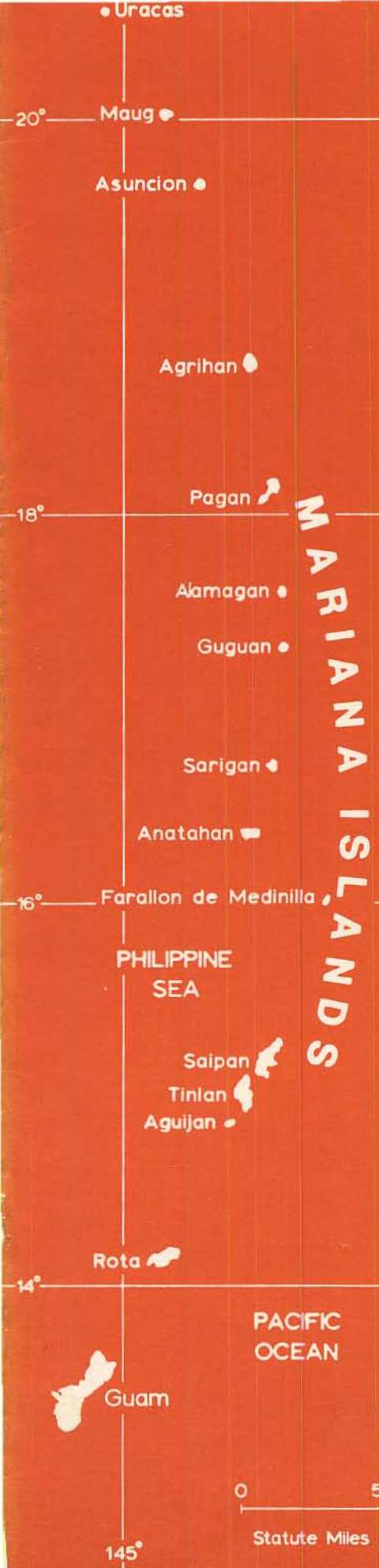

NITROGEN UPTAKE BY TROPICAL FRESHWATER MACROPHYTES

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Water Resources Research Center

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OFFICE OF WATER RESEARCH AND TECHNOLOGY

OWRT PROJECT NO. A-014-GUAM

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Project Completion Report

for

NITROGEN RETENTION, REMOVAL, AND TURNOVER RATES IN AQUATIC MICROCOSMS IN RELATION TO THE DEVELOPMENT OF AQUACULTURE SYSTEMS FOR WATER QUALITY IMPROVEMENT

OWRT Project No. A-014-Guam, Grant Agreement No. 14-34-0001-9012

Principal Investigator: Stephen G. Nelson

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ABSTRACT

The kinetics of nitrogen uptake were examined for three species of aquatic macrophytes which are common on Guam. Nitrate-nitrogen and ammonium-nitrogen uptake by Pistia stratiotes were monitored in response to a wide range of substrate concentrations. First order kinetics of nitrate-nitrogen uptake were examined for Pistia stratiotes, Hydrilla verticillata and Microspora sp. Uptake rates were higher after twenty-four hours of exposure to the nitrate source than immediately after exposure. The rate of uptake of nitrate-nitrogen by P. stratiotes was greater in the light than in the dark. Nitrate uptake followed a pattern which could be described by the Michaelis-Menton expression. Rates of ammonium-nitrogen uptake were similar in the dark and in the light. Ammonium-nitrogen uptake response to substrate concentration appeared to be linear. For any given dissolved nitrogen concentration, the rate of ammonium-nitrogen uptake was greater than the rate of nitrate-nitrogen uptake.

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INTRODUCTION

In recent years there has been increased interest in the use of aquaculture systems for water purification. This interest has stimulated research which has indicated that aquaculture systems can be effectively employed to reduce nutrient loads in both marine (Ryther, 1977) and freshwater systems (Wooten and Dodd, 1976; Gordon, 1977; Ball, 1977). Aquaculture systems have also proven to be cost-effective when compared to other treatment methods (Henderson and Wert, 1976). Aquaculture as a means of wastewater treatment may be especially well-suited to tropical areas because of their relatively constant environmental conditions.

Aquatic macrophytes have often been suggested as major components of aquaculture systems for water purification (Ehrlich, 1966; Culley and Eps, 1973; Boyd, 1975; Wooten and Dodd, 1976) and have proven highly effective at reducing the concentrations of nutrients in water (Rogers and Davis, 1972; Boyd, 1976; Glandon and McNabb, 1978). The relations between the rate of nutrient uptake by aquatic macrophytes and the ambient nutrient levels are poorly understood although such information would be useful in understanding the function of aquatic plants in aquaculture systems for water purification. Toetz (1971) showed that the macrophyte Ceratophyllum takes up nitrate only during the day but that ammonium was taken up during both the day and the night. He suggested that a large biomass of aquatic macrophytes may have a significant impact on the nutrient dynamics of aquatic ecosystems

(Toetz, 1971, 1973) and that the plants may even compete with microorganisms for NH_4^+ which, in turn, may reduce the rate of nitrification.

In many aquatic plants, nutrient uptake is mediated by enzymes in the cell membrane known as permeases. Therefore, relations between the rate of uptake (V) and nutrient concentration ([S]) in many cases can be adequately described by the Michaelis-Menton expression:

$$V = \frac{V_{max}[S]}{K_s+[S]} \qquad (1)$$

where V_{max} is the maximum rate obtained and K_s is the substrate concentration when $V = \frac{V_{max}}{2}$. A knowledge of V_{max} and K_s enables comparisons between species under specific environmental regimes.

For uptake kinetics which can be described by the Michaelis-Menton expession, the plot of V on [S] can be divided into three regions. At low substrate concentrations the response is almost linear. This is the region of "first-order" kinetics, where V is directly related to [S]. At high substrate concentrations, V is independent of [S]. This is the region of "second-order" kinetics, where $V = V_{max}$. At intermediate substrate concentrations the response is intermediate and is termed "mixed order" kinetics.

Toetz (1973) reported that the kinetics of ammonium-nitrogen (NH_4^+-N) uptake by Ceratophyllum were poorly described by the Michaelis-Menton expression. He suggested that NH_4^+-N uptake may be accomplished by a passive-diffusion process. Toetz (1971, 1973) also suggested that NH_4^+-N may be a more important source of nitrogen

for aquatic plants than NO_3^--N.

The purpose of this study was to examine the kinetics of nitrogen uptake for several common freshwater macrophytes on Guam. We examined the "first-order" nitrate-nitrogen uptake kinetics for three species. These were a floating macrophyte, Pistia stratiotes, a submerged macrophyte, Hydrilla verticillata and a filamentous alga, Microspora sp. We examined the kinetics of nitrate-nitrogen and ammonium-nitrogen uptake over a wide range of substrate concentrations for P. stratiotes. The objective was to provide information which would be useful in the design of aquaculture systems for ammonium and nitrate removal and which would contribute to our understanding of the nutritional ecology of freshwater macrophytes.

METHODS AND MATERIALS

Freshwater macrophytes were collected from the field and cultured at the University of Guam Marine Laboratory for this study. Three species were examined, Hydrilla verticillata, Microspora sp. and Pistia stratiotes. Both H. verticillata and Microspora sp. were cultured in an outdoor tank supplied with rainwater. Pistia stratiotes was cultured either in a cement raceway or in a recirculating system made of plywood. Both of these latter systems were supplied with Mangilao, Guam, well water.

All uptake experiments were carried out in an environmental chamber. Either 400 or 200 footcandles of illumination were supplies by both incandescent and flourescent lights during nitrogen uptake determinations in the light. For the uptake experiments, from 0.02 to 0.17 grams dry weight of aquatic macrophytes were placed

in jars which contained 200 ml of water with known concentrations of dissolved nitrate or ammonium. Mean temperatures ranged from 25 to 31°C. Nitrate and ammonium concentrations were determined with Orion specific-ion electrodes. Both the nitrate electrode and the ammonia electrode have been shown to be reliable within the ranges of concentrations used in this study (Beckett and Wilson, 1974; Shechter and Gruener, 1976). Nitrate was supplied as KNO_3 and ammonium was supplied as NH_4Cl. The jars containing the plants were gently aerated with compressed air throughout the incubation periods in order to assure an even distribution of nutrients. After the jars had been in the environmental chambers for approximately five minutes, an initial water sample was taken from each. The experimental protocols for the nitrate and ammonium-uptake experiments are described below.

First Order Kinetics of Nitrate Uptake

The study of first-order nitrate-nitrogen (NO_3^--N) uptake by the three species of macrophytes was undertaken under the conditions described above with 400 footcandles of illumination. Corrections were made for evaporation by the use of control jars without plants. Three water samples were taken during a 48-hour incubation period, and the nitrate-nitrogen concentrations in the samples were determined. The initial samples were taken immediately after the plants were placed in the jars. The second and third samples were taken after 24 and 48 hours of incubation respectively. The mean rate of uptake and the mean substrate concentration for each 24-hour period were calculated.

Saturation Kinetics

A wide range of nitrate concentrations was examined for Pistia stratiotes incubated both at 200 footcandles of illumination and in dark bottles. Substrate concentrations of up to 40 mg NO_3^--N·l^{-1} were used. This allowed the determination of the maximum rates of uptake (V_{max}) and substrate affinity (K_s) for plants which were incubated in the light and in the dark. Both V_{max} and K_s were determined by calculating the line-of-best-fit by the least squares method (Snedecor and Cochran, 1969) for the plot of [S] versus [S]/V. The resulting line had a slope equal to V_{max} and a Y-intercept equal to $-K_s$ in accordance with the linear form of the Michaelis-Menton expression:

$$[S] = V_{max}([S]/V) - K_s \qquad (2)$$

Ammonium Uptake by Pistia

Similar procedures were used to estimate the rate of ammonium-nitrogen uptake by P. stratiotes. The rates of uptake were determined under both light (200 footcandles) and dark conditions. Since the rate of ammonium-nitrogen uptake was very rapid, an incubation period of only 4.5 hours was used. Corrections for ammonium loss other than uptake by the plants were made by the use of controls without plants. The control jars contained ammonium concentrations over the range of initial ammonium concentrations in the experimental jars.

After the uptake trials each plant was dried overnight at 50°C and weighed to the nearest 0.01 mg on an electronic balance. Uptake rates were expressed as mg NH_4^+-N·g^{-1}·h^{-1}.

RESULTS AND DISCUSSION

First-Order Nitrate-Nitrogen Uptake Kinetics

The relations between the rate of uptake of nitrate-nitrogen and the substrate concentration for the three macrophytes are indicated in Table 1. For all three species there was relatively little dependence of the rate of uptake on substrate concentration for the first 24-hour period. The correlation coefficients for the regressions of rate of uptake on substrate concentration ranged from 0.45 to 0.78 for this period as shown in Table 1. For the second 24-hour period there was a strong dependence of uptake rate on substrate concentration. The correlation coefficients for these regressions ranged from 0.86 to 0.92, indicating that a linear model adequately described the relations between rate of uptake and substrate concentration.

During the first 24 hours of incubation, rates of nitrate-nitrogen uptake were low in comparison to those of the subsequent 24-hour period. This lag indicates that the uptake systems were being induced. The patterns of uptake during the first and second 24-hour intervals indicated that the uptake systems had been induced and were reponding to increases in substrate concentration in a linear fashion. Representative plots of uptake rate of substrate concentration are shown for P. stratiotes for the first and second day of incubation in Figures 1 and 2 respectively.

Nitrate-Nitrogen Uptake Kinetics

For other aquatic macrophytes, it has been shown that an enzyme, nitrate reductase, mediates the uptake of nitrate (Joy,

Table 1. Regression statistics describing the relation of first-order uptake of nitrate-nitrogen (mg NO_3^--N·g^{-1}·h^{-1}) to mean substrate concentration (mg NO_3^--N·l^{-1}) for several aquatic macrophytes.

Species	Conditions	Day	Correlation Coefficient	Slope	Y-intercept	N
Pistia stratiotes	light	1	0.4481	0.0048	0.0011	15
Pistia stratiotes	light	2	0.8598	0.0469	0.0469	15
Hydrilla verticillata	light	1	0.4847	0.0095	0.0164	47
Hydrilla verticillata	light	2	0.9151	0.0470	-0.0376	47
Microspora sp.	light	1	0.7843	0.0350	-0.0011	24
Microspora sp.	light	2	0.9061	0.0729	-0.0603	24

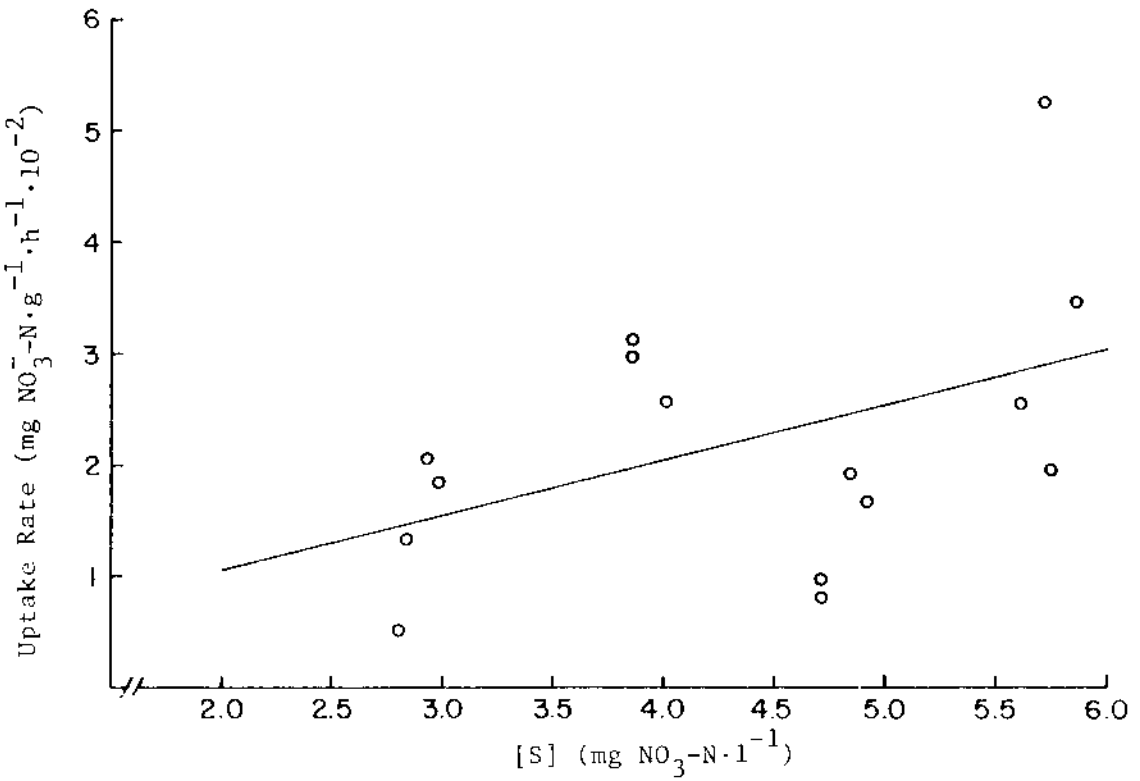

Figure 1. Nitrate-nitrogen uptake by Pistia stratiotes in relation to average substrate concentration ([S]). Uptake rates were determined during the first 24-hour period of incubation.

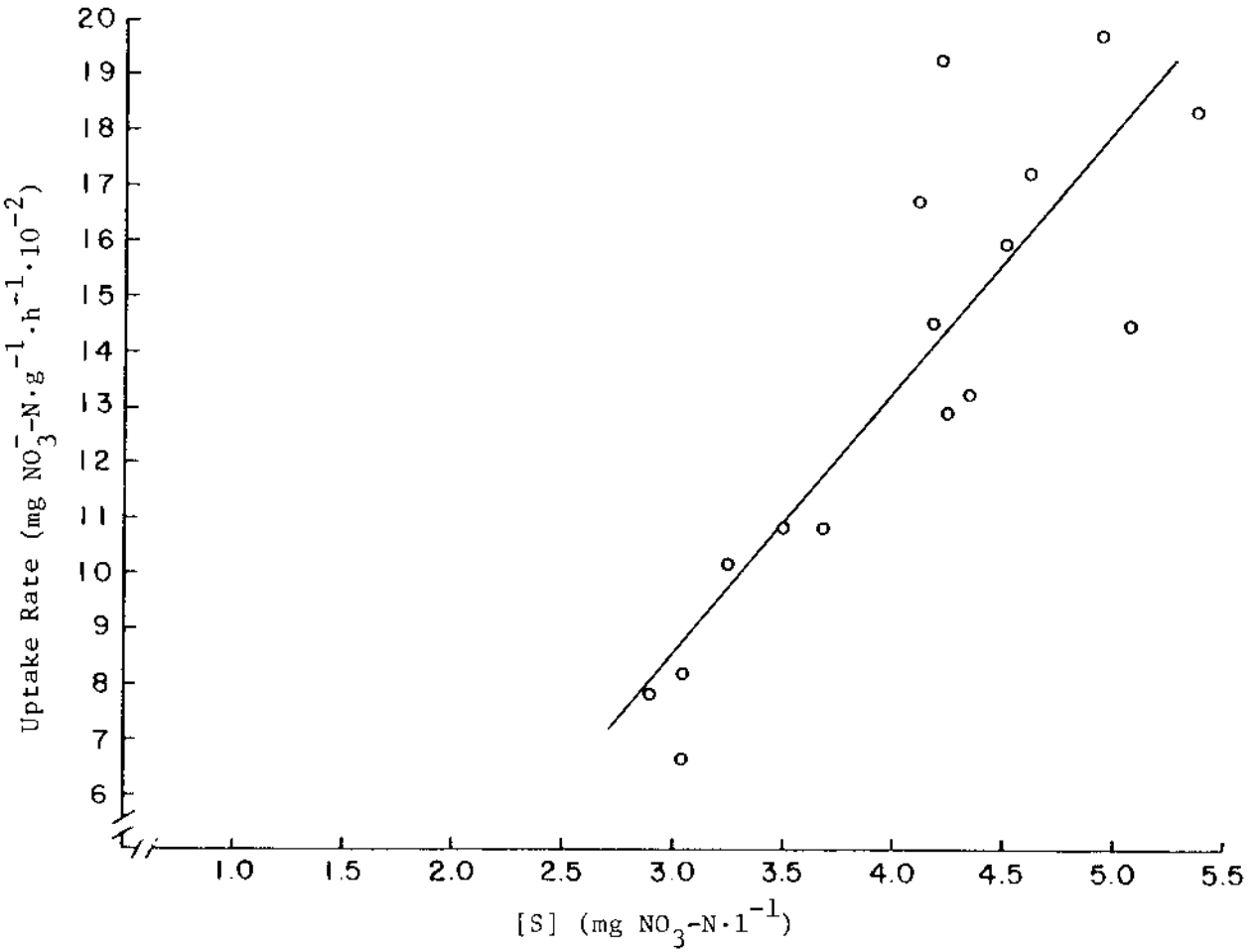

Figure 2. Nitrate-nitrogen uptake by Pistia stratiotes in relation to average substrate concentration ([S]). Uptake rates were determined during the second 24-hour period of incubation.

1969; Ferguson, 1969). Studies on the duckweed Spirodelia oligorhiza have shown that plants grown in a NH_4^+-N medium developed nitrate reductase when transferred to media containing only nitrate as a nitrogen source (Ferguson, 1969). In those studies the nitrate reductase reached peaks of activity after approximately 48 hours of exposure to nitrate as the sole source of nitrogen. Joy (1969) found that in the duckweed Lemna minor the nitrate reductase system was fully induced after 24 hours of exposure to a nitrate source. Our results suggest a similar occurrence for the species which we examined.

We examined the kinetics of nitrate-nitrogen uptake by P. stratiotes over a wide range of substrate concentrations in order to determine the maximum rate of uptake (V_{max}) and the substrate affinity (K_s) according to the Michaelis-Menton expression. The rate of uptake was monitored after the plants were exposed to high nitrate concentrations for 24 hours when the uptake system was fully operational. The relations between uptake rate and substrate concentration for plants in the light and in the dark are shown in Figure 3. The patterns depicted can be adequately described by the Michaelis-Menton expression. Similar general patterns of uptake in relation to substrate concentration are shown in the light and the dark, but the rate of uptake is much reduced in the dark. The plots of [S] on [S]/V for nitrate-nitrogen uptake by P. stratiotes in light and dark are shown in Figure 4. These plots were used to estimate V_{max} and K_s. The results of the analysis are shown in Table 2. The maximum rate of uptake (V_{max}) was higher in the light (0.2430 mg NO_3-N$\cdot g^{-1}\cdot h^{-1}$) than

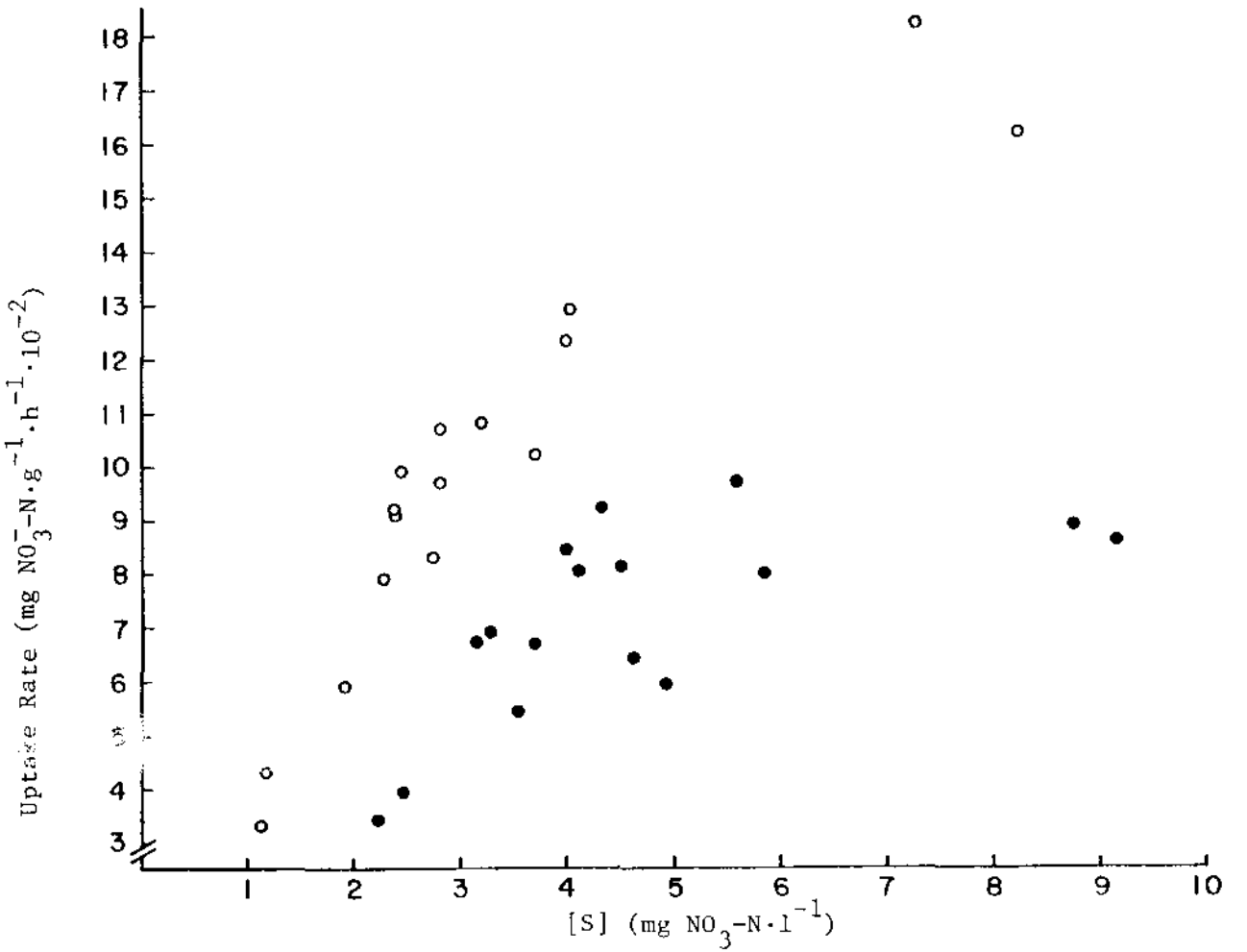

Figure 3. Nitrate-nitrogen uptake by Pistia stratiotes in relation to average substrate concentration ([S]). Light incubation is indicated by open circles (o) and dark incubation is indicated by closed circles (•).

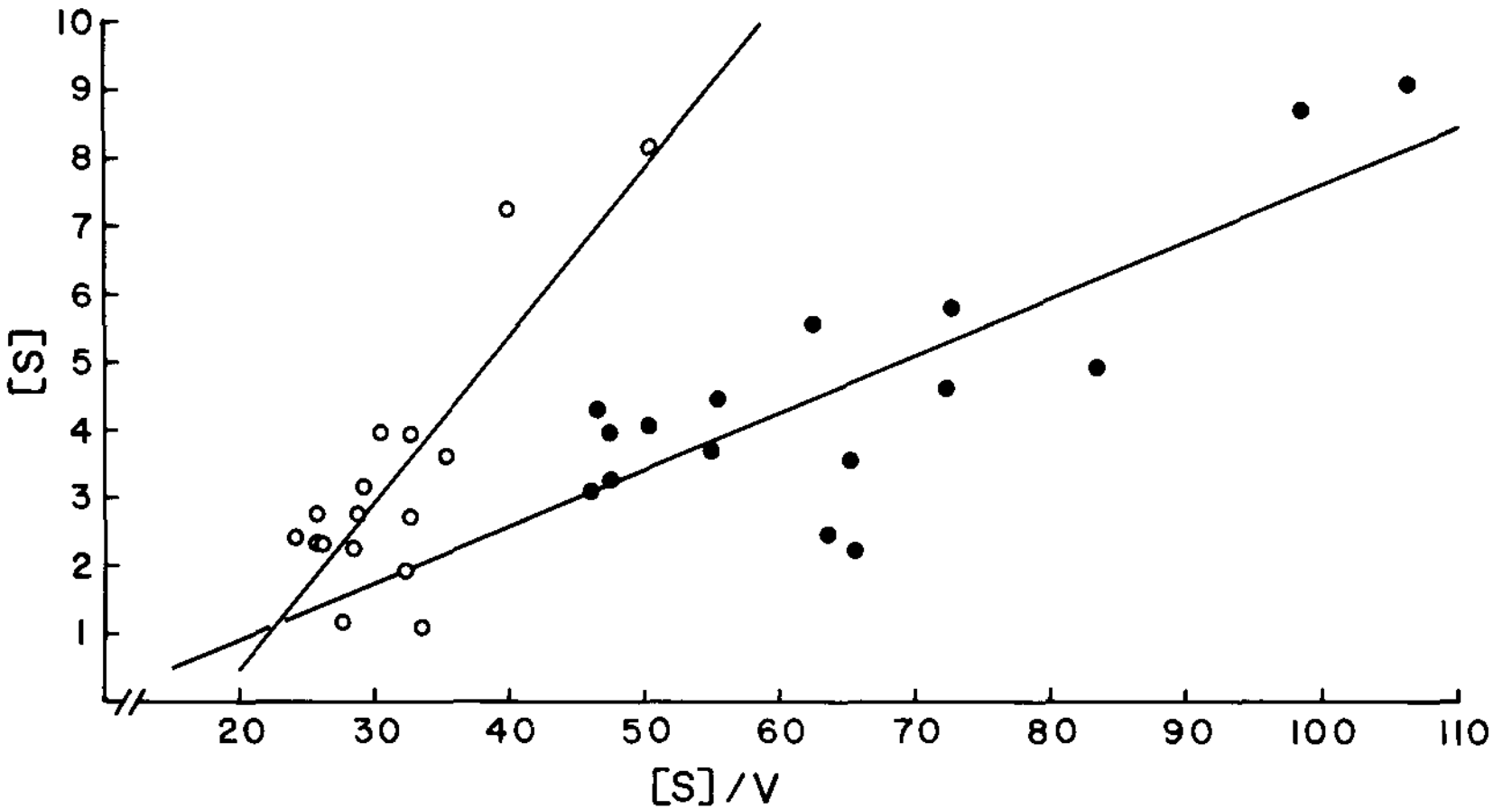

Figure 4. Plot of average substrate concentration ([S]) versus the ratio of average substrate concentration to uptake rate $(\frac{[S]}{V})$ for Pistia stratiotes. Light incubation is indicated by open cirles (o), and dark incubation is indicated by closed circles (•).

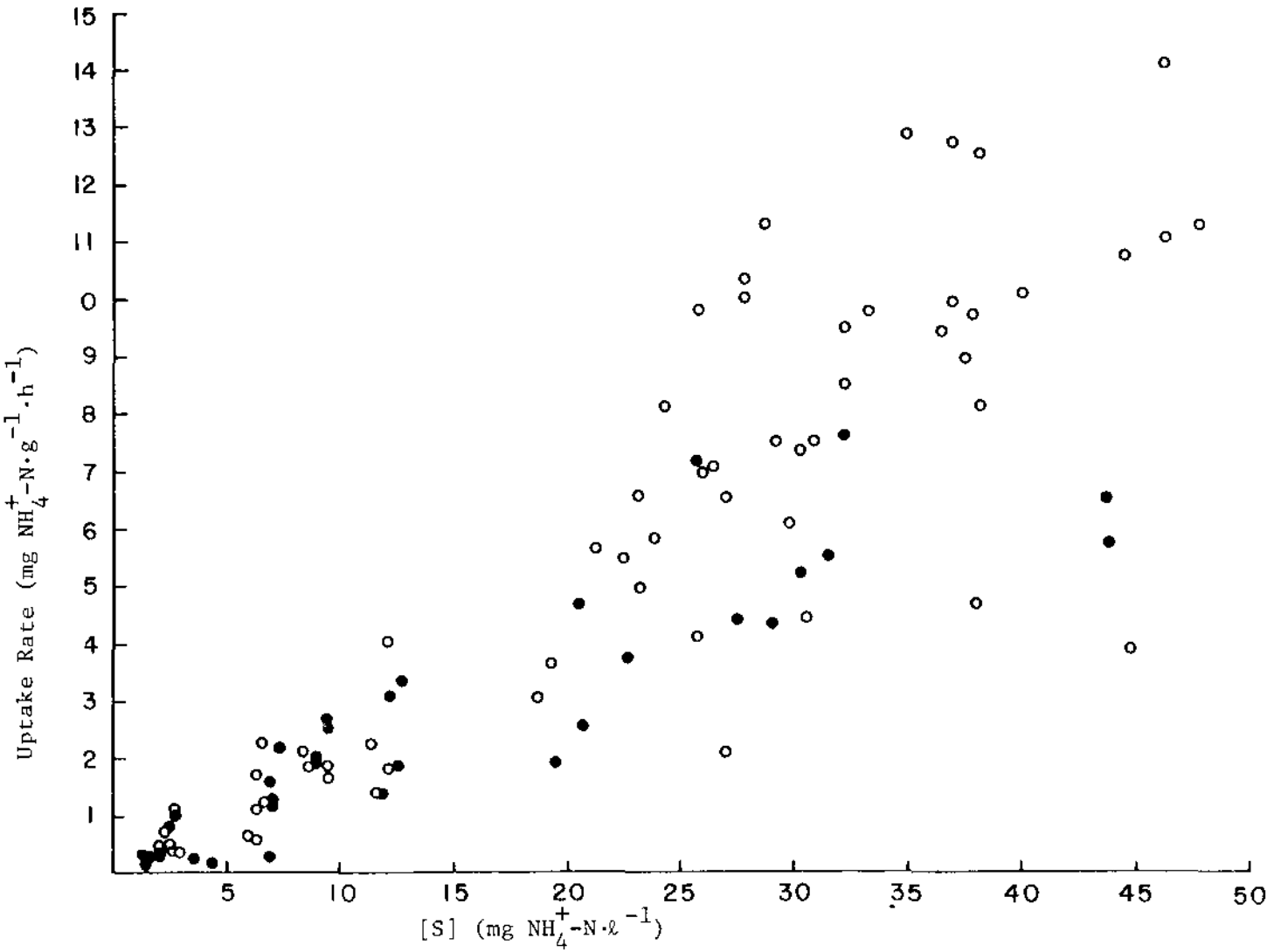

Figure 5. Plot of ammonia-nitrogen uptake rate versus average substrate concentration ([S]) for light-incubated (o) and dark-incubated (•) Pistia stratiotes.

Table 2. Regression statistics used to estimate parameters of the Michaelis-Menton equation* describing nitrate-nitrogen uptake by *Pistia stratiotes*.

Species	Conditions	Correlation Coefficient	V_{max}	K_s	N
Pistia stratiotes	light	0.8173	0.243	4.385	16
Pistia stratiotes	dark	0.7818	0.083	0.743	16

*Based on the linear form $[S] = V_{max}([S]/V)-K_s$.

in the dark (0.0831 mg NO_3-N·$g^{-1}h^{-1}$). This suggests that energy may be needed for nitrate-nitrogen uptake. The substrate affinity is less in the light than in the dark. This is evidenced by a higher K_s value in the light (4.3851) than in the dark (0.7430).

Ammonium-Nitrogen Uptake by Pistia stratiotes

The plot of the rate of ammonium-nitrogen uptake on average substrate concentration is shown in Figure 5 for P. stratiotes. Rates of uptake were the same for plants incubated in the light or in the dark. The uptake rate is dependent on the substrate concentration even up to very high substrate concentrations. The plot of uptake rate versus substrate concentration indicates that the ammonium uptake kinetics cannot be adequately described by the Michaelis-Menton equation. In fact, a linear model adequately describes the relation between rate of uptake and substrate concentration ($r = 0.8361$, $p<0.05$). This suggests that ammonium-nitrogen may be taken up by diffusion rather than by an active transport system. A similar pattern of ammonium uptake was suggested by Toetz (1973) from his studies on Ceratophyllum sp. However, McRoy and Alexander (1975) concluded that ammonium uptake by the roots of the submerged macrophyte Carex aquatilis exhibited a hyperbolic response to substrate concentration which could be described by the Michaelis-Menton expression. From in situ studies of ^{15}N uptake, Toetz (1971) reported that rates of ammonium uptake were lower in the dark and suggested that ammonium uptake was dependent on photosynthesis to

some degree. Data from the present study indicate that for P. stratiotes the rates of ammonium uptake are not dependent on light.

Turnover Times

Calculations of substrate turnover times for P. stratiotes with nitrate or ammonium as a nitrogen source are shown in Table 3. Calculations were made separately for conditions of light and dark for nitrate uptake, but the data were combined for calculation of turnover values based on ammonium uptake. The turnover times are expressed in hours and are calculated for a mass of plants of 1 gram dry weight in a constantly-stirred volume of one liter. The assumption is made that the nutrient input is equal to the rate of uptake in each case (after Toetz, 1973). For any substrate concentration of nitrogen, the turnover time is less for NH_4-N uptake than for NO_3-N uptake. This indicates that the plants are more efficient at removal of ammonium-nitrogen than removal of nitrate-nitrogen. The disparity between these turnover times is greater at the higher substrate concentrations.

Implications

Evidence suggests that ammonium-nitrogen may be a major nitrogen source for freshwater macrophytes. The relative importance of ammonium-nitrogen would be magnified in low nutrient waters or those which support high densities of aquatic animals (i.e. aquaculture systems).

Table 3. Substrate turnover times (hours) calculated for a range of substrate concentrations (mg $N \cdot l^{-1}$). Assumptions are that the volume of the container is one liter, that it contains one gram dry weight of Pistia stratiotes, that the rate of nutrient input is equal to the rate of uptake and that the system is constantly stirred.

Substrate Concentration	Turnover Time (hours)		
	Based on light uptake of NO_3^--N.	Based on dark uptake of NO_3^--N	Based on uptake of NH_4^+-N
25.600	123.4	317.0	4.3
12.800	70.7	163.0	4.3
6.400	44.4	86.0	4.3
3.200	31.2	47.5	4.3
1.600	24.6	28.2	4.2
0.800	21.4	18.6	4.2
0.400	19.7	13.8	4.2
0.200	18.9	11.4	4.2
0.100	18.5	10.1	4.2
0.050	18.2	9.5	4.1
0.025	18.1	9.2	3.9

This hypothesis is supported by the observation that P. stratiotes takes up ammonium-nitrogen more rapidly than it takes up nitrate-nitrogen at any substrate concentration. Also, ammonium-nitrogen is taken up at a high rate during both the day and the night, while the rate of uptake of nitrate-nitrogen is much reduced at night.

Other studies have shown that ammonia can both inhibit the formation of nitrate-reductase in aquatic plants (Joy, 1969) and prevent the assimilation of nitrate by plants which display a high level of nitrate-reductase activity (Ferguson, 1969). This suggests that nitrate would not be utilized to any extent until ammonium-nitrogen in the media was depleted. From our data and those of others (Ferguson, 1969; Joy, 1969) it appears that there is a lag time in the formation of the nitrate-uptake system after exposure to elevated nitrate concentrations. In the case of P. stratiotes the uptake systems appear to be fully active after 24 hours.

From our data it can be seen that aquatic plants can effectively reduce dissolved nitrogen loads in freshwater systems whether this dissolved nitrogen is in the form of nitrate-nitrogen or ammonium-nitrogen. The plants are more efficient at taking up ammonium than nitrate, especially at high dissolved nitrogen levels at which nitrate-uptake systems become saturated.

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