

Absorption and Translocation

Submerged, as well as emergent, higher aquatic plants can absorb nutrients through roots and translocate them to the shoot. In a study of 209 species from a diversity of habitats, Shannon (1953) found root hairs on 195 species. He concluded that most aquatic plants produce abundant root hairs and thus increase the surface area for absorption. The roots could then function as in terrestrial plants in uptake of water and solutes.

Arber (1920) speculates that macrophytes translocate water and minerals from roots to shoots. Sculthrope (1967) concludes that only a transient flow of water and dissolved salts might occur from roots to leaves in submerged and floating-leafed plants.

Several investigators consider the reduced root systems of sub-

merged macrophytes to be relic and functionless in relation to mineral uptake and translocation. Myriophyllum spicatum, Potamogeton perfoliatus and P. lucens roots do not play an important role in ion absorption and translocation (Waisal and Shapira, 1971); however, all four elements (Rb, Na, Cl and P) investigated were absorbed by the roots and some acropetal translocation did occur. These authors believe roots of aquatic macrophytes are of the greatest importance as a production site for certain growth hormones, as is true for many terrestrial plants.

The movement of carboxyl-labeled 2-4-dichlorophenoxyacetic acid (2,4-D-1-^{14}C) in Potamogeton pectinatus is primarily basipetal (Aldrich and Otto, 1959). This conclusion is based on comparison of plants exposed to herbicide in the roots and leaves. These findings are contrary to those reported by Earl et al., (1951) for the movement of 2,4-D-^{14}C in alligator weed (Althernanthera philoxeroides) where acropetal translocation was greatest.

Studies on absorption and translocation of radioactive herbicides in the submerged plant, water-stargrass, and in an emersed species, alligator weed, suggests movement can occur in both directions (Funderburk and Lawrence, 1962 and 1963). Herbicides applied to the foliage of alligator weed exhibited little movement. In the latter study ^{32}P was shown to move in both directions, but upward movement was greater in both species.

Frank and Hodgson (1964) found little or no basipetal translocation in ^{14}C-labeled 2,3,6-trichlorophenylacetic acid (fenac) in Potamogeton pectinatus. Both root and shoot treatments were conducted for 24 to 96 hours. Limited acropetal translocation occurred.

The results from radioactive herbicide studies generally support

the contention that roots are important sites of absorption and subsequent upward translocation of compounds can occur. More direct evidence to support this position has been supplied by two recent studies. McRoy and Barsdate (1970) studied absorption of radioactive phosphate in eelgrass (Zostera marina), an important emergent in estuaries. Absorption occurred through both leaves and roots. Labeled P was rapidly translocated to other plant parts. Findings from in situ experiments indicate that phosphate, which traveled to the leaves, was then released into the surrounding water. Bristow and Whitcombe (1971) performed both short-term and long-term laboratory experiments on phosphate absorption by three species of aquatic plants (Myriophyllum brasiliense, M. spicatum and Elodea densa) similar to those conducted by McRoy and Barsdate (1970). Most of the phosphate was absorbed by the roots and not from the medium surrounding the shoot with little downward translocation. In Elodea, upward translocation was independent of ambient concentration around the upper stem. Apparently, phosphorus was not excreted by the leaves. In another study, Bristow (1969) proposed that CO_2 may be absorbed by roots of submerged species and translocated to the leaves, particularly when low concentrations of CO_2 exist in water bathing the leaves.

Lake Sediments: A Source of Nutrients

The sediments of several Wisconsin lakes are rich in both exchangeable and soluble ammonia-nitrogen (Konrad et al., 1970, and Keeney et al., 1970). Total and available concentrations of inorganic nitrogen tended to be high in all the sediment profiles. Sediments of eutrophic lakes invariably had much higher concentrations of soluble ammonia than

sediments of oligotrophic lakes.

Nitrogen and phosphorus budgets were developed for a small eutrophic lake in Connecticut by Frink (1967). The upper centimeter of sediment from this lake was found to contain a total nitrogen content of 44,000 kg or more than ten times the annual input from the watershed. He concluded that the sediments are a vast reservoir of nutrients capable of supporting plant growth.

Keeney (1972) emphasized the importance of lake sediments as a potential source of nitrogen to lake waters. In Lake Mendota, Wisconsin approximately 50 to 200 kg of nitrogen per hectare exist in the top ten centimeters of sediment. Gehler (1969), cited by Keeney, (1972), has found the interstitial water in the sediments to be generally much higher in soluble nitrogen than the over-lying water.

Excretion of Nutrients

Wetzel (1969) investigated factors affecting photosynthesis and excretion of dissolved organic matter (DOM) by aquatic macrophytes. Experiments were conducted on plants grown from seeds in axenic culture. The rate of photosynthesis and excretion of DOM by Najas flexilis was studied in relation to a variety of inorganic interactions. Wetzel postulates that the excreted DOM of macrophytes may be used by epiphytic populations on the plant before DOM enters the surrounding medium.

A subsequent study by Wetzel and Manny (1972) was conducted on the secretion of dissolved organic carbon (DOC and dissolved organic nitrogen (DON). The DOC and DON produced by Najas flexilis ranged up to 25 per cent of the photosynthetically-fixed carbon. The DOC consisted of simple, easily decomposed organic compounds. The DOM, either as per

cent secreted or as mass (kg^{1} C yr^{-1}) contributed by macrophytes, was equivalent to or exceeded that produced by the phytoplankton in a hard-water lake in Michigan.

Live algae and aquatic weeds with abundant nitrogen (N) and phosphorus (P) do not share their nutrients with other algae grown in N or P-limited media (Fitzgerald, (1970). Nitrogen and phosphorus in rocks and lake mud is not readily available for use by algae (Fitzgerald, 1970). Once these elements become a part of the sediments, they are not readily returned in substantial amounts of lake waters by chemical or physical processes (Fitzgerald, 1970).

Summary

Macrophytes represent significant components of certain aquatic ecosystems and contribute to their character in several ways. Light penetration is recognized as a major factor limiting the growth of hydrophytes. The deposition of enriched sediments may contribute to macrophyte production; whereas, water chemistry is highly correlated in many instances with the growth of aquatic plants. Physical and chemical characteristics of lake muds apparently influence the development of vegetation.

Controversy exists as to the role of roots in aquatic plants. Many species are known to produce extensive root hairs and appear to be capable of absorption of solutes. Studies on herbicide uptake and translocation reveal movements from roots to leaves and vice versa. Roots of terrestrial plants produce growth substances used by the shoot, and this may be a major function of roots in submergent aquatic plants. Recent studies of nutrient uptake indicate roots are functional organs

of absorption, particularly of phosphate.

Lake sediments are rich in exchangeable and soluble ammonia, particularly the upper layers. Interstitial water is generally higher in soluble nitrogen than the overlying lake water. Once removed from the sediments, nitrogen may be made available to other forms through excretion. The DOM excreted by a hydrophyte represents a sizeable proportion of the photosynthetically-fixed carbon in one lake. Once nitrogen and phosphorus are incorporated into the sediments, they are not returned to the overlying water in substantial quantities.

CHAPTER III

METHODS AND MATERIALS

Experimental Organism and Collection Site

Potamogeton nodosus Poiret was selected as the experimental organism for the following reasons: availability through the growing season, receptiveness to laboratory maintenance and the relative ease in collection and processing. Identification of the species was accomplished using keys in Muenscher (1944).

Toetz (1971) described the lake where plants were collected and field observations were made. It is located in central Oklahoma: area 5.4 hectares, mean depth 2 m. Two extensive *Potamogeton* beds are present in the lake. The one located near the dam at the north end of the lake served as the source of plants. *Najas* sp., *Ceratophyllum* sp. were interspersed with *P. nodosus*. All plants were collected at depths less than 1 m.

Field Procedures

To characterize the natural habitat and to assist in delineating laboratory conditions, certain chemical and physical parameters were measured: temperature, pH, and ammonia concentrations of both lake water and sediments, light penetration, and depth of water at the collecting site (Table I).

TABLE I

CHEMICAL AND PHYSICAL PARAMETERS MEASURED AT THE COLLECTION SITE GIVEN AS MEAN VALUES OR RANGE

Type of Measurement	Lake Water		Interstitial Water	
	Number of Determinations	Parameter Value	Number Determinations	Parameter Value
Physical				
Water Depth	(2)	0-1.5 m	-	-
Light Penetration*		1 m	-	-
Temperature	(9)	24°-31°C	-	-
Chemical				
pH	(5)	8.1	(4)	7.5
pH**		8.4	(1)	7.9
Ammonia (NH_4-N)	(2)	4.2 µg/l	(2)	85 µg/l
Nitrate		no data	(1)	not detected
Nitrite	(1)	not detected	(1)	not detected

* Light was observed to penetrate to the sediments within the collection site during the study: Toetz (1971) recorded mean secchi disk readings for the lake of 1 m.

** Lake water pH expressed as mean of 12 monthly measurements by D. Toetz (personal communication) and interstitial water pH determined by the Soils Laboratory, Oklahoma State University (7-21-72).

Temperature was measured at irregular intervals during July, August and September at 0.5 m, using a mercury thermometer. Light was observed to penetrate to the sediments within the collection site.

Water for chemical analyses was collected in polyethylene bottles from within the Potamogeton community, returned to the laboratory and filtered through a 0.45 μ millipore filter. Chemical analyses were either accomplished on the same day or the water samples were frozen and analyzed at a more convenient time. Sediments and interstitial water samples were collected, using a simplified core sampler similar to that described by Schneider (1969) and placed in polyethylene centrifuge bottles. Interstitial water was extracted from the sediments by centrifugation at 16,300 g for ten minutes followed by filtration of supernatant (Konrad, et al., 1970).

Plant Collection and Pre-Incubation Treatment

Approximately sixty Potamogeton plants were removed from the sediments at a depth of 0.5 meter, placed in plastic buckets containing lake water and returned to the laboratory for cleaning on the initial day of the laboratory experiment. Plants selected had one or more healthy leaves and several roots: mostly young plants were selected. Each plant was washed with a spray of tapwater and hand cleaned to remove sediments and periphyton. This procedure removed approximately one-half of the attached materials from the leaves. Excess rhizomes were removed from each plant to reduce the mass of storage tissue in proportion to the mass of roots.

Experimental Apparatus

The apparatus designed for this investigation was a variation of that used by Frank and Hodgson (1964). It incorporates the two-compartment concept and permits a continuous purging with the CO_2 enriched air in the root-rhizome compartment (Figure 1). The apparatus housing the roots and rhizomes consisted of an amber glass jar fitted with a hard plastic lid. An air inlet tube extended to near the bottom of the jar and an outlet tube extended below the surface of the lid. Ordinary wide-mouthed gallon jars housed the root-rhizome containers and served as the stem-leaf compartments. The two compartments were partitioned by a seal around the stem of the plant with a plasticine material, Terostat Type VII.

Culture Medium

The selection of a culture medium was based on results obtained from the chemical analysis of interstitial water and data on water chemistry of the lake obtained during 1971 (D. Toetz, personal communication). The medium used contained all of the ions found in the interstitial water and lake water (Table II) except nitrate. One hundred and twenty liters of Wetzel's medium II (Toetz, 1973) were prepared for stem-leaf compartments. Three additional carboys of the medium were prepared for root-rhizome compartments and enriched with 595, 2975 and 5950 µg of 99% $^{15}NH_4$-N respectively. These enrichments represent ambient, five and ten times ambient concentrations found in the interstitial water of the sediment (Table 1). The medium used in the stem-leaf compartment was adjusted to 8.5; while the medium used in the root-rhizome compartment was adjusted to a pH of 7.5 to simulate *in situ* conditions (Table 1).

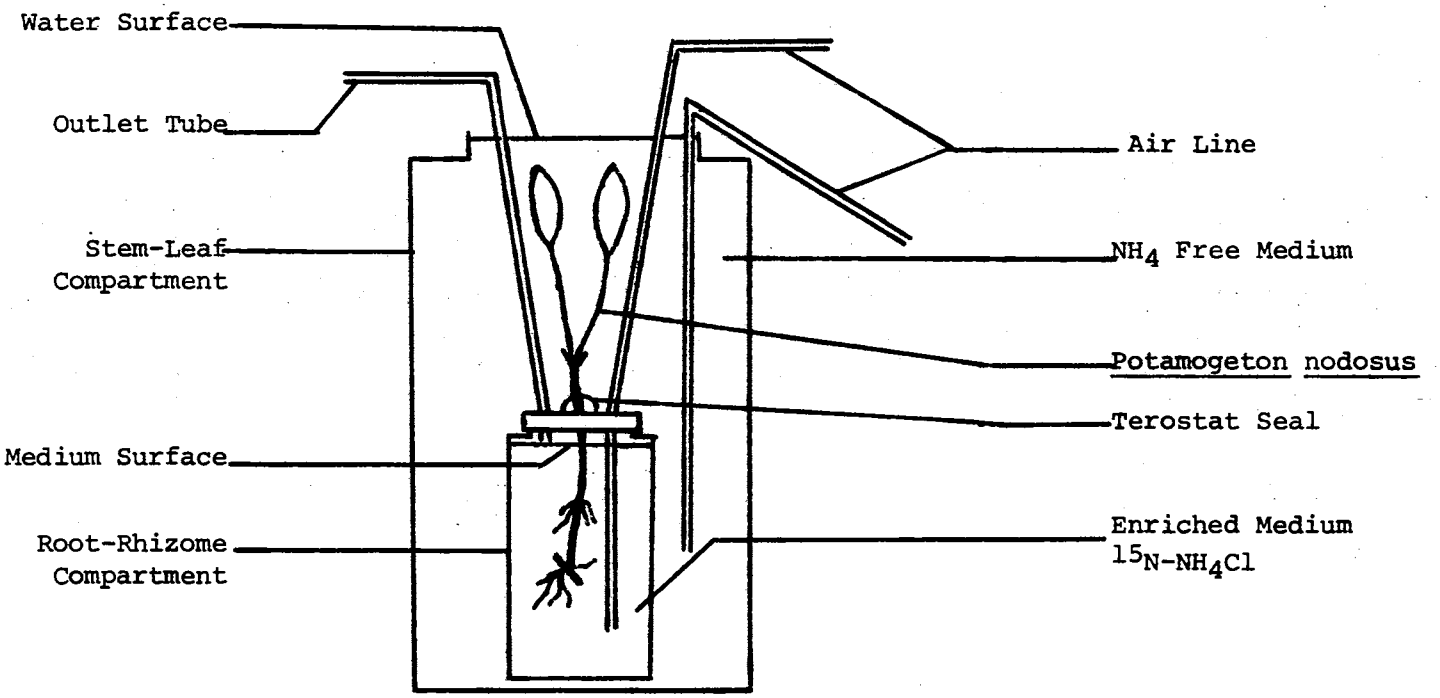

Figure 1. Two Compartment Experimental Vessel for the Laboratory Uptake Experiment

TABLE II

CHEMICAL COMPOSITION OF INTERSTITIAL WATER (n=1) COMPARED TO CHEMICAL COMPOSITION OF LAKE WATER (n=12) (TOETZ, 1971)

	Interstitial HOH		Lake HOH
Constituents	mg/l	meq/l	meq/l
Ca	47.3	2.4	0.86
Mg	19.9	1.7	0.84
Na	27.3	1.2	0.84
K	5.6	0.0	-
Cl	18.0	0.5	0.43
SO_4	12.0	0.3	0.06
NO_3	4.0*	-	-
Total Dissolved Solids	350.0	-	-
HCO_3	-	-	2.06
Hardness ($CaCO_3$)	205.0	-	-

* Represents the apparent lower limit of detection for the procedure used.

(-) Those parameters were not measured or computed

All adjustments of pH were accomplished by the addition of 2 N HCl.

Testing H_o1

The laboratory experiment involved incubation of plants with roots exposed to enriched media containing ^{15}N labeled ammonia. Three series of experimental vessels (Figure 2) were placed in a controlled environment chamber. Each series consisted of eleven vessels, nine experimental and two controls. These were designated as "A", "B", or "C" and numbered one through nine; each of the experimental vessels housed one plant. Root-rhizome compartments were filled with ^{15}N enriched medium. Series "A" vessels were enriched to ambient concentration, 85 µg NH_4-N/l, "B" with five times ambient and "C" with ten times ambient. The root-rhizome compartments were sealed before positioning the assembled apparatus into the gallon jars. The stem-leaf compartments were then filled with nitrogen-free medium. Duplicate controls in each series with no plants were used to detect changes in pH and the concentration of NH_4.

Both compartments of each apparatus were aerated with ammonia-free air enriched with carbon dioxide by attaching an ammonia trap (0.1M $ZnCl_2$ solution) and a saturated solution of $NaHCO_3$ in sequence to four headers that were used to serve the thirty-three vessels (Figure 3).

Three series of nine plants were incubated under fluorescent light (2500 foot candles) and controlled temperature (26°C day and 22°C night $\pm$ 2°C) for 2, 5 and 10 days. These temperatures are near the range of 24° - 31°C (Table 1) measured for the lake water. The photoperiod was 12 hours. At the end of each incubation interval, nine plants, three from each series were removed and treated as described below. Temperature of the medium was measured during incubation and pH deter-

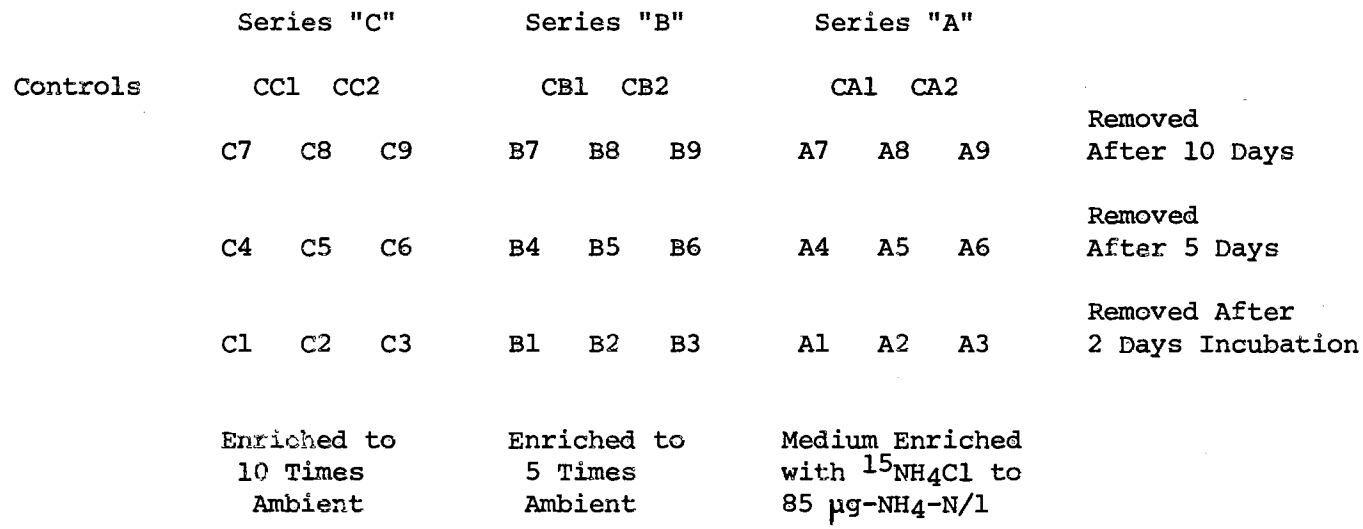

Figure 2. Experimental Design for Potamogeton nodosus for Ten Day Laboratory Experiment Conducted 8-25-72/9-4-72. Numbers and Letters Represent the Arrangement of Plants in the Controlled Environment Chamber

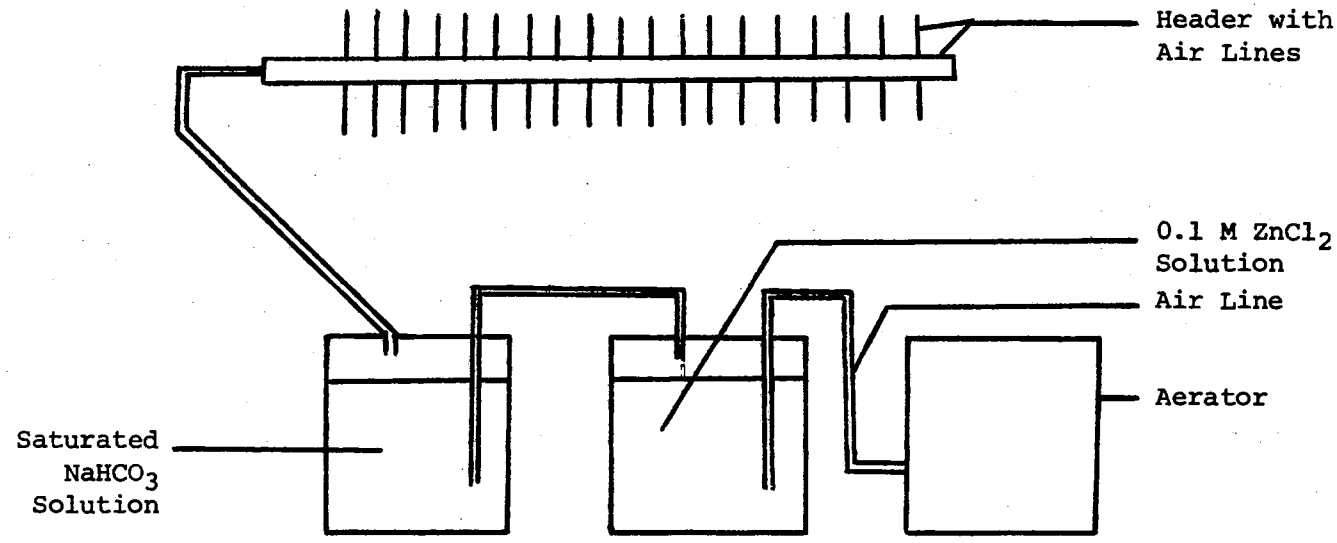

Figure 3. Ammonia Filter, Carbon Dioxide Source and Aeration System

minations were made on aliquots from both compartments at the end of each incubation period. Subsamples of the medium from each vessel were stored at 5°C for NH_4 analysis. A rough approximation of the relative uptake by the root-rhizome tissue in the partitioned containers was derived from the difference between the initial concentration of ammonia and concentration of ammonia at the end of each incubation interval.

Stems and leaves were detached from the root-rhizomes by cutting at the base of the stems. Each organ was rinsed with tap water, dipped twice in deionized distilled water, and placed on paper towels to air-dry for one hour at room temperature. Subsequently, each plant was sectioned into pieces about 5 cm long. Stems, leaves, roots and rhizomes were placed into tared aluminum pans and weighed to determine fresh weight. Pans and contents were then placed in drying ovens at 105°C. After a minimum of forty-eight hours, they were reweighed to determine the dry weight. Samples were stored in desiccators until they could be converted into elemental nitrogen (N_2).

Chemical Analysis

Samples of lake water and interstitial water removed from the sediments were collected from within the Potamogeton beds and analyzed by the Soils Laboratory, Oklahoma State University, for total alkalinity and concentration of major anions and cations. Ammonia determinations of lake and interstitial waters were accomplished using Solórzano's method (1969). The methods of Mullin and Riley (1955) and Strickland and Parsons (1968) were used for analysis of nitrate and nitrite.

Analysis of the medium for NH_4 before and after incubation was performed using direct Nesslerization as outlined in APHA (1960).

All pH measurements were made by a Beckman Zeromatic pH meter.

Nitrogen Content and Isotope Ratio Analysis

Analysis for nitrogen content and the % ^{15}N in tissues of the plants required the conversion of organic nitrogen to N_2. Four sections of root-rhizome, stem and leaf organs of plants not exposed to ^{15}N provided an estimate of per cent nitrogen present in dry matter of each organ. A Coleman Nitrogen Analyzer II was used to measure the mass of N in a preweighed mass of dry organ. The mean % N was multiplied by the dry weight of the organs of experimental plants to obtain the mass of N in each plant part.

Isotope ratio analysis was performed on samples of the experimentally treated root-rhizome, stem or leaf organs after conversion to N_2 with a Coleman Nitrogen Analyzer II. Gas samples were pumped into break-seal tubes, sealed and stored until mass spectroscopy could be performed. The % ^{15}N present in the plant tissue was calculated, using the formula, 100/2R+1, where R is the ratio of the 28 peak to the 29 peak determined by mass spectroscopy (Bremmer, 1965). Organs of plants not exposed to ^{15}N were processed in the same way and used as isotope ratio blanks. Mass spectrometry measurements were performed on a CEC 21-110B mass spectrometer in the Chemistry Department, Oklahoma State University.

To establish statistical limits for ammonia uptake, 95 per cent confidence intervals were computed on the following basis: The mean % ^{15}N and its standard deviation was calculated for six blanks. Uptake was considered significant at the 95% level, if % ^{15}N measured was in excess of the upper confidence level. The difference between the upper

confidence level of the blanks and the % ^{15}N of an experimental determination is the % ^{15}N excess in the tissue. Mean % ^{15}N of blanks and their standard deviations are contained in Appendix D.

Specific rates of uptake were computed using the following expression:

$$A_f \, N_t / A_i \, N_t \, T$$

where A_f = atom per cent excess of ^{15}N in the tissues; N_t = mass N in the tissue exposed to the isotope; A_i = per cent ^{15}N of the NH_4 in the medium initially; and, T = number of days of incubation. (Toetz, 1971).

An estimate of translocation (transport index) was calculated for each plant where uptake was statistically significant. The formula used to derive these estimates was modified from that employed by Bristow and Whitcombe (1971) for phosphorus uptake and is defined as follows:

$$\text{transport index} = \frac{\%\ ^{15}N \text{ excess / mg N stems + leaves}}{\%\ ^{15}N \text{ excess / mg N root + rhizome and stems + leaves}} \times 100$$

Field Experiment Designed to Test H_o2 and H_o3

In mid September, 1972, a field experiment was initiated to test hypotheses H_o2 and H_o3, i.e. that P. nodosus does not absorb NH_4 from the sediment nor translocate it. The experimental performances deemed essential to test these hypotheses are in essence the same; consequently, they serve a dual purpose.

A modification of the in situ study of phosphate uptake in eelgrass by McRoy and Barsdate (1970) was employed. A pilot study of sediment enrichment was conducted to determine the feasibility of this technique. The upper 25 cm of sediment from the study site was

collected and transported to the laboratory. Sediment and lake water were added to two containers and allowed to equilibrate for 48 hours. Syringes (3 ml capacity) with 2.5 cm needles were used to inject varying amounts of aqueous dye solution (food coloring) into the sediments at several depths. Injections of 0.5 ml of dye at 6-7 cm below the sediment surface revealed no backflow. This technique was subsequently employed.

Three areas within the Potamogeton community near the dam at the north end of the lake were selected; the approximate distance separating these areas was 5 and 2 meters. No effort was made to randomize selection of plants. Rather, vigorous, healthy-appearing plants were selected. From each area three experimental plants were identified by placing a wooden dowel into the sediments about 5 cm away. Control plants with considerable roots and rhizomes attached were removed from the sediments; the rhizome of each was attached to the base of a wooden dowel just above the sediment surface.

Syringes containing 0.5 ml of ^{15}N-labeled NH_4Cl having a concentration of 2.88 mg $^{15}NH_4$-N/ml were then placed into the sediments near the base of each experimental plant at a depth of 7 cm. The plunger of the syringe was then depressed. The syringes were left in position for the duration of the experiment; however, only four actually remained intact and the others were washed ashore. At the end of 3, 7 and 12 days, one experimental and one control plant from each of the three study areas were retrieved and processed as above.

CHAPTER IV

RESULTS AND DISCUSSION

Results

Laboratory Experiment (8-25-72/9-3-72)

Isotope ratios of stem, leaf and root-rhizome organs for twenty-seven experimental plants were determined by mass spectroscopy. The % ^{15}N of six blanks was 0.342 $\pm$ 0.139 at the 95% confidence interval. Enrichment of tissues with ^{15}N was considered significant when the % ^{15}N was greater than 0.481 % ^{15}N, the upper end of the confidence level for the blanks. Estimates of the percentage of nitrogen in dry matter of tissues were derived (Appendix C) and the mean values were: leaf tissues = 2.63; stems = 1.88; and, root-rhizome (Rt-Rh) = 1.75%.

Table III reveals ^{15}N enrichment commonly occurred at all levels of substrate concentration and during each incubation interval. Only three root-rhizome tissues were not enriched. Stem or leaf tissues were enriched in at least one of the three replicates for each treatment except those incubated two days at the lowest level of substrate. Generally, enrichment was more frequent in all tissues of plants incubated for five or ten days in higher concentrations of substrate. For example, all replicates incubated five days at 10X ambient concentration were enriched. In most instances where leaves were enriched, stems were also enriched; conversely, five stem samples but not corresponding leaves were enriched.

TABLE III

P. NODOSUS ROOT-RHIZOME (Rt-Rh), STEM (S) and LEAF (L) TISSUES ENRICHED BY EXPOSURE OF ROOTS AND RHIZOMES TO MEDIA CONTAINING ^{15}N LABELED NH_4Cl AT THREE CONCENTRATIONS AFTER TWO, FIVE AND TEN DAYS: ENRICHMENT IS EXPRESSED AS % ^{15}N EXCESS. ALL TREATMENTS WERE PERFORMED IN TRIPLICATE AND IDENTIFIED AS A 1-9, B 1-9 AND C 1-9

Incubation Intervals		Substrate Concentration (µg NH_4-N/l)										
		Ambient = 85			5X Ambient = 425			10X Ambient = 850			
		Rt-Rh	S	L		Rt-Rh	S	L		Rt-Rh	S	L
2 Days	(A1)	-	-	-	(B1)	0.004	-	-	(C1)	-	-	-
	(A2)	1.081	-	-	(B2)	no data	-	no data	(C2)	0.410	0.072	-
	(A3)	0.267	-	-	(B3)	2.957	0.040	0.022	(C3)	no data	0.248	-
5 Days	(A4)	0.948	-	1.202	(B4)	-	-	-	(C4)	11.188	0.365	0.165
	(A5)	1.061	-	-	(B5)	2.878	0.831	0.015	(C5)	12.274	0.804	1.119
	(A6)	1.549*			(B6)	2.025	0.179	-	(C6)	4.013	0.093	0.165
		0.334**	-	-								
10 Days	(A7)	0.997*			(B7)	0.718	-	-	(C7)	5.023	1.130	0.336
		0.599**										
	(A8)	1.042	1.393	no data	(B8)	1.287	0.125	-	(C8)	6.583	-	no data
	(A9)	0.664*	-	-	(B9)	1.953	-	-	(C9)	13.230	0.451	3.552
		0.197**										

(-) No uptake observed at the 95% confidence level (Appendix D)
* Root tissue only
** Rhizomes only

Specific rates of uptake of NH_4 were computed for P. nodusus during the laboratory experiment. Uptake rates are highly variable ranging from 0.0 to 15.04 µg NH_4-N (mg N Rt-Rh day)$^{-1}$ at the ambient level of substrate, 0.0 - 14.93 µg NH_4-N (mg N Rt-Rh day)$^{-1}$ at 5X ambient to 0.0 - 40.08 µg NH_4-N (mg N Rt-Rh day)$^{-1}$ at 10X ambient (Table IV). Plants grown at the 10X ambient substrate level for five and ten days had the highest uptake rates. There was a tendency for uptake rates to increase with higher levels of substrate and longer incubation periods; however, this trend is not without exception, i.e., the highest uptake rate observed at 5X ambient level was detected after two days incubation (Table IV).

Upward translocation occurred when stem and/or leaf organs became enriched (Table III). Table IV compares uptake and the % of transport for replicates under different conditions. At ambient substrate levels only two of nine plants translocated labeled N; however, these represent the highest percentages translocated among all plants studied. At 10X ambient level of substrate, translocation was observed in six plants; the percentage transported was substantial and ranged from 21.3 - 47.8 (Table IV). About half of all plants observed translocated the labeled N. Figure 4 illustrates substantial translocation occurred at all levels of substrate.

For comparative purposes transport indexes were derived, using the formula given in Chapter III. Nitrogen content, root-rhizome dry weight, number of leaves per plant and transport indexes are presented in Table V. No relationships are apparent between biomass estimates or N content and enrichment or between biomass estimates and the transport index. The number of leaves per plant could not be correlated with the

TABLE IV

RATE OF UPTAKE AND TRANSLOCATION OF P. NODOSUS CULTURED IN THREE DIFFERENT SUBSTRATE (NH_4Cl) CONCENTRATIONS FOR TWO, FIVE AND TEN DAYS (UPTAKE RATES ARE EXPRESSED AS μg NH_4-N (mg N ROOT-RHIZOME DAY)$^{-1}$ AND TRANSLOCATION AS PERCENTAGE OF STEM-LEAF ^{15}N ENRICHMENTS TO TOTAL ENRICHMENT)

Incubation Intervals	Replicates (Identified by No.)	Substrate Concentration					
		Ambient*		5X Ambient		10X Ambient	
		Uptake	% Trans.	Uptake	% Trans.	Uptake	% Trans.
2 Days	(1)	0.0	0.0	0.02	0.0	0.00	0.0
	(2)	5.47	0.0	no data	0.0	2.83	26.8
	(3)	0.97	0.0	14.93	6.5	1.68**	no data
5 Days	(4)	15.04	87.3	0.00	0.0	31.13	27.4
	(5)	2.14	0.0	8.28	29.8	40.08	38.1
	(6)	0.90	0.0	4.78	14.4	9.96	21.3
10 Days	(7)	0.65	0.0	0.73	0.0	8.08	37.2
	(8)	5.46	81.5	1.47	11.5	6.65	0.0
	(9)	0.23	0.0	1.97	0.0	25.60	47.8

* Ambient concentration of substrate was 85 μg NH_4-N l^{-1}

** Rates computed from % ^{15}N excess in stem tissue only

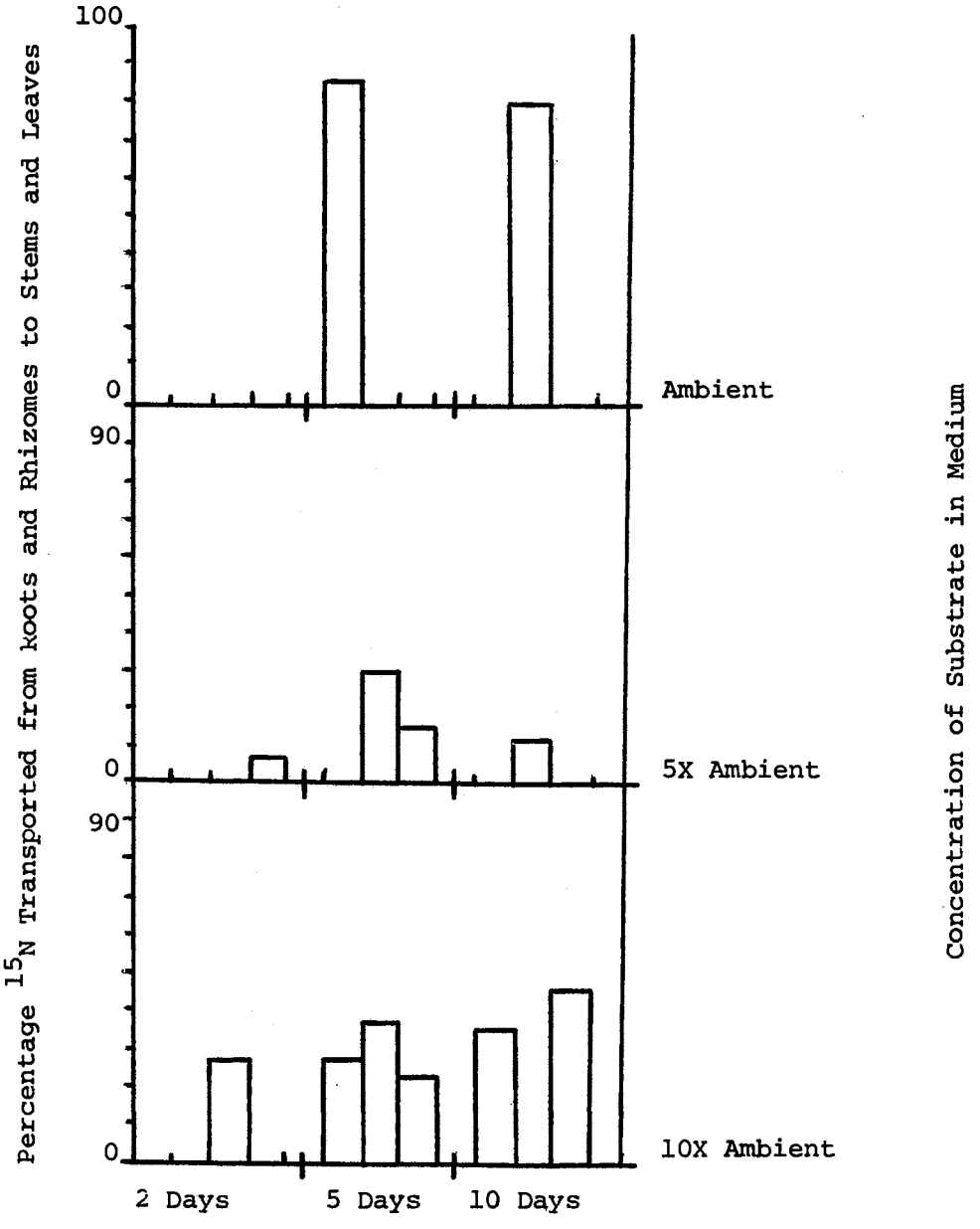

Figure 4. Percentage Transported Derived from the Stem and Leaf ^{15}N Enrichment and Total Enrichment

TABLE V

TOTAL ^{15}N ENRICHMENT, N CONTENT, DRY WEIGHT OF ROOT-RHIZOME TISSUES, NUMBER OF LEAVES AND TRANSPORT INDICES FOR *P. NODOSUS* CULTURED AT DIFFERENT CONCENTRATIONS OF SUBSTRATE AND TIME INTERVALS

Substrate Concentration	Incubation Interval (Days)	^{15}N Enrichment* (μg ^{15}N)	N Content (mg N Plant^{-1})	Biomass Rt-Rh (Dry Wt mg)	No. of Leaves	Transport Index**
	2	0.00	4.29	33.51	3	0.0
		5.32	1.63	28.12	2	0.0
		1.32	7.15	39.48	4	0.0
	5	81.48	8.52	62.05	4	64.1
Ambient		14.08	12.47	75.81	5	0.0
		3.34	10.28	42.73	5	0.0
	10	5.91	10.34	52.58	3	0.0
		40.06	8.22	40.66	6	74.6
		6.60	5.81	167.29	4	0.0
	2	0.03	15.38	40.39	5	0.0
		0.00	0.84***	54.20	2	0.0
		34.78	9.08	62.82	5	2.9
	5	0.00	10.74	71.74	4	0.0
5X Ambient		37.94	6.77	52.91	3	26.3
		22.79	8.80	54.06	4	9.1
	10	8.43	4.99	67.07	4	0.0
		10.66	4.90	41.88	4	10.3
		26.45	8.22	77.41	4	0.0
	2	0.00	13.47	19.81	3	0.0
		5.18	11.94	52.84	3	16.1
		2.08***	4.84	35.83	3	no data
	5	129.64	15.99	48.08	5	4.5
10X Ambient		91.19	3.79	26.26	3	16.1
		36.62	5.81	42.46	3	6.9
	10	123.96	10.68	88.60	6	26.4
		153.45	7.86	133.20	2	0.0
		124.44	2.98	28.06	2	27.8

* Derived by multiplying % ^{15}N excess by mg N X 10^3
** Computed using the formula defined in Chapter III
*** Computed from stem tissue only

transport index. The number of leaves per plant could not be correlated with the transport index. Dry weights of root-rhizome tissues appear to be unrelated to uptake.

The production of growth of new roots was observed in several plants during the laboratory experiment. The degree of growth of new roots related to uptake in that there was a tendency for uptake to increase with time and seven of nine plants incubated for ten days had new roots develop. Uptake also occurred in the two plants with no roots.

An attempt was made to estimate the rate of NH_4 uptake by measuring NH_4 depletion in the medium in each root-rhizome compartment. The decrease of NH_4 concentration would be an indirect measure of uptake. Analysis of NH_4 by direct Nesslerization gave extremely variable results and could not be used. Some constituent(s) present in Wetzel's modified medium apparently interferred with the Nessler's reagent and prohibited normal color development.

Measurements of the pH for each compartment were made at the end of each incubation interval. A summary of these determinations is presented in Appendix B. The pH decreased slightly in the stem-leaf compartments, while a small increase generally occurred in the root-rhizome medium.

In Situ Experiment (9-15-72/9-27-72)

The in situ uptake experiment, performed in mid-September, 1972, consisted of nine experimental plants with roots and rhizomes exposed to $^{15}NH_4$ enriched sediments and nine control plants.

Enrichment was determined with the same statistical limit used for analysis of laboratory treated plants. The % ^{15}N excess and N

content of individual tissues where enrichment occurred are presented in Table VI. These data show enrichment occurred in three of the nine experimental plants. At the end of three days one plant had roots and rhizomes enriched. Both stems and leaves of another plant were enriched after seven days but roots and rhizomes were not collected. All organs were enriched in a third plant after twelve days. Higher enrichments were detected in stems and leaves than in root-rhizomes when enrichment is expressed as % ^{15}N enrichment/mg N plant (Table VII). None of the nine control plants were enriched.

Transport in the plant incubated twelve days was 56.8%. This percentage was exceeded in the laboratory in only two cases, i.e., the unusually high percentages estimated for plants incubated for 5 and 10 days at ambient concentrations of NH_4.

Discussion

Tables III and IV contain data showing % ^{15}N excess in both stems and leaves of plants tested under laboratory conditions. The frequency with which enrichment occurred and the magnitude reported here provide ample evidence for rejection of the null hypothesis H_o1.

The *in situ* uptake of ^{15}N through roots or rhizomes in *P. nodosus* has been demonstrated by the data compiled in Table VII. According to these data, acropetal translocation did occur during the *in situ* experiment. Null hypotheses H_o2 and H_o3 are rejected.

Where quantitative data are available, results of upward translocation of N in *P. nodosus* compare favorably with or exceed that for P in *Myriophyllum spicatum* (Bristow and Whitcombe, 1971 and Waisel and Shapira, 1971), *M. brasiliense* and *Elodea densa* (Bristow and Whitcombe,

TABLE VI

PLANT TISSUES ENRICHED BY IN SITU UPTAKE OF ^{15}N LABELED NH_4 FROM SEDIMENT (EXPRESSED AS % ^{15}N EXCESS) AND N CONTENT OF THE PLANT TISSUE: THREE REPLICATES USED FOR EACH INCUBATION INTERVAL

Tissues Enriched	Incubation Intervals					
	3 Days		7 Days		12 Days	
	% ^{15}N Excess	mg N	% ^{15}N Excess	mg N	% ^{15}N Excess	mg N
Root-Rhizome	0.356	~~0.~~082	no data	no data	0.256	2.468
Stem	0.000	3.693	1.030	1.214	0.408*	22.209
Leaves	no data	no data	1.021	2.230	0.061**	33.360

Controls not enriched

* Leaf biomass subdivided and % ^{15}N excess estimates were averaged

** One subsample, the second not analyzed

TABLE VII

ENRICHMENT OF *POTAMOGETON NODOSUS* WITH ^{15}N EXPRESSED AS $\mu g\ ^{15}NH_4$-N/mg N PLANT^{-1}

Plant Tissues	Incubation Interval 3 Days	7 Days	12 Days
Root-Rhizome	3.55	no data	3.56
Stem	0.00	10.30	4.07*
Leaf	0.00	10.21	0.61

* Averaged results of two subsamples

1971) and Zostera marina (McRoy and Barsdate, 1970).

No data on the translocation of N in other macrophytes is available for comparisons. However, studies in non-rooted forms, such as Ceratophyllum demersum, indicate significant amounts of NH_4 are assimilated in nature (Toetz, 1971).

Although ^{15}N absorption in P. nodosus occurred in most plants observed, the data on uptake rates show considerable variability. Several factors may have contributed to the difference observed. Ion absorption is affected by pH in certain aquatic plants (Olson, 1953); however, the changes in pH in certain aquatic plants (Olson, 1953); however, the changes in pH observed here are insignificant and not correlated with uptake.

Air enriched with CO_2 was passed through the root-rhizome medium to promote growth (Bristow and Whitcombe, 1971). The aeration system posed some difficulties, i.e., the air tubes were of such small bore that they were frequently blocked. Mechanical manipulations on each experimental vessel where blockage occurred were made with each visit to the growth chamber. Fluctuation in the concentration of CO_2 may account in part for variability in NH_4 uptake.

Variation was also observed in the magnitude and frequency of upward translocation. The production of new leaves and roots within the stem-leaf compartment might logically contribute to translocation through an increased demand for nutrients.

Additional axillary roots or new leaves developed in the two plants with high transport percentages grown in ambient substrate. More than 50% of the plants, which did not translocate the label had leaves in poor condition and lacked new leaves. Four plants where ^{15}N trans-

ported was above 40% had new growth, i.e., one mature leaf to as many as seven new immature leaves. Some plants remained submerged for the duration of the laboratory experiment, while others had floating leaves. It is reasonable to conclude that transpiration was nil for those submerged.

Leakage between compartments was primarily restricted to the passage of medium from the stem-leaf compartment into the root-rhizome chamber. Movement in this direction could have affected the results only by dilution of the enriched root-rhizome medium. In one instance leakage in to the root-rhizome compartment was obvious, since the medium completely filled this vessel. Under these conditions stems and leaves could have assimilated the label from the surrounding medium. The enrichment that occurred in this plant (stems and leaves) was comparatively low (% ^{15}N excess in stems, 0.093, and in leaves, 0.165).

This study was conducted near the climax of the growth period (August and September) and a large proportion of the Potamogeton bed appeared necrotic and heavily grazed. Some attention was given to the selection of healthy-appearing plants; however, observable differences did exist among the plants collected. Undoubtedly, the most important factor causing variation in both uptake and translocation was the age and condition of the plants.

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary and Conclusions

This investigation was conducted to determine the ability of roots and rhizomes of Potamogeton nodosus, an emergent macrophyte, to extract NH_4 from enriched aqueous medium and lake sediment and to transport it upward to the stems and leaves. Two major experiments were conducted to test these hypotheses. The first was accomplished under controlled laboratory conditions to determine if and to what extent translocation of ^{15}N occurred. Specific uptake rates were derived. The second experiment on P. nodosus was performed in situ to measure the uptake of NH_4 from lake sediments and translocation of the label to stems and leaves.

In the laboratory, plants were incubated in enriched aqueous medium with roots and rhizomes exposed to three different concentrations of ^{15}N labeled NH_4 for 2, 5 or 10 days. Almost 90% showed uptake of NH_4 by roots and rhizomes, and approximately 50% had the isotope in stem and leaf organs.

Specific uptake rates for plants with enriched organs were high-variable, ranging from 0.02 to 40.08 µg NH_4-N (mg N Root-Rhizome day)$^{-1}$. Uptake rates were highest for plants grown in the 10X ambient level of substrate.

Data from the field experiment indicated that roots, rhizomes,

stems and leaves become enriched with ^{15}N injected as labeled NH_4 into the sediments.

The data presented in Tables III, IV, VI and VII permitted the rejection of all three null hypotheses. Therefore, it was concluded that P. nodosus can absorb ^{15}N through their root-rhizome organs and, subsequently, translocate it to the stems and leaves under both laboratory and field conditions.

The relative importance of NH_4 uptake from the sediments and its role as a nutrient for this species has not been established. However, if significant amounts of NH_4 are assimilated by rooted aquatic plants, then the nitrogen flux between these compartments (inorganic N and organic N) represents an important recyclic pathway in many aquatic ecosystems. It may well be that nutrients which accumulate in bottom sediments as eutrophication proceeds, constitute a vast reservoir for support of macrophyte growth (Frink, 1967).

Recommendations

The plants studied were selected from an aging population, and this factor was recognized as a possible source of variability in the results. A similar study conducted during the early stages of growth would be of value. Other studies should be accomplished to determine if P. nodosus shoots (stems and leaves) are functional in nutrient (NH_4) uptake from lake water. Another possibility is that nutrients may be cycled from the sediments through plants and excreted into the surrounding waters. In testing these hypotheses a possible recyclic pathway might be delineated.

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APPENDIX A

GLOSSARY OF TERMS

Terms are defined from the following sources: Odum (1959), Prescott (1968), Kormondy (1969), and Webster's Seventh New Collegiate Dictionary, or as they are used in the context of this paper.

Acropetal translocation

Acropetal translocation is the movement of dissolved solutes from the base of the plant upward.

Basipetal translocation

Basipetal translocation is the movement of dissolved solutes from the foliage downward.

Ecosystem

An ecosystem consists of the biotic community (all of the populations occupying a given area) and the non-living environment functioning together as a unit or system.

In situ

In situ is the natural or original position.

Isotope ratio analysis

Isotope ratio analysis is the quantification of one isotope relative to another, in this case, the ratio of nitrogen -14 to nitrogen -15.

Macrophytes and hydrophytes

Aquatic macrophytes and hydrophytes will be used synonomously to refer to higher plants that grow in water, either as submerged or emergent forms.

Nutrient cycling

Nutrient cycling refers to the movement of elements in ecosystems.

Per cent ^{15}N excess

The per cent ^{15}N excess is the positive difference between the mean per cent ^{15}N in the blanks and the per cent ^{15}N of an experimental determination.

Periphyton

Periphyton refers to the entire attached community growing on substrates such as higher plants.

Specific uptake rate

The specific uptake rate is the mass of sbustrate absorbed per unit of biomass doing the absorbing per unit time.

Uptake rate

Uptake rate is the mass of substrate absorbed by an entity per unit time.

APPENDIX B

pH MEASUREMENTS FROM THE LABORATORY EXPERIMENT MADE AT THE CONCLUSION OF EACH INCUBATION INTERVAL AND EXPRESSED AS A RANGE OF THE THREE REPLICATES AND TWO CONTROLS

Experimental Groups	Initial	2 Days	5 Days	10 Days
Stem-Leaf Compartments				
"A" Series	8.5	8.25-8.35	8.30-8.30	8.35-8.45*
Controls	8.5			8.25,8.30
"B" Series	8.5	8.35-8.40	8.25-8.25	8.25-8.45
Controls	8.5			8.30,8.35
"C" Series	8.5	8.35-8.35	8.25-8.35	8.20-8.35
Controls				8.15,8.35
Root-Rhizome Compartments				
"A" Series	7.5	7.40-7.45	7.30-7.60	7.25-7.65
Controls	7.5			7.65,7.80
"B" Series	7.5	7.55-7.55	7.10-7.55	7.10-7.30
Controls				8.10,8.20
"C" Series	7.5	7.45-7.50	7.45-7.55	7.30-8.00
Controls				8.25,7.55

APPENDIX C

ESTIMATES OF PER CENT NITROGEN IN LEAF, STEM AND ROOT-RHIZOME TISSUES DERIVED BY CONVERSION OF ORGANIC NITROGEN TO ELEMENTAL NITROGEN AND VOLUMETRIC MEASUREMENT

Four Samples of Leaf Tissue

%N	1.70
	2.67
	3.02
	3.13

mean = 2.63

Four Samples of Stem Tissue

%N	2.21
	2.06
	1.63
	1.61

mean = 1.88

Four Samples of Root-Rhizome Tissue

%N	2.39
	1.39
	2.15
	0.99

mean = 1.75

APPENDIX D

ISOTOPE RATIO OF BLANKS BY MASS SPECTROSCOPY AND CALCULATIONS OF THE STANDARD DEVIATION

Blank No.	Isotope Ratio	
	X	X^2
812	0.3648	0.133
826	0.3629	0.132
921	0.3961	0.157
923	0.2859	0.082
925	0.4095	0.168
926	0.2323	0.054
	$\Sigma X = 2.0515$	$\Sigma X^2 = 0.726$

$$n = 6$$

$$\overline{X} = 0.3415$$

$$s^2 = \frac{X^2 - \frac{(\Sigma X)^2}{n}}{n-1}$$

$$s^2 = \frac{0.726 - \frac{4.209}{6}}{5} = \frac{0.726 - 0.701}{5}$$

$$s^2 = \frac{0.025}{5} = .005$$

$$s = \sqrt{.005} = 0.071$$

Upper limit of 95% CI $= \overline{X} + 1.96\ s$
$= 0.3415 = 1.96\ (0.071)$
$= 0.3415 = 0.139 = \underline{0.481}$

Where: n = number of blanks
X = isotope ratio of each blank
$\overline{X}$ = mean
s^2 = variance $= \frac{X^2 - (\Sigma X)^2}{n-1}$

$$s = \sqrt{\frac{X^2 - \frac{(\Sigma X)^2}{n}}{n-1}}$$

s = standard deviation
CI = Confidence Interval

95% CI $= \overline{X} \pm 1.96\ s$

VITA

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Doctor of Education

Thesis: UPTAKE AND TRANSLOCATION OF ^{15}N BY POTAMOGETON NODOSUS

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Biographical:

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