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A NUTRIENT BASIS FOR THE DISTRIBUTION OF THE LEMNACEAE

The University of Michigan PH.D. 1983

A NUTRIENT BASIS

FOR THE DISTRIBUTION OF THE LEMNACEAE

by
David Michael Docauer

A dissertation submitted in partial fulfillment
of the requirements for the degree of
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Doctoral Committee:

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CHAPTER I

INTRODUCTION

Central to modern ecology is the study of the causes of species diversity and the distribution of species in the environment. Zoologists can visualize complex food webs, a multitude of distinct resources and a large number of behavioral responses to the environment to explain animal diversity, but what can explain the great diversity of plant species? Most plants require a suitable temperature regime, light, moisture, CO_2, and some 20-odd inorganic nutrients to survive. Even considering adaptations to soil structure, humidity, temperature and pH, the great number of species that exist on this planet is astonishing.

Coexistence in terrestrial environments under potentially competitive circumstances is thought to be due to niche separation, in which species requirements vary slightly over time and space (Hutchinson, 1958; Stewart and Levin, 1973). The coexistence of plant species has been variously explained by the separation of niche by resource gradients (Pielou, 1974; Sharitz and McCormick, 1973), patchy environments (Weins, 1976; Jalloq, 1975; and Sarukhan and Harper, 1973), unstable environments (Green, 1974), effects of succession (Hutchinson, 1941; Keever, 1950), reversion to early successional stages (Haizel and Harper, 1973), various competitive life strategies (Stearns, 1976; Marshall and Jain, 1969), or repro-

ductive strategies (Geises, 1976; Harper and Ogden, 1970). On the other hand, some authors state that the colonization and extinction rates of species in habitat patches can cause species coexistence (Slatkin, 1974; Levins and Culver, 1971).

It is evident that these explanations of coexistence depend heavily upon niche separation in a heterogeneous environment. However, species with almost identical niches may limit each other to live under restricted ranges of physiological conditions, giving the appearance of niche separation. This shrinking of the fundamental niche to the restricted or realized niche is found in two Rumex species under the influence of grass species (Putwain and Harper, 1970), in the restriction of Sedum smallii to shallow dry soil by Minuartia uniflora (Sharitz and McCormick, 1973), and in the restriction of Lemna minor to cool oligotrophic pools by Lemna gibba in Czechoslovakia (Rejmankova, 1975).

The physiological restriction of the fundamental niche may be of great importance in aquatic environments, where the movement of the water mass tends to destroy habitat patches. The pelegic habitat of phytoplankton has long been assumed to have significant variation only in the vertical direction, occurring over distances of several meters (Hutchinson, 1941; Reynolds, 1976; and Ruttner, 1952). Some studies have indicated the existence of gross horizontal patches several kilometers in diameter (Lund, 1965; Verduin, 1951). However, Hutchinson (1961) points out that very small volumes of water contain scores of species and it seems unlikely that such large patches, if they contained the few species allowed by classical exclusion theory, could account for this diversity.

Hutchinson (1958) proposed the n-dimensional hypervolume to account for species coexistence. It consists of n species co-existing on n potentially limiting resources, each species being a specialist at utilizing low concentrations of one resource. Yet phytoplankton diversity often seems to outstrip the possible number of potentially limiting nutrients (Hutchinson, 1961). Hutchinson (1961) added a non-equilibrium theory to explain this abundance of species stating that the resource composition of a patch changes before any species can gain dominance. Reynolds (1976) proposed that successional changes produced by the formation of a weak stratification of the epilimnion in Crose Mere maintained a high diversity. Occasional summer turnover of the epilimnion destroyed the vertically layered patches, and returned the system to early successional stages. This encouraged the coexistence of newly developing species with remnant populations.

Reductions in the number of potentially limiting nutrients due to increases in their concentration can cause the number of species able to coexist in an aquatic system to be decreased. For example, artificial phosphorus fertilization has been shown to reduce the diversity of the phytoplankton community such that it consists of only a few blue-green algal species (Schindler, 1976). Similarly, alleviation of nutrient pollution can cause an increase in species diversity (Sonzogni et al., 1975). It therefore appears that nutrient concentrations or ratios may be involved in a mechanism of species coexistence and competitive exclusion.

A mechanism for coexistence or exclusion based upon the kinetics of nutrient utilization for reproduction has been proposed by

Tilman (1976, 1977). According to this hypothesis, one species will be more efficient at the utilization of a nutrient for growth than the other species. When the nutrient is limiting, the efficient species reduces the concentration to the point that the inefficient species cannot maintain the steady state growth rate neccessary to maintain it in the system. Another species may be more efficient in the utilization of another nutrient. When that nutrient is limiting, the second species can decrease it to concentrations at which the other species is excluded from the system. When the two nutrients are potentially limiting, the nutrient concentrations decrease under the influence of both species until a balance point is reached, where each species is limited by the nutrient for which the other species is most efficient. This causes coexistence.

Titman (1976), Tilman (1977) and Tilman and Kilham (1976) described the efficiencies of two diatoms growing on phosphate and silicate using the Monod model and a variable internal stores model. The Monod model describes a species growth rate, which depends on external concentrations of nutrients, and its parameters can be determined by batch culture methods. The variable internal stores model integrates uptake of external nutrients, described by Michaelis-Menten uptake kinetics in short term batch cultures, and subsequent growth upon internal stores of the nutrients as determined in steady state experiments. The latter method is more difficult to apply, as it requires the determination of four parameters, while the Monod model only requires two.

Describing batch culture results with the Monod model, Titman (1976) found that Asterionella formosa was most efficient for growth

on phosphate, having the lowest half saturation value K. Cyclotella meneghiniana was most efficient at silicate. Tilman predicted that A. formosa would predominate at high Si/P ratios and C. meneghiniana at low ratios, with the two species coexisting at intermediate ratios. The results of steady state competition experiments over a nutrient ratio gradient and the distribution of these two species in relation to ambient nutrient concentrations in Lake Michigan agreed with these predictions.

Tilman and Kilham (1976), Tilman (1977) and Kilham (1978) applied the variable internal stores model to the prediction of steady state competitive outcome. They concluded that the Monod model is a reasonable representation of the two step variable internal stores model, and that while the Monod model is much simpler to apply, it also gives a more precise prediction of competitive outcome at steady state. On the other hand, the application of the variable internal stores model is not limited to steady state conditions as it takes into account luxury consumption.

The K values alone cannot be used as a predictive tool if the maximal growth rates (μ_{max}) of the two species are significantly different. The less efficient species, with the higher K, could also have a larger μ_{max} and it would predominate at high nutrient levels. A predictive method that takes the growth rate into account was developed by Tilman (1982). In this graphical method (Figure 1) the supply of one resource increases along each axis. Zero net growth isoclines are drawn for the nutrient supply rates at which each species maintains an equilibrium between growth and mortality. The intersection of the isoclines of the two species is the point at which

Figure 1

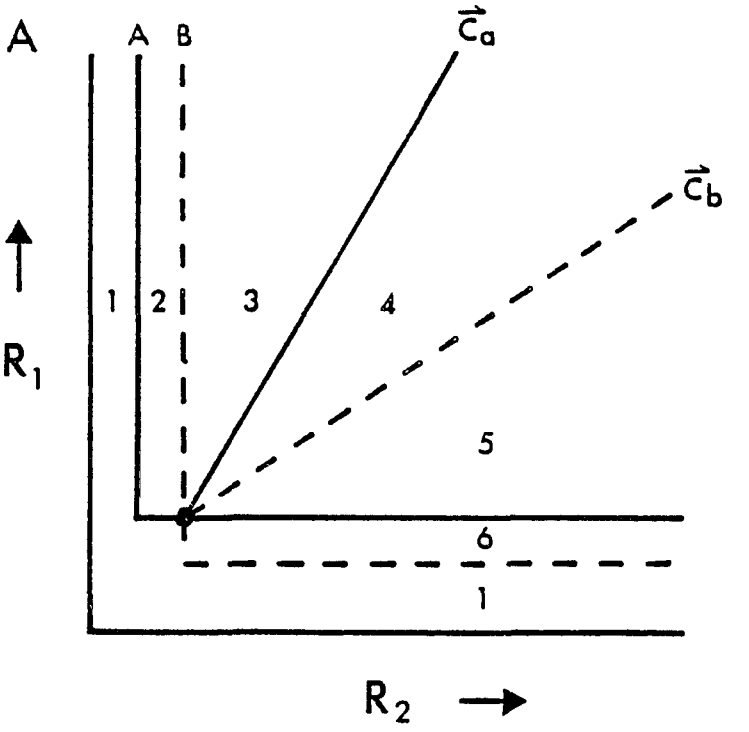
Competitive prediction figures after Tilman, 1982.

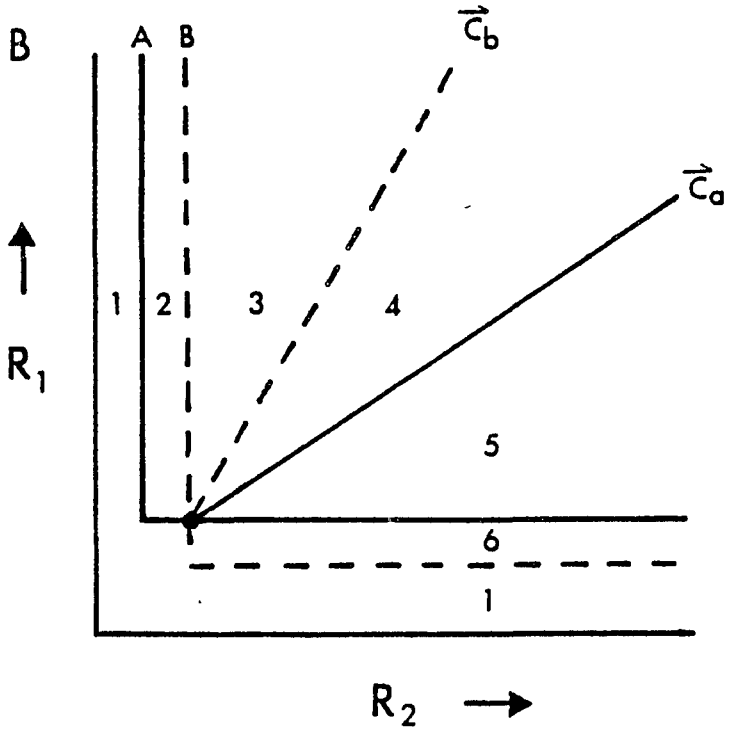
Resource supply, R_1 or R_2, increases along each axis, forming a field of resource supply ratio points. The lines labeled A and B are zero net growth isoclines (ZNGI) for species A and B. A ZNGI is the collection of all nutrient supply points where the mortality rate, set by the flow rate of the chemostat, is balanced by the growth rate at equilibrium. The placement of ZNGI's can be determined from the growth kinetics of the species on each nutrient. The lines labeled C are the consumption vectors for each species, the slope of which is determined by the minimal cell quota ratio for the two nutrients. The point at the intersection of the ZNGI's is the point at which the species will theoretically coexist.

Figure 1A is the case for stable coexistence. Species A excludes species B when conditions originate in areas 2 and 3. Species B excludes species A in areas 5 and 6. The two species coexist in area 4. Both species dilute from the chemostat in area 1.

Figure 1B is the case for an unstable coexistence. Note that the the relation of the consumption vectors is reversed in relation to Figure 1A. The species would not coexist in area 4, and the prediction of outcome in this area is more difficult.

FIGURE 1





they coexist. The nutrient conditions that will lead to this point are described by the area between the consumption vectors, C, for the two species. These vectors describe the direction the nutrient conditions will be forced by each species' nutrient consumption, and the vectors are determined by the cell nutrient quota ratios.

Nutrient conditions outside the consumption vectors will lead to exclusion of species A in areas 5 and 6, and species B in areas 2 and 3. This occurs when nutrient conditions are reduced by one species to concentrations at which the other species cannot maintain the steady state growth rate and is thus diluted from the chemostat.

When the nutrient conditions are below the isoclines of both species, as in area 1, both species are diluted from the chemostat.

Figure 1A shows the case for a stable coexistence point. Each species consumes relatively more of the nutrient that is more limiting to its own growth. Figure 1B shows the case of an unstable coexistence point. Each species consumes relatively more of the nutrient that is less limiting to its own growth. It seems contradictory that a species would consume more of a nutrient for which it has a smaller K than the other species. This can be explained by a species having a large cell quota for the nutrient for which it has an extremely efficient uptake apparatus.

This type of predictive figure not only describes natural populations at steady-state, but it can help predict the direction in which equilibrium systems will develop in approaching equilibrium between periodic disturbances.

Tilman's results show that each apparent niche might contain two or more species by a physiological division of the niche if two

or more resources are potentially limiting. This increases the number of niches, and therefore species, in a habitat as compared to the classical perception of that habitat. Tilman's hypothesis helps us envision n number of species coexisting, if each possesses the lowest K for one resource, on n potentially limiting resources as Hutchinson proposed in 1958. On the other hand, not many of the 20-odd essential plant nutrients could be expected to be potentially limiting at the same time in any one habitat patch, hence not very many species would be expected to coexist.

Tilman (1982) and Tilman, Kilham and Kilham (1982) proposed that habitat heterogeneity can allow more than two species to coexist on just two potentially limiting nutrients. Consider placing many species upon a predictive figure such as Figure 1. There would be a number of coexistence points at each of which a species pair could coexist. If a habitat has many patches whose nutrient variability encompasses all of these coexistence points, then all of the species will coexist. If the habitat variability remains the same, but the nutrient concentrations are increased the nutrient range will be much farther from the origin and the conditions encompassed will lead to the coexistence of fewer species. At extremely high nutrient concentrations only one or two species would exist. This may be the mechanism by which lake phytoplankton communities can be reduced to a few blue-green algal species by increasing the phosphate/nitrate ratio (Schindler 1976).

Tilman's resource based competition hypothesis has great potential for explaining species diversity and distribution. It is only now being applied to the algae, and there has not been any

comparable study with higher plants.

In the present study, members of a family of floating aquatic plants, the Lemnaceae, were chosen to test whether nutrients can determine species distributions for vascular plants and whether this effect may be interpreted by Tilman's resource competition hypothesis (Titman, 1976; Tilman, 1977, 1981, 1982; Kilham and Tilman, 1979; and Kilham, 1978).

These plants occur in relatively homogenous habitats, ponds, which facilitate the separation of factors determining species distribution. The family is found in a pattern of association and dominance that indicates non-random factors influence their distribution. As a whole, the family dominates the habitat; therefore, interactions will be between species that are morphologically and physiologically similar, hence reducing the complexity of comparing dissimilar organisms and rendering any physiological adaptation more meaningful. Members of the family are also simple morphologically and each individual plant is small compared to most vascular plants, making the culturing and the measurement of growth easier and more exact.

The study of nutrient competition in vascular plants is complicated by soil heterogeneity and the interaction of soil with nutrients and roots. Most studies of competition in terrestrial plants have passed over nutrient mechanisms in favor of other possibilities. The complications caused by soil in terrestrial plants and rooted aquatics are avoided in the Lemnaceae, which do not root in the soil, but float free on or just under the surface of the water. Demonstration of a nutrient limitation mechanism for coexistence and exclusion in the Lemnaceae will be a step in the demonstra-

tion of such a mechanism in higher plants and hopefully will encourage study of such a mechanism in terrestrial systems.

Species should evolve appropriate nutrient K values to allow coexistence under normal conditions if they operate under the nutrient competition mechanism. They would then be expected to be found regularly in multi-species assemblages in nature. Such assemblages of the Lemnaceae are often found in Minnesota (Jacobs, 1947) and in Michigan (this study). However, the environment may have excesses or deficiencies of nutrients that would be expected to disturb nutrient ratios enough to cause competitive exclusion of all but one favored species. Jacobs (1947) noted that Lemna minor is often found in dense and extensive monocultures. In the present study L. minor, which turns out to be a mixture of two ecotypes, or two species according to Landolt (1975) (L. minor and L. turionifera = Lemna complex), was most often found in monoculture. Jacobs found that at times Wolffia columbiana is in such predominance as to appear as a monoculture, while Wolffia punctata never dominates the community. I have observed the opposite situation for these two species, possibly indicating some regional difference in conditions. While Spirodela polyrhiza occasionally dominates a community, it is rarely found in a strict monoculture. When it is not associated with L. minor, S. polyrhiza is associated with W. columbiana. These association patterns indicate that species responses to pond conditions may determine their distributions.

In an alternative explanation of the various assemblages of the Lemnaceae in nature, Jacobs (1947) proposes that they result from the characteristics of the formation and germination of overwintering

buds (turions). If overwintering strategy were the only factor influencing the maintenance of species in the habitat, each species would develop a strategy to maintain itself, and a mixed assemblage of all species would be predicted. Though such assemblages are common, the existence of many ponds dominated by one or two species denotes that pond conditions also affect species distribution. Monoculture ponds develop the greatest biomass, and mixed ponds usually have a thin cover of plants, or the plants may only occur as marginal bands. This indicates that as nutrient availability increases, evidenced by higher biomass, species diversity decreases, a situation predicted by nutrient based competition theory.

Keddy (1976) attributed the distribution of L. minor and L. trisulca among Canadian shield lakes to equilibrium between immigration rates and extinction due to random events. These were only marginal populations and the plants never obtained enough biomass to dominate the habitat. Although these marginal populations are common around lakes in the United States, the dominance of many ponds by the Lemnaceae and the species patterns exhibited denote that other factors are involved in influencing the species distribution.

Factors such as light (Jacobs, 1947; Keddy, 1974 and Rejmankova, 1975), water temperature (Landolt, 1957 and Rejmankova, 1975) and pH (McLay, 1974) may affect the distribution of the Lemnaceae, so an analysis of these factors has been included in the present study.

There is some evidence that nutrients do affect the species distribution of the Lemnaceae. Hillman (1961) attributes the seasonal succession from L. minor and L. gibba to W. punctata to a reduction

in nutrient concentrations. Landolt (1957) indicates that L. gibba is found in waters with an average of 1.5 times as much nitrogen and 24.8 times the phosphate of waters in which L. minor is found. Rejmankova (1975) states that in Czechoslovakia, L. gibba was found in eutrophic conditions and L. minor seemed to be limited to more oligotrophic forest and mountain ponds. He attributed this dominance to the simple mechanics of the boat-shaped L. gibba fronds overriding the flat L. minor fronds. Yet, when grown separately on moderately high nutrients, L. gibba maintains higher relative growth rates and its fronds are not inflated unless metal micronutrients are mobilized by chelators. This indicates that mechanical overriding may only be an auxiliary to nutrients in determining competitive outcome.

Competition experiments with the Lemnaceae have resulted in more or less complete exclusion of one species by another (Clatworthy and Harper, 1962; Wolek, 1979; Rejmankova, 1975; and Keddy, 1976). The exclusion experienced in these experiments may have resulted from the use of excessive nutrient concentrations common in experiments with the Lemnaceae. The nutrient concentrations were so high that they saturated the nutrient interaction mechanism which balances growth rates of the species, and one species dominated the community. The results of the different competition experiments do not agree as to the order of the relative competitive abilities of these species. These contradictory results may stem from the use of different nutrient ratios, pH values, light intensities or physiologically distinct clones.

The information on natural species associations, nutrient concentration preferences of the species and the contradictory results

of the competition experiments indicates that a nutrient mechanism for coexistence or exclusion may operate in the Lemnaceae.

The present study determines the growth kinetics of the five floating species of the Lemnaceae commonly found in Michigan. Results will demonstrate whether or not these characteristics can be used to predict the outcome of competition between these species, and whether or not the nutrient levels associated with the species assemblages in the field are consistent with predictions made from the nutrient kinetic studies.

CHAPTER II

FIELD METHODS

In the field study, plant assemblages were determined and ambient nutrient concentrations were measured during the growth seasons from 1979 to 1982. Rain samples were taken in 1982 to measure the magnitude of this potentially important nitrogen source. A transplant-competition experiment was started in 1982. A total of 31 ponds are included in this study, of which ponds 14, 17, 18, 30, 29 and 41 were sampled intensively.

Pond Descriptions

The ponds utilized in this study are located northwest of Ann Arbor, Michigan, many of them in the Pinkney State Recreation Area. Figure 2 is a map of their locations.

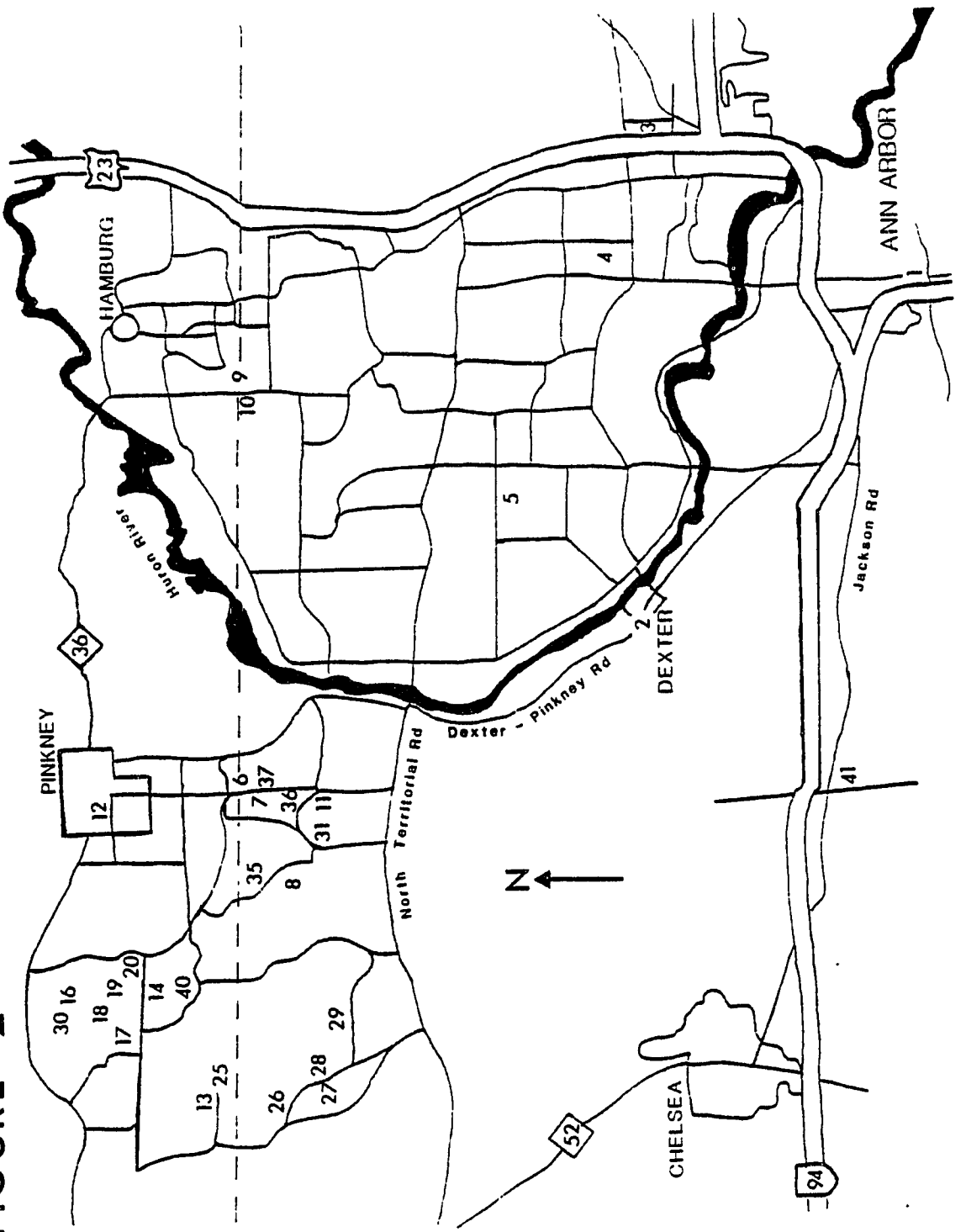
Most of the ponds are circular to rectangular with linear dimensions ranging from 15 to 100 meters. Several ponds were smaller than this (numbers 4, 6, 10, 19, and 36), while others were larger (numbers 2, 9, 20, and 25). Certain ponds were actually small open areas of large marshes (numbers 17, 27, 28, 40 and 35).

The ponds were generally 15 to 45 cm deep when full. Certain of the shallower ponds may dry up for a short time in the fall (numbers 7, 10, 36 and 37) but their bottom material usually remains moist over this period. Some ponds are deeper. Ponds 2 and 12 are

Figure 2

A map locating ponds utilized in this study in Washtenaw and Livingston Counties northwest of Ann Arbor, Michigan.

FIGURE 2


HAMBURG
PINKNEY
DEXTER
ANN ARBOR
CHELSEA
Huron River
Dexter - Pinkney Rd
North Territorial Rd
Jackson Rd
23
36
52
94
N

dammed streams of 3-5 m maximum depth. Pond 25 is 1.5 m deep. Ponds 4, 27, 29 and a portion of pond 41 are roadside ditches 1-2 m deep.

Most of the ponds have an organic sediment layer 15 to 60 cm deep. Ponds 1 and 29 have exceptionally shallow organic sediment layers, while pond 12 has a sandy bottom. Ponds with exceptionally deep organic sediments that precluded wading, i.e. over 120 cm, are ponds 6, 13, 16 and 30.

Surface water flow into most of these ponds was minimal. Ponds 2 and 12 have significant flow rates all year, and ponds 17, 18 and 20 have a constant flow in the spring which is reduced to an intermittent trickle in the summer. The latter three ponds are in a series flowing from pond 17 to pond 20 and their elevations are controlled. In this area of glacial till, subsurface flow may be significant, but the magnitude of the flow was difficult to quantify and was not measured.

Ponds were chosen that were fully or mostly exposed to the sun. The only ponds receiving shade for most of the day were ponds 4 and 10. Ponds 7, 8, 11, 14, 16, 30, and 31 were shaded on the south shore by trees.

The species of Lemnaceae found in each pond and their relative dominance are given in Table I. Although most of the ponds were chosen because they had a complete cover of Lemnaceae, some had a thin but complete cover and others had a band of plants around the pond's margin as noted in Table I. Ponds 12, 18, and 25 had no Lemnaceae and were chosen as controls. Though these were termed "no plant" ponds, they contained large masses of submerged species.

TABLE I

Plant Distributions Between the Study Ponds

Pond	Location	Lemnaceae	Type of Cover	Species
1	Maple + Liberty Rds	Wp>>>Wc>Lt	Marginal	Ltr
2	Dexter Mill Pond	Lt>Sp>>>Wp	Marginal	Cd, Ed, Ps, Ch
3	Gleanor Hall Rd	Lt>>>Sp	Covered	Ltr, A
4	Helner Rd	Lt	Covered	Ltr, Rf
5	Gregory Rd	Lt	Covered	Rf, A
6	Toma Rd N	Wp>>>Lt[a]	Covered	Ch, Ltr, Uv
7	Toma Rd W	Lt>>>Sp	Covered	
8	Silver Hill Rd	Lt=Wc>Wp	Covered	Rf
9	Merrill + Sheldon Rds	Lt=Sp	Marginal	Cd, Ltr, Uv, Pb,
10	Merrrill Rd	Lt	Covered	
11	Stinchfield Rd S	Wp>>>Lt=Wc[b]	Thin Cover	Cp, Ltr, Rf, A
12	Mower Rd	NONE		Cd, Ec
13	Kaiser Rd N	Wp>Sp	Marginal	Ch, A
14	Doyle Rd	Lt=Wp>Wc	Covered	Ltr
16	Bart Pond GR	Sp>Wc>>Lt	Marginal	Ch, Cd, A
17	SW Swamp GR	Sp=Wc=Lt>Wp	Thin Cover	Cd, Ltr
18	Crane pond GR	NONE		Ch, Cd, Ps, Ec, Ns, A
19	E Marsh Gr	Wp=Lt>Sp[c]	Thin Cover	Uv, Rs
20	E Marsh Gr	Lt=Sp	Marginal	Ltr
25	Kaiser Rd S	NONE		Cd, Ec, Ns, Ps, Uv
26	Bartell Rd	Lt=Wp	Covered	

TABLE I (Continued)

Pond	Location	Lemnaceae	Type of Cover	Submerged Species
27	Goodband Rd W	Lt=Wp=Wc	Marginal	Cd, Pc, Uv
28	Goodband Rd E	Sp	Marginal	Cd, Pc, Uv
29	Northlake Rd	Wp>>>Lt	Covered	Pl
30	George Pond N	Sp>Wc	Thin Cover	Cd, Ch, A
31	Dexter Town Hall Rd	Lt=Wp	Covered	
35	Silver Hill Rd N	Lt>>>Sp	Covered	
36	Stinchfield Rd N	Lt	Covered	
37	Toma Rd E	Lt	Covered	
40	Patterson Rd	Lt	Covered	
41	Dancer Rd	Lt	Covered	

[a] Complete plant cover 1979, 1981 and 1982; sparce 1980.
[b] Rare 1979, sparce 1980, half covered 1981, covered 1982.
[c] Covered 1979 (Wp only), sparce 1980, half covered (mixed) 1981 and 1982.

Abreviations:

N, E, S and W = Directions of the compass.
G.R. = University of Michigan George Reserve

Lt = Lemna turionifera + Lemna minor
Sp = Spirodela polyrhiza
Wp = Wolffia punctata
Wc = Wolffia columbiana
Ltr = Lemna trisulca (submerged)

A = Algal masses
Cd = Ceratophyllum demersum
Ch = Chara species
Cp = Caltha palustris
Ec = Elodea canadensis
Ns = Najas species
Pc = Potamogeton crispus
Ps = Potamogeton species
Rf = Riccia fluitans
Rs = Ranunculus species
Uv = Utricularia vulgaris

Pond Water Sampling

Samples for water analysis were collected by wading into ponds and reaching into undisturbed water. Samples were collected in acid washed polyethylene bottles. Initially, half-liter bottles were used with nylon mesh (325 μm) held over the opening to exclude plants from the sample. This method was found to be tedious and, because of the time needed to take a sample, allowed the samples to be contaminated by bottom material disturbed by wading. The use, in 1982, of small (150 ml) wide mouthed bottles without screening helped reduce contamination by bottom materials. The bottle was submerged upside down and inverted at the proper depth. The bottle could be quickly capped underwater before material disturbed from the bottom or submerged plants from the surface could reach it. When there was less that 15 cm of water, screening was necessary and the sample was taken as close to the surface as possible. In all cases, air was excluded from the bottles before capping.

At least two samples per pond were taken at each sampling. In ponds that were thermally unstratified, partially covered with plants or very shallow, two samples were taken from just below the surface, ca. 3 meters apart. In ponds partially covered with plants, one sample was taken from the open, and one from the plant covered areas. If the pond was thermally stratified and depth permitted, surface and sub-thermocline samples were taken.

Dialysis Sampling

In 1982, dialysis tubes, cut in 30 cm lengths, tied off and

filled with distilled water, were used to sample selected ponds. The tubes were suspended by twine between two stakes, with one tube just under the surface of the water, and another just below the surface of the unconsolidated bottom material. The dialysis tubes were allowed to come to equilibrium for seven days. The tubes were then removed, one end wiped clear of any film, rinsed, cut with scissors, and emptied into a sample bottle.

Dialysis sampling has the advantage of sampling only dissolved substances. This method is particularly helpful in sampling the bottom sediments. This method also integrates nutrient conditions over time rather than representing an instantaneous condition.

pH Sampling and Analysis

Samples for pH determinations were taken in 50 ml polycarbonate centrifuge tubes stoppered under water. A pencil type combination electrode fitted through a rubber stopper was used to measure pH to minimize exposure to air during collections and measurement. This was important, since the samples were often undersaturated or oversaturated with CO_2 with respect to the expected CO_2 concentration at equilibrium with the air.

Initially, pH was measured in the field with a Beckman model GS field pH meter, but it was found that comparable results were obtained when samples were transported to the lab and the pH taken immediately with an Orion Ionanalyzer 407A. The meter was calibrated and the slope adjusted between pH 7 and pH 4 with standard buffer solutions at room temperature. Samples were brought to room temperature before reading the pH. The electrode was washed after each

buffer and between each sample with 0.2 N sulfuric acid. It was then rinsed with distilled water, until the distilled water reading gave no further change. This minimized contamination from buffers, acid, or other samples.

Temperature and Oxygen Measurement

Oxygen and temperature profiles were taken *in situ* with a Yellow Springs Instruments model 57 oxygen meter. The temperature readings were checked against a mercury thermometer. The oxygen probe was calibrated each day with oxygen free and oxygen saturated water (AAPA 1980). Distilled water was bubbled with air for four hours to ensure saturation. Water was deoxygenated by boiling, and after cooling, the sample was bubbled with nitrogen gas during the oxygen analysis. The error between saturated and zero oxygen meter readings was 0.2 ppm O_2. The calculated saturation oxygen concentration differed from that found with the Winkler method (AAPA 1980) by less than 0.25 mg/l oxygen. The published accuracy of the membrane electrode method is 0.1 ppm oxygen (AAPA 1980).

For convenience the oxygen meter was floated in a plastic tub, and the probe dangled over the side. This allowed readings to be taken at arm's length, away from the disturbance caused by wading. The probe was moved slightly up and down during the readings to provide flushing of the membrane surface, and data were taken only after readings had stabilized.

Conductivity Measurement

Conductivity was measured with a Dionic water tester (Welch,

1948). This machine gives very rough values for conductivity, because of vibrations caused by turning the dynamo. The attributes of this machine include its field use without batteries and a mechanical temperature compensation (the electrode to electrode distance is varied). I used this machine for sentimental reasons because this is the very piece of equipment described by Welch (1948).

Rainwater Sampling

Rain sample collectors were made using polyethylene funnels placed in acid washed polyethylene bottles, and fastened to stakes. A large rain gauge with a funnel radius of 44.5 cm was installed at 1500 Woodland Drive in Ann Arbor, and five smaller collectors of 19 cm radius were placed at Dancer Road pond (41), North Lake Road pond (29), and George Pond (30), Crane Pond (18), and Southwest Swamp (17) in the University of Michigan George Reserve.

Acid washed gauges were erected immediately before a rain, and collected 1-6 hours after the rain ceased. Occasionally insects were found in the gauges. This contamination was recorded and the samples were strained through 325 μm mesh.

Air was excluded from the samples and they were frozen to await analysis within a week.

Rainwater pH was measured in the Ann Arbor samples. Electrodes were standardized and washed as for the pond pH samples, but three consecutive portions of each sample were brought to equilibrium before the final pH was measured in a quiescent sample. This reduced the variable streaming potential at the electrode surface in dilute solutions that accounts for the greater part of rainwater pH

measurement variability (Galloway et al., 1979). The use of concentrated buffers may have introduced a small error caused by extreme differences in ion strength between buffer and sample, but the error should be limited to 0.04 pH (Galloway et al., 1979).

Nutrient Analysis: Filtration and General Procedures

Dissolved nitrate-nitrite, ammonia, phosphate, and total iron were assayed colorimetrically using a Bausch and Lomb Spectronic 100 spectrophotometer. Normally 5 cm spectrophotometer cuvettes were used, but 1 cm and 10 cm cuvettes were used for concentrated and dilute samples respectively. Two replicates of three standards and a blank were analyzed with each batch of samples.

All water used for reagents and standards was distilled in a closed metal still, deionized (Corning Ultra-High Purity cartridge) and redistilled in a vented glass still. Water utilized for the ammonia assay was treated with a strong cation exchange resin (Fischer Rexyn 101-H^+) immediately before use to remove traces of ammonia absorbed from the air during storage.

All glassware used in analysis was rinsed in 2 N hydrochloric acid or soaked in 0.2 N sulfuric acid, followed by three double distilled water rinses.

Where color was present in the samples, blanks were acidified or alkalinized according to the assay and their absorbencies measured at the appropriate wavelength. The yellow color found in many of the samples had the most effect at shorter wavelengths, used for the iron, nitrate, and ammonia determinations, and had a very small effect, less than 0.003 absorbency units with a 5 cm cuvette, at 885

nm used for phosphate determination.

Initially, Gelman 0.45 μm membrane filters were used to filter the samples. These filters became clogged and air became trapped in the plastic filter supports of the Gelman magnetic filter heads, causing excessive filtration times (10-20 minutes) and vacuum (over 50 cm mercury). This led to over 90 percent ammonia loss from the samples. The use of Whatman GF-F glass fiber filters and a plastic Millipore apparatus in 1982 reduced filtration times (less than 2 minutes) vaccuum (less than 10 cm mercury) and ammonia loss (less than 10 percent). The filters were washed with 10 ml 0.02 N sulfuric acid followed by three 100 ml rinses of double distilled water. No contamination was detected from blanks that were filtered.

A precipitate occasionally formed in some of the dialysis samples. This usually dissolved upon the addition of the analytical reagents, but some samples required further sedimentation or filtration to remove residual precipitate before the absorbencies could be measured.

Ammonia

Ammonia was analysed immediately after filtration by the phenol-hypochlorite method of Solorzano (1969) and Strickland and Parsons (1972). Calcium and magnesium, possible sources of interference, are complexed with citrate in this method. Minimum detectable ammonia with a single analysis is 0.1 μM ammonia (Solorzano, 1969; Strickland and Parsons, 1972).

Nitrate-Nitrite

Nitrate was reduced to nitrite with copper treated cadmium and the resulting nitrite was colorimetrically analyzed. Cadmium filings were treated with copper sulfate and the sample was buffered with ammonium chloride after Strickland and Parsons (1972). The filings were added directly to 30 ml of buffered sample in a 125 ml flask that was shaken at 100 rpm on a shaking table for 30 minutes (Mackareth et al., 1978). Twenty-five ml of sample was then carefully poured from the filings such that no cadmium particles were transferred, as copper can inhibit the color reaction (APHA, 1980). The resulting nitrite was analysed by diazotising it with sulfanilamide and coupling it with N-(1-Napthyl)-ethylene-diamine, which formed an azo dye, measurable at 543 nm (Strickland and Parsons, 1972).

The efficiency of nitrate reduction was in excess of 95 %. The nitrite of the sample was not assayed separately, but the high reduction efficiency assured an accurate estimate of the combined nitrate and nitrite present. The samples did not contain substances that interfere with the assay as complete recovery was obtained when nitrate was added to the samples. The minimum detectable limit is 0.8 µM nitrate for the Mackareth et al. (1978) method. In that method, the sample was diluted to 0.10 of its original volume and a 1 cm light path was used. In the present study, the sample was diluted to 0.83 of the original volume and a 5 cm light path was used. Therefore the minimum detectable limit should be comparable to that reported for the Strickland and Parson's (1972) method, 0.05 µM nitrate.

Phosphate

Phosphate was assayed with the molybdate-antimony-ascorbic acid method of Golterman and Clymo (1969), and measured at 885 nm (Strickland and Parsons, 1972). The use of the 10 cm cell made the use of color extraction methods unnecessary. The form of phosphate measured is soluble reactive phosphate, which includes ortho-phosphate and variable amounts of meta-phosphate and weakly bound organic phosphate that are hydrolized by the short exposure to acid at room temperature. Therefore, the actual amount of available phosphate may be less than that determined by the assay (Strickland and Parsons, 1972; Wetzel and Likens, 1979). On the other hand, ortho-phosphate can be lost to the container during storage (Hassenteufel et al., 1963). This can lead to a determined value less than that actually present. Minimum detectable phosphate is 0.03 µM phosphate (Strickland and Parsons, 1972).

Iron

Total inorganic iron was analysed with Hach Ferro-Ver powder pillows (Hach, 1975). This method follows the 1-10 phenanthroline method of standard methods (APHA , 1971) minus the boiling pre-treatment to reduce interference by polyphosphate, cyanide, and nitrite. Hach does not indicate whether their single powder addition contains a component to reduce these sources of interferences. Minimum detectible iron with a 10 cm cell was 0.1 µM iron (APHA, 1971).

Plant Survey

Only qualitative plant data are given here. Observations were made in the field; these included the dominant species present, the approximate importance of each, which species were found in trace amounts and what submerged macrophytes were present. The amount of surface coverage by the plants was also recorded. Color slides of the ponds constitute a permanent record of coverage, but after complete cover in June, they add little information. Close-up photos were also taken, but after complete cover was attained, they could not be used as estimates of biomass, for there were often several layers of plants. These photos may not accurately record species composition, as Wolffia columbiana often was not observable on the surface, but large numbers were found underneath.

The differentiation of Lemna minor and L. turionifera is difficult in the field. Landolt (1975) indicates that L. minor has frond length to width ratios greater than three to two, and L. turionifera has ratios less than three to two, but field specimens are very often at the three to two ratio. Definite identification of L. turionifera is only possible while turions germinate in the spring or form in the fall. At these times L. turionifera has a red spot at the base of the root and a line of red pigmentation between the parent frond and new turion. On the other hand, if a plant does not have this coloration, it cannot be assumed to be L. minor.

It was found that turions could be induced in L. turionifera at temperatures below 25°C if nutrients were limiting. Phosphate limitation elicits a faster and more intense turion formation than

nitrate limitation. Five to ten plants from each pond were shaken in a tube with 5% Clorox (0.26% sodium hypochlorite) for five to ten minutes until the edges turned white, then rinsed in distilled water. Each plant was then transferred into 10 ml of medium in a test tube and incubated at a temperature of 20°C at a light intensity of 50 μ Ein/m^2·s for 16 hours per day. Turions could be distinguished in two to three months. Many plants died due to the Clorox treatment or algal contamination, so the number of determinations per pond varied.

Transplant Experiment

Enclosures (Figure 3) were made of 1 m X 1 m sheets of 4 mil polyethylene film taped into 1 m long tubes with Monsanto general purpose tape. The top of the tube was taped to a 33 cm equilateral triangular frame made from three 2.5 cm wide pieces of plexiglass, with a 0.95 cm hole in each corner (ethylene dichloride was used as a solvent-cement). Loops made of tape were put at the top, middle and bottom of the tube directly below the frame holes. Three 1.3 m bamboo plant stakes were implanted in the pond bottom with their bottom ends forming a 30.5 cm equilateral triangle and their tops angling out. The enclosure was slipped over these, threading the stakes through the loops on the tube and the holes in the triangle. The bottom of the tube was implanted in the bottom, and the top was at least 30.5 cm above the surface of the water. The tube and triangular frame were taped to the stakes. A triangle of 325 μm nylon mesh with holes at the corners for the stakes was tightly lashed over the open top.

Figure 3

Drawing of an enclosure used in the field transplant experiment.

Key:

A. Nylon mesh, 325 μm.

B. Plexiglass strip.

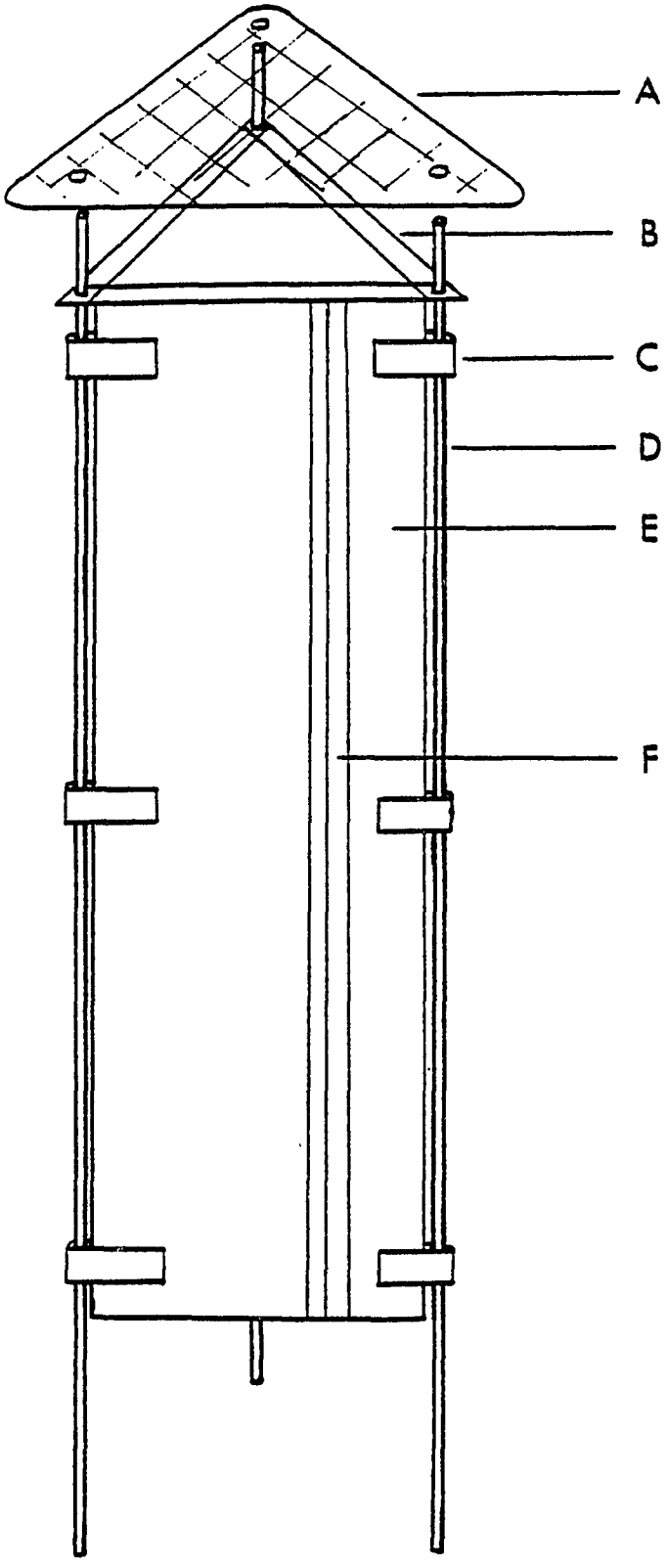
C. Tape loops.

D. Bamboo stakes, 1.3 m long.

E. Polyethylene film, 4 mil thick.

F. Taped seam.

FIGURE 3



The enclosure's open bottom allowed nutrient exchange with the sediment, and the open top allowed hot air to leave and rain to enter. The mesh was necessary to prevent Wolffia and Spirodela turions from splashing over the sides during heavy rains.

The enclosures were put in four locations, pond 41, with Lemna turionifera as a monoculture; pond 29, with Wolffia punctata dominant and only traces of L. turionifera; pond 30, with Spirodela polyrhiza dominant and Wolffia columbiana as a significant proportion of the biomass; and pond 18, which contains no Lemnaceae. Plant inocula for the transplants were taken from these ponds, with the W. columbiana washed from the S. polyrhiza by using a large mesh screen. Each pond had a separate enclosure for L. turionifera, S. polyrhiza and W. punctata as well as enclosures for each pairing of these species.

Enclosures were erected in May, 1982, but continued turion refloatation from the bottom of the pond delayed the initiation of the experiment until mid-June. At that time the pond bottom within the enclosures was stirred and any resuspended turions were removed with an aquarium fishnet. The enclosures were inoculated with plant material sufficient to cover 10 percent of the water surface. In mixed species enclosures the biomasses of the two species were roughly equal. The enclosures were observed and photographed at three to four week intervals. Because of continued addition of turions these experiments should be viewed as transplant experiments where competition with the pond species was reduced, but not entirely eliminated.

CHAPTER III

LABORATORY METHODS

Laboratory experiments were performed to provide information on the optimal conditions for growth as well as tolerance of extremes for temperature, light, and pH for each species. Setting these conditions at optimal values, the growth responses of each species to limiting phosphate and nitrate concentrations were determined in batch and semicontinuous culture. Two competition experiments were run in semicontinuous culture at various nitrogen to phosphate ratios to test whether growth response to the nutrients could be used to predict competitive outcome. General laboratory procedures are given first, followed by experimental procedures.

General Methods

Media

The basic medium (*Lemna* medium) used in laboratory experiments is given in Table II. Double distilled water was used for all media and stock solutions. The major elements were added as separate stock solutions and the metal micronutrients as one solution. The EDTA solution was added first, and the medium is mixed after the addition of each solution. Care must be taken not to add the phosphate and calcium solutions consecutively, as they

TABLE II

Lemna Medium

Compound	Stock Concentration g/l	ml Stock per Liter Media	Final Media Concentration mg/l	mM
Na_2EDTA[a]	20.00	2.0		0.107
$NaNO_3$	25.00	2.0	8.2 N	0.586
K_2HPO_4	3.68	1.0	1.3 P	0.042
KCl	50.00	1.0	26.0 K	0.671
$CaCl_2$	25.00	2.0	18.0 Ca	0.450
$MgSO_4 \cdot 7H_2O$	37.50	0.5	13.5 Mg	0.555
Micronutrients	See below	1.0	See below	
$NaHCO_3$	60.0	1.0[b]	48.0 HCO_3	0.785

Micronutrient Stock:

Compound	Stock Concentration g/100 ml	Final Media Concentration mg/l	mM
$FeSO_4 \cdot 7H_2O$	0.995	2.0 Fe	0.036
$MnCl_2 \cdot 4H_2O$	0.072	0.2 Mn	0.004
$Na_2MoO_4 \cdot 2H_2O$	0.044	0.1 Mo	0.001
H_3BO_3	0.057	0.1 B	0.009
$ZnSO_4 \cdot 7H_2O$	0.044	0.1 Zn	0.002
Na_2EDTA	2.000		0.054

Mix the following separately and add 1 ml per 100 ml micronutrient stock:

$CuSO_4 \cdot 5H_2O$	0.004	$1X10^{-4}$ Cu	$1.6X10^{-6}$
$CoCl_2 \cdot 6H_2O$	0.400	$1X10^{-2}$ Co	$1.7X10^{-4}$
Na_2EDTA	0.400		$1.0X10^{-4}$

[a]If crystallization occurs, add NaOH, but not to a pH above 7.0.

[b]Add $NaHCO_3$ to desired pH, not over 8.0; 1.0 ml gives pH 6.5.

can form an insoluble precipitate. The pH is adjusted to the desired value, usually 6.0, with sodium bicarbonate. The medium is supersaturated with CO_2 after addition of bicarbonate; therefore, it is more acid than when at equilibrium with the air. The initial pH of a medium sample was measured, the sample was then bubbled with air for ten minutes and the equilibrium pH measured. If the equilibrium pH was above 6.5, there could be precipitation during autoclaving due to high pH obtained as CO_2 is driven off.

Precipitation of the medium occurred when the molar ratio of EDTA to micronutrients was 1 to 1. This may be caused by pH increases during autoclaving or plant growth, as the effectiveness of EDTA chelation decreases above pH 8.0 (Dow Chemical Co., 1974). Poor growth of plants was seen with a 10 to 1 ratio of EDTA to micronutrients. De Kook et al. (1973) state that calcium deficiency in Lemna may develop if EDTA is in excess compared to calcium, as it was at the 10 to 1 ratio of EDTA to micronutrients. A 3 to 1 ratio prevented precipitation and allowed normal plant growth, and was used in the Lemna medium.

The Lemna medium was designed to be comparable to natural waters. Bicarbonate was used as a buffer because it is the most common buffer in aquatic systems (Hutchinson 1957). The conductivity of the Lemna medium, 440 μmhos, is comparable to that of the ponds in this study (Table III). The nitrate and phosphate concentrations are within one order of magnitude (higher) than the mean concentrations of 0.185 mM nitrate and 0.003 mM phosphate reported by Thomas (1969) for surface waters of central European lakes.

The successful Chu number 10 algal medium is comparable in

TABLE III

Conductivity of Lemnaceae Ponds, in $\mu mho \cdot cm^{-1}$

Pond	4/81	10/81
P 4	700	1,300
P 29	830	750
P 41		600
P 11	625	805
P 36	575	560
P 26	420	575
P 31	350	355
P 6	305	540
P 27	290	410
P 13	280	430
P 16	275	425
P 16	240	
P 3		280
P 5	270	290
P 5	245	
P 37	240	560
P 30	235	320
P 17		210
P 25	195	290
P 18	175	230
P 9	160	260
P 20	145	
P 20	129	136
P 7	140	210
P 10		109
P 14	96	128
P 19	95	122
P 8	73	78
P 35	85	
	Samples = 25.0	Samples = 25.0
	Mean = 274.9	Mean = 398.9
	SD[a] = 207.6	SD = 275.1
	SE[b] = 83.0	SE = 110.0

[a] Standard Deviation
[b] Standard Error

ionic strength, inorganic nitrogen and phosphate to the Lemna medium (Stein, 1973).

Many common higher plant media such as Knop's and Evan's (Salisbury and Ross, 1969) as well as algal media such as Biejerink's and Bold's media (Stein, 1973) have 5 to 15 times higher ionic strength, 15 to 50 times more nitrogen and 12 to 150 times more phosphate than the Lemna medium. These high concentrations are unnecessary for the Lemnaceae. Growth rates obtained with the Lemna medium equal or exceed those obtained by other experimenters using more concentrated media (Clark, 1926; Fly, 1935; White, 1936, 1937; Jacobs, 1947; Bitcover and Seiling, 1951; Landolt, 1957; Hodgeson, 1970; McLay, 1974, 1976; Krezechowska et al. 1975; Rejmankova, 1975; and Wolek, 1979).

Sodium is avoided in the Knopp, Evans and Biejerink media. Sodium is found in natural waters and the Lemnaceae can tolerate 0.05 to 0.43 M NaCl (Hillman 1961). Thus no attempt was made to avoid sodium in the Lemna medium.

Culture Chamber

Temperature-controlled culture chambers, with fans to distribute temperatures evenly, were used. One or two mercury thermometers on each shelf helped monitor for temperature variations. Each thermometer was standardized against the mean of all of the thermometers. A water bath was used at 32°C and above for greater temperature stability because a slight increase of temperature over the plants' maximum would incapacitate them for the rest of the experiment.

Light Source

Double banks of Sylvania cool white fluorescent lamps provided light for sixteen hours per day. Light was measured with a Li-Cor Quantum Sensor and a Li-Cor Model 185 light meter.

Although the emmission of a Grow-lux lamp more closely fits the photosynthetic pigment absorption curve, the cool white bulb more closely fits the flat curves for incident sunlight and the light probe sensitivity between 400 and 800 nm wavelengths (Fig. 4). The Grow lux lamp will give a greater photosynthetic response per unit of measured light intensity than the cool white bulb or the sun. When comparing lab light intensities to those in the field, less error will be experienced when using cool white as compared to Grow-lux lamps. Although deficient, the cool white lamp has some far-red emmission at 730 nm. This may be sufficient to satisfy any growth-related phytochrome responses present in the Lemnaceae.

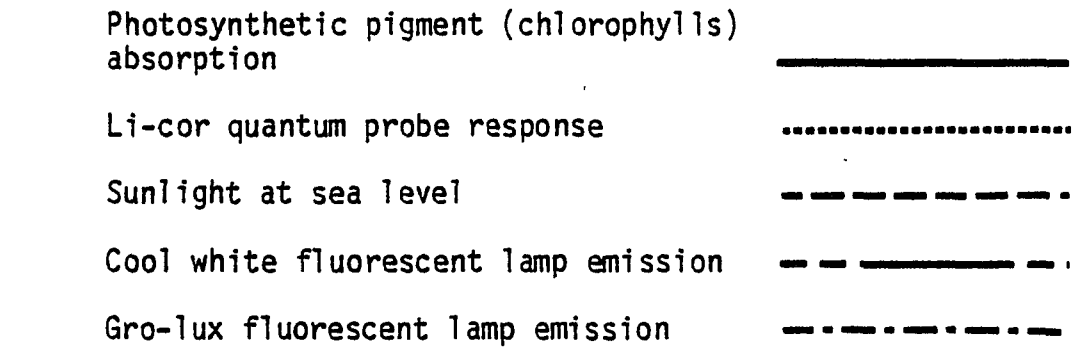
Culture Containers

Most of the experiments were carried out in 250 ml borosilicate glass beakers with borosilicate Petri dish covers. The use of 200 ml of medium in these containers placed the plant growth plane (medium surface) within 5 cm of the light bulbs, providing a light intensity of 250 $\mu Ein/m^2 \cdot s$. One temperature experiment is designated 'low light' because 250 ml Erlenmeyer flasks covered with 50 ml plastic tri-pour beakers were used. The medium surface was 10 cm from the light bulbs in these containers and the plastic covers did not transmit light effectively. This provided a light intensity of

Figure 4

The relative energies of light sources and sensors compared to the absorption curve for photosynthetic pigments.

Key:



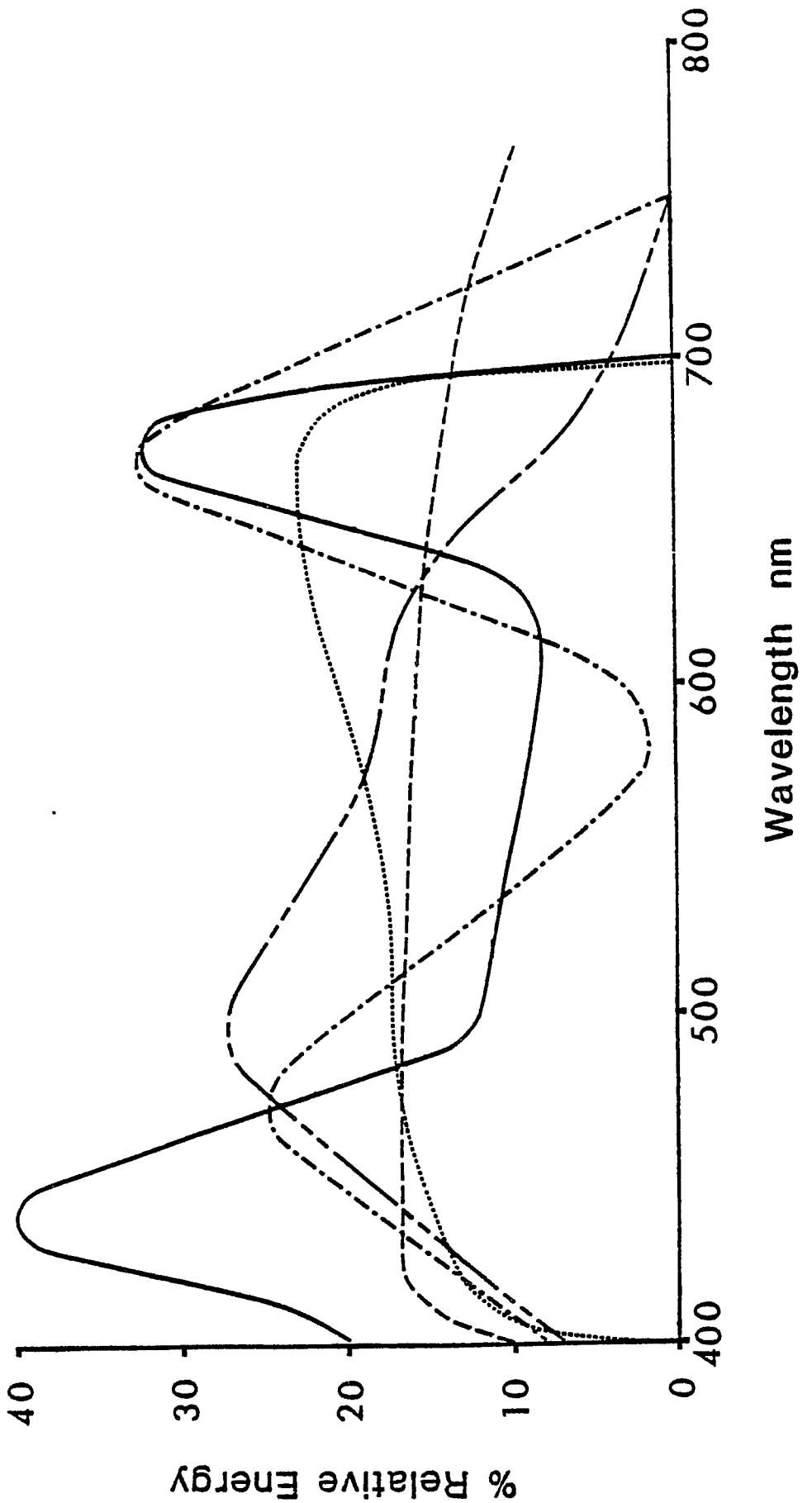

% Relative Energy
40
30
20
10
0
400
500
600
700
800
Wavelength nm

FIGURE 4

only 80-120 $\mu Ein/m^2 \cdot s$ to the plants. The flasks could only accommodate 100 ml of medium, increasing the possibility of nutrient depletion. Wolffia plants also tended to become stranded on the sides of the flasks, but not the beakers.

The proximity of the beakers to the light source did allow the warming of the medium in relation to the incubator air temperature. Therefore, the temperature of the medium was measured during medium replacement.

Culture containers were cleaned by autoclaving them filled with 0.2 N sulfuric acid, and rinsing 3 times with double distilled water. All culture containers, media and implements used in these experiments were sterilized, and sterile microbiological methods were used in transfers. The cultures were axenic at the beginning of the experiments. The lack of available organic nutrients and the high medium replacement rate kept any contaminating bacterial and fungal growth below noticeable levels during the experiments. If a culture vessel became noticeably contaminated with algae, the vesses was removed from the experiment and the other containers were sampled and examined microscopically for contamination.

Plant Cultures

The experimental clones of Lemna minor, Lemna turionifera, and Spirodela polyrhiza came from pond 2, Dexter Mill pond, while Wolffia punctata and W. columbiana came from pond 1 at Maple and Liberty Roads on the west side of Ann Arbor.

Plants were disinfected with Clorox as described in the plant survey section above. Sterility was tested by transferring a

plant and 1 ml of culture medium to separate tubes of a general bacterial medium composed of the Docauer medium plus 0.8% beef extract and 0.8% sucrose.

Plant Counting and Removal and Media Replacement

The experiments, with the exception of a few purely batch experiments, were basically a continuous series of batch experiments designed to keep the plants as near the log phase of growth as possible. The medium was replaced, and the plants reduced to their initial numbers every four days. These experiments shall be termed 'semi-continuous'.

The initial number of plants used were adjusted to provide nearly equal initial biomass between the species. Initial numbers of plants used were 10 - 15 for Spirodela polyrhiza, 19 - 29 for Lemna minor, and 26 - 39 for Lemna turionifera. The number of Wolffia plants needed for equivalent biomass was 150 - 300, as used for nutrient kinetic and competition experiments. Large numbers of Wolffia are difficult to count, so only 75 - 150 were used in temperature and light experiments as equal initial biomass was less important in these. Plants were counted before removal. Growth data are reported as relative daily growth rate (RDGR) and calculated by the equation:

$$RDGR = \frac{\ln N^{t+1} - \ln N^{t}}{t} \qquad (1)$$

where N^{t} is the initial number of fronds, N^{t+1} is the number of fronds after the time interval, and t is the time interval (four days).

Excess Lemna and Spirodela plants were removed with a sterile microbiological loop. The medium was poured out of the 250 ml beakers or removed from the other types of containers using a sterile Pasteur pipette and a vacuum from a faucet aspirator. Wolffia and their medium were removed with a Pasteur pipette and vacuum. To avoid removing all of the Wolffia, a small amount (20 ml) of medium was left in the containers.

Plant Weights

Fresh weights were taken at the end of the experiments. Plants were prepared for weighing by folding them in a Kimwipe cellulose tissue, and blotting them firmly (but not so hard as to rupture the plants) with other tissues that were replaced until no more moisture could be extracted. Before blotting, Wolffia plants were poured with their medium through a tissue in a funnel. The plants were then placed upon a tared piece of glassine paper and immediately weighed to 0.0001 g on a Mettler type 34 electrostatic balance. Significant weight loss owing to dehydration seems to start about 20 seconds after the plant is removed from the damp tissue, so speed was important.

Experimental Procedures

Light

Experiments were done to determine optimal light conditions for the nutrient experiments and to determine whether there were any

significant differences in saturation values that might be ecologically significant. Light intensity was controlled by varying the distance of the cultures from the cool-white fluorescent lamps, the addition of lamps and/or reflectors, or the use of layers of plastic window screen.

Light intensity was measured with the light probe directed perpendicularly toward the light sources. Diffuse light intensity from the bottom and sides of the culture containers was 10-20 $\mu Ein/m^2 \cdot s$, significant only at the lowest light levels.

Illumination experiments for the Lemna and Spirodela species were done at 25, 28 and 31.5°C. Experiments for the two Wolffia species were done at 30°C.

The data were fit to a curve, as used in Michaelis - Menten kinetics (Bliss and James, 1966), by iteration with the Fortran IV program of Hanson, Ling, and Havir (1967). This computer program provided a measure of the variance and an estimate of the light intensity required for half the maximal growth rate. The latter was a convenient and useful quantity to compare efficiency of light utilization between species.

Temperature

The optimal temperature range for growth was determined for each of the 5 species. These experiments also provided information on the maximum tolerable temperature for each species and the effect of low temperature on turion formation.

Various temperatures were provided by the use of a number of culture chambers. The temperature of the medium was measured as noted

above in the Plant Counting and Removal and Media Replacement section.

The light intensity provided in the Lemna and Spirodela experiments was about 100 $\mu Ein/m^2 \cdot s$, somewhat below saturation. These data were designated as 'low light' temperature curves. The saturating conditions in the 25, 28 and 31.5°C light intensity experiments provided data for 'high light' temperature curves. A light intensity of 250 $\mu Ein/m^2 \cdot s$ was provided for all temperature conditions in the Wolffia experiment.

Turions were counted separately and allowed to accumulate in the cooler conditions.

pH

The pH optima for growth of L. minor and S. polyrhiza were determined by the semi-continuous culture method. The medium becomes alkaline as the plants maintain charge balance when utilizing nitrate, therefore, the medium was changed daily and the plants were counted and removed every two days. In this manner, the pH change was limited to 0.1 to 0.3 units per day. The pHs of fresh and spent media were measured daily and these values were averaged to provide an estimate of the ambient pH. Growth rates from two consecutive intervals were averaged to correspond to the four-day intervals of the other experiments.

Sterile sodium bicarbonate (1.5 M) was added to sterile medium to obtain the desired pH, with fine adjustments made with sterile 0.1 N H_2SO_4 and NaOH. To minimize errors caused by disequilibrium CO_2 concentrations, media samples were bubbled before pH measurement and the media were aged before use. Some difficulty was

found in adjusting the pH above 8.0. After the addition of base, the pH would increase, only to decrease to near 8.0 when CO_2 equilibrium with the air was attained. When enough base was added, precipitates formed in the medium and the pH stabilized at a higher value.

An experiment was designed to circumvent the precipitate problems by allowing the plants to force the pH to their maximum tolerable limit while growing on excess nitrate. The medium concentration of nitrate was increased ten-fold, the phosphate two-fold, the micronutrients three-fold and the EDTA three-fold. The medium was not replaced, but plant populations were reduced by 75 percent when they covered the medium surface. Medium samples were taken at intervals to measure pH.

Initially, L. minor died and S. polyrhiza showed poor growth in these media. This was attributed to a calcium deficiency caused by excess EDTA (DeKook et al., 1973). When calcium was added to all the media, growth was initiated in the Spirodela culture.

Turion Germination

The species utilized in this study, excluding L. minor, form overwintering structures called turions. Turions are special buds that form and sink to the pond bottom in the fall. Lemna turionifera and Spirodela turions are morphologically distinct from vegetative plants while Wolffia turions appear to be vegetative plants with negative buoyancy.

Turions may form and germinate in response to temperature and day-length. It was found in this study that severe nutrient limitation, especially of phosphate, caused turion formation, while

resupplying nutrients stimulated germination. Turion formation seemed to be inhibited by high temperatures, and germination seemed to be inhibited by low temperatures. The effect of temperature on the formation of turions of Lemna turionifera and Spirodela polyrhiza was observed in batch temperature experiments, in which the plants became nutrient limited.

To study germination, turions were obtained from nutrient-limited cultures that had been pre-treated at 10° C for two months. At least 20 turions were put in 100 ml of fresh media at each of the temperatures used; 10, 15, 20, 25, and 30° C. Day-length remained at 16 hours. The number of germinated turions was recorded at four-day intervals and the resulting plants removed.

Wolffia Floatations

I observed that both species of Wolffia form turions and sink when in extreme nutrient-limited conditions. In active growth phases, W. columbiana will not sink, even when surface tension is broken by mixing, while approximately 20% of the W. punctata will sink under similar conditions.

W. punctata turions were obtained from actively growing (active) and nutrient-limited (inactive) cultures by stirring the cultures and collecting the plants that remained on the bottom. W. columbiana turions could only be collected from nutrient limited cultures.

Turions were incubated in Docauer medium or in phosphate-free Docauer medium. Each nutrient condition was tested under high (200 $\mu Ein/m^2 \cdot s$) and low (10 $\mu Ein/m^2 \cdot s$) light intensity to represent

light conditions of the pond bottom under small or large populations of Lemnaceae.

Turions were placed in 200 ml of medium in 250 ml beakers and counted. Each day the number of floating plants was counted, then removed. Every week, the number remaining on the bottom were counted to measure any possible growth. When a large flush of floatation occurred, the plants were removed and placed in 200 ml of phosphate-free media and placed under high light. Their growth was monitored every two days. This gave an estimate of the growth potential of the plants using only stored phosphate. The temperature at the high light condition was 28° C, and 26° C at the low light condition.

Batch Nutrients

Initial estimates of the Monod model half-saturation constants for growth (K) on phosphate and nitrate were made for Lemna minor, L. turionifera, and Spirodela polyrhiza using batch culture techniques.

The initial concentrations in the phosphate-limited series were 0.20, 0.55, 1.30, 3.45 and 41.0 μM phosphate, and in the nitrate-limited series were 44, 71, 89, 138 and 600 μM nitrate. The media were assayed to confirm the initial concentration of the limiting nutrient.

Plants were preconditioned by growing them on nitrate- or phosphate-deficient media until growth ceased. This ensured that the internal stores of the nutrient in question were depleted and that subsequent growth became limited by the external nutrient

supply. Each 250 ml Erlenmeyer flask contained 100 ml of medium and was inoculated with three or four plant fronds. The plants were incubated at 25°C and provided a light intensity of 120 $\mu Ein/m^2 \cdot s$.

Fronds were counted every four days and the daily relative growth rate was calculated for each interval. The highest daily relative growth rate for each flask was taken as an estimate of the initial growth rate. The curve of initial growth rates vs. initial concentrations was fitted to a curve (see Light above).

Semi-continuous Nutrients

The semi-continuous culture method was used to correct the nutrient depletion problem caused by the high biomass in batch experiments. This provided a consistent nutrient regime, to which the plants could adjust their growth rate. In contrast to the semi-continuous culture methods of Tilman and Kilham (1976) and Kilham (1978), where the same nutrient concentration was used at various flow rates, different nutrient concentrations at a single flow rate were used in the present study.

The initial media concentrations in the phosphate-limited series were 1.0, 2.3, 4.2, 8.5 and 41.0 μM phosphate, and in the nitrate-limited series were 4.5, 9, 18, 36, 71, and 286 μM nitrate. To provide a relatively constant nutrient environment, one-fourth of the medium was replaced daily.

The initial inocula biomasses for the five species were roughly equal (see plant counting and removal) and the plants were counted and the population was reduced to initial densities at four-day intervals.

The temperature was 28°C and the light intensity was 200 $\mu Ein/m^2 \cdot s$.

Nitrate or phosphate was analyzed in the spent media on the day of plant removal. Fresh media nutrient concentrations were also measured at this time. I developed the following formula to calculate the mean daily concentration of nutrients:

$$\frac{(0.25 \times C_f) + (1.75 \times C_s)}{2} = C_d \qquad (2)$$

where C_f is the concentration of the nutrient in the fresh medium, C_s is the concentration in the spent medium and C_d is the mean daily nutrient concentration. The medium replacement rate is 0.25/day.

Data were taken when the growth rates had stabilized for at least three consecutive time intervals, and were fit to a curve (see Light above).

Biomass Quota

The minimal biomass quota, Q_b (analogous to the minimal cell quota for algae, K_Q, of Tilman and Kilham, 1976 and Rhee and Gotham, 1980) is defined as the minimum internal concentration of the limiting nutrient per unit biomass required to maintain a species with no growth or mortality.

Rhee and Gotham (1980) proposed that cell quotas at low growth rates were good estimates of K_Q, and found the N/P cell quota ratios were equal to the N/P K_Q ratios for the three algal species tested. He stipulated that the maximal growth rate must be equal for both nutrients in order to use the cell quota as an estimator of K_Q.

The Q_b for the Lemnaceae were estimated by dividing the change in the nutrient concentration by the change in biomass for

cultures growing near one-half the maximum growth rate in the semi-continuous culture nutrient limitation experiments. Although these cultures were nutrient limited, their growth was sufficient to produce measurable changes in nutrient concentrations and biomass.

Biomass, expressed as fresh weight, was measured only at the end of the experiment. The initial biomass for the last growth interval (B_i) had to be estimated by the following formula:

$$B_i = e^{[\ln (B_f) - (4 \cdot DRGR_l)]} \qquad (3)$$

where $DRGR_l$ is the daily relative growth rate for the last interval (ΔB_f) is the final culture biomass. The change in biomass for the last interval (ΔB_l) was adjusted to the mean change in biomass (mean ΔB) for the experiment by the formula:

$$\text{mean } \Delta B = \Delta B_l \quad \frac{\text{mean DRGR}}{DRGR_l} \qquad (4)$$

The minimal biomass quota, Q_b, was then calculated by the formula:

$$Q_b = \frac{\text{mean } \Delta N \text{ (}\mu\text{ moles)}}{\text{mean } \Delta B \text{ (mg wet weight)}} \qquad (5)$$

whre ΔN is the change in the limiting nutrient concentration.

Competition

Competition experiments were set up with the same light and temperature conditions and with the same medium replacement rate as in the semi-continuous culture nutrient experiments. This provided a reliable estimate from the nutrient experiments of the limiting nutrient concentration required in the competition experiment.

The species pairs tested against a nitrate/phosphate ratio

gradient were Lemna turionifera and Spirodela polyrhiza, and L. turionifera and W. punctata. Each species was in monoculture as well as in competition at each ratio. Nitrogen/phosphate molar ratios, used were 0.22 (4.5 μM N and 20.3 μM P), 3.3 (20.5 μM N and 6.2 μM P) and 237 (520 μM nitrate and 2.1 μM phosphate).

The initial biomass was approximately equal for all the species. The same number of plants of each species was used in the monoculture and the bicultures. Therefore, the biculture competitive condition had about 2 times the biomass of the monoculture. This was done to accentuate competitive responses.

The plants were counted and the numbers reduced to the initial density every four days. The experiment was continued until the growth rate stabilized for at least three time intervals. The total wet weight biomass of each species in each condition was then measured.

Allelopathy

A crude test for allelopathy between Spirodela polyrhiza and Lemna minor was made. Plants were grown in 200 ml regular medium with an initial pH of 6.0 until their growth slowed as they entered stationary phase. The medium was poured into sterile containers, adjusted to the original concentration of phosphate and nitrate with stock solutions, and the pH was adjusted to 6.0. The medium was then split into two 100 ml portions in separate sterile 250 ml flasks. The flasks were inoculated with 15 Spirodela or 25 Lemna fronds, such that each species was in both types of conditioned media. The medium was not changed and plants were counted every four days.

CHAPTER IV

RESULTS

Light

The half-saturation constants for light (K_L), maximal growth rates (μ_{max}), and their 95% confidence intervals are given in Table IV for three temperatures, 25°, 28° and 31°C. As can be seen, Spirodela polyrhiza has a significantly lower K_L, 19-24 $\mu Ein/m^2 \cdot s$, for light than any other species. This would imply that it utilizes low light intensities more efficiently than the other species. Lemna minor has the next lowest K_L, 25-38 $\mu Ein/m^2 \cdot s$ but it is not significantly lower than that of Lemna turionifera and Wolffia columbiana, which are approximately equal (40-47 $\mu Ein/ m^2 \cdot s$). Wolffia punctata has a significantly higher K_L, of 86 $\mu Ein/m^2 \cdot s$ at 28°C.

Table IV shows that the μ_{max} for W. punctata and W. columbiana are equal, 0.307 and 0.336, and lower than those of the other species, 0.398-0.503 at 28° C. The μ_{max} of S. polyrhiza and L. minor are barely equal at the 95% confidence level. The μ_{max} of L. turionifera is significantly higher than those of the other species. The only significant difference at the three temperatures is for L. minor, whose K_L seems to drop at 31°C (Table IV). This effect is primarily due to a 17% decrease in the maximal growth rate for L. minor between 28° C and 31° C, which is signifi-

TABLE IV

Calculated Half Saturation Constants (K_L) and Maximal Growth Rates (μ_{max}) for Light Experiments. K_L Values as $\mu Ein/m^2s$.

Temperature	Species[a]	K_L	95% Confidence Interval	μ_{max}[b]	95% Confidence Interval
25° C	Lt	43	38- 48	0.404	0.390-0.418
	Lm	38	33- 44	0.392	0.375-0.409
	Sp	19	16- 23	0.332	0.319-0.344
28° C	Lt	47	40- 51	0.503	0.475-0.531
	Lm	35	29- 43	0.437	0.413-0.461
	Sp	24	20- 28	0.398	0.382-0.414
	Wp	86	63-114	0.336	0.297-0.375
	Wc	47	39- 56	0.307	0.290-0.324
31° C	Lt	40	34- 46	0.489	0.468-0.510
	Lm	25	20- 30	0.362	0.345-0.379
	Sp	19	12- 26	0.356	0.332-0.380

[a] Species Abbreviations as in Table I.
[b] Daily Relative Growth Rate, DRGR/ln2 = doublings/day

cant at the 95% confidence level.

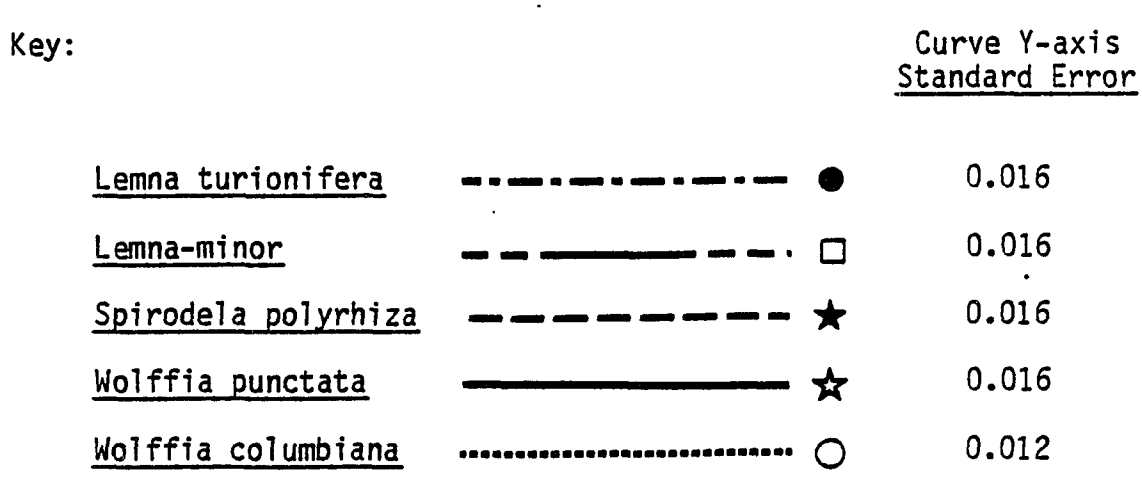
The relative competitive efficiency of the species when light intensities are limiting can be deduced from the curves in Figure 5, and the 95% confidence limit of each curve can be defined by the y-axis (growth rate) standard error. It can be seen that the growth rate of S. polyrhiza is slightly higher than those of the two Lemna species up to a light intensity of 40 $\mu Ein/m^2 \cdot s$, falls below that of L. turionifera at 120 $\mu Ein/m^2 \cdot s$, and below that of L. minor at 350 $\mu Ein/m^2 \cdot s$. W. punctata has a lower growth rate than that of W.columbiana from near zero light to about 150 $\mu Ein/m^2 \cdot s$, above which they become equal. The growth rates of the latter two species are always lower than those of the other three species.

The growth rates in Figure 5 were calculated from the increase in the number of plants. Figure 6 shows that the fresh weight to frond ratios for the two Lemna species and Spirodela increase with increasing light intensity up to about 75 $\mu Ein/m^2 \cdot s$, above which they remain constant.

Evidence of a light-induced species gradient within Pond 14 is given in Table V. These data were taken at 10 A.M. on a hazy sunny day with a sunlight intensity of 1300 $\mu Ein/m^2 \cdot s$. Pond 14 has a larger area that receives constant shade than most of the other ponds in this study. As can be seen, in 1981 there was a species gradient from the sunny center of the pond to the shaded southeast shore of: Wolffia punctata, Lemna turionifera, Wolffia columbiana, Lemna minor, and Lemna trisulca, which is consistent with the K_L values found for these species. Although it was

Figure 5

Growth of the Lemnaceae at various light intensities, cool white fluorescent source, 28° C. Daily Relative Growth Rate / ln2 = doublings per day.



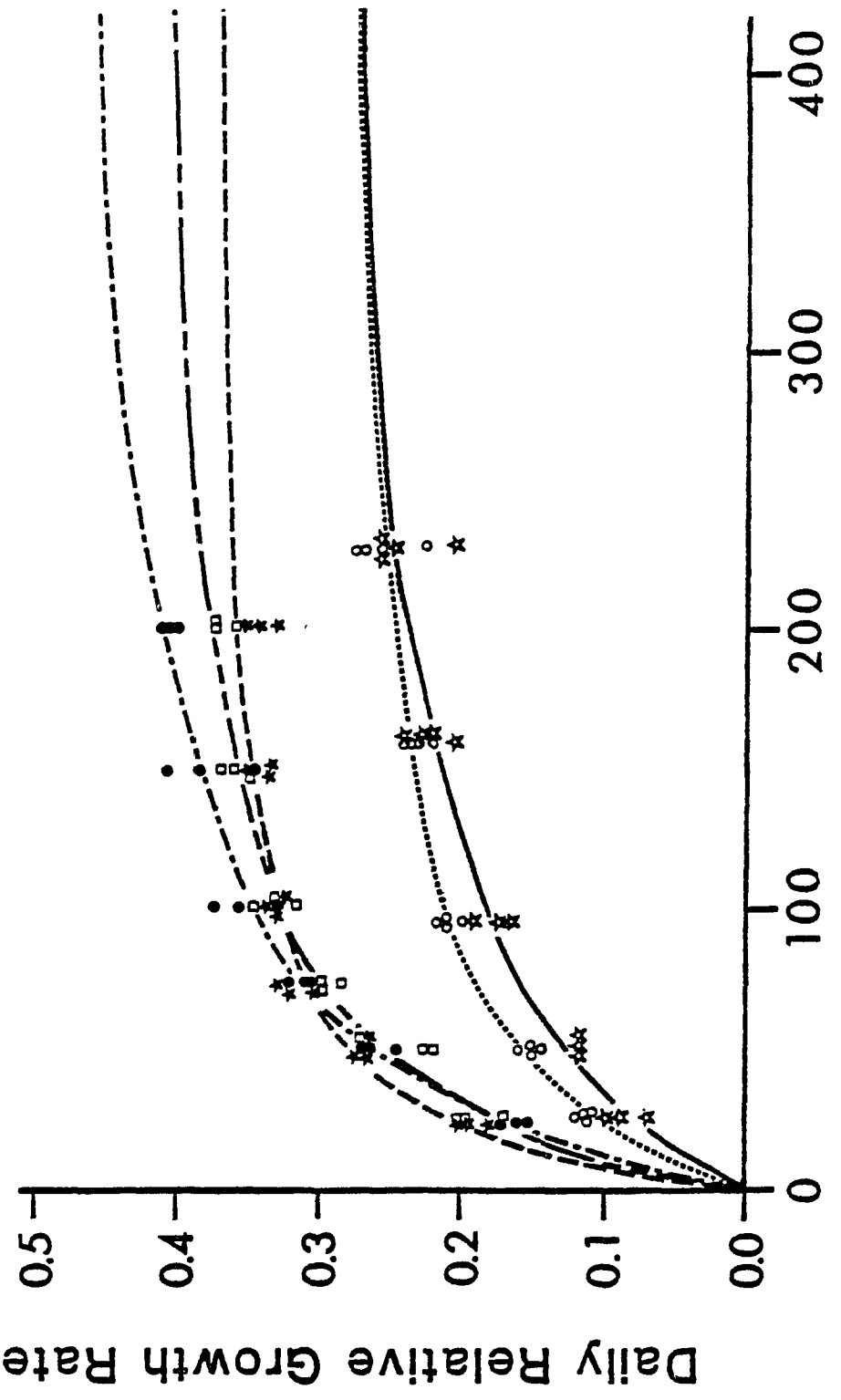

Daily Relative Growth Rate
0.5
0.4
0.3
0.2
0.1
0.0
0
100
200
300
400
Light Intensity µEin/m²s

FIGURE 5

Figure 6

Changes in frond wet weight as a function of light intensity, cool white fluorescent lamp as light source, 28° C. No replicate data.

Key:

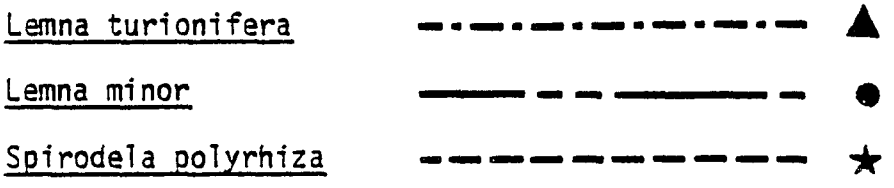


FIGURE 6

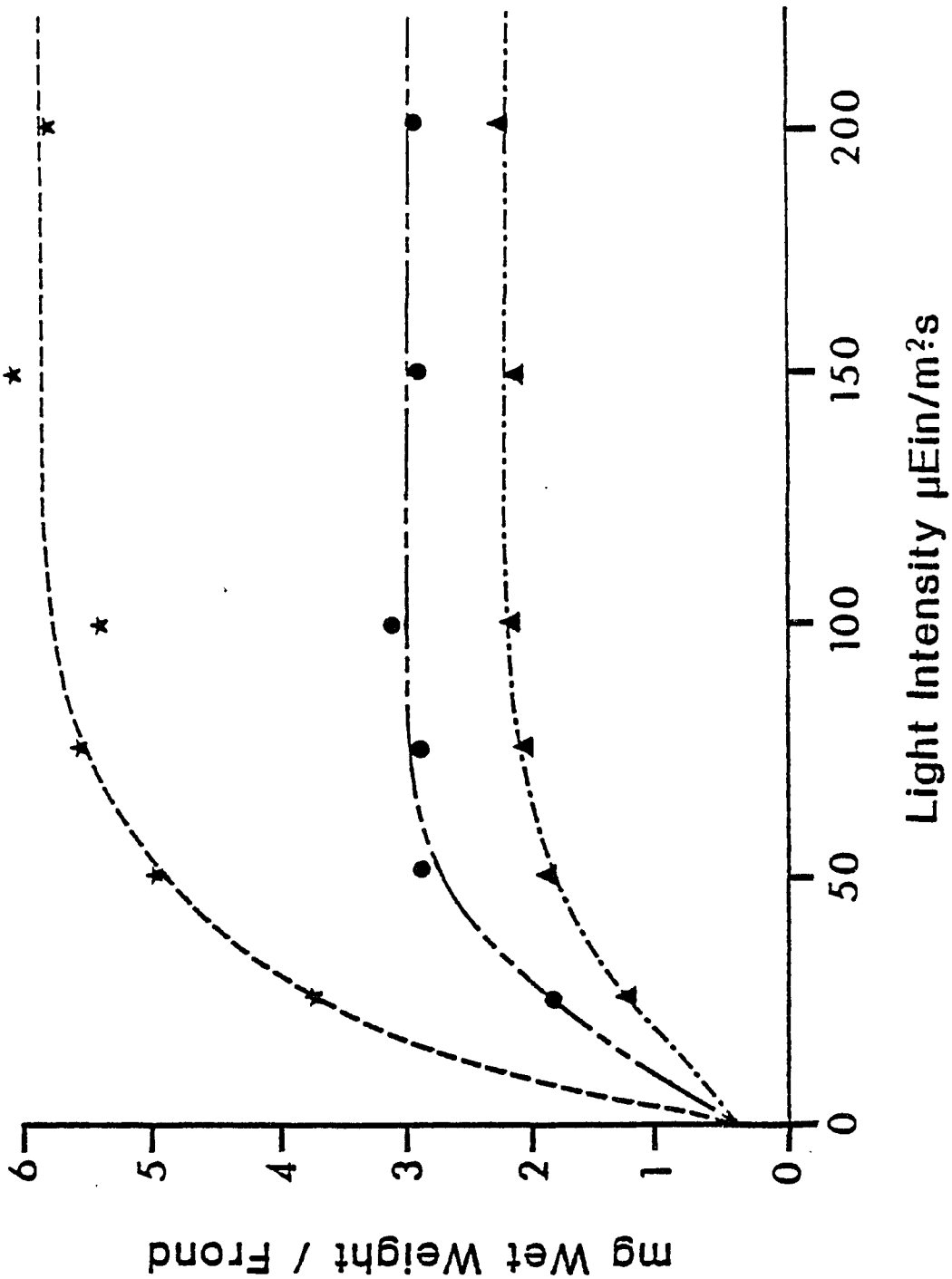

mg Wet Weight / Frond
0
1
2
3
4
5
6
0
50
100
150
200
Light Intensity µEin/m²s

TABLE V

Light vs. Species Gradient, Pond 14; 10 A.M., August 2, 1981

and Plant Gradient, May 20, 1982

Meters From[a] Southeast Shore	Light Intensity $\mu Ein/m^2s$	Species 8/2/81	Species 5/20/82
0	45	Ltr	Ltr
1	55	"	"
2	65	Lm>Wc	Lt=Wc
3	75	"	"
4	110	Wc>Lt	"
5	101	"	"
6	120	"	"
7	135	Wc>Lt>Wp	"
8	145	"	"
9 shade	170	"	"
sun patch	280	"	"
10 shade	170	Lt>Wc>Wp	Wp>Lt
sun patch	410	"	"
11 shade	180	"	"
sun patch	410	"	"
14 shade	230	Wp=Lt>Wc	"
sun patch	510	"	"

[a] Southeast shore is a wooded hill.

Ltr = Lemna trisulca
Lm = Lemna minor
Lt = Lemna turionifera
Wc = Wolffia columbiana
Wp = Wolffia punctata

not tested, the submerged species, L. trisulca, may be the most efficient species at the utilization of low light intensities as it often grows under masses of other Lemnaceae (Keddy, 1976). A similar species gradient was found in 1982.

The position of the L. minor band in 1981 is based upon the isolation from that area of the only L. minor clone from pond 14. There remains the possibility that the Lemna species are distributed evenly at a 1 to 3 ratio of L. minor to L. turionifera.

Temperature

The growth rate response curves for the Lemnaceae to temperature are given in Figure 7. The Y-axis standard error for each curve is given in the legend and can be used to determine the 99% confidence range of each curve. The curves for the two Lemna species and Spirodela are composites of the 'low light' temperature data and the data for saturating conditions in the light experiments done performed at 25, 28 and 31°C. Appendix A shows that these curves fit together smoothly, except at lower temperatures for L. minor. Here, the low light curve would indicate higher growth rates at low temperatures than the extrapolation of the high light curve. All points for the Wolffia curves were obtained under saturating light conditions.

The most notable differences between species in their response to temperature (Figure 7) are in their tolerances to high temperatures. The curves would indicate that growth would cease at 32.5° C for L. minor, 34° C for L. turionifera, 35° C for W. punctata, 36° C for W. columbiana and 38° C for S. polyrhiza. The differences in growth rates between species at these high temperatures are highly

Figure 7

Growth rate of the Lemnaceae at various temperatures. Daily Relative Growth Rate / ln2 = doublings per day.

Key:

		Y-axis Standard Error
Lemna turionifera [a]	●- - -	0.010
Lemna minor [a]	□ ———	0.018
Spirodela polyrhiza [a]	★ - - - -	0.017
Wolffia punctata [b]	☆ ———	0.015
Wolffia columbiana [b]	○	0.014

[a] Curves a composite of high and low light curves as shown in Appendix A.

[b] Curves for high light, 250 μEin/m^2·s.

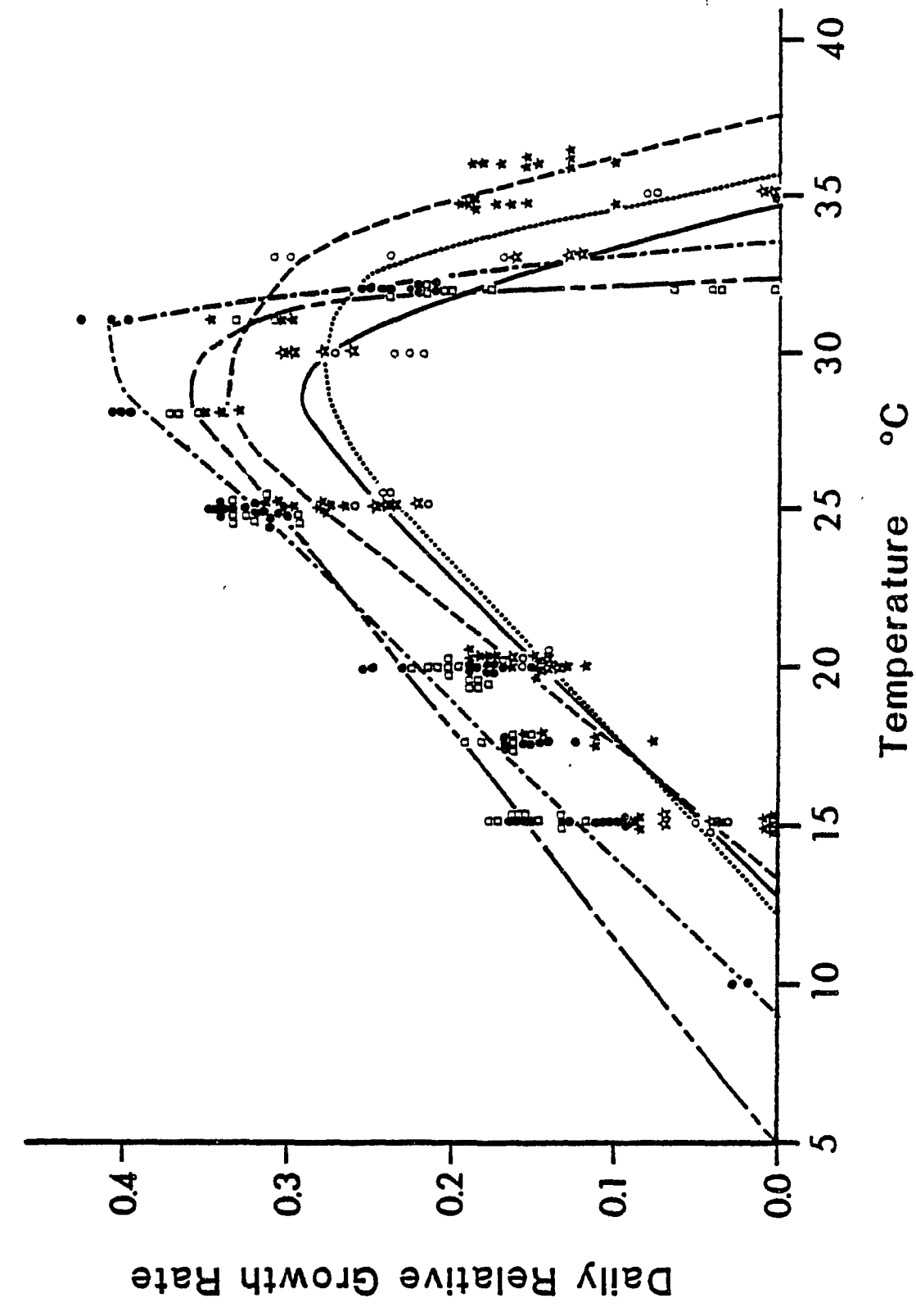

Daily Relative Growth Rate
0.0
0.1
0.2
0.3
0.4
5
10
15
20
25
30
35
40
Temperature °C

FIGURE 7

significant at the 95% level.

Although L. turionifera has the highest maximum growth rate, its rate is exceeded by those of the slow growing W. columbiana and W. punctata above 32.5°C. The growth rate of S. polyrhiza exceeds that of all other species above 32.5°C.

L. minor and L. turionifera have equal growth rates from 15°C to 28°C, but L. minor has a significantly higher rate below 15°C. This is reflected in the estimate (by extrapolation) of the lowest temperature that allows growth, 5°C for L. minor and 9°C for L. turionifera. The other three species do not grow below the 12.0-13.5°C range.

There are few differences in the temperature optima for growth amoung the five species. L. minor and W. punctata have slightly lower optima at 29°C than the other three species at 30°C. The optimal growth ranges for S. polyrhiza and W. columbiana are nearly twice as wide as those for the other three species.

Field temperature data (Appendix B) show seasonal trends, with large day to day fluctuations caused by changing air temperature and cloud cover. The surface temperature differences between ponds on any given day primarily correlate with the time of day the temperature was taken.

Surface temperatures in ponds without Lemnaceae and those with Lemnaceae differ. In the late spring, after the plants have covered the pond surface (Table VI), ponds with the plants were 3.3°C warmer at the surface than ponds without them. The differences in temperature between ponds with and without plants were less during the summer (Figure 8). These 24 hour temperature profiles show that

TABLE VI

Spring Heating of Pond Surface Water,

Under Lemnaceae Plants and in Open Water;

May 6, 1980

Pond	Temperature ^{o} C with plants	Temperature ^{o} C without plants
1		21.2
2	25.2	17.5
8	25.7	24.5
13	23.8	
14	25.1	
15		22.4
16	26.0	
17	23.8	
18		22.4
19		21.0
20		22.8
21		21.2
24		21.5
25		20.7
26	22.0	
27		19.8
28		19.5
Mean	24.5	21.2
SD[a]	1.4	1.8
Samples	7	12
SE[b]	1.1	1.0

[a] Standare Deviation
[b] Standard Error

Figure 8

Diurnal pond temperature profiles for the heavily covered Lemna - W. punctata pond 14, the thinly covered Lemna - Spirodela pond 17, and the "no-plant" pond 18 are given. Data were taken 7/25-26/81, except the am reading was from 8/2/81. Conditions on the evening of 8/1/81 were similar to those on 7/25/81 (Appendix B)

Key:

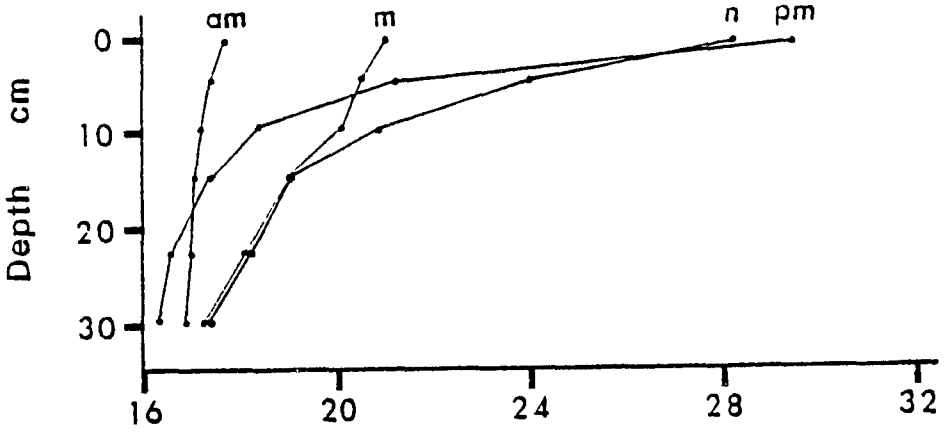
am = early morning, at dawn.

n = noon

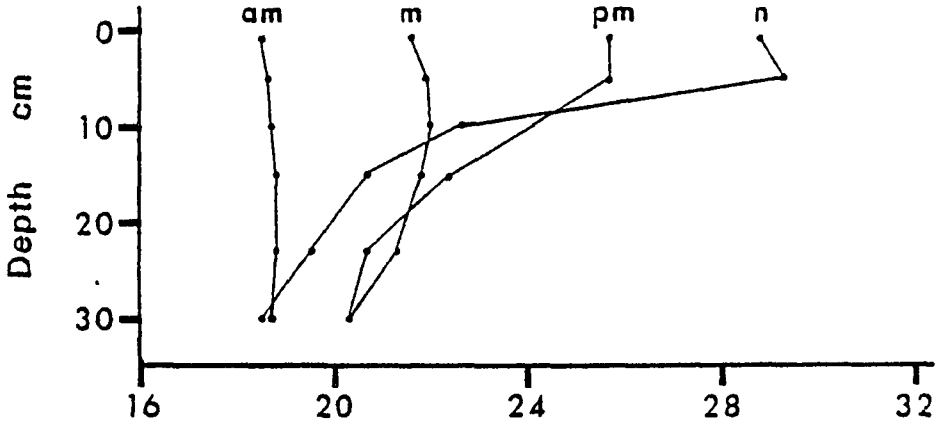
pm = late afternoon, well before dusk

m = midnight

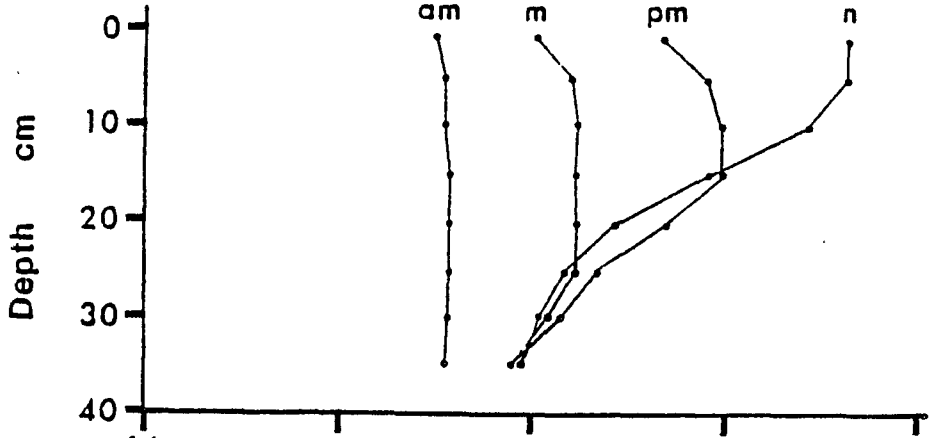
FIGURE 8



Pond 14



Pond 17



Pond 18

the surface of ponds with plant cover become cooler at night than the ponds without plants. Ponds without plants begin the day at a temperature 4 - 5° C warmer than the Lemnaceae ponds, and it takes most of the day for the surface of the Lemnaceae ponds to reach a temperature equal to the plantless ponds.

Maximum surface temperatures are similar for ponds covered with the Lemnaceae, and often exceed 30° C on sunny summer days (Table VII). The air temperature was 25° C to 30° C on the days these measurements were taken, and it might be expected that temperatures above the maximum of 34° C reported here could be obtained on warmer days.

The Lemnaceae ponds are strongly stratified at the surface during the day, but the water can mix owing to surface cooling, especially if nights are cool. During a general warming trend, however, there may be nights when these ponds do not mix. Ponds without plants, on the other hand, have only a weak stratification and should mix nearly every night, being less dependent upon nighttime cooling trends.

Turion Formation and Germination

Spirodela polyrhiza turions formed at 10° C and 15° C in the semi-continuous temperature experiments. At 10° C a few turions were produced by each frond, after which growth ceased. At 15° C turions were produced continuously. Though turions form in nutrient-limited L. turionifera cultures they do not form in response to the low temperatures when the culture medium was renewed every four days.

The number of turions per frond initiated in batch cultures

TABLE VII

Surface Temperatures for Ponds Containing Masses of Lemnaceae; Data Taken Between Noon and 5 p.m., June - August, 1980 and 1981, on Sunny Days

Date	Pond	Temperature ^{o} C
7/23/80	17	28.0
	16	29.0
	16	27.0
7/24/80	19	28.8
	14	30.0
	14	30.0
	13	31.3
	26	28.8
7/31/80	17	27.0
	30	32.0
8/11/80	5	31.0
	8	34.0
	31	34.0
	11	33.0
	7	33.0
	6	32.0
6/ 6/81	8	30.0
	35	29.0
6/ 7/81	14	31.5
	17	31.0
	30	30.4
	16	28.2
6/18/81	32	31.1
	3	33.7
	4	30.3
	38	31.0
	23	29.0
	10	30.3
	9	30.1
	39	27.6
7/12/81	17	32.2
7/13/81	31	33.0
7/25/81	14	25.5
	17	28.8
	14	28.3
8/ 1/82	14	29.2
	17	30.2
Mean		30.25
Standard Deviation		2.07
Samples		37.00
Standard Error		0.07

Figure 9

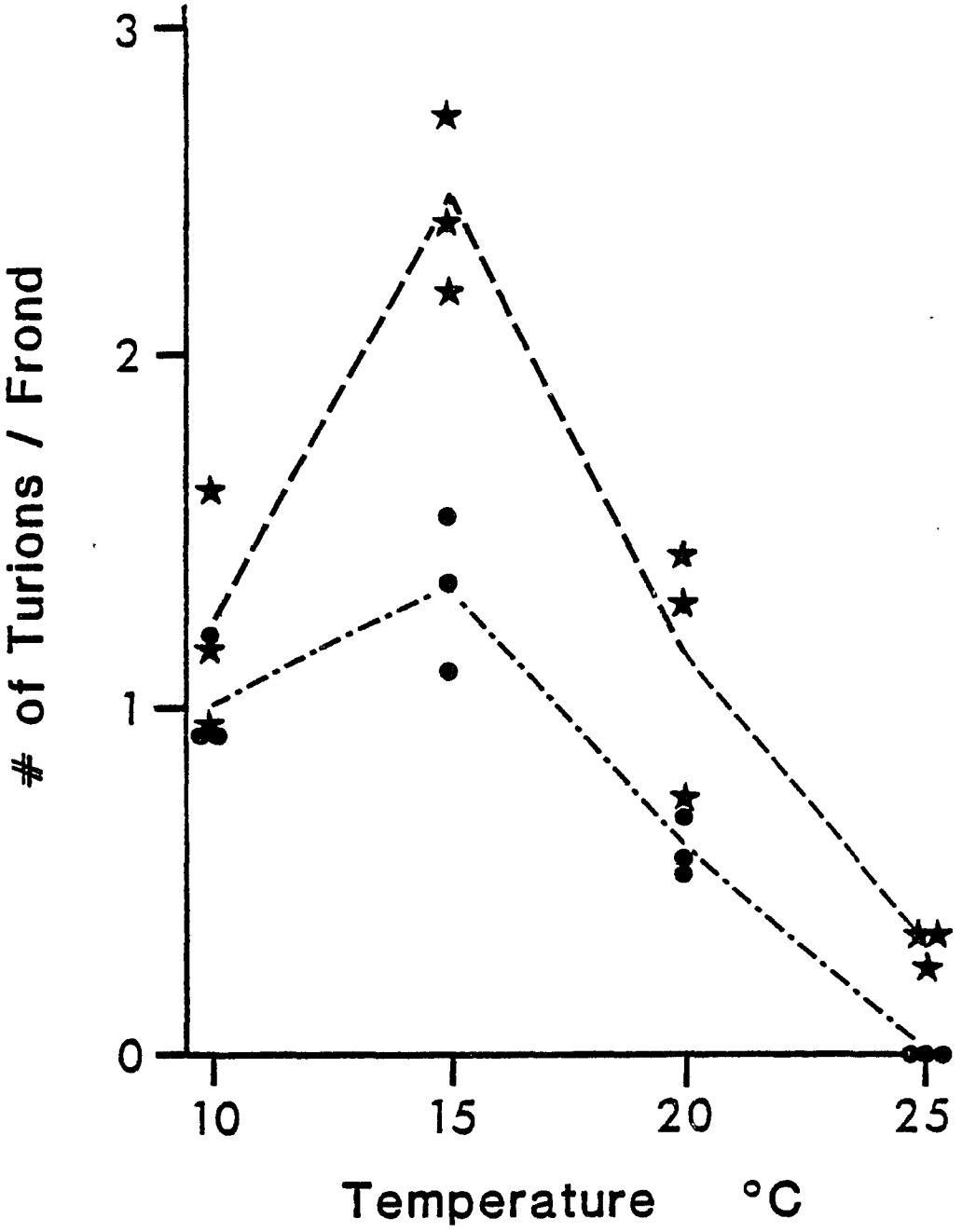
The effect of temperature upon the formation of turions in nutrient limited batch cultures.

Key:

<u>Lemna turionifera</u> -·-·-·-·-·-·- ●

<u>Spirodela polyrhiza</u> ———————— ★

FIGURE 9



at various temperatures is shown in Figure 9. Both species have peak production of turions at 15° C where S. polyrhiza produces two turions per frond and L. turionifera produced one turion per frond. Turion formation is inhibited at 25° C in L. turionifera, but not in S. polyrhiza. The effect of daylength on this phenomenon was not investigated.

Temperature also affects turion germination, as shown in Figure 10. L. turionifera began germinating at a low rate at 10° C and reached 100% germination between 15° C and 20° C. S. polyrhiza turions did not germinate below 20° C, obtained 60% germination at 25° C, and 100% germination at 30° C. There is a 7 - 10° C difference between the germination temperatures of these two species, and L. turionifera would be expected to germinate first in the spring. Table VIII gives the mean bottom temperatures taken immediately after germination of the turions in the field. L. turionifera turions had germinated in large numbers by the time the bottom temperature was 11.1° C. Wolffia punctata began germination by the time the bottom temperatures reached 14.° C and S. polyrhiza began germinating when the bottom temperature was 17.9° C, but massive germination had only occurred in the ponds at 20 - 21°C. These field observations agree well with results of the laboratory experiments.

Lemna minor - Lemna turionifera Differentiation

Table IX gives the results of the differentiation of L. minor from L. turionifera by the formation of turions by the latter species in nutrient-limited cultures. As can be seen, 83% of all the clones collected were L. turionifera. Ponds 7, 8, 11, 17, 26 and

Figure 10

The effect of temperature upon the germination of turions formed at 25° C and placed in complete culture media for one month.

Key:

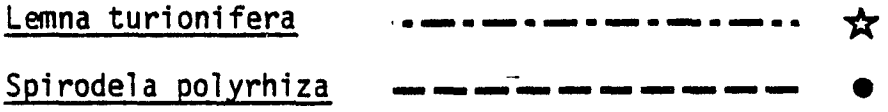


FIGURE 10

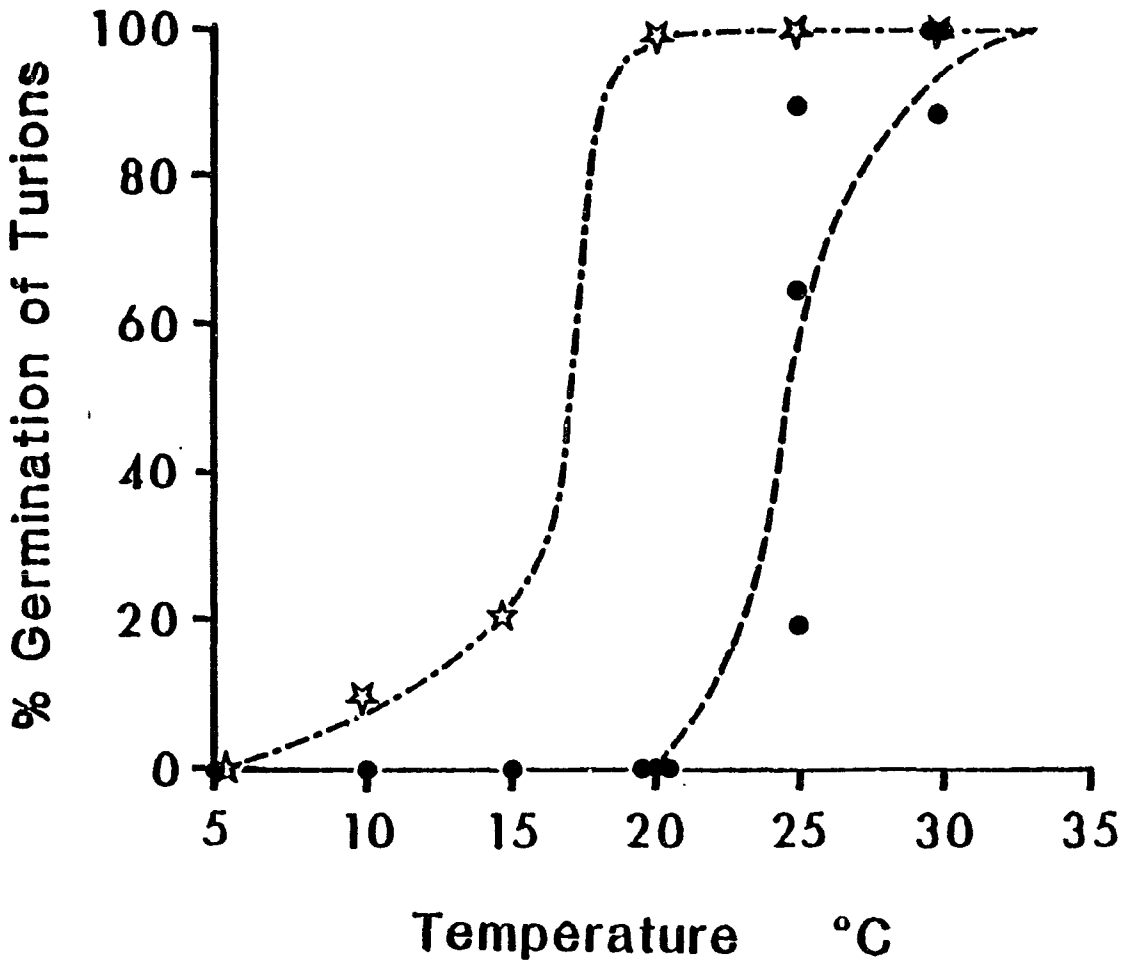

% Germination of Turions
100
80
60
40
20
0
5
10
15
20
25
30
35
Temperature °C

TABLE VIII

Observed Bottom Temperatures

During Germination of the Lemnaceae in the Field, 1980 - 1982

	Mean Bottom Temperature ^{o} C	Standard Error	Number of Observations
Lemna turionifera	11.1	1.7	10
Wolffia columbiana	14.2	1.4	3
Spirodela polyrhiza	17.9	1.7	12

TABLE IX

Lemna minor - *Lemna turionifera*: Differentiation of Pond Clones by Turion Formation in Culture

Pond	Clones of Each Species[a] Lt	Lm	% Lt	Number of Determinations
3	6	1	86	7
4	3	3	50	6
5	7	1	88	8
6	3	2	60	5
7	6		100	6
8	5		100	5
9	2	3	40	5
10	5	2	71	7
11	5		100	5
14	3	1	75	4
16	5	1	83	6
17	8		100	8
19	5	2	71	7
23	6	1	86	7
26	6		100	6
29	4	1	80	5
31	7	1	88	8
36	3	1	75	4
39	4		100	4
41	5	1	83	6
Ponds with fewer than three determinations	21	3	88	24
TOTAL	119	24		143
Percent	83	17		

[a] Species abbreviations as in Table I.

39 show no L. minor, while most show a small percentage of the species. In only three ponds does L. minor approach 50% of the total Lemna populations. In one of these ponds, 6, it is a trace species among W. punctata. In the other two ponds, 4 and 9, it forms a large part of the Lemna mass that covers the ponds. It should be noted that these are the only two ponds in this study which are entirely shaded all day and develop a complete cover of plants.

These determinations were made in nitrogen limited-culture. It was subsequently found that L. turionifera turions form more rapidly in phosphorus-limited culture.

pH

Figure 11 gives the results of the semi-continuous culture pH experiment between S. polyrhiza and L. minor. Both species have low growth rates below pH 4.0 and above 9.5, and a broad optimal growth range from pH 5.0 to 8.0. Above pH 7.5, the growth rate of L. minor falls, while that of S. polyrhiza apparently rises until it begins to fall at pH 8.5. The curves are again equal by pH 9.0. S. polyrhiza may have an advantage between pH 7.5 and 8.5. A separate growth rate standard error is calculated for this pH range because the varience is higher than for the other pHs. The two species' growth rates betweem pH 7.5 and 8.5 did not differ significantly at the 95% level, but do at the 90% level.

Another experiment was performed in which the plants force the pH up to their tolerance limit via their uptake of nitrate. The results of this experiment are given in Figure 12. W. columbiana increased the pH to 10.5 while the L. turionifera and W. punctata

Figure 11

The effect of pH upon the growth of the Lemnaceae, 25° C, 200 $\mu Ein/m^2 \cdot s$. Daily Relative Growth Rate / ln2 = doublings per day.

Key:		Curve Y-axis Standard Error	Y-axis Standard Error pH 7.5-8.5
Lemna minor	——— – – – ●	0.012	0.026
Spirodela polyrhiza	– – – – – – – ★	0.013	0.025

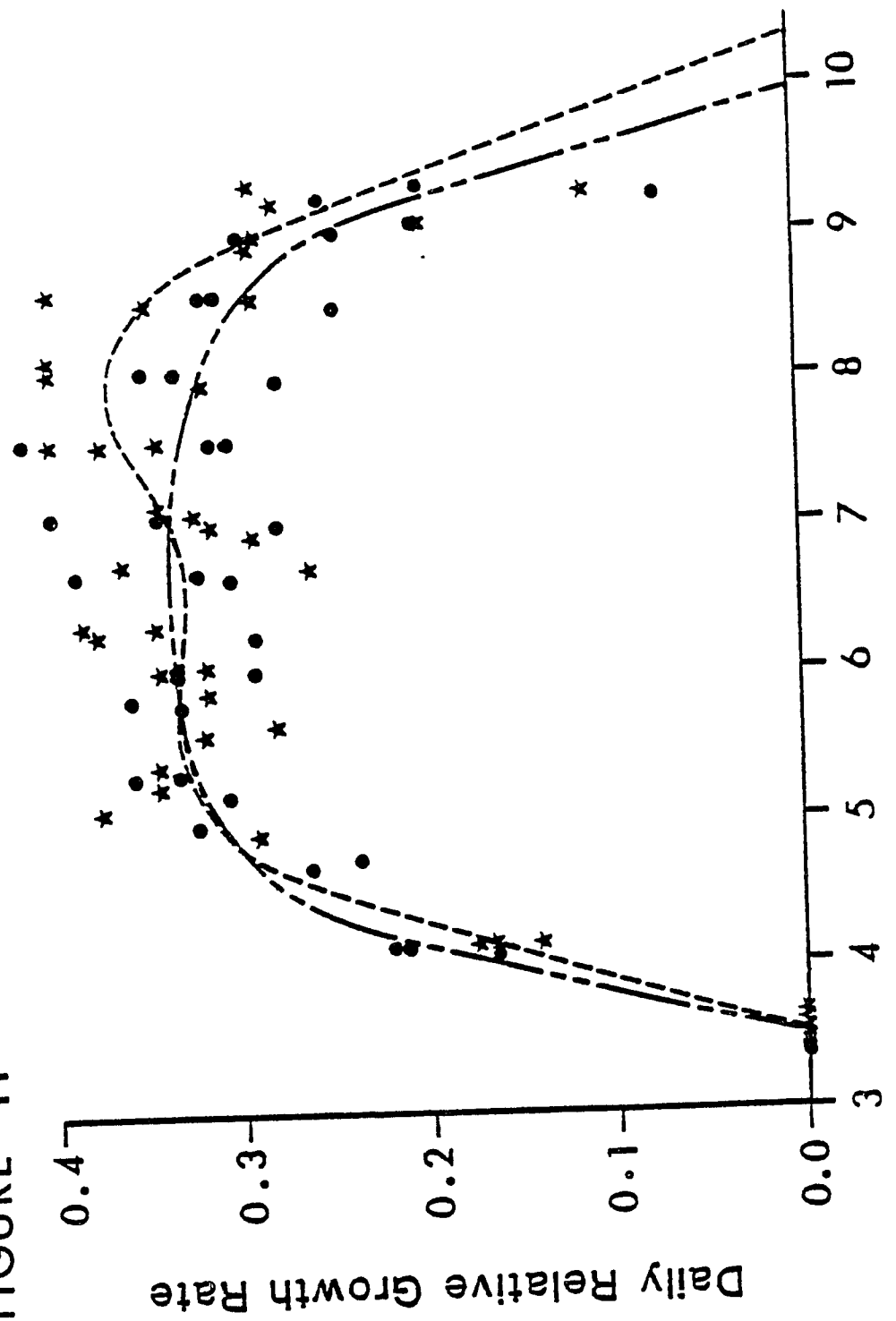


FIGURE 11

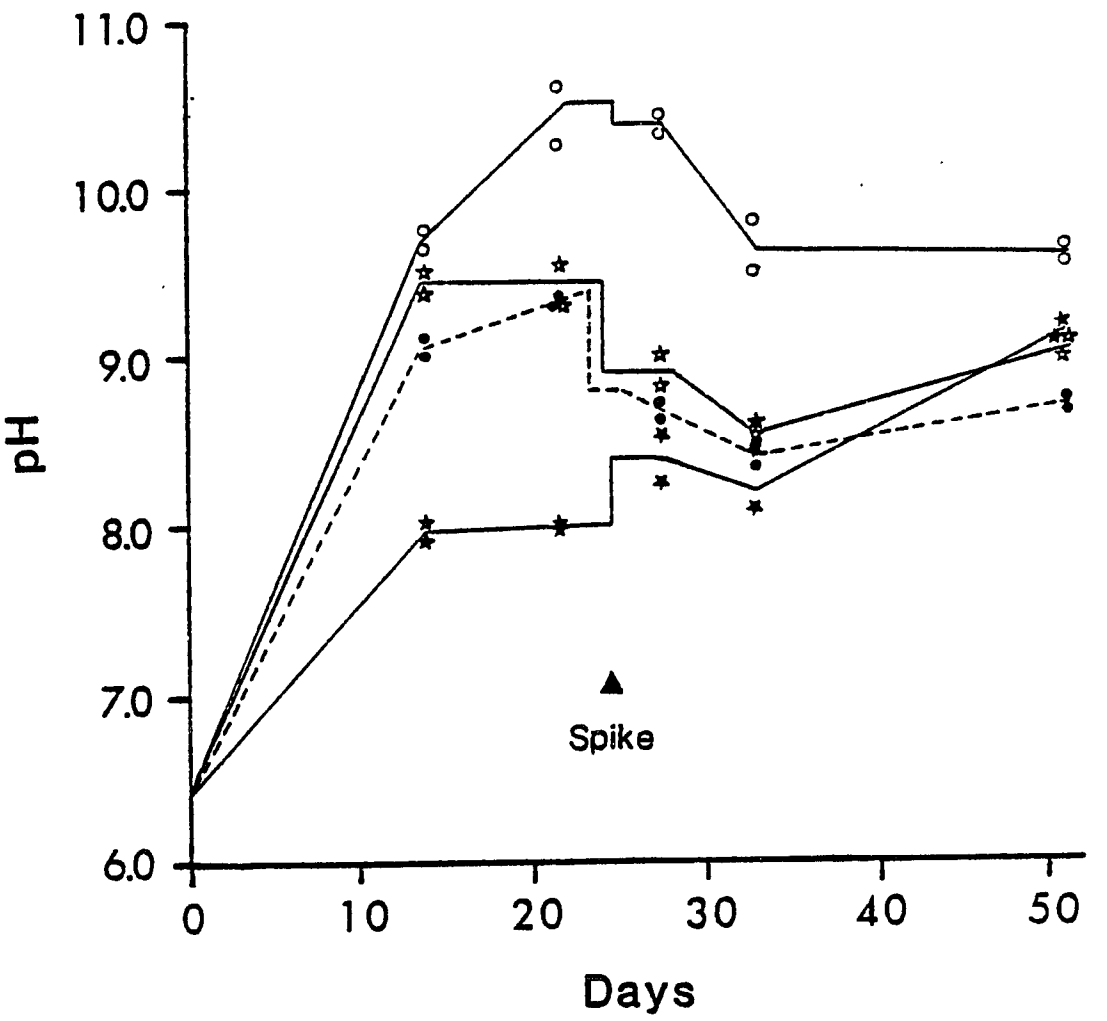
Figure 12

The maximum pH attained in cultures allowed to grow until pH became limiting. Spike consisted of 0.4 ml Calcium stock / 200 ml media.

Key:

Lemna turionifera	●
Spirodela polyrhiza	★
Wolffia punctata	☆
Wolffia columbiana	○

FIGURE 12



media only reached a pH of 9.5. The growth of S. polyrhiza was inhibited, which was alleviated by enrichment with calcium on the twenty fourth day. The pH of the media for the other species decreased when calcium was added. Therefore, only post-enrichment data are utilized to determine a comparable maximum pH value for each species. Only the data from the last sampling date are utilized for S. polyrhiza as its growth was still slow on previous sampling dates.

The maximum pH values (Table X) were 9.88 for W. columbiana, 9.17 for S. polyrhiza, 8.83 for W. punctata and 8.60 for L. turionifera. As the pH of the S. polyrhiza medium was still increasing on day 50 when the experiment had to be terminated, the maximum pH for this species may be higher than indicated here. The maximum pH for W. columbiana is significantly higher than for all other species, and S. polyrhiza has a significantly higher maximum pH than L. turionifera at the 95% confidence level.

The pond pH ranges associated with the Lemnaceae are shown in Table XI. S. polyrhiza and W. columbiana are found at pH 8.75 and 8.30, respectively, pH values only exceeded by "no-plant" ponds. L. turionifera and W. punctata are found in ponds near neutrality. Ponds of mixed S. polyrhiza and L. turionifera are at a pH intermediate to that preferred by each species. S. polyrhiza is strongly differentiated from L. turionifera (and therefore L. minor) and W. punctata at the 95% level while the 95% confidence limits for W. columbiana encompass all the species.

Figure 13 shows diurnal pH variations for three ponds. The pH remains below 7.0 all day in the thickly covered Lemna-W. punctata pond (14). The thinly covered Spirodela-Lemna pond (17) shows an

TABLE X

Maximum Culture pH Experiment,

Mean pH After Spike

Species[a]	pH	SE	N
Lt	8.60	±0.14	6
Wp	8.83	±0.20	6
Wc	9.88	±0.32	6
Sp	9.17	±0.22	2[b]

[a] Species abbreviations as in Table I.

[b] As Sp growth had been delayed, its pH was still increasing at the end of the experiment. Only the last set of points was used.

TABLE XI

Pond Surface pH Ranges for the Lemnaceae

1980 and 1981; June - August Data

	NP[a]	Sp[b]	Sp-Lt[c]	Lt[d]	Lt-Wp[e]	Wp[f]	Wc[g]
Mean	8.92	8.75	7.84	7.02	6.87	7.34	8.30
SD[h]	0.82	0.67	0.63	0.49	0.44	0.57	1.49
Samples	10	10	13	15	15	7	6
SE[i]	0.51	0.42	0.56	0.25	0.22	0.43	1.22
Δ Ponds	4	4	7	13	6	3	3

[a] Ponds with no plants.
[b] Ponds with Spirodela polyrhiza.
[c] Ponds with Spirodela polyrhiza and Lemna turionifera.
[d] Ponds with Lemna turionifera.
[e] Ponds with Lemna turionifera and Wolffia punctata.
[f] Ponds with Wolffia punctata.
[g] Ponds with Wolffia columbiana.
[h] Standard Deviation
[i] Standard Error

Figure 13

Diurnal pond pH profiles for the heavily covered Lemna - W. punctata pond 14, the thinly covered Lemna - Spirodela pond 17, and the "no-plant" pond 18 are given. Data were taken 7/25-26/81, except the am readings are from 8/2/81. Conditions on the evening of 8/1/81 were similar to those on 7/25/81 (Appendix B).

Key:

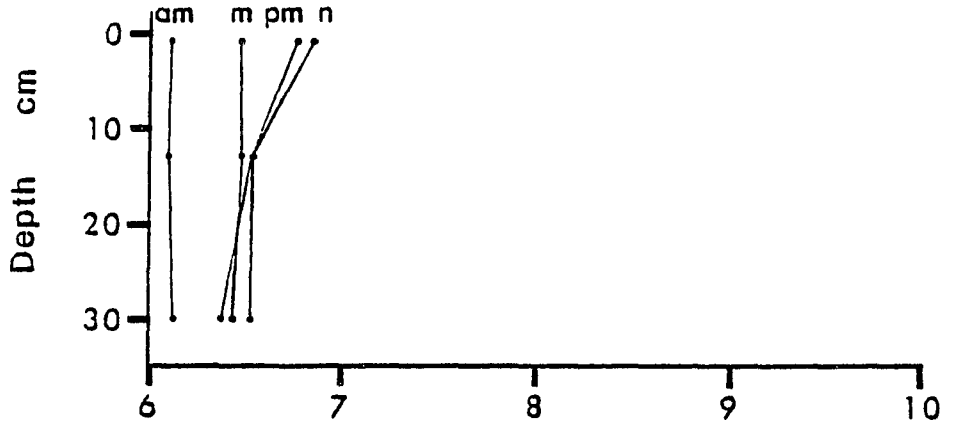
am = early morning, at dawn.

n = noon

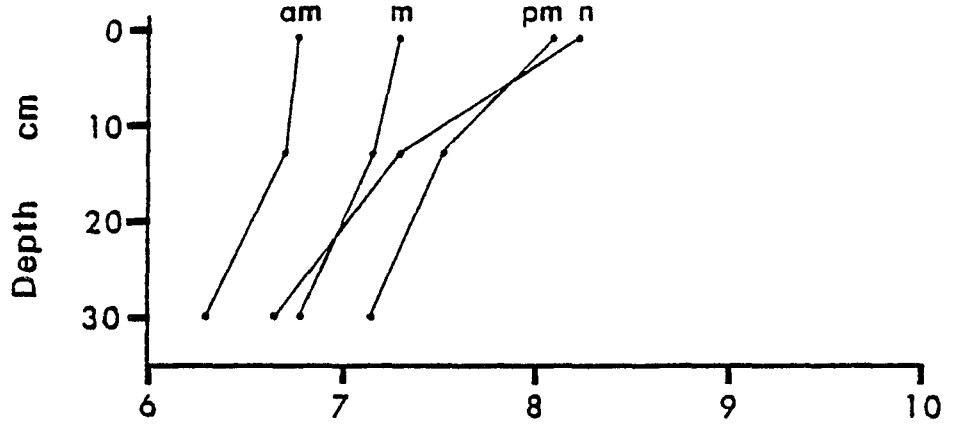
pm = late afternoon, well before dusk

m = midnight

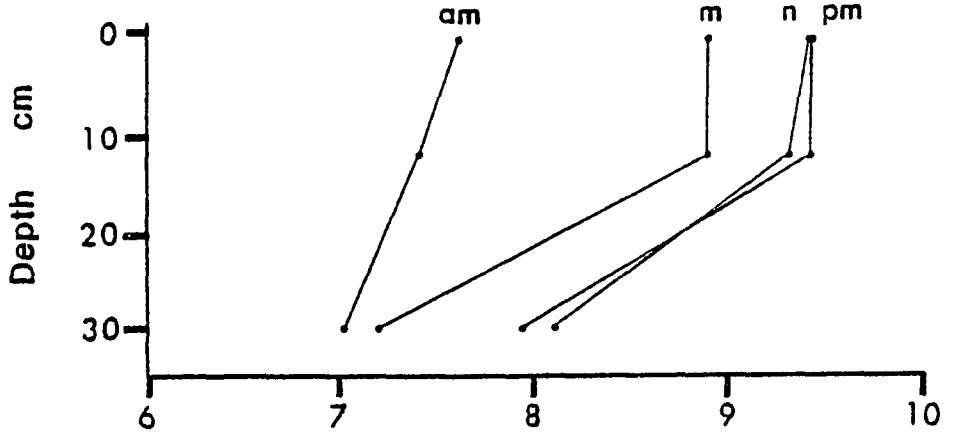
FIGURE 13



Pond 14



Pond 17



Pond 18

increase at the surface of 1.5 pH units from dawn to dusk, reaching a maximum pH of 8.0. The 'no plant' pond (18) shows the greatest surface pH increase of two units, reaching a maximum of 9.3. The pH varies less and remains lower at the pond bottoms and in ponds with a more complete cover of Lemnaceae.

Oxygen

High pH values are correlated with high oxygen concentrations. Figure 14 shows diurnal oxygen profiles for three ponds.

Pond 18 has no Lemnaceae, but has a thick growth of Chara, with smaller amounts of Potomogeton species and Elodea canadensis mixed in. This growth reaches up to the 20 cm depth, where an oxygen maximum occurs during the afternoon. The water is supersaturated with oxygen by the late afternoon, and the water column stays well oxygenated all night except for the bottom, which is usually below 1 ppm oxygen.

Pond 17 contains a loose layer of L. turionifera and S. polyrhiza and has masses of Ceratophyllum demersum underneath. This pond has a high oxygen concentration in the surface 15 cm during the day, but this decreases to below 1 ppm at night. In the lower 15 cm, oxygen is always below 2 ppm, and decreases to zero at night.

In pond 14, a pond that develops a thick cover of L. turionifera and W. punctata, the only significant oxygen is at the surface (0 to 2.5 cm), where oxygen may reach 3 ppm during the day. The oxygen concentration is nearly zero at every other depth, and the surface oxygen also decreases to less than 0.5 ppm during the night.

Figure 14

Diurnal pond oxygen profiles for the heavily covered Lemna - W. punctata pond 14, the thinly covered Lemna - Spirodela pond 17, and the "no-plant" pond 18 are given . Data taken 7/25-26/81, except the am readings are from 8/2/81. Conditions on the evening or 8/1/81 were similar to those on 7/25/81 (Appendix B).

Key:

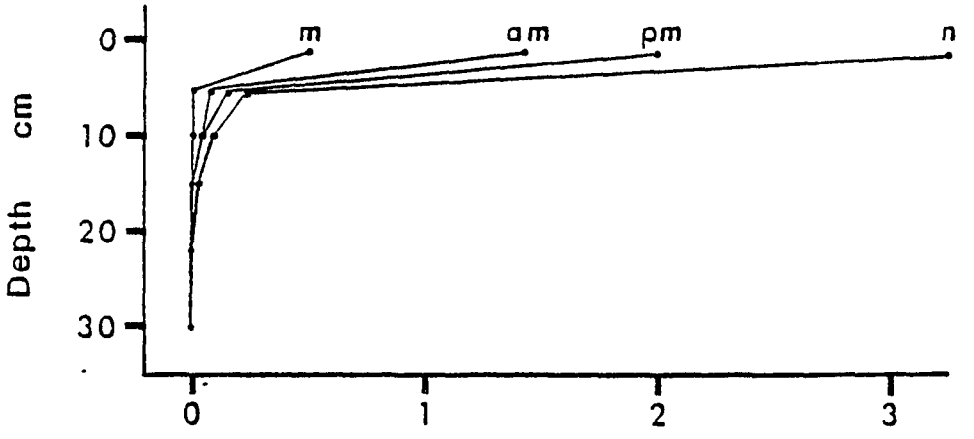
am = early morning, at dawn.

n = noon

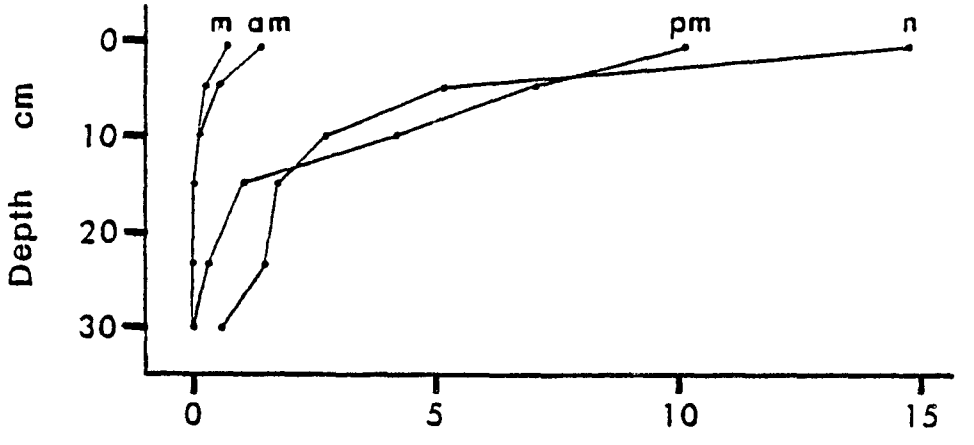
pm = late afternoon, well before dusk

m = midnight

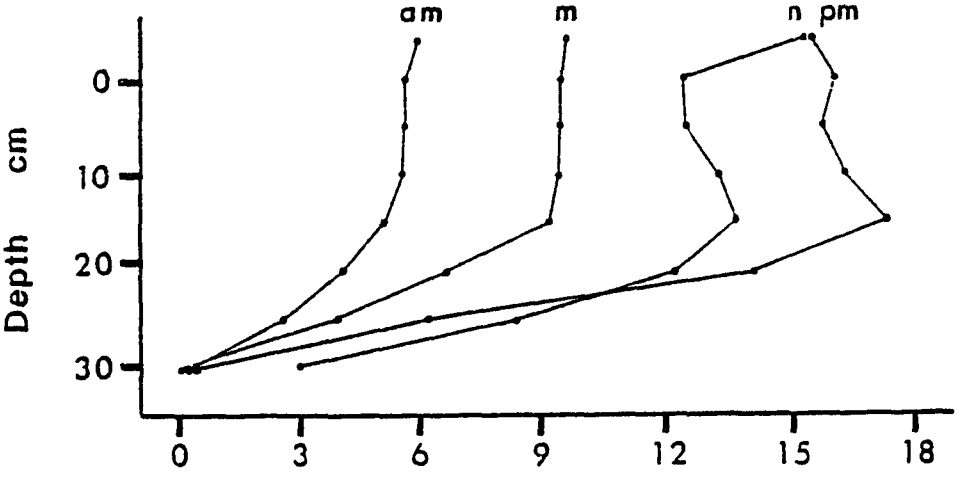
FIGURE 14



Pond 14



Pond 17



Pond 18

Iron

In the pH experiment, a strong precipitate was noted above pH 8.0, the range where S. polyrhiza may gain a growth advantage. Subsequent analysis of the unused media showed that the phosphate concentration was decreased 50%, and the iron concentration was decreased 99%. This indicates that iron depletion due to precipitation as hydroxides at high pH could be the basis for the growth responses in the pH experiments. Therefore, iron was analyzed for field samples.

Data are given in Table XII for total filterable iron in the six ponds most intensely studied. In the spring, all ponds had approximately the same surface iron concentration, ranging from 0.29 to 0.75 µM with the Lemna pond (41) having the smallest, and the Spirodela pond (30) having the largest concentration.

The iron concentrations increased in all the ponds during the summer. As indicated by the iron increase factor (Table XII), the increase in surface iron is less, 1.7 and 3.0 times, in the Spirodela (30) and the W. punctata ponds (29) than in the other ponds with increase factors of 7.4, 8.4, 10.0, and 36.4 for the Lemna (41), "no-plant" (18), Lemna-Spirodela (17) and the Lemna-W. punctata (14) ponds, respectively. By late summer the iron concentrations ranged from 0.9 to 3.3 µM in ponds 17, 29, 30, and 41, which were equal within the 95% confidence limit of assay error. Pond 14 had the highest iron concentration, 24.0 µM, which was not suprising as it had a heavy Lemnaceae cover and was mostly anaerobic (Figure 14). Pond 18 also had a significantly high iron concentration of 3.8 µM.

TABLE XII

Seasonal Concentrations of Filterable Iron in Six Ponds, 1982. In μM Fe ± SE.

Pond	Species	Sample Depth	Spring April α May	Early Summer June	Late Summer August	Iron[a] Increase Factor
29	Wp[b]	S[c]	0.30±0.25[e] (6)[f]	0.56±0.33 (4)	0.90± 0.28 (2)	3.0
		B[d]	0.31±0.15 (3)	0.82±0.72 (2)	1.20± 0.56 (2)	3.9
14	Lt-Wp	S	0.66±0.40 (4)	2.01±1.30 (6)	24.00± 4.46 (2)	36.4
		B		3.24±2.56 (6)	22.50± 2.96 (2)	
41	Lt	S	0.29±0.18 (6)	2.00±0.22 (2)	2.15± 0.98 (2)	7.4
		B	0.54±0.32 (4)	1.89±1.48 (2)	10.65±21.20 (2)	19.7
17	Lt-Sp	S	0.33±0.32 (4)	2.67±1.41 (8)	3.30± 1.96 (2)	10.0
		B	1.08±0.16 (2)	4.98±2.08 (8)	12.60± 0.00 (2)	11.7
30	Sp	S	0.75±0.17 (6)	1.63±0.81 (6)	1.25± 0.14 (2)	1.7
		B	1.83±0.14 (2)	2.19±0.57 (6)	3.20± 0.00 (2)	1.7
18	NP[g]	S	0.46±0.35 (6)	1.88±1.59 (4)	3.82± 0.14 (2)	8.4
		B	1.14±0.40 (2)	1.63±1.15 (4)	3.65± 0.14 (2)	3.2

[a] Iron increase factor = late summer concentration / spring concentration.
[b] Species abbreviations after Table XI.
[c] Surface samples.
[d] Bottom samples
[e] Standard error
[f] Number of samples
[g] No plants.

This "no-plant" pond had high pH values and high oxygen concentrations (Figures 13 and 14) which would be expected to precipitate iron.

Because finely colloidal iron could have penetrated the glass fiber filters, dialyzable iron might have been a more dependable assay for soluble iron than total filterable iron. Analysis of the May 30, 1982 dialysis samples showed that the concentration of dialyzable iron was generally less than the filterable iron in samples taken concurrently. The proportion of dialyzable iron are 0.09 and 0.13 for the Lemna-W. punctata (14) and the "no-plant" (18) ponds, 0.37 and 0.39 for the Lemna-Spirodela (17) and Spirodela (30) ponds, 0.63 for the Lemna (41) pond and 1.0 for the W. punctata (29) pond (Table XIII).

Dialyzable iron concentrations are estimated from the seasonal filterable iron concentrations (Table XIII). In June and August, the Spirodela pond (30) has the lowest dialyzable iron concentrations, 0.49 and 0.64 μM. The Lemna pond (41) has significantly higher dialyzable iron concentrations of 1.34 to 1.44 μM. The Lemna-Spirodela pond has iron concentrations (0.98 to 1.22 μM) that are intermediate and equal to those of the single species ponds at the 95% confidence limit.

The "no-plant" pond (18) has low summer dialyzable iron concentrations in June and August of 0.24 and 0.50 μM, which is expected in this highly oxygenated pond of high pH. The W. punctata pond (20) has moderately low dialyzable iron concentrations of 0.56 go 0.90 μM. The Lemna-W. punctata pond (14) starts out with smaller and ends up with larger dialyzable iron concentrations, 0.18 to 2.16 μM. This may be owing to a change in the proportion of dialyzable

TABLE XIII

Seasonal Filterable Iron Data

Adjusted to Dialyzable (Soluble) Iron

by a Proportional Factor.

In μMFe, with Mean and Standard Error Given.

Pond	Species	April-May[a]	June[b]	August	Proportion Dialyzable[c]
29	Wp[d]	0.30±0.25[e]	0.56±0.33	0.90±0.28	1.00
14	Lt-Wp	0.06±0.04	0.18±0.12	2.16±0.40	0.09
41	Lt	0.19±0.11	1.34±0.14	1.44±0.61	0.63
17	Lt-Sp	0.12±0.12	0.98±0.52	1.22±0.73	0.37
30	Sp	0.29±0.07	0.64±0.33	0.49±0.05	0.39
18	NP[f]	0.06±0.04	0.24±0.21	0.50±0.00	0.13

[a] Plant mass not providing a solid cover in this time period.
[b] The time period that the proportion factor was determined.
[c] Proportion factor = dialyzable Fe / filtered Fe.
[d] Species abbreviations as in Table XI.
[e] Standard error.
[f] "No plant" pond.

iron caused by the large increase in iron over the summer. The June value of 0.18 μM iron is the most accurate, as this was the period that the proportion of dialyzable was determined.

Phosphate

The curves for growth of the Lemnaceae on limiting phosphate in batch culture are given in the Figure 15. In this experiment, L. turionifera has a higher growth rate than S. polyrhiza at low phosphate concentrations. The two growth rate curves are not significantly different at the 95% confidence limit. The halfsaturation constants for phosphate, Kp, were 0.37 μM for L. turionifera and 0.95 μM for S. polyrhiza, which were significantly different at the 90%, but not the 95% confidence limits (Table XIV) . The μ_{max}, 0.399 and 0.440 for the two species, respectively, are not significantly different at either confidence limit (Table XIV).

The semi-continuous experiments (Figure 16) show that L. turionifera grows more rapidly than the other species at all phosphate concentrations. This relationship is significant below 1 μM phosphate at the 95% confidence limit. L. minor and S. polyrhiza have growth rate responses to phosphate that are not significantly different, even at the 50% level. The growth responses to phosphate of the two Wolffia species are significantly different from the other species, but not from each other at the 95% level. W. columbiana has a significantly higher growth rate than W. punctata below 3 μM phosphate at the 90% confidence limit.

In semi-continuous culture, L. turionifera has the smallest Kp, 0.35 μM, essentially the same Kp as in the batch experiment (Table

Figure 15

Growth of the Lemnaceae under phosphate limiting conditions in batch culture, 28° C and 250 uEin/m^2·s. Daily Relative Growth Rate / ln2 = doublings per day.

Key:		Curve Y-axis Standard Error
Lemna turionifera	-·-·-·-·- ●	0.046
Spirodela polyrhiza	———— ★	0.049

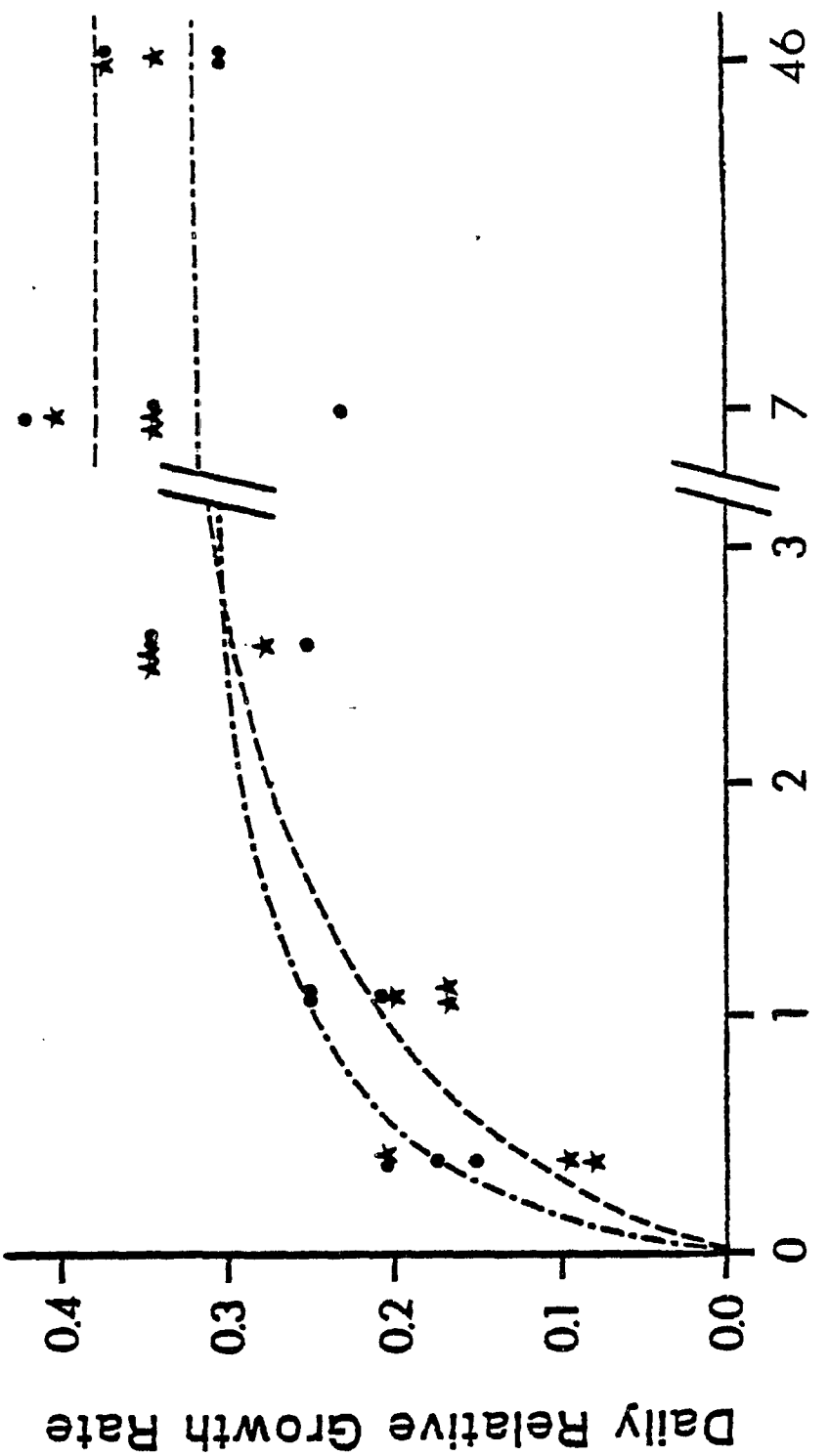

Daily Relative Growth Rate
0.0
0.1
0.2
0.3
0.4
0
1
2
3
7
46
µM Phosphate

FIGURE 15

TABLE XIV

Calculated Half-Saturation Constants (K_P)

and Maximum Growth Rates (μ_{max})

for Batch and Semicontinuous Phosphate Growth Experiments

Batch:	K_P µM P	90% Confidence Interval	μ_{max}[a]	90% Confidence Interval
L. turionifera	0.37	0.21-0.55[b]	0.339	0.311-0.367
S. polyrhiza	0.95	0.65-1.23[b]	0.400	0.364-0.435
Semicontinuous:		**95% Confidence Interval**		**95% Confidence Interval**
L. turionifera	0.35	0.33-0.38	0.443	0.427-0.459
L. minor	0.45	0.41-0.50	0.426	0.409-0.444
S. polyrhiza	0.46	0.41-0.51	0.415	0.396-0.434
W. punctata	5.52	2.68-9.67	0.274	0.214-0.334
W. columbiana	1.48	0.89-1.72	0.211	0.195-0.228

[a] Daily Relative Growth Rate, DRGR/ln2 = doublings/day.
[b] 95% confidence intervals overlap.

Figure 16

Growth of the Lemnaceae at limiting phosphate concentrations in semi-continuous culture, 28° C, 250 µEin/m^2s. Daily Relative Growth Rate / ln2 = doublings per day.

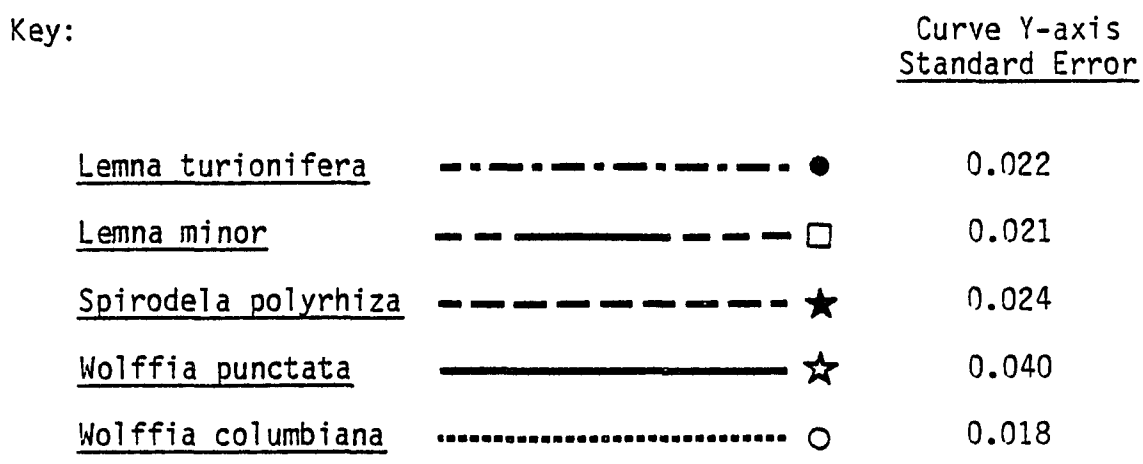
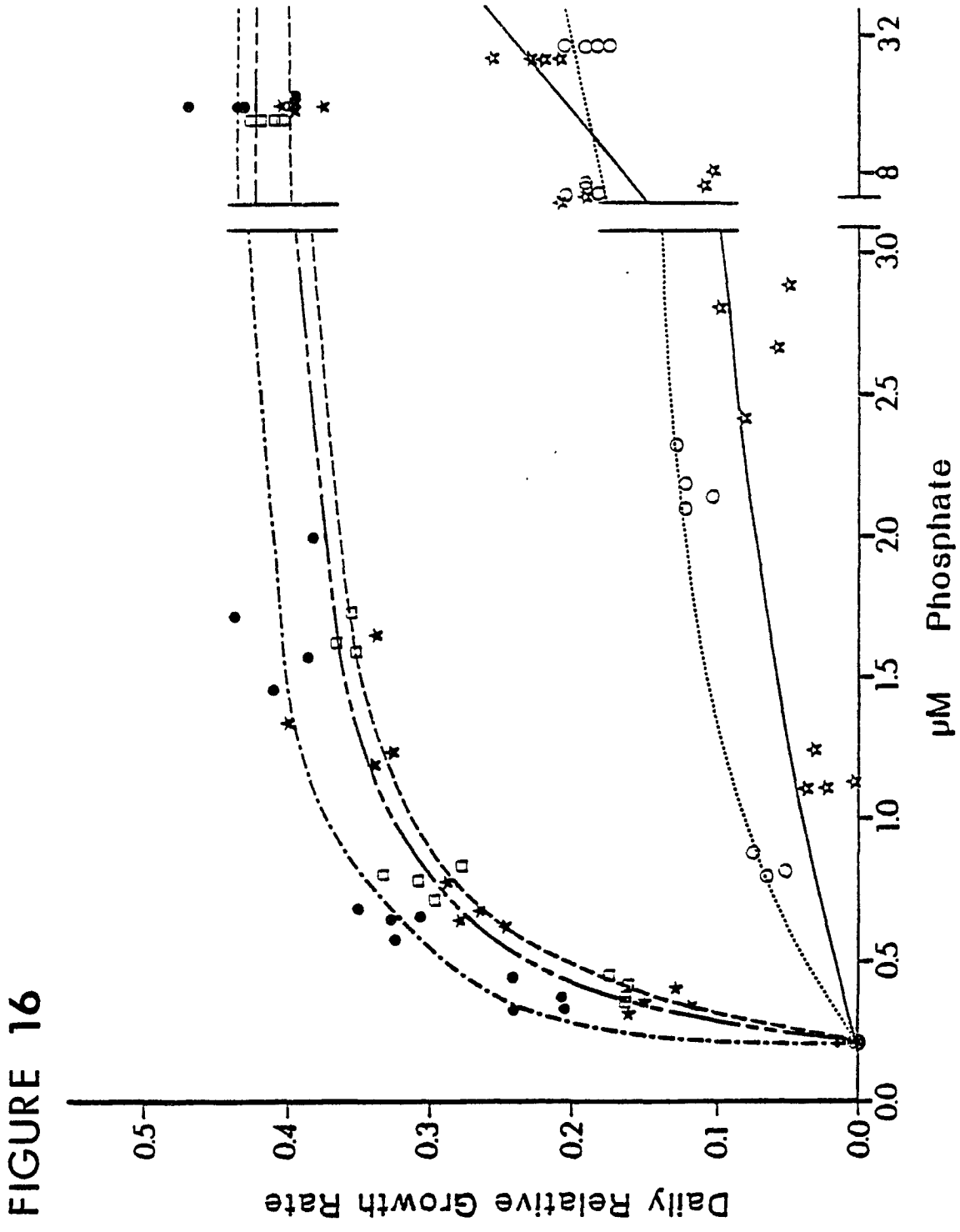


FIGURE 16


Daily Relative Growth Rate
0.0
0.1
0.2
0.3
0.4
0.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
8
32
μM Phosphate

XIV). The Kp for S. polyrhiza and L. minor were equal, 0.46 and 0.45 μM phosphate, and significantly larger than the Kp of L. turionifera at the 95% confidence limit. W. columbiana had a Kp of 1.48 μM, and W. punctata had a Kp of 5.52 μM, with each differing significantly from the Kp of all the other species at the 95% confidence limit.

The μ_{max} for L. turionifera, L. minor and S. polyrhiza ranged from 0.415 to 0.443 (Table XIV), and were equal at the 95% confidence limit. The μ_{max} for W. punctata was 0.274. and for W. columbiana was 0.211, equal to each other but lower than the other species at the 95% confidence limit.

Table XV gives the mean pond surface phosphate concentrations according to the species assemblages. The S. polyrhiza ponds have higher phosphate concentrations, a mean of 0.92 μMP, than L. turionifera ponds with a mean of 0.36 μMP. W. punctata ponds have very low concentrations, 0.09 μMP. These values differ significantly at the 95% confidence limit.

The phosphate concentrations in L. turionifera - S. polyrhiza mixed ponds are intermediate, 0.75 μM, but did not differ significantly from the concentrations in ponds with monocultures of these two species. The L. turionifera-W. punctata mixed ponds also had intermediate phosphate concentrations (0.227μM) as compared to the the ponds with monocultures of these species. In this case, the mixed ponds differed at the 95% level from the W. punctata ponds, but not the L. turionifera ponds.

The L. minor/L. turionifera species ratio was largest in ponds with a mean of 0.87 μMP, but the small number of samples makes the variance too high for meaningful statistical differences. W.

TABLE XV

Phosphate Concentrations in Surface Samples from Lemnaceae Ponds. Spring and Summer, 1981 - 1982.

Species[a]	Mean µM P	SE	Number Of Samples	Number Of Ponds
Sp	0.922	0.292	25	2
Sp-Lt	0.751	0.314	35	7
Lt	0.358	0.075	28	6
Lt-Wp	0.227	0.070	26	5
Wp	0.093	0.026	36	5
Lm	0.868	0.753	4	2
No Plants	0.141	0.045	30	4

[a] Plants in ponds after abbreviations in Table XI.

columbiana is not included in the table as it is never found as a large proportion of the total plant mass, and is usually found with S. polyrhiza or occasionally with L. turionifera. Therefore, the phosphate concentrations at which W. columbiana will be found tend to be higher than those where L. turionifera is found.

Nitrate

In nitrogen-limited batch culture, Figure 17, S. polyrhiza has a higher growth rate than L. turionifera. This relationship is barely significant at the 50% level, so the growth responses can be considered equal. Though the half-saturation constant for nitrate, K_N, for S. polyrhiza, 21.5 μM, was smaller than the K_N for L. turionifera, 30.9 μM, they differed significantly at the 75%, but not at the 85% confidence limit (Table XVI).

The semi-continuous experiment (Figure 18) showed that L. turionifera had the higher growth rate, but it is not significantly different from that of S. polyrhiza even at the 75% level. L. minor is intermediate to these two species. Above 5 μM nitrate W. columbiana has a lower growth rate than the three former species. Below 5 μM nitrate its growth rate equals or exceeds that of the other three species, which normally are faster growing. W. punctata again has the smallest growth rate when nitrate is limiting, but above 30 μMN, its rate equals that of W. columbiana.

In semi-continuous culture, L. turionifera, L. minor and S. polyrhiza have K_N values near 5 μMN, and did not differ, even at the 75% level (Table XVI). These values were much smaller than found in the batch experiments. W. punctata has a significantly larger K_N

Figure 17

Growth of the Lemnaceae on limiting nitrate concentrations in batch culture, 28° C and 250 μEin/m^2·s. Daily Relative Growth Rate / ln2 = doublings per day.

Key:		Curve Y-axis Standard Error
Lemna turionifera	-·-·-·-·-·- ●	0.024
Spirodela polyrhiza	——————— ★	0.037

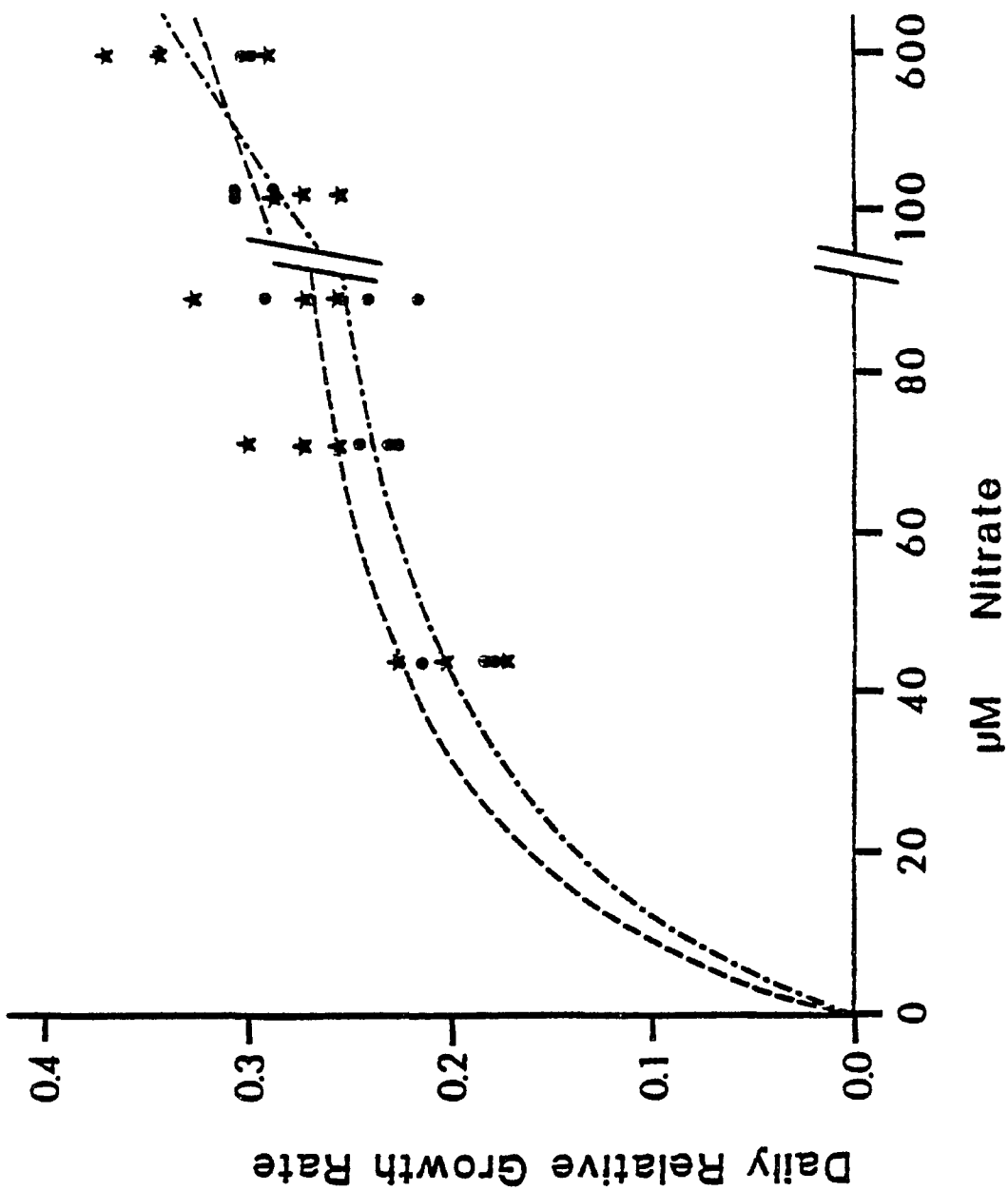

Daily Relative Growth Rate
0.0
0.1
0.2
0.3
0.4
0
20
40
60
80
100
600
µM Nitrate

FIGURE 17

TABLE XVI

Calculated-Half Saturation Constants (K_N)

and Maximum Growth Rates (μ_{max})

for Batch and Semicontinuous Nitrate Growth Experiments

Batch:	K_N μM N	75% Confidence Interval	μ_{max}[a]	90% Confidence Interval
L. turionifera	30.9	26.8-35.2[b]	0.345	0.317-0.373
S. polyrhiza	21.5	16.6-16.9[b]	0.334	0.298-0.369
Semicontinuous:		**95% Confidence Interval**		**95% Confidence Interval**
L. turionifera	5.4	4.9- 5.9	0.442	0.422-0.463
L. minor	5.0	4.6- 5.5	0.394	0.371-0.418
S. polyrhiza	5.5	5.0- 6.2	0.374	0.351-0.397
W. punctata	10.0	8.0-12.1	0.240	0.205-0.238
W. columbiana	3.1	2.5- 3.7	0.197	0.183-0.209

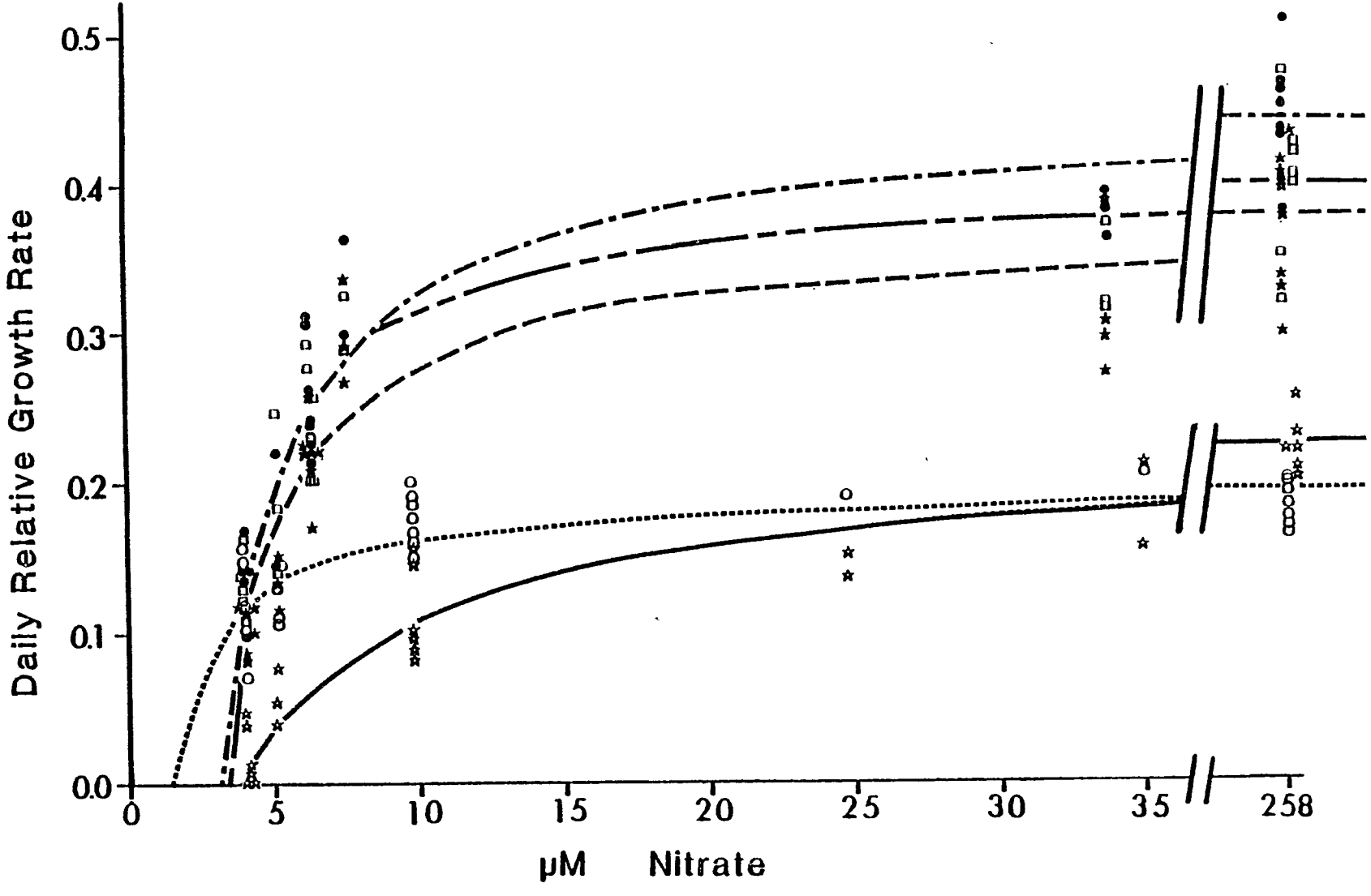
[a] Daily Relative Growth Rate, DRGR/ln2 = doublings/day.
[b] 85% confidence intervals overlap.

Figure 18

Growth of the Lemnaceae at limiting nitrate concentrations in semi-continuous culture, 28° C, 250 μEin/m^2·s. Daily Relative Growth Rate / ln2 = doublings per day.

Key:		Curve Y-axis Standard Error
Lemna turionifera	●—·—·—·—	0.038
Lemna minor	□— — —	0.041
Spirodela polyrhiza	★— — —	0.043
Wolffia punctata	☆———	0.025
Wolffia columbiana	○·····	0.023

FIGURE 18


Daily Relative Growth Rate
0.5
0.4
0.3
0.2
0.1
0.0
0
5
10
15
20
25
30
35
258
µM Nitrate

of 10 μMN, while W. columbiana has a significantly smaller K_N of 3.1 μMN at the 95% confidence limit.

The μ_{max} in semi-continuous culture (Table XVI) for L. turionifera, L. minor and S. polyrhiza ranged from 0.374 to 0.442 and were equal at the 95% confidence limit. The μ_{max} of the two Wolffia species are significantly smaller than the μ_{max} of the other species. W. columbiana has a lower μ_{max} than W. punctata at the 95% confidence limit. This was why the growth rate of W. punctata approaches that of W. columbiana at 30 μM nitrate.

All of the ponds had equal surface concentrations of combined soluble inorganic nitrogen (nitrate, nitrite and ammonia) at the 95% level, except for the W. punctata ponds, which had a lower nitrogen concentration of 2.2 μMN (Table XVII). The concentrations in the W. punctata ponds do overlap those of the W. punctata - L.turionifera mixed ponds and the "no plant" ponds.

Rainwater

Rainwater can be an important source of combined soluble nitrogen (NH_4, NO_2 and NO_3) as indicated in Table XVIII. The mean total inorganic nitrogen in rainfall is 52 μMN, much higher than the nitrogen concentrations found in the surface waters of these ponds. This indicates that rainfall is capable of significantly increasing the inorganic nitrogen supplies in these ponds. The concentration of phosphate in the rain about equals that of the pond water and would have little effect. The iron concentration is much lower than that of the ponds, and may have a dilution effect as long as rainfall exceeds evaporation.

TABLE XVII

Combined Soluble Inorganic Nitrogen (NO_2+NO_3+NH_4) in μM N for Lemnaceae Ponds, 1982.

Species[a]	Mean	SE[b]	Number of Samples	Number of Ponds
Lt	11.8	5.9	28	6
Lt+Wp	4.3	3.4	18	4
Wp	2.2	1.1	14	3
Sp-Lt	17.3	12.9	15	1
Sp	16.7	10.0	16	1
No plants	6.0	4.9	16	1

[a] Plants in ponds, abbreviations after Table XI.
[b] Standard error.

TABLE XVIII

Rainwater Nutrient Data, May 30 - July 26, 1982

Values in μM

	NH_4	$NO_3 + NO_2$	CN[a]	PO_4	Fe
Mean	13.7	39.95	52.4	0.40	0.09
SD[b]	7.4	15.3	13.3	0.23	0.14
Samples	16	16	16	14	6
SE[c]	7.7	3.7	6.6	0.12	0.11

[a] Combined soluable inorganic nitrogen (NH_4, NO_2 and NO_3).
[b] Standard deviation.
[c] Standard error.

Dialysis Samples

The nutrient concentrations, as determined by dialysis sampling are given in Table XIX. Few samples were available for each sample site, so the determination of statistical differences is not, for the most part, possible. The data do show that the combined soluble inorganic nitrogen at the surface ranges from 2.4 to 7.4 µM, and did not differ significantly from pond to pond.

The surface iron concentrations were significantly smaller in the S. polyrhiza pond (0.79 µM) than in the L. turionifera pond (1.22 µM) at the 95% confidence limit. The "no-plant" pond had less iron, 0.45 µM, than the L. polyrhiza pond at the 85% limit. The other ponds have iron concentrations equal to or higher than those of the L. turionifera pond. These relationships agree with the filterable iron data adjusted to dialyzable iron.

As with iron, the phosphate concentrations were lower in the dialyzed samples than in the filtered samples. Unlike iron, the percent decrease of concentrations in the dialyzed samples were roughly equal, about 50%, in all ponds. The exception was pond 30, the S. polyrhiza pond, where the percent decrease was 75%. This may have been caused by a possible error during analysis. Omitting the questionable datum would have been sufficient to cause the percent reduction to rise from 50% to 75%. Other than the S. polyrhiza pond, the same relationships hold for dialyzed and filtered phosphate between ponds.

The smallest combined nitrogen concentration , 4.2 µM, was found at the surface and the bottom in pond 29, the W. punctata pond,

TABLE XIX

Dialysis Samples: May 30, 1982; June 8, 1982; and August 1, 1982.

Concentrations in μM with SD, Two to Five Samples Each.

Pond	$NH_4+NO_3+NO_2-N$	PO_4-P	Fe
41 Lt[a]			
Surface	2.4[b]-NA[c]	0.26± 0.03[d]	1.22± 0.21
Bottom	43.9±39.5	18.30±25.20	58.30±76.30
14 Lt-Wp			
Surface	6.1± 6.4	0.16± 0.09	3.85± 6.12
Bottom	9.5± 0.9	0.55± 0.75	183.30±18.60
29 Wp			
Surface	4.8± 2.4	0.12± 0.15	1.22± 0.81
Bottom	4.2± 0.5	0.85± 0.89	88.81±53.10
17 Lt-Sp			
Surface	6.6± 4.0	0.47± 0.29	1.34± 0.90
Bottom	64.3± 6.3	11.72±13.31	50.70±42.40
30 Sp			
Surface	3.3± 2.7	0.18± 0.12	0.79± 0.28
Bottom	73.1±54.9	6.50± 5.31	3.11± 1.56
18 No plant			
Surface	3.6± 3.8	0.06± 0.01	0.45± 0.14
Bottom	11.7± 8.8	0.32± 0.38	68.13±97.30

[a] Species abbreviations as in Table XI.
[b] Mean.
[c] Not available; only one sample.
[d] Standard deviation.

which was equal to the surface concentration. In all the other ponds there is a larger concentration of combined nitrogen at the bottom than at the surface. The next smallest combined nitrogen concentrations at the bottom, about 10 μMN, are found in ponds 14 and 18, the L. turionifera - W. punctata and "no plant" ponds. The other three ponds all had very high nitrogen concentrations of 40 to 70 μMN. The bottom interstitial phosphate concentrations were very small, ranging from 0.55 to 0.85 μM, in ponds 18, 14 and 29: the "no-plant", L. turionifera - W. punctata and W. punctata ponds. The other three ponds containing L. turionifera and/or S. polyrhiza had high phosphate concentrations of 6 to 18 μMP.

The only pond bottom interstitial water deficient in iron was pond 30, the S. polyrhiza pond, which only had 3 μMFe. The other ponds, including the "no plant" pond, had much higher iron concentrations of 60 to 90 μMFe. The W. punctata - L.turionifera pond (14) had an even higher concentration of 183 μMFe.

Wolffia Flotation

In the high-phosphate medium (Figure 19A), almost 100% of the W. columbiana plants, originally from inactive cultures, return to the surface within three days in the high light conditions, but only 10% ever float in the low light conditions. It took 9-12 days for most of the W. punctata in high light to refloat, and 20-30 days for the W. punctata plants in low light to float. Plants from active cultures tended to float 2 to 3 days earlier than those from inactive cultures.

Reflotation in phosphate-free media is shown in Figure 19B.

Figure 19

Wolffia reflotation as a function of time. Wolffia that sank from actively growing and inactive cultures were incubated in high and low phosphate media and high and low light. The plants were counted and removed as they floated. The percent of the original inoculum having already floated is plotted against time. Figure 19A is for high phosphate media, and Figure 19B is for phosphate-free media.

Key:

Figure 24a: High Phosphate Media

Wolffia punctata (inactive) - high light ○

Wolffia punctata (active) - high light ◉

Wolffia punctata (inactive) - low light □

Wolffia punctata (active) - low light ×

Wolffia columbiana (inactive) - high light ●

Wolffia columbiana (inactive) - low light ★

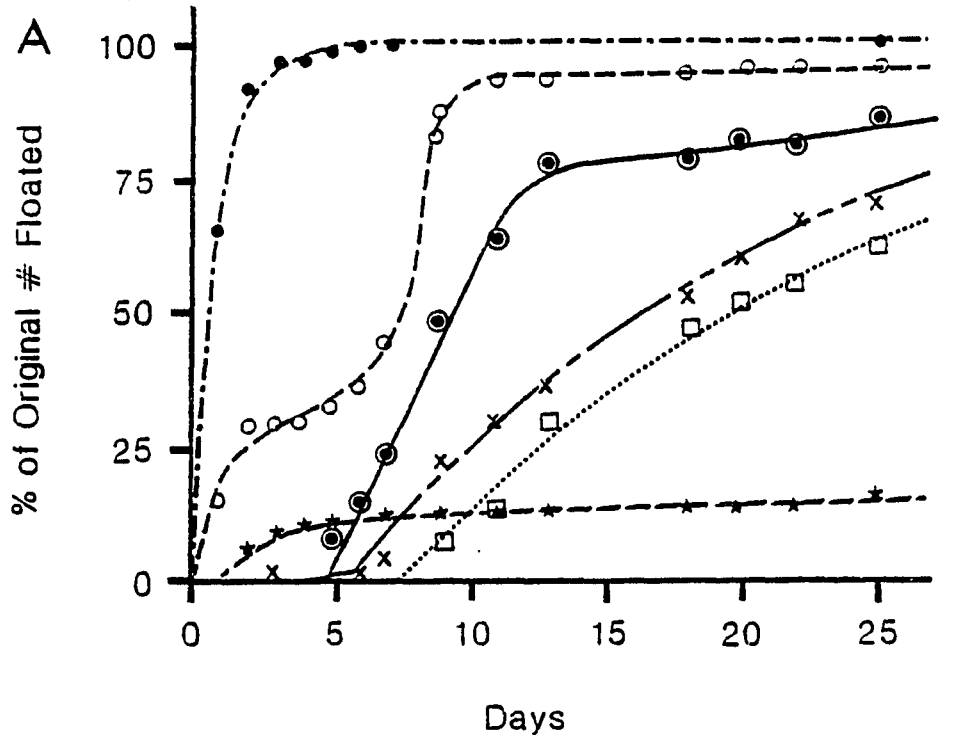
Figure 24b: Phosphate-free Media

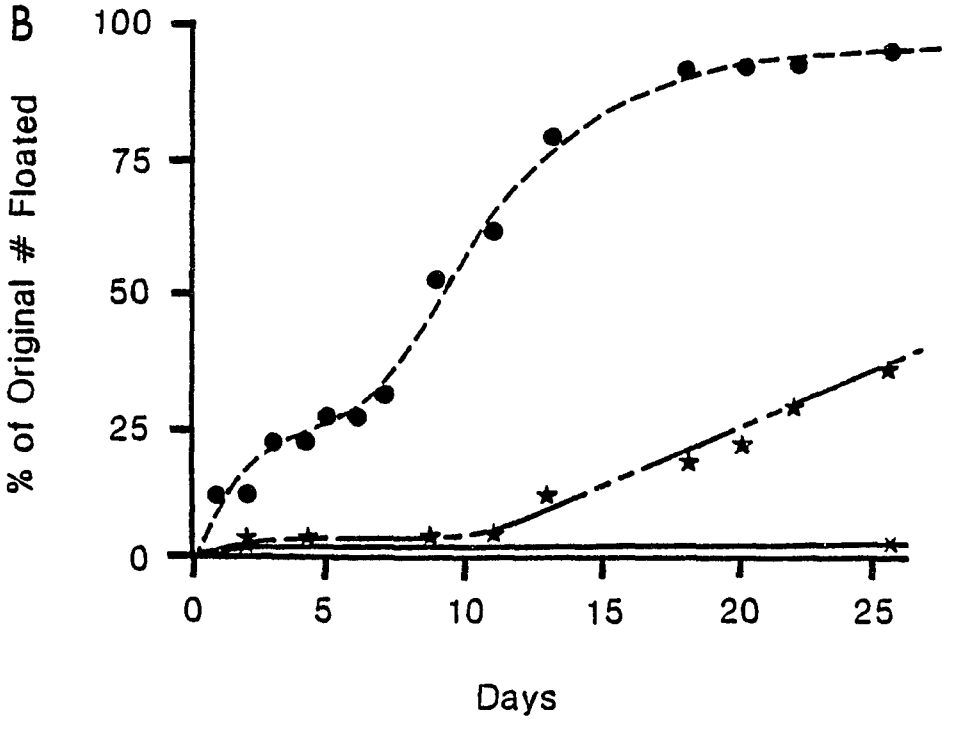
Wolffia punctata (active) - high light ●

Wolffia punctata (active) - low light ★

Wolffia columbiana (inactive) - low light ×

FIGURE 19


A
100
75
50
25
0
% of Original # Floated
0
5
10
15
20
25
Days


B
100
75
50
25
0
% of Original # Floated
0
5
10
15
20
25
Days

Only W. punctata plants from active cultures refloated, and at only half the rate as in the high phosphate concentrations. Again, the flotation rate was slower at low light than at high light intensities.

The growth of refloated plants in a phosphate-free medium was given as an estimate of the growth potential of stored nutrients (Table XX). W. punctata plants, originally from active cultures, increased their numbers 6.3 times in a phosphate-free medium after refloating in a high-phosphate medium, while they only increased 3.6 times after refloating in a phosphate-free medium. Plants originating from inactive cultures and refloated in a high-phosphate medium increased their numbers 3.0 times when reflotation occurs in high light, compared to an increase of 6.4 times when reflotation occurs in low light.

W. columbiana only refloated in high phosphate media. Although most of the plants refloated within 3 days, they only increased their numbers 2.6 times in a phosphate-free medium.

Biomass Quotas

The biomass quotas, Q_b, of nitrogen and phosphate, the Q_bN/Q_bP ratios, and the K_N/K_P ratios for each species are given in Table XXI. The species, listed in the order of increasing Q_bN values, are: W. columbiana (0.08 μM/mg), L. minor (0.12 μM/mg), L. turionifera (0.14 μM/mg), S. polyrhiza (0.14 μM/mg) and W. punctata (0.17 μM/mg). Listed in the order of increasing Q_bP, the species are: L. turionifera (0.006 μM/mg), L. minor (0.008 μM/mg), S. polyrhiza (0.011 μM/mg), W. columbiana (0.016 μM/mg) and W. punctata

TABLE XX

Growth of Freshly Floated Wolffia punctata and Wolffia columbiana in Phosphate-Deficient Media.

Plants	Flotation Media	Flotation Light Intensity	Population Increase Factor	Mean	SD[a]
Inactive Wolffia punctata	HiP	Hi	3.00 2.41 3.55	2.99	0.57
		Lo	7.33 5.51	6.42	1.29
Active Wolffia punctata	HiP	Hi	6.25		
		Lo	5.55 7.00	6.27	0.73
	LoP	Hi	3.33 3.60 3.33	3.56	0.32
		Lo	4.00		
Inactive Wolffia columbiana	HiP	Hi	2.48	2.62	0.19
		Lo	2.75		
	LoP	Hi	0.00(Died)	0.00	

[a] Standard deviation.

TABLE XXI

Biomass Quotas (Q) and Ratios (Q_bN/Q_bP)
and Half-Saturation Constant Ratios (K_N/k_P)
for Five Species of the Lemnaceae.
Biomass Quotas are Measured at Half the Maximum Growth Rate
Quotas as μ moles/mg Wet Weight.

Species	Q_b N[a]	Q_b P[b]	Q_bN/Q_bP	K_N/K_P
Lemna turionifera	0.142	0.0060	21.5	16.9
Lemna minor	0.120	0.0084	14.3	11.8
Spirodela polyrhiza	0.140	0.0108	13.0	13.9
Wolffia punctata	0.169	0.0176	9.6	3.0
Wolffia culumbiana	0.083	0.0164	5.1	1.6

[a] N = Nitrate
[b] P = Phosphate

(0.018 μM/mg).

The ranking of the species according to increasing Q_bN/Q_bP ratios of their biomass quotas is: W. columbiana (5.1), W. punctata (9.6), S. polyrhiza (13.0), L. minor (14.3) and L. turionifera (21.5). Though some of the species have equal biomass quotas, none seem to have equal Q_bN/Q_bP ratios. These same relationships are also reflected in the K_N/K_P ratios for these species, except that S. polyrhiza has a higher ratio (13.9) than L. minor (11.8). Even though W. punctata has a low N/P ratio, its biomass quotas are higher than for any other species.

Competition

In the limiting phosphate condition, N/P = 150, L. turionifera had nearly twice the growth rate (Table XXII) of S. polyrhiza when they are grown together in competition. L. turionifera also inhibited growth of S. polyrhiza 42%, while S. polyrhiza inhibited L. turionifera only 18% as compared to growth in monocultures. These differences were significant at the 95% level.

When both phosphate and nitrogen were moderately limiting, N/P = 2.7, the differences noted in the phosphate-limited media were smaller, but still significant at the 95% level.

In the nitrogen-limited condition, N/P = 0.2, while the growth rate of L. turionifera in competition (0.141) was still significantly higher than the growth rate of S. polyrhiza (0.129) at the 95% level, the inhibition of each species by the other was equal, about 23%.

The final fresh weight data (Table XXIII) show the same

TABLE XXII

Competition for Nitrogen and Phosphorus

by Lemna turionifera and Spirodela polyrhiza: Growth Data

N/P[a]	Species	DRGR[b]	±SE[c]	n[d]	% of Monoculture	±SE
150	Lt	0.191	±0.007	3		
	Sp	0.162	±0.014	3		
	Lt(Sp)[e]	0.157	±0.009	9	82.2	±4.9
	Sp(Lt)	0.094	±0.018	9	58.0	±11.1
2.7	Lt	0.297	±0.011	4		–
	Sp	0.229	±0.032	4		
	Lt(Sp)	0.230	±0.015	12	77.4	±5.1
	Sp(Lt)	0.153	±0.008	12	66.8	±3.5
0.2	Lt	0.277	±0.029	4		
	Sp	0.197	±0.016	4		
	Lt(Sp)	0.212	±0.024	12	76.5	±8.7
	Sp(Lt)	0.153	±0.029	12	77.7	±14.2

[a] Nitrogen to Phosphorus ratio.
[b] Daily Relative Growth Rate, DRGR/ln2 = doublings/day.
[c] Standard deviation.
[d] Number of replicates.
[e] Growth data for L. turionifera in competition with S. polyrhiza, species abbreviations as in Table XI.

TABLE XXIII

Competition for Nitrogen and Phosphorus

by Lemna turionifera and Spirodela polyrhiza.

Final fresh weight data.

N/P[a]	Species	Wet Wt g Mean	SE[c]	Mean % of Monoculture	SE
150	Lt	0.1694			
	Sp	0.1699			
	Lt(Sp)[b]	0.1486	±0.0100	88	±6
	Sp(Lt)	0.0714	±0.0124	42	±7
2.7	Lt	0.1727			
	Sp	0.1751			
	Lt(Sp)	0.1661	±0.0116	96	±7
	Sp(Lt)	0.1398	±0.0348	74	±9
0.2	Lt	0.1639			
	Sp	0.1854			
	Lt(Sp)	0.1406	±0.0128	86	±7
	Sp(Lt)	0.1288	±0.0070	70	±4

[a] Nitrogen to Phosphorus ratio.
[b] Growth data for L. turionifera in competition with S. polyrhiza, species abbreviations as in Table XI.
[c] Standard error.

general trend as do the growth rate data. In competition at N/P = 150, the difference in inhibition between the species was 46% for fresh weight data, as compared to 24% for the growth rate data. On the other hand, the inhibition of the two species does not quite become equal in the limiting nitrogen condition, though there is a trend in that direction. Meanwhile, the fresh weights of the two species while in competition are equal except in the phosphate limiting condition. Here L. turionifera has twice the biomass of S. polyrhiza. The wet weights of the two species in monoculture are equal, while the relative growth rate of L. turion ifera was always greater.

The results for competition between L. turionifera and W. punctata for phosphate and nitrate are given in Table XXIV. The growth rate results were so extreme that final wet weight determinations were not neccessary. At all three N/P ratios L. turionifera grows in competition as if W. punctata were not present. In fact, there is some evidence that the presence of W. punctata stimulates L. turionifera. In competition, W. punctata does not grow in the limiting phosphate condition and grows very slowly, with a daily relative growth rate of about 0.013 in the other two conditions. In monoculture, W. punctata grew very poorly in the limiting phosphate condition, but grew near half its maximum growth rate (0.13 to 0.14) in the other two conditions.

These results show that L. turionifera out-competes S. polyrhiza for phosphate, but that the two species can coexist under limiting nitrogen conditions. L. turionifera is competitively superior to W. punctata for either nitrogen or phosphate.

TABLE XXIV

Competition for Nitrogen and Phosphorus

by *Lemna turionifera* and *Wolffia punctata*: Growth Data

N/P[a]	Species	DRGR[b]	±SE[c]	n[d]	% of Monoculture	±SE
150	Lt	0.191	±0.007	3		
	Wp	0.005	±0.007	3		
	Lt(Wp)[e]	0.207	±0.013	3	108.3	±7.9
	Wp(Lt)	0.005	±0.005	3	200.0	±115.5
2.7	Lt	0.297	±0.025	4		
	Wp	0.137	±0.020	4		
	Lt(Wp)	0.300	±0.026	4	101.0	±8.8
	Wp(Lt)	0.012	±0.017	4	8.8	±12.4
0.2	Lt	0.277	±0.029	4		
	Wp	0.125	±0.027	4		
	Lt(Wp)	0.291	±0.015	4	105.1	±5.4
	Wp(Lt)	0.015	±0.004	4	12.0	±3.2

[a] Nitrogen to Phosphorus ratio.
[b] Daily Relative Growth Rate, DRGR/ln2 = doublings/day.
[c] Standard error
[d] Number of replicates
[e] Growth data for *L. turionifera* in competition with *W. punctata*, species abbreviations as in Table XI.

TABLE XXV

A Test for Allelopathy

Between Lemna turionifera and Spirodela polyrhiza.

Values as Log-Normal Daily Relative Growth Rates.[a]

Source of Media[b]	Plant Tested	
	S. polyrhiza	L. turionifera
S. polyrhiza	0.260	0.301
L. turionifera	0.239	0.275
Fresh	0.197	0.212

[a] Daily Relative Growth Rate, DRGR/ln2 = doublings/day.
[b] N and P added to media to offset depletion.

Allelopathy

The only species pair tested for allelopathy was *L. turionifera* and *S. polyrhiza*, and the result is presented in Table XXVI. There was no evidence of inhibition of either species by the other. In fact, the conditioned medium had a stimulatory effect when compared to fresh media. Medium conditioned by *S. polyrhiza* may be slightly more stimulatory.

Field Transplants

The results of the transplant experiment, originally intended to be a field competition experiment, are given in Table XXVI. The results were roughly estimated in the field as the percentage of the enclosure surface covered by each species. Generally, a species either disappeared from an enclosure, barely maintained its population, or grew to cover the surface. Several enclosures failed as their sides were apparently cut by some organism. The results were confounded by the fact that species tended to refloat from the bottom and grow in cages they were to be excluded from. The refloating of plants was the least problem in the *L. turionifera* pond since most of the turions had surfaced before the middle of June. *S. polyrhiza* turions had surfaced by the end of June. The problem was most extreme in the *W. punctata* pond, where they seemed to surface throughout the experiment.

The most definite growth response was in pond 18, the "no plant" pond, where no species grew. *W. punctata* disappeared overnight, presumably eaten by small fish fry observed in some of the

TABLE XXVI

Transplant Experiment

Pond/ Species	Enclosure Inoculum[a]	End of June	End of July	End of August
41/Lt	Lt	10%Lt[b]	10%>Lt[b]	No plants
	Sp	10%Sp+40%Lt[c]	10%Sp+50%Lt	2%Sp+98%Lt
	Wp	10%Wp+50%Lt[d]	20%Wp	Enclosure lost
	Lt-Sp	20%Lt+10%Sp	No plants	Enclosure lost
	Lt-Wp	30%Lt+10%Wp	50%Lt+20%Wp	99%Lt+1%Wp
	Sp-Wp	5%>Sp+5%Wp	1%>Wp+1%Sp	Enclosure lost
29/Wp	Lt	10%Lt[b]	1%Lt+80%Wp	100%Wp
	Sp	10%Sp+5%Wp	1%>Sp+80%Wp	1%>Sp+99%<Wp
	Wp	50%Wp	100%Wp	100%Wp
	Lt-Sp	10%Sp+10%Lt+20%Wp	20%Lt+50%Wp	100%Wp
	Lt-Wp	10%Lt+50%Wp	10%Lt+90%Wp	100%Wp
	Sp-Wp	10%Sp+50%Wp	5%>Sp+95%<Wp	1%>Sp+99%<Wp
30/Sp	Lt	30%Lt+30%Sp[e]	100%algae	Not observed
	Sp	80%Sp[f]	1%>Sp[g]	Not observed
	Wp	10%Wp+30%Sp[e]	1%>Wp[g]	Not observed
	Lt-Sp	25%Lt+45%Sp	100%Sp	Not observed
	Lt-Wp	30%Lt+5%Wp	1%>Wp+Chora	Not observed
	Sp-Wp	30%Lt+5%Wp	20%Sp+1%>Wp[g]	Not observed
18/ No plants	Lt	10%Lt	1%>Lt	Not observed
	Sp	10%Sp	1%>Sp	Not observed
	Wp	None[h]	None[g]	Not observed
	Lt-Sp	10%Lt+10%Sp	5%Lt+1%>Sp	Not observed
	Lt-Wp	Enclosure lost	Enclosure lost	Not observed
	Sp-Wp	10%Sp[h]	1%>Sp	Not observed

[a] Inoculum = 10% of surface; 5% for each species if two species were used, species abbreviations as in Table XI.
[b] Roots were tangled, and most of the plants were pulled below the surface.
[c] Reduced L. turionifera to 5% of the surface.
[d] Removed all L. turionifera.
[e] Removed S. polyrhiza.
[f] Removed one-half of S. polyrhiza.
[g] An algal mass covered the remainder of the surface.
[h] W. punctata disappeared overnight.

cages. The numbers of L. turionifera and S. polyrhiza remained virtually unchanged for the first month, and were only reduced in the second month. No observations were made of this pond in the third month.

In the other ponds, only large populations of the species native to each pond developed in the enclosures. The non-native species maintained their populations for a month, after which they declined. The exception was W. punctata in pond 41, the L. turionifera pond. Here, the W. punctata population was growing slowly through the second month, but the third month's observation was lost due to failure of the enclosure.

Of the species native to the ponds, W. punctata was the most consistently successful. By the end of the experiment in its own pond, 29, it attained 100% cover in all six enclosures. This might be attributed to the reflotation of plants originating from the bottom. In their own ponds, L. turionifera attained 100% cover in only two enclosures and S. polyrhiza in only one enclosure. Note that these two species did not attain appreciable biomass in cages they had been inoculated as monocultures.

In the enclosures where plant masses did not develop, an algal scum covered the surface or submerged macrophytes grew to the surface as in ponds 18 and 30, or the bottom became oxidized (rust brown) and covered with algae as in pond 41.

CHAPTER V

DISCUSSION

Light

There were significant differences in the efficiency of light utilization between the species of the Lemnaceae. Spirodela polyrhiza has the lowest K_L, therefore the highest efficiency, for light, around 20 - 25 $\mu Ein/m^2 \cdot s$, while Wolffia punctata has the highest K_L for light, at 86 $\mu Ein/m^2 \cdot s$. These values are significantly different from those for L. minor, L. turionifera, and W. columbiana, which fall between 35 - 47 $\mu Ein/m^2 \cdot s$. Even though the latter three species do not have significantly different K_L values, Lemna minor has lower K_L values than L. turionifera or W. colombiana. The species approach light saturation growth rates at light intensities roughly four times higher than their K_L values. These light saturations are of the same order of magnitude as that found by White (1936-37) of 100 $\mu Ein/m^2 \cdot s$ for Lemna sp. and by Krezechowska et al. (1975) of 150 $\mu Ein/m^2 \cdot s$ for L. minor.

According to the K_L data, one would predict that S. polyrhiza would outcompete the other species in shady habitats, while W. punctata would be at an extreme disadvantage. As the maximum growth rates are not equal, notably higher for L. turionifera and low for the Wolffias, a direct comparison of growth rates over a light gradient (Figure 5) between species must be made. S. polyrhiza

has a weakly significant growth rate advantage at low light levels, but the higher maximal growth rate of L. turionifera limits this advantage to light levels below 50 μEin/m^2·s. W. punctata's low growth rate compounds its low efficiency for light, but above 300 μEin/m^2·s, its growth rate equals and eventually exceeds that of W. columbiana.

Though there are seemingly significant differences between these species in their utilization of light, one must ask whether these differences are biologically significant, and whether they affect species distributions.

The range in which S. polyrhiza has a growth advantage is below 50 μEin/m^2·s, about 3% of direct sunlight, an intensity expected near shore under overhanging brush, or in small forest ponds heavily shaded all day. The only two heavily shaded ponds to develop a complete cover of plants contained Lemna, not Spirodela. These were the only ponds in which L. minor approached parity with the population of L. turionifera. As the two Lemna species have virtually identical growth rates at low light intensities, some factor other than light must be determining species distribution in these shady ponds. Though only two shady ponds were studied, many were noted, and all shady ponds that contained appreciable plant masses contained Lemna sp. It would be interesting to determine the L. turionifera / L. minor species ratios for these ponds.

The other ponds that developed a complete plant cover have the greater part of their surfaces exposed to direct sunlight for a great portion of each day. Thus, the light available is in excess of the 300 μEin/m^2·s level, above which the maximum intrinsic

growth rates determine the differences between species, and L. turionifera has the advantage. If light limits species distribution between ponds, L. turionifera should dominate all ponds. Though L. turionifera does dominate many ponds, there are many mixed ponds, W. punctata-dominated ponds, and a few S. polyrhiza-dominated ponds. Thus, some factor other than light is responsible for inter-pond distributions of the Lemnaceae.

There is the possibility that intra-pond gradients of light could produce species gradients. In ponds with tall trees to the south, a strip of pond along the southern shore could be maintained below the 300 $\mu Ein/m^2 \cdot s$ level for a large part of the day. At such low light levels, the Lemnaceae do react to light differentially. Evidence of a species gradient in response to a light gradient is given in Table V for pond 14. In 1981, the species order, from the center of the pond to shore, was W. punctata, L. turionifera, W. columbiana, L. minor, and L. trisulca. This is the same gradient which would be predicted from K_L data (Table IV), with L. trisulca taking the place of S. polyrhiza. S. polyrhiza may have been excluded from this pond by factors other than light. L. trisulca is also very efficient at utilizing light (Keddy, 1976), as it floats below the surface of the water and often grows well under three or more layers of floating Lemnaceae. In pond 14, it is found under the other plants throughout the pond, but near the southwest shore, it forms thick masses over which no other Lemnaceae are floating. Thus, it grows alone in areas where other Lemnaceae cannot grow, obstensibly sively because of the low light conditions. In 1982, the gradient was generally repeated, but W. columbiana and L.

turionifera were more evenly distributed over the range where they coexisted, and no determinations were made to distinguish L. turionifera and L. minor.

The change from W. columbiana to W. punctata domination occurred both years when the average light intensity between shade and sun patches exceeded 300 $\mu Ein/m^2 \cdot s$. This corresponds to the light intensity above which W. punctata grows faster than W. columbiana in my experiment (Figure 5). It should also be noted that when a W. punctata layer is disturbed, often a layer of W. columbiana is revealed underneath. Thus, W. columbiana may gain an advantage in nature over W. punctata in low light situations.

The Lemnaceae, as a group, seem adapted to low light intensities with light saturations occurring at about 10 to 20% of full sunlight. This is somewhat lower than the 20 to 30% of full sun saturation level for shade-adapted terrestrial plants (Shirley, 1929; Kramer and Decker, 1944; Zelitch, 1971) but comparable to the saturation of three diatoms (Talling, 1957) at 5 to 14% of full sunlight. The Lemnaceae are well below the light saturation levels of 30 to 60% for sun-loving crop plants (Shirley, 1929; Kramer and Decker, 1944; Zelitch, 1971; Bjorkman, 1973), or the 100%+ sunlight saturation of C_4 plants (Black, 1973; Zelitch, 1971; Bjorkman, 1973). These facts seem to indicate that the Lemnaceae are well-adapted to living in the shade of reeds (grasses and sedges) and trees at pond edges. This may seem incongruous for a family whose members often grow to cover ponds exposed to full sunlight.

The reason for this low light adaptation may be related to maintenance of marginal populations of Lemnaceae in otherwise hostile

ponds. Submerged plants may force the pH up during photosynthetic CO_2 uptake while decreasing nutrient concentrations through uptake and/or precipitation. There may also be fish present, providing significant herbivory upon Lemnaceae. At the pond margins, roots of the Lemnaceae can reach the nutrient-rich soils of low pH while reeds protect the plants from fish herbivory. While colonizing a pond, the Lemnaceae may need to tolerate low light levels at pond edges. This might explain their relatively low light requirements.

Even though there are species differences in growth rate at low light intensities, light does not seem to determine species distribution of the Lemnaceae between ponds. There is some evidence that species-light gradients can form in mixed species ponds. The generally low light saturation levels of the family may be an adaptation to aid the invasion of "no plant" ponds by allowing the colonization of their shores.

Temperature

The temperature optima for the five Lemnaceae species tested fall near 30° C (Figure 7). The optimum for L. minor of 27 to 30.5° C agrees well with the 29° C optimum found by Ashley and Oxley (1935) for that species. Of the other species, S. polyrhiza has the widest and highest optimum, extending from 25 to 33° C, while that of W. colombiana is comparable from 27 to 33° C. L. turionifera has the narrowest optimum, with an increasing growth rate to 29° C and a rapid decrease above 30° C. All of the species of the present study would be classified in the higher temperature range for the family by Landolt (1957). According to Landolt (1957), S. oligorhiza, L.

valdivana and L. trisulca had optima in the lower temperature range near 23° C. The latter species is the only low temperature species found in my study ponds, but since it is a submerged species, it was not included in this study.

Temperature optima found by Landolt (1957) for the Lemnaceae are wider and lower than mine. This may be because he performed his experiments at 50 $\mu Ein/m^2 \cdot s$, well below light saturation, and the results are comparable to my low light temperature curves. Landolt could find no species differences for temperature optima or maximum tolerance unless he added 1 to 2% sugar to the medium. I achieved the same results as Landolt for the maximum tolerable temperature by increasing light intensity to near saturation. In my experiments, the temperature optima were narrower and higher than Landolt's. Because Landolt's plants were light-limited, they could not reach their maximum growth potentials at optimum temperatures. Thus, his experiments showed no distinct optima until sugar was added. Cessation of growth at the maximum tolerable temperature may be due to enzyme denaturation, as suggested by the sharp decrease in growth rates.

The five species studied here have such overlapping optima that it is only at very high temperatures, over 32° C, do the growth rate relationships between the species differ from the maximum intrinsic growth rate relationships. The maximum intrinsic growth rate relationships are as follows: L. turionifera > L. minor > S. polyrhiza > W. punctata > W. columbiana. Above 32° C, these relationships change and are reflected in the maximum temperature tolerances, determined by extrapolating the descending growth curves

to zero growth. The maximum tolerances are as follows: S. polyrhiza, 38° C; W. columbiana, 36° C; W. punctata, 35° C; L. turionifera, 33° C; and L. minor, 32.5° C.

The maximum tolerable temperatures I found for L. minor, L. turionifera, and S. polyrhiza agree well with the results of Landolt (1957). My values are generally 1° C higher than Landolt's, possibly because I actually took the temperature of the culture media rather than the culture chamber temperature. The closeness of the maximum tolerable temperature for S. polyrhiza , 37° C as reported by Landolt (1957) and 38° C reported here, supports the validity of my extrapolation of the curves to zero growth.

It should be noted that all of Landolt's species were generally similar as to growth rate response of different strains to temperature, except for Lemna minor. Strains from Europe, the southern U.S., and the California coast were homogeneous, with maximum temperature tolerance agreeing with my results. Three clones from the eastern U.S. (Maryland, New Jersey, and Pennsylvania), had a maximum temperature tolerance of 35° C, above that of L. minor or L. turionifera in either of our studies. This ecotype might be a separate or mis-identified species. It does indicate that investigators, even when studying such clonal species, should test the physiological characteristics of their local species rather than depend upon published data for that species collected elsewhere. One should also test many strains from each locale, although this was not accomplished in this study. As Landolt's data indicate, ecotype distribution is clinal and not random.

The differential maximum tolerable temperatures may cause

a selective effect between species under the heat of the full summer sun. Hillman (1961) indicated that temperatures over 30° C are easily attained in masses of Lemnaceae under full sun. Table XII shows that on sunny summer days the temperature in the surface centimeter of plants and water was often over 30° C and occasionally reached 34° C. The days these data were taken had maximum air temperatures of 30° C. Higher plant mass temperatures would be expected on warmer days, a common experience in July and August. Also, the measurement method mixed plants and water. Casperson (1956) showed that with incandescent light of 5 to 6% the intensity of full sunlight, the fronds of S. polyrhiza could be 1 to 3° C warmer than the media. This effect would not be so great in my laboratory experiments run with cool white fluorescent bulbs deficient in infared light, but would be expected in sunlight. Lemnaceae plants in ponds may be exposed to temperatures near the 38° C tolerance of S. polyrhiza in late summer. Therefore, in late summer, the growth rate of less tolerant species, L. minor and L. turionifera, should decrease, while the growth rate of more tolerant species, S. polyrhiza and W. columbiana, would be maintained. This is potentially important for the two latter species since they normally have lower growth rates than L. turionifera.

Most of my ponds containing a continuous cover of Lemnaceae receive full sunlight over most of their surfaces for a large portion of the day. The effect of surface heating by the sun should then be similar for all the ponds and maximum temperature tolerances would not be expected to affect the species distributions between ponds.

Temporal succession within a pond already supporting mixed

populations might be influenced by temperature and assist in maintaining certain species, namely, S. polyrhiza and W. columbiana, in those ponds. Rejmankova (1975) postulated that overheating of the duckweed mass is the primary reason L. gibba populations die off in July in South Moravian fish ponds. Landolt (1957) found a maximum tolerable temperature for L. gibba comparable to that of L. turionifera; so, the mechanism of population reduction proposed by Rejmankova is plausible. If other species are present when the growth rate of the temperatureintolerant species' slows, and die-off occurs, they may gain a growth advantage, utilizing released nutrient resources, and a succession may occur.

Jacobs (1947) described a succession in Minnesota ponds that corresponds well to one determined by high summer temperatures and species maximum temperature tolerances. L. trisulca takes advantage of early spring, when other Lemnaceae species are still dormant on the bottom, by having a low temperature optimum. L. minor, which has the lowest temperature tolerance in my study, grows rapidly during the early summer until presumably limited by high temperatures in the late summer. S. polyrhiza, with the highest tolerance, germinates relatively late, as does L. turionifera, and grows to dominance during the hot summer months. Wolffia columbiana also has a high temperature tolerance, and develops late in the summer.

In certain of my ponds (17 and 27) S. polyrhiza seems to compete well with the Lemna complex. Here, it also appeared that the Lemna complex was dominant in the spring, while S. polyrhiza was dominant by the late summer. This further supports the hypothesis that maximum temperature tolerances may determine seasonal succession

within ponds that otherwise have conditions favorable to more than one species.

I also observed in several ponds that L. turionifera was dominant in the spring while W. punctata was dominant by late summer. This might appear to be a temperature tolerance effect, but this is unlikely since the difference between the maximum temperature of the two species is only 1.0° C. Hillman (1961) reported the same phenomenon, but ventured that W. punctata dominated when nutrients became depleted.

The argument that maximum tolerances may affect seasonal succession depends upon extrapolation of laboratory conditions to field conditions. The temperature in the laboratory was constant day and night. The 24 hour temperature profiles of ponds 14 and 17 show that though pond surfaces may become very warm during the day, they cool dramatically during the night. The decrease may be 10 or 11° C. The effect of the night cooling on maximum tolerable temperatures must be considered. Landolt (1957) found that temperature periodicity had no effect on growth rate, and that the growth rate could be predicted from the average of the day and night temperatures. If maximum temperature tolerance is determined by enzyme denaturation, nighttime cooling should not affect the mechanism of species succession caused by summer heating. Nightime cooling is important in the vertical mixing of the water column, and can affect the nutrient supply to the surface.

L. minor appears to have a significantly higher growth rate (0.06) at 10° C than L. turionifera (0.02; Figure 7). Hodgeson (1970) found the growth rate for L. minor in sunlight to be 0.179 at 12.5° C,

a much higher growth rate than indicated from my results for this species. This difference is not likely to have been caused by a systematic experimental error since the maximum growth rates for both studies were approximately equal. In my study, however, the 10° C data for L. minor were lost, and the value for this temperature was determined from an extrapolation of the combined "high" and "low" light temperature curves. An extrapolation of the "low" temperature curve (Appendix A) would provide a higher estimated growth rate of 0.10. Therefore, L. minor may have a greater growth advantage over L. turionifera than Figure 7 indicates. Landolt (1957) found that L. minor (his L. minor II) had a higher growth rate below 20° C than L. turionifera (his L. minor I). Landolt found that L. minor grew slowly at 4° C, while L. turionifera did not grow.

L. minor would then have an advantage in the early spring or in ponds shaded all day, where surface heating by the sun does not occur. The only heavily shaded ponds I found that had heavy surface cover of Lemnaceae contained the Lemna minor - Lemna turionifera complex. Though Jacobs (1947) occasionally found S. polyrhiza in shaded woodland ponds, he usually found L. minor in these ponds. Rejmankova (1975) stated that L. minor was limited to small forest, alpine, or spring-fed ponds, i.e. cool habitats, by L. gibba. It can be seen from Table XV that although L. turionifera dominates this complex with 83% of the total, in three ponds L. minor approaches parity. In one of these ponds, 6, W. punctata is dominant and the Lemna complex comprises a small percentage of the community. The other two ponds, 4 and 9, are the only two heavily shaded ponds for which this determination was made. Even though L. minor would have a

great growth advantage over L. turionifera in spring when temperatures are around 15° C, in the summer when shaded ponds might be 20 to 25° C, their growth is equal, leading to a 50 - 50 ratio. In ponds exposed to full sun, the day temperature remains above 25° C, where L. turionifera has a definite advantage. Thus, the high proportion of L. minor in the Lemna complex appears to be related to lower temperatures, resulting from low light intensities. Lemna minor may be further aided in low light ponds by its lower half-saturation constant for light.

High temperature tolerance differences between the Lemnaceae species could cause the seasonal succession observed in mixed species ponds. Relatively high growth efficiency at low temperatures may give L. minor an advantage in shady ponds. This is the only observed effect of temperature, and indirectly, of light, upon inter-pond species distribution.

Turions

An early start of growth in the spring can be advantageous to a species, especially if its temperature optimum is low. According to Landolt (1957), L. trisulca can grow below 8° C. Jacobs (1947) observed that this plant produced its biomass in the spring before the other species cover the surface.

Jacobs (1947) claims that L. minor over-winters by vegetative plants having their roots entangled in the bottom litter during the late summer drought, and is submerged when the ponds refill with fall rains. He observed them already growing on the pond surface in early May, before S. polyrhiza turions had germinated. Thus,

L. minor, which grows well at low temperatures, is able to grow early in the spring when other species are not present to compete with it. L. minor does not have to depend upon a germination cue and the disappearance of starch to float; it needs only to produce an oxygen bubble by photosynthesis and float to the surface.

Once at the surface, the plant mass temperature will be above that of pond surface water. Table XIII shows that the surface temperature in the plant masses is 3° C higher than the surface temperature in open water in early May in Michigan. With the actual plants having a temperature even a few degrees higher than the water (Casperson, 1956), one can see that the temperature of the plants can approach the optimum range above 25° C even when the ponds are still quite cool. This is a further incentive to starting growth early.

Though Jacobs (1947) observed the germination of L. turionifera turions only while S. polyrhiza turions were germinating, I have found L. turionifera turions germinating two weeks to a month before those of S. polyrhiza. The confusion may be due Jacobs inability to recognize L. turionifera as a separate ecotype, much less a separate species, and to the presence of L. minor in the pond confusing the observation of Lemna turion germination. The laboratory germination of L. turionifera at a lower temperature, 10° C, than S. polyrhiza, which begins germination above 20° C, is shown in Figure 13. Similar results were found for the pond bottom temperatures at the first observation of germinated turions (Table XIV). L. turionifera germinates first, when the bottom temperature is 11.1° C; W. punctata at 14.2° C, and S. polyrhiza at 17.9° C.

There is a strong correlation between species for the time of growth initiation in the spring and their temperature optima and maximum tolerances. The order of growth initiation would be: L. trisulca, L. minor, L. turionifera, W. punctata, and S. polyrhiza. L. trisulca and L. minor have low temperature optima and tolerance, while L. turionifera is intermediate, W. punctata higher, and S. polyrhiza the highest. Thus, S. polyrhiza may postpone its initiation of growth to minimize competition with the earlier species until temperature conditions become optimal. W. columbiana and L. perpusilla also seem to do this in Jacobs' ponds (1947). Thus, the higher a species' temperature optimum and tolerance are, the later it seems to initiate growth. This accentuates temporal succession of the mixed species ponds.

Turion formation is of some interest as relative efficiencies of formation may be important in determining the relative number of each species able to germinate in the spring.

Guppy (1895) believed that high temperatures were required for turion formation since S. polyrhiza formed turions in August. Though noting the same pattern, Jacobs (1947) found that turion formation occurred whenever conditions allowed excess photosynthate to be stored as starch. Dense starch accumulation is responsible for turion sinking (Jacobs, 1947; Landolt, 1957; and Hillman, 1961). Jacobs found turion formation when light and temperatures were high, when low nighttime temperatures offset high daytime temperatures, when temperatures were low, when CO_2 partial pressure was high, or when nitrogen was limiting. Hillman (1961) attributed turion formation studied the formation of S. polyrhiza turions under nitrate

limitation, and their subsequent germination with nitrate addition. I have found nutrient depletion to be a very consistent method of obtaining turions as long as the temperature is below 30° C. Phosphate limitation is more effective than nitrogen limitation in causing L. turionifera turions to form. In my turion formation experiment (Figure 9) S. polyrhiza, and to a lesser extent L. turionifera, had maximum turion formation at 15° C, and little turion formation above 25° C. This generally agrees with my qualitative field observations. Although S. polyrhiza forms some turions in the summer, the flush of turion formation seems to be in September.

The turion formation results show that S. polyrhiza can form twice as many turions per vegetative frond as L. turionifera. Jacobs indicates that each S. polyrhiza frond has the bud potential to form up to six turions. As the one month duration of this experiment included an initial growth period when turions did not form, continuing the experiment could provide evidence for a greater turion to frond ratio. S. polyrhiza may be able to produce more over-wintering units than L. turionifera, giving it a possible numerical advantage in the spring, to offset the later start of germination.

In the semi-continuous culture temperature experiment where nitrogen was not allowed to become limiting, S. polyrhiza fronds seemed to produce turions continuously at 15° C, while only one or two turions per frond were produced at 10° C, and none were produced at 20° C or above. Thus, nitrate limitation apears to promote turion formation at higher temperatures.

S. polyrhiza vegetative growth stops below 15° C (Landolt,

1957), the temperature where the most turions are formed, and above which cold-treated turions germinate. This denotes a tight interfacing of these three processes in S. polyrhiza.

If a species, such as L. minor or L. turionifera, could cover a pond surface while the pond temperatures were low, around 15°C, it might be able to postpone or reduce the germination of S. polyrhiza or the Wolffias. This would appear to give an undue advantage to L. minor and all ponds should be covered with this plant. First, consider that the over-wintering of L. minor seems to be a physical process, beset by chance. It is not known what percentage of the population actually manages to adhere to the bottom in the fall. As has been shown here, L. turionifera is expected to produce one turion, and S. polyrhiza more than two per frond. Thus, L. turionifera should have the same, and S. polyrhiza double, the over-wintering population as its late season population. Secondly, though L. minor vegetative plants are more cold hardy than those of S. polyrhiza, the turions of S. polyrhiza are even more cold hardy (Jacobs, 1947). If a winter is harsh, fewer L. minor plants will survive than the turions of the other species. It has been my observation that L. minor makes up a small percentage of the Lemna assemblage in Michigan and that it shows up only sparsely before the L. turionifera germination flush that almost immediately covers the pond surfaces in the late spring. Landolt (1957, 1975) indicates that L. minor is dominant in the southern U.S., where winters are mild and plants may remain active in some areas all winter. L. minor would be expected to continue to grow all winter while the growth of other species decreased (L. turionifera), stopped (S.

polyrhiza and the Wolffias), or was directed towards turion formation. Thus, in the South, L. minor may maintain a more consistent cover than the other species. However, all ponds are not dominated by L. turionifera in the North or L. minor in the South; hence, other factors, such as water chemistry or competition, may be more important.

The order of growth initiation in the spring complements high temperature tolerances in determining seasonal succession in mixed species ponds. The higher over-wintering capability of L. turionifera may allow it to dominate over L. minor in the North. However, the greater turion production of S. polyrhiza might counteract the earlier germination and superior growth characteristics of L. turionifera.

pH and Iron

L. minor and S. polyrhiza grew between pH 3.5 and 10.0 (Figure 11). Both species show a wide plateau of optimal growth, between pH 5 and 8 for L. minor and pH 5 and 9 for S. polyrhiza. This range is much wider than found in early field (Hicks, 1932; Moyle, 1948) and laboratory (Clark, 1926; Deuber, 1925) pH experiments with the Lemnaceae. My results are very similar to those of McLay (1976), who used similar culture media and methods. McLay's curves are of similar shape to mine, but the pH range of both species extends one unit higher for his data.

The growth rates shown for L. minor and S. polyrhiza in Figure 14 are quite similar up to pH 7.0, above which the growth rate of L. minor declines; the growth rate of S. polyrhiza increases slowly up to 8.5, after which it declines rapidly. The growth rate

difference is maximal at pH 8.5, but is not significant at the 95% confidence level estimated by standard errors. The difference is significant at the 90% level, indicating a possible superiority of S. polyrhiza at these high pH levels. McLay also found the growth rate of S. oligorhiza higher than that of L. minor at pH 10, but since all of the other points of the curves corresponded, he concluded that there was no significant difference between the growth curves for the two species. Though McLay used a different Spirodela species, the similarity of our results indicates a possible tolerance of Spirodela sp. to high pH.

A comparison of the limiting pH attained in batch culture (Figure 15) showed L. turionifera attained the lowest pH, with W. punctata, S. polyrhiza and W. columbiana in order of increasing pH. As L. minor was killed by severe calcium deficiency due to the excess EDTA in this experiment, no direct comparison with the other species is possible. Landolt (1975) did indicate that the mean field pH found for L. minor was 0.6 pH unit lower than for L. turionifera; thus, it would be expected to have the lowest tolerance of high pH of these five species. Though the S. polyrhiza medium had a lower attained pH than W. columbiana, the plants were still actively growing at this point, which may have been caused by the inhibitory effect of the initially high EDTA/calcium ratio. As L. turionifera, W. punctata, and W. columbiana were not affected by the calcium withholding effect of the excess EDTA, they had reached their limiting pH and had ceased growth by the end of the experiment. S. polyrhiza may have had the potential of reaching or exceeding the final pH of W. columbiana. This information indicates that the two Lemna species

are more limited by high pH than S. polyrhiza and W. columbiana. W. punctata has an intermediate tolerance to high pH. More comparative work on the tolerance of these species to high pH is needed owing to the few replicates used in this experiment.

Field data, shown in Table XI, support the high pH tolerance relationships found in the laboratory experiment. In order from the lowest to the highest mean pH, the species are: L. turionifera, W. punctata, W. columbiana, and S. polyrhiza. No separate mean was calculated for L. minor, as it was always found mixed with L. turionifera. The mean pH of S. polyrhiza was 8.75, significantly higher than the mean pH of 7.07 for L. turionifera ponds. Mixed ponds had an intermediate pH. W. columbiana also had a high mean pH of 8.30, but its range was broad and overlapped both S. polyrhiza and L. turionifera. While S. polyrhiza seems limited to high pH, W. columbiana seems better able to coexist with species at all pH values.

The high pH of S. polyrhiza ponds equals that of ponds without Lemnaceae that are full of submerged species. The pH of natural waters is controlled by the bicarbonate system (Hutchinson, 1957), and since many submerged aquatics take up HCO_3^- they can force the pH of the water to high values during photosynthesis. S. polyrhiza does not form as thick or continuous a cover as L. turionifera, allowing submerged aquatics, especially Ceratophyllum demersum, to develop. This accounts for the high pH in S. polyrhiza ponds. Evidence for submerged species causing the high pH levels in some ponds is provided by the diurnal fluctuation of pH shown by three ponds (Figure 13). The minimum pH of 7.64 occurs just before dawn in pond 18, a pond without Lemnaceae, but full of submerged species.

The pH rises nearly two units to 9.46 by late afternoon. Fluctuations in pH follow the photosynthesis cycle in this pond. Pond 17, containing a loose mixed cover dominated by S. polyrhiza and underlain by the submerged species Ceratophyllum demersum, has less pH fluctuation, 1.5 units, and a lower maximum pH of 8.22. The pH profile of a pond with tight plant cover of L. turionifera and W. punctata, pond 14, shows little diurnal change, of 0.7 units, and the maximum pH is below 7.0.

As the pond surface becomes covered, underwater photosynthesis is reduced, causing a damping of the diurnal pH cycle. In ponds with a complete surface cover several plant layers thick, as often happens in L. turionifera ponds, little diurnal fluctuation in pH is expected and the pH remains low. High pH appears in ponds where the main source of CO_2 for photosynthis is the bicarbonate system rather than the atmosphere. The near neutral pHs found for the Lemnaceae by Hicks (1932) and Moyle (1948) indicate that they may have allowed the pH samples to come to equilibrium with the air during measurement.

High pH levels have been shown, in this study, to exclude all the species of the Lemnaceae, as McLay (1974) proposed for the exclusion of L. perpusilla from portions of Lake Los Carnos containing thick growths of submerged macrophytes. A somewhat less extreme pH will allow species tolerent of high pH, such as S. polyrhiza and W. punctata, to dominate. It is not known whether the association of the loose covering of S. polyrhiza and submerged aquatics such as C. dermersum are at a stable equilibrium, or just a stage in the colonization or abandonment of the pond by the Lemnaceae.

The lower pH values at the bottom of the densely covered pond 14 (Figure 13) indicate that the maintenance of pH may be provided by bacterial action, especially CO_2 and organic acid production, in the thick organic muck. The loosely covered S. polyrhiza pond, 17, maintained a pH below 7.14 near the bottom while the Lemnaceae-free pond, 18, attained a minimum pH of 7.0 at the bottom just before dawn. If the Lemnaceae ever entirely covered these two ponds, killing the submerged plants, the pH would be expected to decrease to values associated with heavily covered ponds such as in pond 14. As high pH may exclude the Lemnaceae, a dense cover of Lemnaceae will lower the pH. This offers an interesting mechanism of pond colonization by the Lemnaceae involving an intermediate S. polyrhiza stage.

The response of species to high pH has been shown to be correlated with the species distribution of the Lemnaceae. Laboratory investigations have shown that pH is the causative agent of the correlation, but it would be desirable to determine the mechanism of this effect. Since many plants can withstand higher external pH levels than the Lemnaceae, the possibility that this effect is due to direct hydroxide toxicity is remote. Nutrient availability is known to be affected by pH. The effect of pH on nutrient uptake enzymes aside, high pH can cause the precipitation of certain nutrients, such as calcium, magnesium, phosphate and the metal micronutrients (Hutchinson 1957). Iron is extremely susceptible to precipitation due to oxidation at high pH, especially at the high oxygen concentrations associated with high pH in these ponds. It is possible that the precipitation of iron, or another metal micronutrient, is responsible for the inhibitory effect of high pH on the Lemnaceae.

The low pH optima, in the range of 4.7 to 6.0, for growth of the Lemnaceae found by early workers (Hicks, 1932; Deuber, 1925; and Clark, 1925) is associated with using inorganic iron sources without the utilization of chelators. Deuber (1925) increased the range of the pH optima to near 7.0 by the use of ferric citrate. Bitcover and Seiling (1961) also increased the growth rate of the plants between pH 7 and 8 by increasing the citrate level. Fly (1935) found that organic iron complexes allow a higher pH optimum, and that the optimum pH range can be varied according to how much iron is made available. A great increase in the pH range of the Lemnaceae was exhibited when EDTA was added as a chelator and the medium was replaced often as in the present study and that of McLay (1976). As noted, the maximum tolerated pH found by McLay (1976) was 1 pH unit higher than that found in this study. McLay also used ten times more iron than I did, which may correspond to the ten-fold increase in the hydroxide ions tolerated. Both of us noted color changes and precipitate in the media above pH 8.0 due to chelator failure and iron oxidation. This is strong evidence that the upper pH limit observed in the laboratory studies of the Lemnaceae is caused by the lack of iron availability. Data from Deuber (1925) indicate that the K_{Fe} for S. polyrhiza is on the order of 0.2 μM Fe^{++}, but to my knowledge, no comparative study of growth kinetics on iron has been made for the Lemnaceae.

Pond filterable iron concentrations, shown in Table XII, are generally low in the spring, between 0.3 and 1.0 μM Fe, and increase throughout the summer. The increase in iron is most extreme for ponds containing appreciable amounts of L. turionifera, by a factor

of about 10. Although the mixed L. turionifera - W. punctata pond had the largest factor, 36.0, the pure W. punctata pond had a factor of only 3.0. The S. polyrhiza pond had the smallest factor, 1.7, indicating that iron supply may be lower in this pond.

Data from dialysis bags buried in, and at equilibrium with, the bottom muck (Table XIX) do show that the S. polyrhiza pond has a much lower iron concentration than any other pond, only about 6% that of most of the other ponds. The W. punctata - L. turionifera pond has an extremely high bottom iron content, which is consistent with its extreme increase in iron concentrations in the pond during the growing season. High iron concentrations in the bottom muck are directly correlated with high supply rates in these ponds, indicating that iron is easily mobilized from bottom muck, even in the highly oxidized "no plant" pond.

Although the increase factor is less in the S. polyrhiza ponds, the actual iron concentrations are much lower in the L. turionifera ponds in the spring, and they equal or only slightly exceed the concentrations in the S. polyrhiza ponds in the summer. Thus, during the growth period, iron in the L. turionifera ponds is at most equal to that in the S. polyrhiza ponds. This would imply that L. turionifera is more efficient at iron utilization and that iron is not operating as the mechanism of the pH effect upon distribution.

The answer to this contradiction may be found in the "no-plant" pond, 18. This pond develops high pH and oxygen concentrations, especially during the day, which should precipitate iron, causing low iron concentrations in the pond water. Yet the iron concentration in the spring is moderately high, 0.46 μM Fe, and it

increases by a factor of 8.4, reaching 3.82 μM Fe in late summer. This exceeds the concentration in all ponds except pond 14. This situation might be explained by the presence of oxidized iron colloids suspended in the water. These would be relatively unavailable to plants, but a significant proportion could penetrate the glass fiber filters.

Samples taken with dialysis bags (Table XIX) show lower iron cocentrations. In June, dialysis samples and grab samples were taken concurrently, and a correction factor between filtered and dialyzed samples was calculated. It is not known whether this factor is constant over the whole season. As the mean dialyzed iron from three summer sample dates generally agree with the corrected values (Table XIII), the use of the correction appears to be valid. The exception is pond 14, where the summer dialyzed iron is extremely variable and higher than that calculated. It is probable that as the season progresses, the proportion of soluble iron increases in this pond. The calculated dialyzed iron concentrations should be most accurate for the early summer, as the correction was determined during this important growth period.

The "no plant" pond has a very low correction factor, only 13% of the filterable iron being dialyzable, or soluble. This indicates that most of the iron is oxidized and colloidal. The soluble iron concentrations in this pond are lower than those in the other ponds, as expected from the high pH and oxygen levels.

The L. turionifera - W. punctata mixed pond, 14, has a correction factor as low as that of the "no plant" pond, but the great increase of filterable iron causes a high calculated soluble iron

concentration by late summer. On the other hand, the pure W. punctata and L. turionifera ponds had the largest proportion of soluble iron with correction factors of 1.00 and 0.63, respectively. Both ponds containing S. polyrhiza had intermediate correction factors just lower than 0.40. This indicates that the oxidation of iron is greatest in the "no plant" pond and least in the ponds with dense L. turionifera and W. punctata cover, with S. polyrhiza ponds intermediate. This would be expected from observation of the thin cover of S. polyrhiza ponds and underlying submerged vegetation.

S. polyrhiza ponds should then have less available iron than L. turionifera ponds, as is shown in Table XIII. Although the L. turionifera ponds begin the season with less iron, by the time plants have thinly covered the surface in June, the iron concentrations are significantly higher than in the S. polyrhiza pond, with the mixed ponds having intermediate iron concentrations. This distribution denotes that the soluable iron concentration is the mechanism of the pH effect and that S. polyrhiza should have a lower K_{Fe} than L. turionifera.

L. turionifera may compensate for the very low iron concentrations in the pond during the spring by storing enough iron, while dormant on the bottom, to undergo several doublings when it surfaces. As the ponds become more completely covered with the plants, the pH and O_2 concentration decrease, allowing iron to be mobilized from the sediments.

In summary, while even highly oxidized "no-plant" ponds can build up high iron concentrations, much of this is oxidized colloidal iron and is not readily available to the plants. The distributions

of S. polyrhiza and L. turionifera show that high pH and low iron favor S. polyrhiza, indicating that iron limitation is the mechanism of the high pH effect in the field as was indicated by laboratory studies. A comparative study of the K_{Fe} for the species of the Lemnaceae would be desirable since this study shows that iron is important in the distribution of at least two of the species.

Nutrients

If nutrient competition is an important species interaction for the Lemnaceae, and if this competitive outcome is based upon nutrient kinetics, each species is expected to have a significantly higher efficiency, i.e. lower K, than the other species for growth on one nutrient. One also would expect ponds with a low concentration of a nutrient to be dominated by the species with the smallest K for that nutrient.

Phosphate

L. turionifera is highly efficient at the utilization of phosphate. It has the lowest K_P (0.37 μM P), as well as a significantly higher growth rate than any other species at all phosphate concentrations. This may explain this species' dominance in the environment. It is almost always present, at least in trace quantities, in ponds supporting the Lemnaceae. It is also the species most commonly dominating ponds, and it attains higher standing crops than the other species. This is understandable, as phosphate is considered to be the most common limiting nutrient in the U.S. (Hutchinson, 1957), and when it is plentiful, L. turionifera has an advan-

tage, because it has the highest intrinsic growth rate, as shown in all of my experiments. Only when some other factor becomes extremely limiting, such as the high pH and low iron of S. polyrhiza ponds, is it completely excluded.

S. polyrhiza and L. minor had equal Kp values, near 0.46 μM P, while the two Wolffia species had much higher values. The results indicate that the order of species ability for successful growth on limiting phosphate is as follows: L. turionifera > S. polyrhiza = L. minor >> W. columbiana >> W. punctata.

Field phosphate data (Table XV) show that L. turionifera is found at a mean phosphate concentration very close to its Kp, while S. polyrhiza dominated ponds have a significantly higher mean phosphate concentration, as is predicted by the Kp data. The mixed L. turionifera - S. polyrhiza ponds have intermediate phosphate levels. This is the same type of situation found between these two species for iron and high pH, with S. polyrhiza being more efficient. It appears that there is a balance in nutrient competition for phosphate and iron between these two species, with L. turionifera favored at high Fe/P ratios, S. polyrhiza favored at low Fe/P ratios, and both species coexisting at intermediate Fe/P ratios.

Since W. columbiana is usually associated with S. polyrhiza, it would be found at higher phosphate concentrations than L. turionifera. The two L. minor-dominated ponds also have very high phosphate concentrations, but the small number of samples makes statistical treatment difficult.

The phosphate kinetic data satisfactorily predict the phosphate concentrations at which these four species are found. The

problem is that the distribution of W. punctata does not fit the prediction. W. punctata is found in ponds with extremely low phosphate concentrations, one-fourth the K_P of L. turionifera, and 50 times lower than its own K_P. The phosphate concentration equals that of "no-plant" ponds. Mixed L. turionifera - W. punctata ponds also have intermediate phosphate levels. This would appear to be the distribution expected if W. punctata were most efficient at phosphate, but it is the actually the least efficient. This contradiction must be resolved, or the dependency of species distribution upon nutrient kinetics is refuted.

Nitrate

Although W. columbiana has a significantly lower K_N than the other species (3.1 μM N), its maximum growth rate is about half that of the two Lemna species and Spirodela. Therefore, it only has an advantage over these species at very low nitrogen concentrations, below 3 μM nitrogen, i.e., at very slow growth rates, below 0.10 daily relative growth rate. As the lowest nutrient concentration used in these experiments was 4.5 μM nitrogen, resolution of the growth rate curves becomes poor at concentrations small enough for W. columbiana to have an advantage. It may be surmised that such a low K_N would not have evolved unless there were an incentive of a growth advantage, so these data indicate that W. columbiana has the highest growth rates at very low nitrogen concentrations.

The two Lemna species and S. polyrhiza have equal K_N values, near 5 μM nitrogen. Their growth rate curves are also not significantly different when limited by nitrogen. Equality of inefficient

species appears to be reasonable. It can be presumed that the closely related species of the Lemnaceae originally had similar capabilities, and when W. columbiana evolved a great efficiency at nitrogen utilization, there was little reason for another species to develop in that direction.

Although the K_N for W. punctata and the Kp for W. columbiana are two to three times higher than the values for the other inefficient species, this may result from their rootless condition and the lack of constant mixing in the cultures. The K values for the Wolffia species may really be equal to the other inefficient species. Taking rootlessness into account, W. punctata is still less efficient at phosphate, as its Kp is four times higher than that of W. columbiana. It is somewhat interesting that W. columbiana could overcome the disadvantage of having no roots in these experiments, demonstrating such a low K_N. If the experiment were repeated with very low nitrogen concentrations and constant mixing, significantly higher growth rates might be obtained for W. columbiana than for the other species.

Since the Lemna and Spirodela species have roots, the formation of depleted zones should be less extreme, and a better estimate of nutrient concentrations should be attained. White (1937) tested Lemna species in semi-continuous culture with a twelve hour media replacement cycle and constant media mixing. His data show a K_N of 7.77 μM nitrogen with a 95% confidence interval of 5.5 to 10.1 μM nitrogen, overlapping those of both Lemna species in my experiments. This not only demonstrates that the lack of media mixing is not limiting for the rooted species, but also that there is strong agreement between White's results and my own.

The field data do not show any of the expected correlations of species with nitrogen concentration (Table XVII). W. columbiana is not found dominating any pond, and since it is usually associated with S. polyrhiza, it seems to be associated with high nitrogen rather than low nitrogen. There are ponds with low nitrogen, but these are populated by W. punctata. This contradicts the expected results, as the species with a low K_N does not occur in the low nitrogen ponds, where the species with a high K_N is found instead.

The concentration of nitrogen in the W. punctata ponds is 2.2 uM nitrogen, almost the same concentration as the K_N for W. columbiana. This concentration of nitrogen would appear small enough to be limiting, as was the case for L. turionifera, whose K_P equals the mean phosphate concentration at which it occurs in nature. One would expect W. columbiana to grow in these ponds.

It may be that nitrogen is not limiting because of a consistent but periodic input of nitrogen. Table XVIII demonstrates that rain is a significant source of nitrogen, with a mean concentration of 52 μM nitrogen in samples collected near the ponds. At least 2.5 cm of rain per week can be expected in southern Michigan until some time in July. This corresponds to the main growing season for the Lemnaceae. This much rain in such shallow waters could raise the nitrogen concentration as much as 5 μM nitrogen, equivalent to the K_N of the inefficient species. Because the rain enters the pond from the surface, the plants probably experience much higher concentrations and may sequester a large portion of the nitrogen before it enters the pond proper.

The alleviation of nitrogen limitation by rain may also

explain Jacobs (1947) and my observation, that W. columbiana tends to increase its population later in the season, at the time of the late summer drought, than the other species in mixed ponds.

Thus, competition for nitrogen is relieved and W. columbiana will not be able to predominate in ponds with a low nitrogen supply from the sediments. In more arid regions, W. columbiana might be allowed to dominate such ponds.

The Wolffia punctata Contradiction

This study has shown that L. turionifera is most efficient in the utilization of phosphate, that W. columbiana is most efficient for nitrogen, and that S. polyrhiza is probably most efficient for iron. This information has been found consistent with the field data except for the fact that W. punctata, the least efficient species in the utilization of these nutrients, is found in ponds with the lowest concentrations of all three nutrients.

The balancing of competitive success by nutrient competition assumes a species is efficient in using one nutrient, and less efficient in using other nutrients. It would dominate ponds where that one nutrient is more limiting than the others. If all nutrients were equally limiting, all species would coexist. For one species to dominate such ponds, it must be most efficient at all nutrients. This would be a super-species and it would exclude the other species to marginal populations unless they developed non-nutrient competitive strategies. If, on the other hand, all nutrient concentrations were so low that no species could grow, none would develop an appreciable biomass.

W. punctata defies all of these scenarios: it is not a super-species, since it dominates relatively few ponds compared to L. turionifera, and it has relatively high K values for the nutrients studied, yet it covers ponds with very low nutrient concentrations. This species must be using an alternative strategy to overcome nutrient deficiencies in these ponds.

Although all of the species, except L. minor, can sink to the bottom of culture vessels after undergoing extreme nutrient limitation, W. punctata sinks independant of nutrient status in laboratory cultures. When the plant surface tension is broken in active cultures of W. punctata and W. columbiana, all W. columbiana plants return to the surface, but 20 to 30% of the W. punctata plants sink to the bottom of the flask. I also observed that wading into ponds containing W. punctata at the surface, mixed the plants throughout the water column. If a large proportion of the W. punctata population sinks to the bottom during a periodic disturbance, such as rain storms, it might be able to take up nutrients from the relatively nutrient-rich sediments and store them. If the plants then refloat in a relatively short time, they may grow in situations where low nutrients concentrations exclude the other species of the family. Although it is known that many species in the Lemnaceae have a yearly sinking cycle to survive winter conditions, no evidence of a short sinking cycle has been reported. This study demonstrates that sunken W. punctata plants, when placed in nutrient media, can return to the surface from ten days to one month later, depending upon conditions.

The mechanism for sinking for such short term is not known, but as the plants sank more readily when nutrient-limited and

no inactive plants refloated in the phosphate-free medium, it appears that nutrients may be involved. Possibly, it is as simple as Jacobs' (1947) theory for S. polyrhiza turions, which suggests that when the photosynthesis products accumulate, as when nutrients limit growth, starch accumulates and the plant sinks. Instead of requiring temperature cues, as in S. polyrhiza, W. punctata "turions" may refloat when available nutrients and the appropriate temperature allow growth, which in the dark would use up starch. In the light, when nutrients were available, plants from inactive cultures that refloated actually seemed to be newly formed plants, as there were more plants on the bottom at the end of the experiment than in the original inoculum. Photosynthesis may maintain starch reserves in the parent plant, while its offspring do not have excess starch and thus float.

Under high light conditions, reflotation of W. punctata takes 8-12 days. This would allow most of the population to cycle to the bottom within one month, assuming a conservative sinking proportion of 25% per disturbance, and at least four disturbances per month in the form of storms. In the spring, when plants on the pond surface are scarce, and the light intensity is high at the bottom, W. punctata would require a high turnover rate to cover the surface before submerged species could develop. Plants which remained on the bottom and whose progeny floated to the surface could be more efficient in covering the surface quickly. This might be a strategy used by W. punctata, as these plants continued to appear at the surface in the transplant enclosures well into the summer in the W. punctata pond.

Low light conditions delay reflotation, as plants in complete nutrient media take up to one month to refloat. This may be

an adaptive feature. When W. punctata has already covered the surface of the pond and subsurface light is reduced, it does not require a high turnover rate to exclude submerged species. Remaining at the bottom longer may be a strategy to maximize nutrient storage for inactive plants (see Table XX). The slower refloating plants in low light intensity have about twice the growth in phosphate-free media, increasing 6.42 times, than do plants in high light, which refloat rapidly.

All active plants which refloated in complete nutrient media (high and low light) can also increase about 6 times in phosphate-free media, indicating that this is a probable maximum value resulting from nutrient storage. If the mass of overwintering W. punctata turions stored enough nutrient for this much growth, and if in the first month the whole population could return to the sediments for a similar store of nutrients, it is obvious that these plants have the potential to cover the pond surface quickly in the spring.

Active plants which refloated in phosphate free media increased about 3.5 times, a measure of the amount phosphate stored prior to sinking, and sufficient to allow reflotation. This may be the reason why plants which sink from active cultures attain their maximum flotation three days before the inactive plants in nutrient media.

All of the W. columbiana plants refloat immediately in high light, while only a few refloat in low light. No viable plants refloat in low phosphate conditions. The refloated plants in the high phosphate conditions increase less than 3 times in phosphate-free media, indicating little phosphate storage. This species, of course, would not be expected to take advantage of the elevator

mechanism, as it does not sink readily from nutrient-limited cultures.

A proposed mechanism by shich W. punctata may circumvent nutrient limitation in extremely nutrient poor ponds, which excludes other species of the Lemnaceae is now breifly sumarized. W. punctata may sink, take up nutrients from the bottom muck and refloat within ten days to a month. When sufficient light reaches the bottom, the turnover rate is rapid, and some plants may reproduce vegetatively on the bottom. The progeny rise to the surface and maintain a high growth rate until the surface is covered. When little light reaches the bottom, the turnover rate slows, taking about one month, but phosphate storage will be maximal. Iron and other metal micronutrients may be similarly stored, while nitrogen is available from the rain, making its storage less important. Although vertical movement of phytoplankton in the water column may allow them to take advantage of ideal conditions, and higher plant shoots often grow toward light, or roots grow toward nutrients or moisture, W. punctata may be the first reported case of an entire higher plant regularly migrating to obtain nutrients.

Competitive Prediction

The information from the Monod nutrient growth curves for the Lemnaceae cannot be used directly for precise prediction of competitive outcome in the manner of Tilman (see Chapter I), because the competition experiments of this study did not obtain steady state. However, these consecutive batch experiments operated at such low nutrient concentrations that nutrients were depleted during each period, they approached equilibrium on an imaginary

zero net growth isocline. Therefore, the relative growth rates of the two species will be used as indicators of the direction in which the system's steady-state lies.

Figure 20 shows the four types of predictive figures obtained when the zero net growth isocline for each species is set arbitrarily, but in relation to its efficiency of growth at a low concentration of each nutrient. The nutrient concentrations at which each species maintains the same low growth rate, such as a 0.05 daily relative growth rate, can be determined from Figure 16 for phosphate and Figure 18 for nitrate. The relative order of increasing nutrient concentration can be used to set the relative position of the phosphate zero net growth isoclines in Figure 20. The relationships of the zero net growth isoclines would then be: L. turionifera < S. polyrhiza = L. minor << W. columbiana << W. punctata. For nitrogen the relationships would be: W. columbiana < L. turionifera = L. minor < S. polyrhiza << W. punctata. For iron, the species' zero net growth isocline relationships deduced from pH data would be S. polyrhiza = W. columbiana < L. turionifera = L. minor = W. punctata.

Figure 20A shows the conditions for coexistence between two species based upon a balancing of growth according to their relative efficiency in the utilization of two nutrients. Each species is more efficient at one of the nutrients. The stable coexistence point is obtained when the Q_b ratios between the two species is identical to the the K ratio (Table XXI). In other words, one species will have the highest Q_b and the highest K ratios. Each species will thus utilize more of the nutrient for which it is least efficient. Thus, the coexistence is stable, as each species limits itself

Figure 20

Competitive prediction figures for the Lemnaceae.

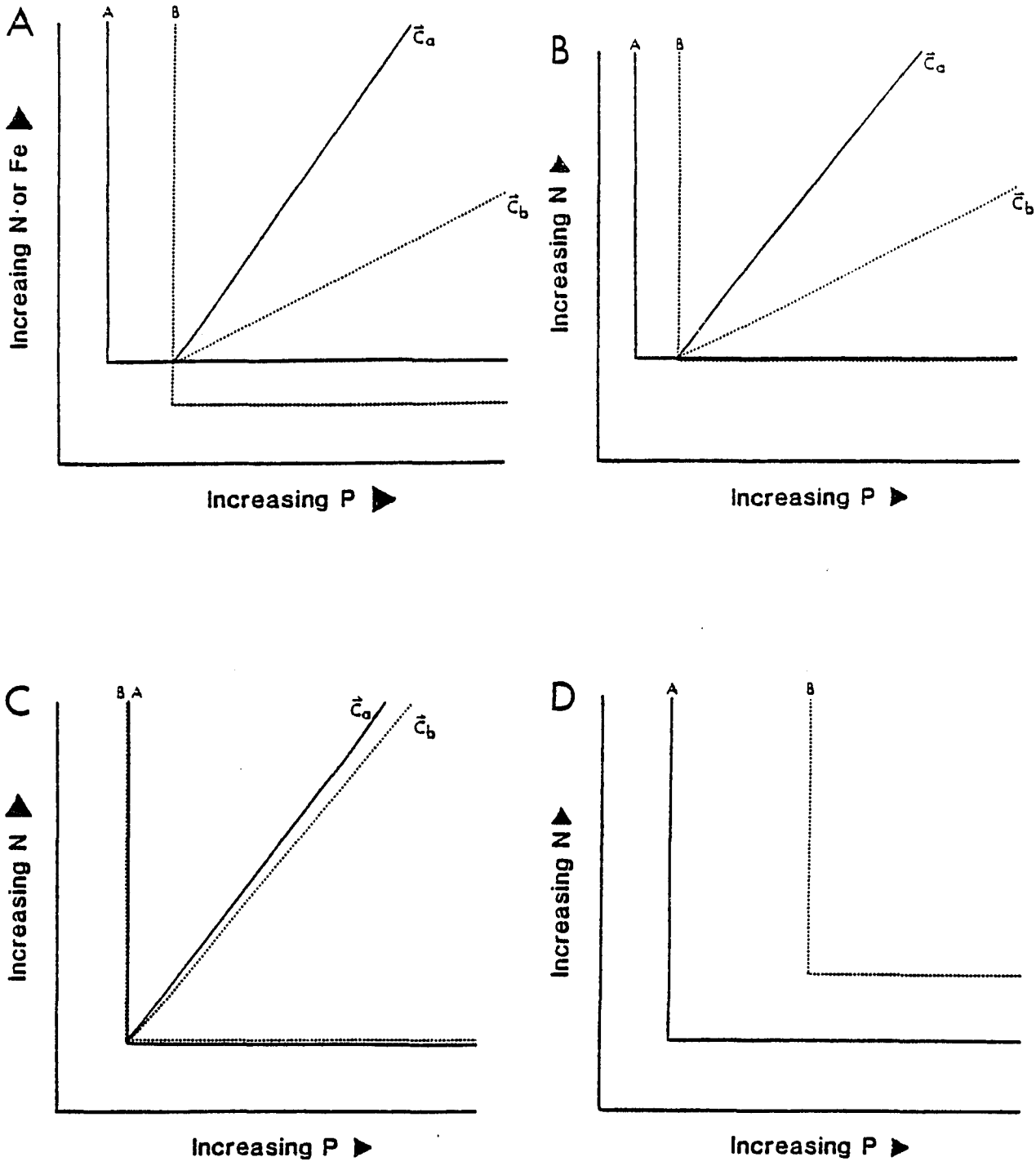
Figure 20A: For nitrogen and phosphate, Wolffia columbiana as species B dominates in limiting nitrogen conditions against Lemna turionifera, Lemna minor or Spirodela polyrhiza, as species A. The "A" species dominates in limiting phosphate conditions and coexistence occurs at intermediate conditions. For iron and phosphate, L. turionifera as species A dominates in limiting phosphate conditions and S. polyrhiza will dominate in iron-limiting conditions. These species coexist at intermediate conditions.

Figure 20B: For nitrogen and phosphate, L. turionifera as species A dominates under phosphate-limiting conditions and S. polyrhiza as species B can only coexist with L. turionifera under limiting nitrogen conditions.

Figure 20C: S. polyrhiza, species A, and L. minor, species B, have nearly superimposed curves, leading to the prediction of coexistence at all nitrogen and phosphate supply points that allow growth.

Figure 20D: Wolffia punctata as species B will always be excluded by any of the four other species when in competition for nitrogen and phosphate.

FIGURE 20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before it limits the other species.

Species pairs that would fit Figure 20A, with species A listed first, are as follows. For nitrogen vs. phosphate they are: L. minor - W. columbiana, L. turionifera - W. columbiana, S. polyrhiza - W. columbiana. For iron vs. phosphate the species pair is L. turionifera - S. polyrhiza. These species should coexist under balanced nutrient conditions such as would fall between their consumption vectors. Dominance of one species or the other will be occur when conditions fall outside the consumption vectors. Specifically, in the case of L. turionifera - W. columbiana, L. turionifera would dominate by excluding W. columbiana under phosphate-limiting (high nitrogen/ phosphate ratio) conditions, while W. columbiana would exclude L. turionifera under nitrogen-limiting, (low nitrogen/phosphate) ratios. This is the situation found by Tilman (1977) for Asterionella formosa - Cyclotella menenghiana competing for silicate and phosphate.

In competition for nitrogen and phosphate W. columbiana would dominate under limiting nitrogen conditions and S. polyrhiza or L. minor would dominate under phosphate-limited conditions. Intermediate nutrient conditions should allow coexistence.

In competition for iron and phosphate, S. polyrhiza would dominate under iron limitation and L. turionifera would dominate under phosphate-limited conditions. The species should coexist at intermediate conditions.

Figure 20B would represent the species pairs L. turionifera - S. polyrhiza and L. turionifera - L. minor with limiting nitrogen and phosphate. Here, the species' zero net growth isoclines of the

species differ for phosphate, but not for nitrate. Under phosphate limiting conditions, L. turionifera would exclude the other species, but coexistence would only be certain where the nitrogen zero net growth isoclines coincide. It is impossible to predict which initial concentration would lead to coexistence, and the best that can be said is that as nitrogen becomes more limiting, the species will tend more toward coexistence. S. polyrhiza or L. minor will never exclude L. turionifera on a nitrogen/phosphate gradient.

Figure 20C shows competition between L. minor and S. polyrhiza for nitrogen and phosphate. Close scrutiny of the relative zero net growth isoclines and consumption vectors shows little real difference between these species. Therefore, Figure 25C presents two superimposed curves, and coexistence at all points on a nitrogen/phosphate ratio would be predicted. The Q_b nitrogen/phosphate ratio is higher for L. minor, while the K nitrogen/phosphate ratio is higher for S. polyrhiza, which is an indication of an unstable equilibrium. This is trivial in this case as the zero net growth isoclines are identical for the two species, and no matter how nutrient concentrations are decreased, the coexistence or decimation of both species will result.

Competition of any of the species vs. W. punctata is shown in Figure 20D. As W. punctata has a higher zero net growth isocline (lower efficiency) for both nutrients, the curves do not intersect and W. punctata will always be excluded.

These predictive figures can be applied to laboratory or field. tuations. If one could devise a steady-state competition experiment with these plants, or measure the steady-state parameters

in the field, more precise predictions could be made by providing numerical scales for each axis and by determining the zero net growth isocline lines from the nutrient growth curves for the known mortality rate. Unfortunately, steady state experiments with the Lemnaceae would be very difficult and tedious, and they would be nearly impossible with terrestrial plants. Measurement of mortality rates in the field is difficult, and there is no dependable method of measuring field nutrient supply rates.

Competition

The competition experiments were started before the kinetic experiments of the five species were completed. The choice of species pairs was based on earlier crude experiments, which did not include the Wolffia species, and on field data.

Field data showed L. turionifera at lower phosphate than S. polyrhiza, although the nitrogen data were confusing. Early experiments indicated S. polyrhiza had a lower K_N. These two species were chosen to compete on a nitrogen/phosphate ratio gradient. Subsequent data showed L. turionifera to be more efficient at phosphate, but that both species were equal in their utilization of nitrogen. Therefore, this pair would operate on a nitrogen/phosphate predictive figure represented by Figure 25b. L. turionifera would exclude S. polyrhiza at high nitrogen/phosphate ratios, but the best S. polyrhiza could attain would be coexistence, as nitrogen became more limiting at low nitrogen/phosphate ratios.

The results of competition of these two species, measured as growth rates, at three nitrogen and phosphate ratios are given in

Table XXII. When in competition at high nitrogen/phosphate ratios (phosphate limited), L. turionifera has nearly twice the growth rate of S. polyrhiza, while the growth rate of Spirodela is much more depressed than the growth rate of L. turionifera. This system shows a trend toward the exclusion of S. polyrhiza by L. turionifera when limited by phosphate.

At the lowest nitrogen/phosphate ratio, the growth rate of S. polyrhiza is still lower than that of L. turionifera, but they are approaching equality. Furthermore, each species depresses the growth of the other species by the same amount. This system is tending toward coexistence of the two species under limiting nitrogen conditions. The final wet weight data in this experiment agree with the above data, but the results exaggerate the superiority of L. turionifera with low phosphate and the equality of the species with low nitrate concentrations. The experimental results fit the predicions in Figure 20B.

Field data for W. punctata had indicated that this species was found in ponds of lower phosphate than L. turionifera. As its high K values for both nitrogen and phosphate had not yet been determined, it was placed in competition with L. turionifera for these two nutrients. As predicted in Figure 20D, L. turionifera was superior at all nitrogen/phosphate ratios (Table XXIII). In fact, it grew as if W. punctata were not present. Though W. punctata could not even grow in monoculture under the phosphate-limiting conditions, it did grow in monoculture in the intermediate and nitrogen-limiting conditions. In competition, its growth rate was depressed by 90%, indicating its quick exclusion under these condi-

tions. As has been noted, W. punctata avoids direct competition for nutrients in the field by the sink/float strategy.

In general, the results of these non-steady state competition experiments confirm the predictions made by a method that assumes competition will come to steady state. The measure of competitive success in these non-steady state experiments was differential growth rate. This is an indicator of the direction in which the system is moving on its way to a steady state. Even though this system is not at steady state, a general idea of where that steady state will lie, in relation to species success, can be gained from it.

It may be argued that the outcome resulted from systematic error, that the proper species pairs were not tested. After all, the competitive superiority of L. turionifera over W. punctata at all nitrogen/phosphate ratios might be predicted from its higher intrinsic growth rate. But L. turionifera also has a higher intrinsic growth rate than S. polyrhiza, yet S. polyrhiza was able to approach equity with L. turionifera under nitrogen-limiting conditions, as predicted.

It thus seems likely that the competitive outcome in these experiments indeed depends on the kinetic mechanism of nutrient uptake. It would be desirable to test species pairs, such as L. turionifera and S. polyrhiza for phosphate and iron or L. turionifera and W. colombiana for nitrogen and phosphate, whose predictions would allow for the exclusion of either species as well as coexistence.

Equilibrium in Nature

To apply the competitive prediction to a natural system,

one must assume that the nutrient supply and demand, as well as plant growth and mortality, come to steady-state. One might argue that Lemnaceae ponds are yearly batch cultures where, at best, equilibrium is just being approached when winter weather destroys the population and reduces all the species present to equally low population densities. In this case, nutrient conditions are never allowed to approach concentrations low enough to exclude one species or the other. Thus, coexistence might be predicted as the nutrient competitive process does not affect the species diversity.

The above argument assumes that winter reduces the populations of each species to such low densities that they are practically equal. This is, however, not the case. Most of these species have developed complex overwintering mechanisms, and there is a large biomass carryover to the spring. Ponds are often covered by plants within a week or two after turion germination is initiated. Inspection of plants from these ponds at that time shows that most are freshly germinated overwintering forms. Thus, the apparent early growth spurt may be a misconception caused by the prolonged germination of considerable biomass carryover from the fall. It is reasonable to assume that the overwintering capabilities of these plants are similar, and that the relative number of turions of each species which germinate in the spring reflects the relative number present in the fall. The exception to this assumption is L. minor, which has no overwintering structure and depends upon random events for overwintering. Its population can be decimated by adverse overwintering conditions, as is evidenced by its minor position in northern U.S. flora and frequent dominance in southern U.S. flora. This species

has been shown in Michigan to gain an advantage from low temperature conditions of shaded ponds, but has not been shown to have any superiority for any of the three nutrients studied. Therefore, it is not expected to participate in nutrient competition except as a victim.

The efficient overwintering strategies of these species may allow nutrients conditions to elicit gradual species shifts over a period of years. The large biomass carryover also allows populations to increase quickly to densities that will provide a significant draw on the pond's nutrient supply, intensifying nutrient competition.

Population densities seem to stabilize in midsummer. Although Rejmankova (1975) attributes this to high temperatures, it could also be explained by the attainment of equilibrium between nutrient based growth rates and mortality. The differentiation between high temperature and low nutrient concentrations as the cause of late summer population stagnation deserves further study. In the case of a nutrient equilibrium, mortality could be from self-shading as in the thick masses of plants in L. turionifera ponds, or from invertebrate herbivory, which has not been quantitatively assessed for this family. Duck and fish herbivory may not factor into mortality that leads to equilibrium populations as the former is seasonal and sporadic, and the latter seems to lead to the exclusion of the Lemnaceeae from the ponds. In some ponds forming a thin cover, such as the S. polyrhiza pond, the equilibrium growth rate may be very low due to extremely limiting nutrients, giving the appearance of stagnation after the initial recruitment of germinated turions and growth on stored nutrients.

The nutrient that appears most limiting in each pond seems to reach an equilibrium concentration shortly after the plants cover the pond surface. Thus phosphate in L. turionifera ponds remains at a low, potentially limiting level all summer, while iron concentration remains relatively constant in S. polyrhiza ponds and all nutrients remain low in W. punctata ponds. Non-limiting nutrients, especially iron in L. turionifera ponds, may show an increase over this period. Though this may not appear to be an equilibrium situation, the non-limiting nutrient concentrations may be high enough that the plants will perceive the nutrient ratio as at equilibrium.

From the above discussion it seems valid to assume that Lemnaceae ponds are capable of equilibrium conditions, and that resource competition theory can be applied. It is always possible that nutrient conditions may be altered, as by human activity, causing a disequilibrium and species shift. A disaster, such as a particularly hard winter, or particularly heavy duck herbivory in the fall, may reduce populations to such low densities that nutrient competition will be relieved between the species. In both cases, the intrinsic growth rate is so high for the Lemnaceae that equilibrium conditions might be approached within one season. Therefore, even with so few ponds and a few years of observation, it is probable that a great proportion of the ponds observed are at or near equilibrium conditions. However, some ponds were noted in an apparent species succession during the period of the study, and are discussed in the section on succession below.

Transplant Experiments

The results of my laboratory studies predict the field distribution of species, in relation to nutrient concentrations, actually found. To test whether the species - nutrient relationship found in the field is coincidental and caused by random factors, field transplant experiments were executed. If the observed species distribution is determined by random factors, plants should be able to grow in ponds in which they were not originally found. If pond conditions, in this case surmised to be nutrients, determine the species distributions, then species of Lemnaceae should not grow in ponds in which they are not naturally found.

Large populations only developed in ponds for which that particular species was endemic; the other species did not grow, or disappeared (Table XXVI). No species grew in the "no plant" pond. Hence, the results of the transplant experiment support the determination of species distribution based upon nutrient conditions.

In some enclosures, the species endemic to the pond disappeared as algae and/or submerged plants developed. This demonstrates the delicate balance between a Lemnaceae pond and a "no plant" pond. Failure to cover a pond quickly in the spring could cause exclusion of the entire family.

Wolffia punctata was the only species to attain a complete cover in all enclosures in its native pond owing to the continued recruitment of plants from the bottom during the experiment.

There is the possibility that W. punctata might have survived in one cage in the L. turionifera pond had the cage not been destroyed.

At the end of the second month, two cages with W. punctata had shown significant growth, but by the third month, the monoculture cage was lost, and in the other W. punctata apparently succumbed to competition with L. turionifera. This is consistent with the observation that W. punctata can survive in ponds with very low nutrients via the elevator mechanism, but if nutrientconcentrations are high enough to allow other species to grow at the surface, W. punctata will be excluded. W. punctata may not have survived in the S. polyrhiza pond because the excessive algal mat formation and the mass of submerged plants interfered with the elevator mechanism. In the "no plant" pond, the Wolffia plants were eaten by fish soon after inoculation.

Thus, results of the transplant experiments are consistent with the nutrient based distribution of the Lemnaceae proposed in this dissertation.

Allelopathy

The possibility of allelopathic interactions must be considered in any competitive circumstance. Allelopathic interactions have been demonstrated for terrestrial plants such as Juglans nigra and Encelia farimosa (Rice, 1974). Demonstration of allelopathic effects in aquatic environments has been less definite, possibly due to the diluting effect of the water. Rice (1974) gave evidence for compounds produced by Chlorella vulgaris that were both allelopathic and autotoxic. Whether these substances have a significant effect in algal species distribution and succession in nature remains to be shown.

Wolek (1979) claims to have observed an allelopathic inhibition of Spirodela polyrhiza by Wolffia arrhiza. However, S. polyrhiza inhibited its own growth to the same degree. This indicates that the effect may not be a meaningful inter-species allelopathic effect. Also, the effect cannot be separated from nutrient competition, as Wolek used diluted media and did not replenish nutrients after the conditioning growth period. In fact, the growth rate of plants on fresh media showed an initial increase, then rapid decline as would be expected as nutrient depletion occurred. Plants grown on conditioned or carbon-purified conditioned media do not have the initial increase in growth rate, just a rapid decline. This denotes nutrient limitation, as Wolek admits. He concludes that the nutrient competitive stress produced by Spirodela on Wolffia was stronger than the allelopathic stress produced by Wolffia on Spirodela. No allelopathic compound was identified or isolated.

In my allelopathy experiment, Spirodela polyrhiza and Lemna turionifera had a stimulatory effect on each other when each was grown in nutrient replenished conditioned media when compared to growth on fresh media. Both S. polyrhiza and L. turionifera grew slightly better in media conditioned by S. polyrhiza, but the differences were not significant. The lack of allelopathic interaction was reflected in the competition experiment where the dominance of L. turionifera over S. polyrhiza was relieved when phosphate was increased and nitrate limited. Wolffia punctata was also unable to affect the growth rate of L. turionifera under either nitrate- or phosphate-limiting conditions, as expected from its very high K values for these nutrients. This indicates any allelopathic effect

is weaker than the effect of competition for limiting nutrients for these species.

In addition, L. turionifera and W. punctata are often found in equal proportions in many ponds, indicating that allelopathic effects are absent or weak. On the same basis, allelopathy would not be expected between W. columbiana and S. polyrhiza, or between W. punctata and W. columbiana, as these species pairs are also often found together in co-dominant situations. L. minor L. turionifera, L. turionifera - W. columbiana, L. turionifera S. polyrhiza, and S. polyrhiza - W. punctata are species pairs occasionally found together as co-dominants, which may be explained by the rarity of conditions to allow these assemblages to occur. The possibility of allelopathy still appears remote in these cases.

Therefore, allelopathic inhibitions within the Lemnaceae have yet to be demonstrated, and if they exist, they probably are not as strong as competitive interactions owing to limiting nutrients.

Succession

In this study I have viewed Lemnaceae ponds as static entities, each having certain conditions which allow particular species or species mixes to survive. An intriguing question is whether there is a succession from ponds with submerged macrophytes to Lemnaceae ponds, and/or whether there is a pattern of species succession within the Lemnaceae.

To invade a pond, the Lemnaceae must contend with hostile conditions caused by populations of submerged plants and algae. Submerged plants can, by taking up CO_2 for photosynthesis, force

the pH up to 10.0 or more during the day. The Lemnaceae have been found to be intolerant of such high pH (this study, Hicks, 1932; Jacobs, 1947; Moyle, 1949 and McClay, 1974). The high pH also contributes to nutrient depletion due to phosphate and iron precipitation. The submerged plants intensify this depletion by nutrient uptake into the often sizable biomass.

Lemnaceae species that invade ponds with well-developed submerged plant populations must be able to compete under these conditions. Wolffia punctata is a likely candidate to initiate transition to a Lemnaceae pond. Though it is inefficient at the utilization of both phosphate and nitrate, and possibly iron, it has the ability to take nutrients directly from the bottom with its floating-sinking cycle. W. punctata does not seem to have the pH tolerance of some of the other species. If pH intolerance is due to iron precipitation and low iron concentration, as indicated in these experiments, then W. punctata can overcome it.

There is strong evidence for the takeover of ponds by W. punctata. In pond 29, the pond with the most consistent and thick W. punctata cover, the cover is not so thick as to exclude light from the bottom since a species of linear leaved Potomogeton develops. In two of the other ponds, 6 and 19, W. punctata cover was sporadic, completely covering the ponds some years and having sparse populations other years. In 1979 pond 11 contained an extremely sparse mixed species community, but W. punctata had completely covered it in 1980. Information taken from these four ponds may indicate that W. punctata is involved in a constant takeover struggle with submerged plants.

Spirodela polyrhiza and Wolffia columbiana may be able to

invade ponds with submerged vegetation since these species tolerate high pH, but they would require significant concentrations of nutrients, particularly phosphate, at the surface of the pond. Several ponds were invaded by W. columbiana in Massachusetts after they began receiving alkaline sewage effluents (Burk et al., 1976). While the sewage provided adequate nitrogen and phosphate, its alkalinity may have reduced the available iron. From the results of this study, this condition is predicted to favor S. polyrhiza and W. columbiana over L. turionifera.

Jacobs (1947) observed L. minor (probably L. turionifera) becoming established in newly created ponds. These ponds were created by human disturbance of the environment. Nutrient concentrations were most likely high because of soil leaching and decay of terrestrial plant debris in these newly formed ponds. There were no established submerged plants in these ponds, which, if present, would have raised the pH and inhibited the two Lemna species. Conditions were ideal for the species with the highest intrinsic growth rate, L. turionifera. The colonization of newly created ponds obviously differs from the invasion of ponds with established submerged plant populations.

As ponds age a nutrient-rich muck develops from years of accumulation and decay of plant matter. When submerged plants dominate the pond, the oxidized conditions caused by their photosynthesis reduces the availability of nutrients in the water overlying the rich sediments. When the Lemnaceae take over a pond, completely covering the surface with their fronds, the pond water becomes anaerobic and these nutrients become available at the surface.

As iron availability seems more affected by oxidizing condi-

tions than phosphate, the onset of anaerobic conditions would be favorable to species which are inefficient at iron utilization. Thus, L. turionifera might be favored once a pond is colonized by one of the other species. A succession of Lemnaceae species from pioneering species such as W. punctata, and possibly S. polyrhiza and W. columbiana, to the "climax" species L. turionifera might be envisioned.

Although there is no direct evidence of this succession at this time, there is some circumstantial evidence that may be explained by it. The most common and most dense Lemnaceae populations are those of L. turionifera. As nutrients become more available, L. turionifera takes advantage of its superior intrinsic growth rate to develop a large biomass. This large biomass may keep the phosphate concentrations at the surface low, even in the face of increased supply in the anaerobic conditions. This is evidenced by the ponds dominated by L.turionifera having phosphate concentrations equal to the K_p of this species. This would inhibit all the other species because they have higher K_p values.

The takeover of W. punctata ponds by L. turionifera can be implied from plant distribution and nutrient data. The four W. punctata ponds, 29, 6, 11, and 19, all had L. turionifera scattered sparsely across them. There are also many ponds where both species make up a significant portion of the population, i.e., ponds 14, 26, 27, and 31. These mixed ponds have intermediate nutrient levels between the low nutrient W. punctata ponds and the higher nutrient L. turionifera ponds. These ponds may represent stages in a succession from W. punctata to L. turionifera.

The takeover of Spirodela ponds by L. turionifera is sup-

ported by the fact that several ponds with nearly 100% L. turionifera cover had small amounts, 1-2%, of Spirodela. Two of these ponds, 7 and 3, showed a decline in S. polyrhiza over the four years they were observed.

The invasion of W. punctata ponds by L. turionifera may be delayed where the sediments are thin and are relatively nutrient-poor, as in pond 29. Thus, W. punctata ponds may persist for years before their sediments build up enough nutrients to allow L. turionifera to invade.

S. polyrhiza (and W. columbiana by association) may delay or prevent the invasion of L. turionifera by coming to equilibrium with submerged plants. If S. polyrhiza does not form a tight cover, it allows submerged plants to survive. The resulting high pH will inhibit L. turionifera. S. polyrhiza is normally found as a thin covering on ponds containing submerged plants, usually Ceratophyllum demersum.

Evidence of W. punctata colonizing ponds with established submerged plants, and L. turionifera colonizing newly created ponds is given. S. polyrhiza and W. columbiana may be pioneering species under special circumstances. Succession of Lemnaceae ponds to a climax of L. turionifera is proposed. This would be an interesting situation as the invasion of the pioneer species would be responsible for converting a hostile habitat to habitat suitable for L. turionifera.

Summary

Species differences were found in the growth efficiencies

of the five species at different light intensities and temperatures. Spirodela polyrhiza grows better than the other species under low light or high temperatures and Lemna minor grows better at low temperatures. Light did not seem to affect the inter-pond distributions, but may cause intra-pond species gradients when mixed assemblages are present. Low temperature may allow Lemna minor to increase its proportion relative to Lemna turionifera in shaded, cool forest ponds. Otherwise these two factors have little effect upon species distribution in nature.

The species most tolerant to high temperatures was S. polyrhiza, while L. minor was the least tolerant. Temperatures that occur in the plant mass during the summer are high enough to inhibit the less tolerant species. A seasonal succession in mixed ponds of L. trisulca, L. minor, L. turionifera, W. punctata, W. columbiana and S. polyrhiza can be explained by these data.

The species with the lowest half-saturation constants for nutrients are Lemna turionifera for phosphate and Wolffia columbiana for nitrate. Wolffia punctata was the least efficient species for these two nutrients. Efficiency at low iron concentrations was inferred from tolerance of high pH, as is the case for Spirodela polyrhiza and Wolffia columbiana.

The outcome of competition experiments between Lemna turionifera and Wolffia punctata for nitrogen and phosphate showed exclusion of the latter at all nitrogen/phosphate ratios, while competition between Lemna turionifera and Spirodela polyrhiza for these nutrients showed exclusion of Spirodela polyrhiza at high nitrogen/phosphate ratios and coexistence at low ratios. These results are consistent

with predictions made from the growth kinetic data, but the other competitive pairs have not been tested.

The field distributions of Lemna turionifera and Spirodela polyrhiza in relation to iron and phosphate are consistent with predictions based upon their nutrient kinetics, with L. turionifera occurring at higher iron/phosphate ratios, and S. polyrhiza at lower iron/phosphate ratios. The distribution of the five species in relation to nitrogen do not correlate with the expected results. This is attributed to the periodic input of large amounts of nitrogen in rainfall.

Contrary to competitive theory, W. punctata is grows in large numbers on ponds with extremely low concentrations of all three nutrients, yet to grow to such large numbers in these ponds, this inefficient species must have a source of nutrients. It is proposed that this species sinks to the bottom of the pond to absorb nutrients, and rises replenished in a week to a month ready to grow at the surface. Laboratory experiments have shown that they are able to undergo such a cycle, but it has not been demonstrated in the field.

The possibility of allelopathic interactions being stronger than competitive interactions is thought to be remote.

Ponds may undergo a succession from submerged plants to L. turionifera ponds by an intermediate W. punctata stage. S. polyrhiza or W. colombiana may also be intermediate stages under special circumstances.

This study has shown that the nutrient kinetic characteristics of the Lemnaceae were such that species coexistence or exclu-

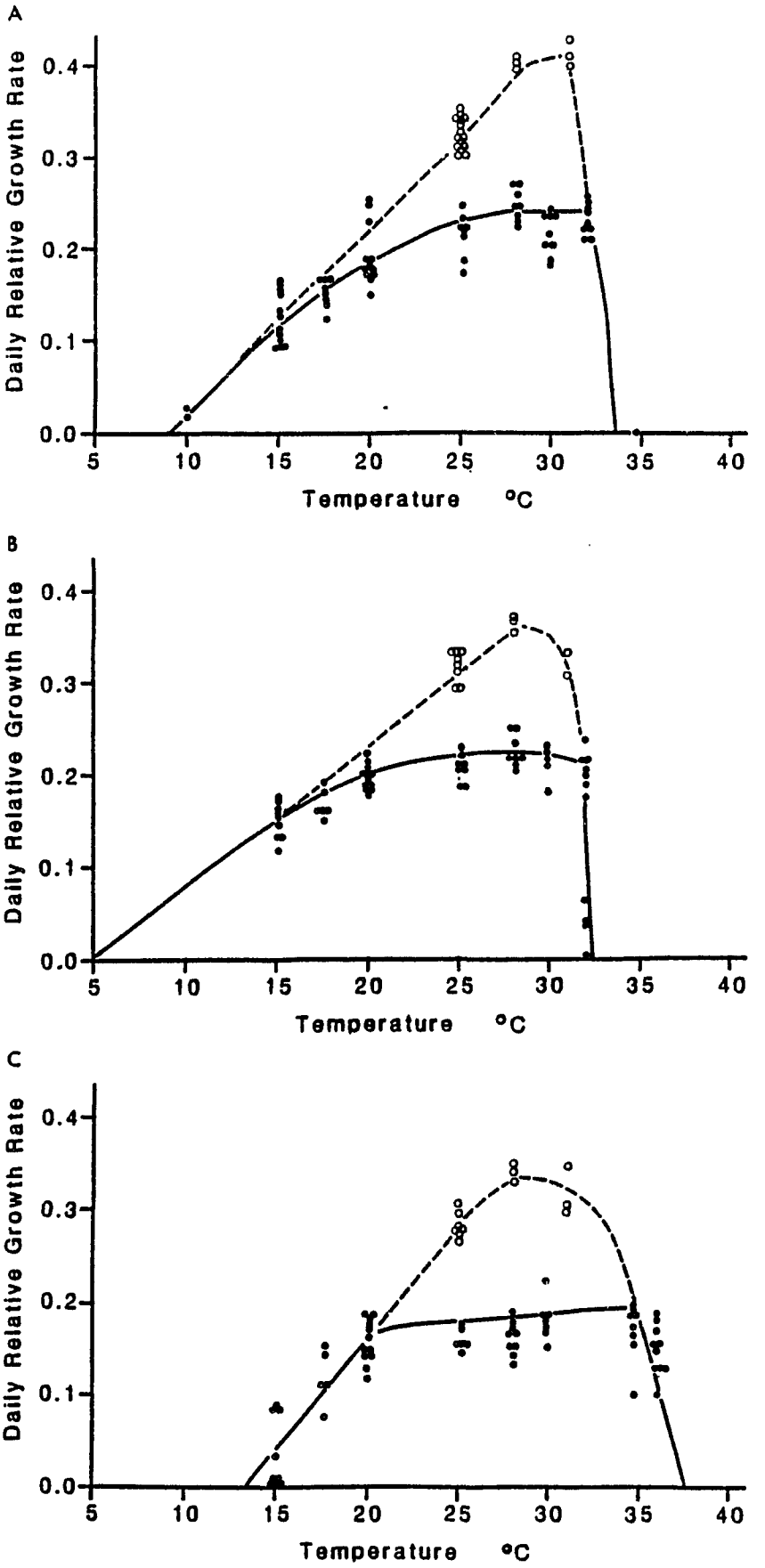
sion could be determined by phosphate, iron and nitrogen via a mechanism consistent with Tilman's Resource Competition Hypothesis. Though lab and field data confirm this situation for iron and phosphate, one species, Wolffia punctata, avoids nutrient competition by migration to the sediments for nutrients and is therefore found in ponds deficient in all three nutrients. This may be the first reported case of a higher plant moving itself, in entirety, to obtain better growth conditions.

APPENDICES

Appendix A

High light (200 μEin/m^2s) temperature curves are shown superimposed upon low light (<100 μEin/m^2s) curves for three species; A) Lemna turionifera, B) Lemna minor, C) Spirodela polyrhiza. High light curves are represented by broken lines and open circles while low light curves are represented by solid lines and circles.

APPENDIX A


A
Daily Relative Growth Rate
0.0
0.1
0.2
0.3
0.4
5
10
15
20
25
30
35
40
Temperature °C
B
Daily Relative Growth Rate
0.0
0.1
0.2
0.3
0.4
5
10
15
20
25
30
35
40
Temperature °C
C
Daily Relative Growth Rate
0.0
0.1
0.2
0.3
0.4
5
10
15
20
25
30
35
40
Temperature °C

APPENDIX B

FIELD TEMPERATURE, OXYGEN AND pH DATA

Date	Pond	Depth cm		Temperature ° C	Oxygen ppm	pH
5/6/80 11am Sunny 18° C	1	0.0		21.2		
		30.0		18.7		
5/6/80 noon	2	0.0		25.2		
		30.0		16.5		
5/6/80	6	0.0		22.5		
		30.0		17.5		
5/6/80 1pm	7	0.0		22.8		
		30.0		20.8		
5/6/80 1:30pm	11	0.0		22.5		
		30.0		20.4		
5/6/80 2pm	8	0.0	(Plants)[a]	25.7		
		30.0		17.5		
		0.0	(Open)[b]	24.5		
		30.0		17.8		
5/6/80 2:30pm	14	0.0		25.1		
		30.0		18.9		
5/6/80 3pm	17	0.0		23.8		
		30.0		21.8		
5/6/80 3:15pm	18	0.0		22.4		
		0.0		22.4		
		30.0		21.1		
		30.0		22.4		
5/6/80 3:30pm	16	0.0		26.0		
		30.0		18.5		
5/6/80 4pm	20	0.0		22.8		
		30.0		21.0		
5/6/80 4:30pm	13	0.0		23.8		
		15.0		20.5		

[a] Taken from under plant mass.
[b] Taken from open water.

Date	Pond	Depth cm		Temperature ° C	Oxygen ppm	pH
5/6/80 4:45pm	25	0.0 30.0		20.7 20.7		
5/6/80 5:15pm	26	0.0 30.0		22.0 19.4		
5/6/80 5:45pm	27	0.0 30.0		19.8 19.8		
5/6/80 6pm	28	0.0 30.0		19.5 19.5		
5/6/80 6:30pm	5	0.0		18.5		
7/23/80 3pm Pt Cloudy 26 °C	17	0.0 30.0		28.0 22.0		7.50 7.00
7/23/80 4pm	18	0.0 30.0		28.8 26.0		9.40 8.18
7/23/80 5pm	16	0.0 0.0 30.0	(Plants) (Open)	28.0 27.0 25.5		7.67 8.81 6.97
7/24/80 2pm Pt Cloudy 28 °C	18	0.0 30.0		27.0 23.5		9.36 8.90
7/24/80 3pm	19	0.0 30.0		28.8 23.0		7.22 7.04
7/24/80 3:30pm	20	0.0 30.0		31.5 26.0		9.64 7.09
7/24/80 4pm	14	0.0 30.0		30.0 23.5		6.90 6.62
7/24/80 4:30pm	13	0.0 30.0		31.3 25.0		8.91 6.77
7/24/80 5:15pm	25	0.0 30.0		29.5 26.0		7.82 6.95
7/24/80 6pm	26	0.0 30.0		28.8 25.5		6.75 6.40

Date	Pond	Depth cm	Temperature ° C	Oxygen ppm	pH
7/24/80	27	0.0	27.0		7.48
6:30pm		30.0	25.5		7.25
7/24/80	28	0.0	27.8		7.35
7pm		30.0	25.0		6.88
7/31/80	17	0.0	27.0		7.22
2pm		30.0	23.0		6.95
Cloudy 31° C					
7/31/80	30	0.0	32.0		8.75
3pm		30.0	22.0		7.30
8/4/80	5	0.0	31.0		7.20
2pm		30.0	23.0		7.18
Sunny 28° C					
8/4/80	8	0.0	34.0		6.49
2:30pm		15.0	26.0		
		30.0	22.0		6.53
8/4/80	31	0.0	34.0		6.63
3pm		15.0	29.0		
		30.0	22.0		6.85
8/4/80	11	0.0	33.0		7.20
3:30pm		30.0	25.0		6.84
8/4/80	7	0.0	33.0		6.19
4pm		30.0	23.0		6.50
8/4/80	6	0.0	32.0		8.55
4:30pm		30.0	23.0		7.18
8/4/80	12	0.0	25.0		7.68
5pm		30.0	25.0		
10/30/80	5	0.0			6.90
11-5pm					
Sunny	8	0.0			6.60
7° C					
	7	0.0			6.45
	6	0.0			7.40
	14	0.0			7.20
	17	0.0			6.98

Date	Pond	Depth cm		Temperature ° C	Oxygen ppm	pH
10/30/80 (cont.)	30	0.0				7.75
	16	0.0				7.57
	19	0.0				7.10
	18	0.0				7.35
	26	0.0				7.28
	27	0.0				7.14
	29	0.0				7.07
4/16/81 2pm Sunny 20° C	5	0.0		14.8	11.8	7.82
		30.0		14.0	11.7	7.90
4/16/81 3pm	29	0.0		16.0	12.8	7.24
		60.0		15.8	10.4	7.41
4/16/81 4pm	27	0.0		14.0		7.86
4/16/81 5pm	26	0.0		13.5	10.2	7.13
4/18/81 1pm Sunny 16° C	8	0.0		25.0	10.0	6.48
		15.0		13.0	3.3	
		30.0		13.0	3.0	
		45.0		11.0	0.5	6.05
4/18/81 1:30pm	35	0.0	(Plants)	25.3	12.5	6.55
		15.0		11.3	1.0	6.25
		0.0	(Open)	20.0	9.0	
		15.0		15.0	5.0	
4/18/81 2pm	31	0.0	(Plants)[a]	20.3	10.4	6.9
		10.0		18.3	8.1	
		20.0		14.5	2.8	6.95
		0.0	(Open)[b]	19.5	10.0	
		10.0		18.5	8.1	
		20.0		15.5	5.1	
4/18/81 3pm	11	0.0		18.0	11.8	
		22.5		18.2	9.9	8.35
4/18/81 3:30pm	36	0.0		16.5	12.4	7.64
		15.0		16.3	7.0	

Date	Pond	Depth cm		Temperature ° C	Oxygen ppm	pH
4/18/81	7	0.0		20.3	8.1	7.05
4:30pm		15.0		17.9	8.0	
4/18/81	37	0.0		16.0	9.9	7.05
5:30pm		45.0		15.2	8.0	7.10
		45.0		15.5	1.8	7.10
4/18/81	6	0.0		17.5	20.0	8.00
6pm		7.5		17.5	20.0	8.00
		15.0		17.2	9.5	6.95
4/25/81	3	0.0	(Plants)	12.0	20.0	9.25
Noon		30.0		8.7	8.1	
Cloudy		60.0		7.9	0.8	7.35
10° C		0.0	(Open)	8.5	10.4	
		30.0		8.3	8.8	
		60.0		8.3	5.3	
4/25/81	4	0.0		8.7	13.2	7.80
12:30pm		10.0		7.5	4.3	
		30.0		7.0	0.8	
		45.0		7.0	0.8	7.42
4/25/81	10	0.0		9.9	6.8	6.82
2:30pm		15.0		9.7	6.0	
		30.0		9.2	6.2	6.85
4/25/81	9	0.0		9.8	9.8	7.15
3pm		30.0		9.2	9.5	
		60.0		9.2	4.5	
4/27/81	14	0.0	(Plants)	15.2	10.5	6.98
1pm		15.0		10.8	5.8	
Hazy		30.0		8.8	3.7	6.55
15° C		0.0	(Open)	13.5	8.4	
		15.0		10.8	7.0	
		30.0		9.2	7.0	
4/27/81	16	0.0	(Plants)	24.5	16.4	
2pm		7.5		21.5	0.3	
		0.0	(Open)	21.5	14.2	7.91
		15.0		15.0	5.0	
		17.5		14.2	1.6	6.85
4/27/81	17	0.0	(Plants)	18.5	8.5	7.40
2:30pm		15.0		14.4	8.0	
		45.0		11.3	3.0	7.35
		0.0	(Open)	17.8	8.5	
		15.0		12.5	9.0	
		45.0		11.1	8.8	

Date	Pond	Depth cm		Temperature ° C	Oxygen ppm	pH
4/27/81 3pm	18	0.0		16.3	10.0	7.43
4/27/81 3:30pm	30	0.0	(plants)	26.3	20.0	8.87
		15.0		18.0	13.5	
		30.0		14.3	0.8	8.00
		0.0	(Open)	21.0	14.3	
		15.0		18.5	16.2	
		30.0		13.5	20.0	
5/13/81 1pm 15° C	1	0.0		16.5	13.7	8.60
		15.0		16.1	13.6	8.57
		30.0		16.0	13.5	
		45.0		15.6	12.0	8.57
5/13/81 2pm	2	0.0		16.5	9.3	7.57
		7.5		16.0	9.1	
		15.0		14.3	3.8	7.49
6/6/81 3pm 24° C	8	0.0		30.0	7.6	6.45
		7.5		24.6	1.2	
		15.0		21.5	0.6	6.25
		30.0		18.0	0.3	6.18
6/6/81 3:30pm	31	0.0		24.0	4.6	7.32
		7.5		22.8	2.5	
		15.0		21.3	0.9	
		30.0		17.0	0.1	6.75
6/6/81 4pm	35	0.0		29.0	6.9	6.14
		7.5		24.0	1.2	
		15.0		21.2	1.0	
		30.0		19.8	0.1	6.00
6/6/81 5pm	11	0.0		23.0	9.7	8.03
		7.5		24.0	9.2	
		15.0		24.0	7.1	
		22.5		23.5	6.9	
		30.0		22.0	0.4	7.10
6/6/81 6pm	36	0.0		21.0	2.2	7.06
		7.5		21.0	0.7	
		15.0		20.0	0.1	
		22.5		19.2	0.0	6.67
6/6/81 7pm	37	0.0		21.0	1.9	7.07
		15.0		20.0	0.2	
		30.0		18.0	0.1	6.80

Date	Pond	Depth cm	Temperature ° C	Oxygen ppm	pH
6/6/81	7	0.0	22.0	1.7	6.95
8pm		15.0	22.7	0.5	
		30.0	21.5	0.1	
		60.0	18.0	0.0	6.85
6/6/81	6	0.0	20.5	1.9	7.31
8:30pm		7.5	20.3	0.6	
		30.0	20.0	0.5	7.24
6/7/81	14	0.0	31.5	8.0	7.02
3pm		7.5	20.0	1.2	
Sunny		15.0	18.0	0.7	
29° C		30.0	17.0	0.0	6.37
6/7/81	17	0.0	31.0	8.0	8.07
4pm		7.5	24.0	2.4	
		15.0	21.0	1.6	
		30.0	18.7	0.3	
		60.0	17.8	0.0	6.90
6/7/81	30	0.0	30.4	9.9	9.40
4:30pm		7.5	24.1	7.7	
		15.0	20.9	6.9	
		30.0	18.3	1.6	7.90
6/7/81	16	0.0	28.2	10.6	8.84
5pm		7.5	28.2	9.3	
		15.0	26.0	9.2	
		30.0	21.3	0.8	7.20
6/7/81	18	0.0	28.5	11.0	9.37
6pm		7.5	28.5	11.5	
		15.0	28.5	11.4	
		30.0	25.2	12.9	8.69
		45.0	24.0	6.8	
6/7/81	19	0.0	26.6	10.5	7.95
7pm		7.5	26.0	5.9	
		30.0	23.3	2.8	
		22.5	20.3	0.5	
		30.0	18.2	0.0	6.95
6/7/81	20	0.0	26.3	5.5	8.15
7:30pm		7.5	26.9	3.1	
		15.0	26.0	2.3	
		22.5	22.3	0.3	
		30.0	21.0	0.1	7.10

Date	Pond	Depth cm	Temperature ° C	Oxygen ppm	pH
6/7/81	14	0.0	22.5	3.8	
8pm		7.5	21.0	1.7	
		15.0	19.0	0.8	
		30.0	17.0	0.1	
6/11/81	5	0.0	23.7	12.5	7.85
noon		7.5	20.2	9.9	
Pt. Sunny		15.0	17.2	11.2	
25° C		30.0	16.3	9.3	7.37
6/11/81	29	0.0	28.0	6.6	7.17
1pm		7.5	23.7	2.3	
		15.0	21.0	1.9	
		30.0	20.7	1.15	7.13
		35.0	19.9	0.7	
6/11/81	27	0.0	26.8	12.5	8.72
2pm		7.5	23.0	6.2	
		15.0	21.9	5.7	
		30.0	20.0	3.7	7.37
		60.0	17.0	0.1	
6/11/81	26	0.0	26.3	5.6	7.38
3pm		7.5	20.5	0.5	
		15.0	19.7	0.2	
		22.5	17.8	0.1	7.40
6/11/81	13	0.0	26.2	9.5	9.85
4pm		7.5	22.0	7.1	
		15.0	20.2	3.7	
		30.0	19.0	1.6	7.05
		37.5	18.0	0.6	
6/11/81	25	0.0	26.7	17.4	9.53
5pm		7.5	25.0	12.6	
		15.0	23.2	7.2	
		22.5	21.9	5.6	
		30.0	20.7	3.4	8.90
		45.0	19.6	0.8	
6/18/81	3	0.0	33.7	11.7	7.12
1pm		7.5	22.4	2.8	
Sunny		15.0	20.0	0.5	
24° C		30.0	18.4	0.1	6.83
6/18/81	4	0.0	30.3	7.8	6.95
2pm		5.0	21.0	0.2	
		7.5	19.5	0.1	
		15.0	17.8	0.2	
		30.0	17.0	0.3	6.87
		37.5	16.0	0.0	

Date	Pond	Depth cm	Temperature ° C	Oxygen ppm	pH
6/18/81	10	0.0	30.3	9.2	6.9
3pm		5.0	23.3	1.9	
		7.5	22.0	0.7	
		15.0	19.5	0.4	
		30.0	18.8	0.2	6.57
6/18/81	9	0.0	30.1	8.9	7.3
4pm		5.0	26.0	5.0	
		10.0	22.7	1.7	
		15.0	21.3	0.7	
		25.0	20.5	0.7	6.67
7/12/81	17	0.0	32.2	15.1	8.80
2pm		5.0	27.7	3.3	
Hazy		10.0	25.8	1.8	7.10
30° C		15.0	24.1	0.6	
		22.5	22.7	0.1	6.73
		30.0	21.7	0.0	
7/13/81	14	0.0	24.7	4.1	6.72
10am		5.0	23.2	0.9	
Cloudy		10.0	22.5	0.6	6.46
28° C		15.0	21.5	0.4	
		20.0	20.5	0.0	6.25
		30.0	19.9	0.0	
7/13/81	29	0.0	26.7	1.9	7.02
noon		5.0	25.8	2.7	6.82
Pt Cloudy		10.0	25.2	0.9	
		15.0	24.3	0.5	
		20.0	23.7	0.2	6.78
		30.0	23.3	0.0	
7/13/81	31	0.0	33.0	5.7	6.50
2pm		5.0	27.0	0.3	
Sunny		10.0	24.0	0.1	6.45
		15.0	22.7	0.0	
		20.0	21.3	0.0	6.39
7/16/81	7	0.0	27.4	1.0	
2 pm		5.0	26.1	0.1	
Pt Cloudy		10.0	23.9	0.0	
26° C		15.0	22.2	0.0	
		20.0	21.7	0.0	
		25.0	21.3	0.0	
		30.0	20.9	0.0	

Date	Pond	Depth cm	Temperature ° C	Oxygen ppm	pH
7/25/81	14	0.0	25.5	3.3	6.85
12:15pm		5.0	21.3	0.1	
Hazy		10.0	18.3	0.1	
26° C		15.0	17.4	0.0	6.55
		22.5	16.6	0.0	
		30.0	16.3	0.0	6.39
7/25/81	17	0.0	28.8	14.8	8.22
1pm		5.0	25.3	5.2	
		10.0	27.6	2.9	7.30
		15.0	20.7	1.9	
		22.5	19.5	1.5	6.65
		30.0	18.5	0.6	
7/25/81	18	0.0	30.5	15.3	9.44
2pm		5.0	30.5	12.5	
		10.0	29.7	12.4	
		15.0	27.8	13.4	9.32
		20.0	25.8	13.8	
		25.0	24.8	12.2	
		30.0	24.2	8.5	8.10
		35.0	23.8	3.75	
7/25/81	14	0.0	28.3	2.0	6.78
5:30pm		5.0	24.0	0.3	
		10.0	20.9	0.1	6.55
		15.0	19.0	0.0	
		22.5	18.1	0.0	
		30.0	17.3	0.0	6.54
7/25/81	18	0.0	26.7	15.3	9.46
6:15pm		5.0	27.5	15.9	
		10.0	27.9	15.5	9.43
		15.0	27.9	16.5	
		20.0	26.8	17.3	
		25.0	25.3	13.95	
		30.0	24.6	6.35	7.94
		35.0	23.6	1.10	
7/25/81	17	0.0	25.9	10.1	8.10
6:30pm		5.0	25.7	7.0	
		10.0	24.1	3.95	7.51
		15.0	22.4	1.0	
		22.5	20.7	0.2	
		27.5	20.3	0.0	7.15

Date	Pond	Depth cm	Temperature ° C	Oxygen ppm	pH
7/26/81	14	0.0	21.0	0.4	6.48
1am		5.0	20.5	0.0	
		10.0	20.1	0.0	6.48
		15.0	19.0	0.0	
		22.5	18.2	0.0	
		30.0	17.4	0.0	6.43
7/26/81	17	0.0	21.6	0.8	7.30
1:30am		5.0	21.9	0.2	
		10.0	27.0	0.1	7.16
		15.0	21.8	0.1	
		20.0	21.3	0.0	6.78
		25.0	20.3	0.0	
		30.0	20.1	0.0	
7/26/81	18	0.0	24.0	9.7	8.90
2am		5.0	24.8	9.5	
		10.0	24.9	9.5	8.90
		15.0	24.9	9.5	
		20.0	24.9	9.4	
		25.0	24.9	6.85	
		30.0	24.3	4.4	7.2
		35.0	23.6	0.7	
8/1/81	14	0.0	29.2	3.1	6.40
3:30pm		5.0	21.4	0.2	
Sunny		10.0	19.5	0.0	
29° C		15.0	17.8	0.0	6.36
		20.0	16.6	0.0	
		25.0	16.3	0.0	6.25
		30.0	16.2	0.0	
8/1/81	17	0.0	30.2	10.8	8.51
4:30pm		5.0	27.3	5.1	
		10.0	24.8	3.5	7.17
		15.0	22.7	1.8	
		20.0	19.7	0.3	
		25.0	18.6	0.1	6.70
		30.0	18.1	0.0	
8/1/81	18	0.0	29.6	14.5	9.37
5:30pm		5.0	29.7	14.5	
		10.0	29.3	14.9	9.34
		15.0	28.7	15.3	
		20.0	27.3	15.9	
		25.0	25.3	14.9	9.10
		30.0	23.8	1.11	
		35.0	32.2	6.0	

Date	Pond	Depth cm	Temperature ° C	Oxygen ppm	pH
8/2/81	14	0.0	17.7	1.4	6.13
8am		5.0	17.3	0.2	
18° C		10.0	17.2	0.1	6.1
		15.0	17.1	0.0	
		20.0	16.8	0.0	
		25.0	16.7	0.0	6.13
		30.0	16.4	0.0	
8/2/81	17	0.0	18.5	1.35	6.78
7:20am		5.0	18.6	0.4	
		10.0	18.7	0.2	6.71
		15.0	18.8	0.1	
		20.0	18.8	0.0	
		25.0	18.8	0.0	6.30
		30.0	18.7	0.0	
8/2/81	18	0.0	22.0	6.6	7.62
6:30am		5.0	22.2	6.1	
		10.0	22.2	6.1	
		15.0	22.3	6.1	7.41
		20.0	22.3	5.6	
		25.0	22.3	4.7	
		30.0	22.3	3.3	7.04
		35.0	22.2	1.1	
10/2/81	5	0.0	9.3	9.6	7.02
12 noon		7.5	9.5	8.4	
Cloudy		15.0	9.7	7.4	
7° C		22.5	9.7	7.1	
		30.0	9.7	6.2	
		37.5	9.7	5.4	
10/2/81	7	0.0	9.2	6.1	6.42
1pm		7.5	9.2	2.3	
		15.0	8.9	0.7	
		22.5	8.9	0.6	
10/2/81	11	0.0	8.8	4.3	6.38
1:30pm		7.5	8.6	1.9	
		15.0	8.8	0.7	
10/2/81	14	0.0	9.6	4.9	6.51
2:30pm		7.5	9.2	0.8	
		15.0	9.0	0.6	
		22.5	8.9	0.6	
		30.0	9.1	0.5	
		37.5	9.2	0.5	

Date	Pond	Depth cm		Temperature °C	Oxygen ppm	pH
10/2/81 2pm	31	0.0		8.8	4.9	6.72
		7.5		8.6	1.9	
		15.0		8.7	1.4	
		22.5		8.8	0.7	
10/2/81 3:30pm	29	0.0		10.1	5.4	6.95
		7.5		10.1	5.0	
		15.0		10.1	4.8	
		22.5		10.0	4.8	
		30.0		9.9	4.0	
		37.5		9.9	3.5	
		45.0		10.1	1.4	
10/5/81 9 am Sunny 15°C	17	0.0		13.8	12.7	7.02
		7.5		11.3	6.2	
		15.0		10.6	4.9	
		22.5		10.3	4.4	
		30.0		10.2	3.5	
		37.5		10.1	1.6	
10/5/81 2:30pm	30	0.0	(Plants)	13.3	10.8	7.65
		7.5		12.3	11.0	
		15.0		11.8	7.2	
		22.5		11.2	3.2	
		30.0		10.9	1.6	
		37.5		10.9	0.7	
		0.0	(Open)	11.9	7.9	
		7.5		11.8	8.4	
		15.0		11.8	8.8	
		22.5		11.3	9.4	
		30.0		11.1	7.9	
		37.5		11.0	1.1	
10/5/81 1 pm	16	0.0	(Plants)	12.9	7.0	7.10
		7.5		12.3	2.4	
		15.0		12.1	1.4	
		22.5		11.8	0.1	
		0.0	(Open)	12.5	9.4	
		7.5		11.9	8.1	
		15.0		11.8	7.6	
		22.5		11.7	6.8	
		30.0		11.8	1.8	
10/5/81 3 pm	18	0.0		12.1	11.1	7.87
		7.5		12.0	10.9	
		15.0		11.9	11.1	
		22.5		11.9	10.9	
		30.0		12.1	11.0	
		37.5		11.9	10.6	
		40.0		11.9	9.6	

Date	Pond	Depth cm	Temperature ° C	Oxygen ppm	pH
10/5/81 4pm	41	0.0	13.3	5.4	7.31
		10.0	11.7	3.2	
		15.0	10.9	2.8	
		22.5	10.9	1.8	
		35.0	10.9	1.3	
10/9/81 10 am Sunny 7° C	6	0.0	9.6	13.6	
		7.5	9.6	13.8	
		15.0	9.0	10.1	
10/9/81 12 noon	37	0.0	10.6	7.3	7.38
		7.5	9.5	6.5	
		15.0	8.6	5.7	
10/9/81 12:30pm	11	0.0	11.2	5.8	7.32
		15.0	9.5	5.6	
		30.0	9.6	7.8	
10/9/81 1pm	36	0.0	9.9	6.2	7.45
		7.5	9.4	3.4	
10/9/81 2pm	8	0.0	10.5	3.3	6.23
		7.5	8.0	0.7	
		15.0	7.4	0.7	
		22.5	7.2	0.0	
		30.0	7.0	0.0	
10/9/81 3pm	19	0.0	15.8	9.3	6.89
		7.5	12.1	5.7	
		15.0	10.3	5.0	
		20.0	9.7	1.2	
10/15/81 noon 10° C	27	0.0	10.9	4.8	7.20
		7.5	10.7	4.2	
		15.0	10.3	3.2	
		30.0	10.1	2.6	
		45.0	9.8	2.4	
10/15/81 1pm	26	0.0	10.9	1.8	7.07
		7.5	9.8	0.5	
		15.0	9.5	0.5	
		30.0	9.4	0.5	
10/15/81 1:30pm	13	0.0	11.4	5.4	7.35
		7.5	11.3	5.6	
		15.0	11.3	5.7	
		30.0	11.2	4.4	

Date	Pond	Depth cm	Temperature ° C	Oxygen ppm	pH
10/15/81	25	0.0	11.0	6.2	7.36
2pm		7.5	10.8	6.1	
		15.0	10.7	6.4	
		30.0	10.5	6.2	
		45.0	10.3	5.1	
10/16/81	3	0.0	14.9	5.9	6.77
12 noon		7.5	10.6	0.8	
Sunny		15.0	9.1	0.4	
10° C		30.0	8.9	0.2	
10/16/81	4	0.0	13.2	4.6	7.02
1pm		7.5	9.2	0.4	
		15.0	9.2	0.2	
		30.0	9.9	0.2	
10/16/81	10	0.0	13.3	4.6	6.32
2pm		7.5	9.8	0.2	
		15.0	9.1	0.2	
		20.0	8.9	0.2	
10/16/81	9	0.0	13.6	8.8	7.20
3pm		7.5	13.7	8.3	
		15.0	13.5	8.2	
		22.5	13.2	8.6	
		30.0	13.2	8.7	
		32.5	12.7	2.0	
4/19/82	5	0.0	13.0	4.5	7.45
11am		7.5	10.2	6.0	
Sunny		15.0	9.2	5.8	
17.5°C		22.5	8.5	5.8	
4/19/82	7	0.0	15.2	11.5	7.10
12 noon		7.5	14.7	11.2	
		15.0	14.7	11.2	
		37.5	14.3	10.4	
4/19/82	37	0.0	14.5	8.3	7.10
12:30pm		7.5	14.5	8.2	
		15.0	14.2	8.0	
		30.0	12.7	8.1	
		45.0	12.3	7.4	
		50.0	12.3	6.7	
4/19/82	31	0.0	18.5	6.5	6.45
1pm		7.5	15.8	4.6	
		15.0	13.5	4.7	
		22.5	11.7	4.1	
		30.0	11.8	2.5	

Date	Pond	Depth cm	Temperature ° C	Oxygen ppm	pH
4/19/82	8	0.0	21.0	4.0	6.25
2pm		7.5	16.2	2.8	
		15.0	11.6	3.1	
		30.0	10.8	2.8	
		35.0	9.8	2.5	
4/19/82	14	0.0	20.3	9.5	6.58
3pm		7.5	16.8	5.8	
		15.0	13.8	4.9	
		30.0	12.0	4.9	
		45.0	11.5	4.7	
4/19/82	18	0.0	16.5	6.2	7.95
4pm		7.5	16.3	6.2	
		15.0	16.2	6.2	
		30.0	16.2	6.2	
		45.0	16.3	6.2	
		50.0	16.3	5.8	
4/19/82	17	0.0	16.8	4.7	7.35
5pm		15.0	16.8	4.7	
		30.0	16.7	4.7	
		60.0	16.7	3.8	
4/19/82	8	0.0	20.5	11.5	7.38
6pm		7.5	21.0	12.0	
Sunny		15.0	18.0	6.8	
24° C					
4/23/82	41	0.0	13.4	9.4	7.56
1pm		7.5	13.3	9.4	
		15.0	13.0	8.4	
		30.0	12.6	8.6	7.60
		50.0	12.3	8.8	
		75.0	12.6	8.6	
4/23/82	26	0.0	16.0	7.9	7.66
2pm		7.5	15.7	7.9	
		15.0	14.9	7.8	7.67
		20.0	14.8	3.8	
		30.0	12.4	0.2	
4/23/82	29	0.0	16.2	10.6	7.59
3pm		15.0	16.2	10.8	
		30.0	16.0	10.4	
		45.0	15.8	10.2	7.61
		60.0	15.7	9.9	

Date	Pond	Depth cm		Temperature ° C	Oxygen ppm	pH
4/23/82	40	0.0		24.0	6.7	7.50
4pm		7.5		18.5	4.2	
		15.0		15.2	3.4	6.33
		22.5		12.2	2.2	
		25.0		10.7	1.7	
		32.5		9.8	1.0	
5/14/82	29	0.0		25.1	1.6	7.15
noon		7.5		23.9	1.5	
Pt cloudy		15.0		23.6	1.6	
27° C		22.5		22.9	1.6	
		37.5		22.6	1.9	7.29
		45.0		22.3	0.3	
5/14/82	41	0.0		27.7	7.2	7.80
2pm		7.5		25.5	6.0	
		15.0		23.2	7.4	
		22.5		22.1	7.6	
		30.0		21.7	7.0	7.12
		32.5		21.2	2.5	
5/17/82	18	0.0		24.8	9.3	8.43
1pm		7.5		24.7	9.8	
Sunny		15.0		24.7	9.4	
27° C		22.5		24.3	11.0	
		30.0		23.2	14.4	8.98
		40.0		22.7	10.5	
5/17/82	30	0.0		31.3	12.6	9.38
2:30pm		7.5		28.2	11.4	
		15.0		25.2	10.8	
		22.0		23.5	6.4	
		30.0		21.5	1.3	7.30
5/19/82	41	0.0	(Plants)	28.0	5.3	
1pm		7.5		23.8	2.4	
Sunny		15.0		21.3	1.2	
27° C		22.5		20.5	0.3	
		25.0		20.0	0.2	
		0.0	(Open)	25.0	5.4	
		7.5		24.3	5.3	
		15.0		23.2	6.8	
		22.5		22.7	7.2	
		30.0		22.7	7.4	
		32.5		22.4	4.2	

Date	Pond	Depth cm	Temperature ° C	Oxygen ppm	pH
5/19/82	8	0.0	28.8	6.1	
3pm		2.5	28.2	2.2	
		5.0	24.7	0.6	
		7.5	22.8	0.3	
		15.0	19.7	0.1	
		22.5	18.2	0.1	
		30.0	17.2	0.0	
		37.5	15.8	0.0	
		45.0	15.3	0.0	
		55.0	14.7	0.0	
5/20/82	17	0.0	30.7	10.2	
1pm		2.5	28.6	6.4	
Cloudy		5.0	24.9	4.8	
25° C		7.5	22.7	3.9	
		15.0	20.7	2.5	
		22.5	19.3	1.5	
		30.0	18.1	0.3	
		37.5	17.3	0.1	
5/24/82	41	0.0	18.0	6.2	6.90
noon		7.5	16.9	1.65	
Cloudy		15.0	16.5	0.7	
18° C		30.0	16.3	0.2	
		32.5	16.2	0.0	6.90
5/24/82	29	0.0	18.5	2.8	
2pm		7.5	17.8	0.8	
		15.0	17.4	0.5	
		30.0	17.3	0.5	
		45.0	16.9	0.2	
		50.0	17.1	0.0	
5/24/82	18	0.0	18.2	12.8	
4pm		7.5	18.2	12.1	
		15.0	18.2	12.0	
		22.5	18.2	11.4	
		30.0	18.2	7.0	
5/25/82	30	0.0	15.0	7.4	7.35
8am		7.5	15.1	6.4	
Sunny		15.0	15.1	5.8	
16° C		22.5	15.0	1.5	6.70
		30.0	14.7	0.0	
5/25/82	17	0.0	15.3	5.5	7.12
11am		7.5	15.1	3.8	
		15.0	14.7	2.2	
		30.0	14.6	1.0	
		45.0	14.6	0.3	6.86
		50.0	14.6	0.1	

Date	Pond	Depth cm	Temperature ° C	Oxygen ppm	pH
5/25/82	17	0.0	15.3	5.5	7.12
1pm		7.5	15.1	3.8	
		15.0	14.7	2.2	
		30.0	14.6	1.0	
		45.0	14.6	0.3	6.86
		50.0	14.6	0.1	
5/25/82	14	0.0	16.5	8.0	6.7
		2.5	15.4	3.6	
		7.5	14.7	0.5	
		15.0	14.5	0.3	
		22.5	14.4	0.2	6.35
		30.0	14.3	0.1	
5/30/82	18	0.0	22.3	11.2	
noon		7.5	22.3	11.6	
Cloudy		15.0	22.3	11.6	
24° C		30.0	22.3	12.3	
		45.0	21.6	5.2	
5/30/82	30	0.0	21.5	10.9	
2pm		7.5	21.5	9.6	
		15.0	19.5	6.7	
		30.0	14.5	0.8	
6/3/82	17	0.0	16.3	5.1	
10am		7.5	16.3	1.4	
Cloudy		15.0	16.2	1.0	
14° C		30.0	16.1	0.7	
6/3/82	30	0.0	15.7	10.0	
11am		7.5	15.7	5.5	
		15.0	15.4	4.5	
		30.0	14.8	1.0	
		32.5	14.8	0.0	
6/3/82	18	0.0	18.7	9.0	
noon		7.5	18.7	8.8	
		15.0	18.7	8.7	
		30.0	18.8	8.6	
		32.5	18.9	7.4	
6/3/82	14	0.0	15.3	10.8	
1pm		5.0	15.3	4.5	
		7.5	15.2	0.4	
		15.0	15.1	0.4	
		25.0	14.9	0.3	
		27.5	14.9	0.0	

Date	Pond	Depth cm	Temperature ° C	Oxygen ppm	pH
6/3/82	29	0.0	16.3	5.1	
2pm		7.5	16.3	1.4	
		15.0	16.2	1.0	
		30.0	16.1	0.7	
6/3/82	41	0.0	15.7	1.2	
3pm		2.5	15.9	0.5	
		7.5	16.2	0.3	
		15.0	16.2	0.2	
		30.0	16.2	0.1	
		32.5	16.8	0.0	
6/6/82	41	0.0	31.0	5.8	
noon		2.5	28.6	4.0	
Sunny		5.0	24.6	2.1	
24° C		7.5	22.5	1.7	
		15.0	20.0	0.8	
		22.0	16.8	0.6	
		30.0	16.2	0.3	
		37.5	15.8	0.1	
		40.0	15.7	0.0	

APPENDIX C

POND FILTERABLE IRON DATA
in μM Fe

	1981		1982					
Pond	4/16-4/27	6/7	4/19	5/17	5/20-5/25	5/30	6/3	8/1
29	0.70 0.55	0.26 0.45	0.05 0.47	0.00 0.00 0.21 0.26 0.45	0.49		1.02	1.00 0.80
14	1.00 1.00	1.49 0.93	0.39 0.24		2.08 1.67	0.79	5.12	25.6 22.4
41			0.05 0.00	0.57 0.29 0.13 0.81 0.47 0.34 0.68 0.42	1.92 1.36		2.08 2.41	1.8 2.5 2.8
17		1.30 1.52	0.24 0.16	0.11 1.13 1.02 0.82	3.47 4.76 1.98	1.17 1.12	6.27	4.00 2.60
30	1.04 0.81	1.60 0.88	0.82 0.52	0.47 0.82	3.11	0.89 0.62	2.65	1.30 1.20
18	1.07 0.90		0.05 0.36	0.29 1.15 0.06 1.12	2.59 2.82	0.52 0.62 0.86 0.43	3.80 2.39	3.80 3.90 3.70 3.60

APPENDIX D

POND PHOSPHATE DATA
in μM P

Species	Pond	1979 Fall	1980 Spring	1980 Summer	1981 Spring	1982 Spring	1982 Summer
L. turionifera	3	0.45					
	5	0.34 0.44	0.39	0.99	0.49 0.26		
	35				0.49		
	36				0.60		
	37				0.40		
	41					0.18 0.19 0.29 0.35 0.35 0.28 0.47 0.14 0.48 0.15	0.13 0.22 0.42 0.63 0.27 0.12 0.63 0.14
L. turionifera - W. punctata	14	0.44 0.19	0.25		0.16	0.24 0.25 0.10 0.07 0.07 0.22	0.17 0.17 0.20 0.17 0.19 0.19
	19		0.48		0.08		
	26	0.20	0.38	0.96	0.08		
	27	0.19	0.21				
	31				0.10		
W. punctata	1	0.13	0.23				
	6	0.15 0.18	0.10	0.08	0.05		
	11	0.43 0.13	0.12	0.16	0.04		

Species	Pond	1979 Fall	1980 Spring	1980 Summer	1981 Spring	1982 Spring	1982 Summer
W. punctata	13	0.08 0.03	0.19		0.56		
	29	0.06	0.05	0.20	0.01	0.02 0.06 0.08 0.06 0.06 0.06 0.06 0.08 0.05	0.02 0.03 0.04 0.04 0.07 0.04 0.07 0.06
L. turionifera - S. polyrhiza	2		1.09				
	7		4.58	2.31	0.36		
	10	3.40					
	17	0.20 0.23		0.66 1.45	0.32 0.27	0.19 0.18 0.21 0.23 0.45 0.45	0.42 0.24 0.39 0.63 0.16 0.54 0.52 0.70 0.81 1.32 1.04 1.01
	20	0.60		0.57 0.33			
	28	0.12	0.14	0.28	0.60		
S. polyrhiza	16	0.36	0.26	6.79 0.45 1.22	1.18		
	30			3.99 0.36	0.36 0.23	0.09 0.10 0.33 0.32 1.33 1.33	0.34 0.85 0.21 0.40 0.68 1.03 0.21 0.57

Species	Pond	1979 Fall	1980 Spring	1980 Summer	1981 Spring	1982 Spring	1982 Summer
L. minor	4	0.76 0.44			1.96		
	9	0.31					
L. turionifera - W. columbiana	8		2.45	1.50			
"No Plants"	12	0.09 0.08					
	18	0.18 0.20 0.02	0.37 0.13	0.53 0.38	0.01	0.05 0.06 0.20 0.23 0.21 0.20	0.06 0.10 0.06 0.05 0.04 0.03 0.08 0.08 0.08 0.04
	25	0.09	0.30	0.20	0.06		

APPENDIX E

POND SOLUBLE NITROGEN (NO_2, NO_3 and NH_4) in μM N 1982 DATA

Species	Pond	4/18	5/12-5/18	5/30	8/1	8/29
L. Turionifera	41	4.5 4.5	3.6 3.3 5.5 23.3 2.1 4.6 4.5		2.7 4.4 17.2 26.3	
	8	7.8	23.3 21.5			6.9 16.8
	7	5.0 4.0				
	37	0.8 0.5				
	5					21.8 23.0
	40	6.2 7.0				17.6 80.5
L. turionifera-W. punctata	14	3.2 2.9		5.4 0.8	1.0 0.4 0.9 2.3	
	19					1.5 0.9
	31	1.7 1.5				1.0 2.4
	26	2.4 2.0				16.0 30.6
W. punctata	29	4.9 4.8	2.5 6.8 6.6 2.1		0.4 0.7 0.5 0.9	

Species	Pond	4/18	5/12-5/18	5/30	8/1	8/29
W. punctata	6					1.2
						1.0
	11					0.7
						1.4
L. turionifera-S. polyrhiza	17	2.8	1.7	2.8	1.4	
			1.3	2.9	2.2	
			2.1	73.5	5.3	
			2.3	69.7	17.9	
				36.6		
				37.4		
S. polyrhiza	30	13.3	14.0	2.7	5.5	
		8.4	12.8	3.8	2.1	
			66.3	13.5	1.7	
			67.7	13.6	15.6	
				13.0		
				13.3		
"No Plants"	18	4.8	2.5	0.8	0.9	
		4.8	1.1	0.9	0.9	
			0.7	2.1	1.8	
			1.4	31.2	0.7	
				1.4		
				29.5		

APPENDIX F

RAINWATER NUTRIENT DATA
in μM N, P, and Fe

Date	Site	NO_2+NO_3	NH_4	$NO_2+NO_3+NH_4$	PO_4	Fe
5/29/82	Pond 41	25.4	26.3	52.4	0.68	
5/30/82	Pond 18	42.4	7.0	49.4	0.32	
5/30/82	Pond 30[a]	15.4	26.0	41.4	8.10	
5/30/82	Pond 17	51.6	10.7	62.3	0.56	
6/3/82	Pond 17	26.4	22.0	48.4	0.07	
6/16/82 pH = 4.11	Ann Arbor	21.1	6.9	28.0	0.41	0.02
6/28/82	Ann Arbor	58.0	11.2	60.2	0.26	0.37
6/29/82 pH = 3.97	Ann Arbor	23.3	19.4	42.7	0.12	0.00
6/29/82	Pond 30	27.0	23.8	50.8	0.08	0.05
6/29/82 pH = 3.78	Pond 41	43.0	4.4	47.4	0.26	0.07
6/29/82	Pond 29[a]	68.9	8.2	77.1	0.45	2.06
6/29/82	Pond 17[a]	50.4	7.4	57.8	2.90	0.00
6/29/82	Pond 18[a]	53.2	7.6	60.2	0.53	1.20
7/26/82	Pond 41	38.1	13.7	51.8	0.75	
7/26/82	Pond 29	48.4	13.1	61.5	0.37	
7/26/82	Ponds 17, 18, and 30	46.6	12.1	58.7	0.73	

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