# Growth Characteristics of Aquatic Macrophytes Cultured in Nutrient-enriched Water: II. Azolla, Duckweed, and Salvinia[1]

K. R. REDDY AND W. F. DEBUSK[2]

*Seasonal growth characteristics and biomass yield potential of 4 small-leaf, floating, aquatic macrophytes cultured in nutrient nonlimiting conditions were evaluated for central Florida's climatic conditions. Biomass yields were found to be 10.6, 11.3, 16.1, and 32.1 t (dry wt) ha$^{-1}$ yr$^{-1}$, respectively, for azolla (*Azolla caroliniana*), giant duckweed (*Spirodela polyrhiza*), common duckweed (*Lemna minor*), and salvinia (*Salvinia rotundifolia*). Operational plant density was in the range of 10–80 g dry wt m$^{-2}$ for azolla, 10–88 g dry wt m$^{-2}$ for giant duckweed, 10–120 g dry wt m$^{-2}$ for common duckweed, and 35–240 g dry wt m$^{-2}$ for salvinia. Specific growth rate (% increase per day) was maximum at low plant densities and decreased as the plant density increased. Results suggest that small-leaf, floating plants may not be suitable in monoculture biomass production systems because of low biomass yields, but they may be suitable for inclusion in polyculture systems with larger aquatic plants. The high N content (crude protein = 20–33%) of small-leaf, floating plants suggests the use of biomass as animal feed.*

Aquatic macrophytes have been the subject of great interest for the past few years because of their potential uses in wastewater treatment (Boyd, 1970; Cornwell et al., 1977; Sutton and Ornes, 1977) and as a feed supplement for aquatic and terrestrial animal stocks (Culley and Epps, 1973). Biomass harvested from aquatic macrophyte wastewater treatment systems may also be used for the production of gaseous fuels (conversion to energy). The concept of using aquatic plants for water treatment and the harvested biomass as an energy source is gaining attention in the tropical and subtropical regions of the world, where the climate is conducive to plant growth throughout the year.

Several studies have shown that aquatic plants, such as water hyacinth (*Eichhornia crassipes* [Mart] Solms), water lettuce (*Pistia stratiotes* L.), cattail (*Typha latifolia* L.), and bulrush (*Scirpus validus* L.), are capable of treating wastewater (Lakshman, 1979; Reddy et al., 1982; Wolverton and McDonald, 1979; Wolverton, 1982). The possibility of using duckweed and azolla for treating wastewater, especially for phosphorus removal, has been suggested (Sutton and Ornes, 1975; Reddy and DeBusk, 1984a). The success of aquatic macrophyte-based wastewater treatment systems depends on rapid year-round plant growth and nutrient uptake. Very little information is available on the seasonal growth characteristics of aquatic macrophytes cultured in nutrient-enriched waters. DeBusk et al. (1981) showed significant effects of seasonality on the productivity of water hyacinth, duckweed (*Lemna minor* L.), and hydrilla (*Hydrilla verticillata* [L.f.]

[1] Received 23 April 1984; accepted 15 October 1984. Florida Agricultural Experiment Stations Journal Series No. 5928.

[2] Professor and Assistant in Soil Science, respectively, University of Florida, Institute of Food and Agricultural Sciences, Central Florida Research and Education Center, P.O. Box 909, Sanford, FL 32771.

Royle). More recently, Reddy and DeBusk (1984b) also demonstrated the effect of seasonal changes on growth of large-leaf, floating plants, such as water hyacinth, water lettuce, and pennywort (*Hydrocotyle umbellata* L.). The objective of the current investigation was to determine the seasonal growth characteristics of small-leaf, floating plants, such as common duckweed (*Lemna minor* L.), giant duckweed (*Spirodela polyrhiza* L.), azolla (*Azolla caroliniana* Willd.), and salvinia (*Salvinia rotundifolia* Willd.).

## MATERIALS AND METHODS

Aquatic plants used in this study were obtained from the St. Johns River near Sanford, FL. Plants were cultured in 1,000-liter outdoor concrete tanks (1.7 $m^2$ surface area) for a period of 1 yr beginning December 30, 1981. Duplicate tanks were used for each plant type. Two 0.25 $m^2$ floating PVC (polyvinyl chloride) frames were placed in each tank (4 replications per each plant type). Plants were maintained at the same density inside and outside the PVC frames. All plants were stocked at low starting densities and were allowed to grow until maximum densities (no measurable net growth) were reached. At that time, plants were harvested and restocked at starting densities. Starting densities used were: *Azolla,* 8 g dry wt $m^{-2}$; *Lemna* and *Spirodela,* 10 g dry wt $m^{-2}$; and *Salvinia,* 30 g dry wt $m^{-2}$. Shade screens were used to reduce light intensity by about 30% in *Azolla, Lemna,* and *Spirodela* cultures.

All tanks were filled with 700 liters of nutrient medium containing $NH_4$-N = 10.5 mg $l^{-1}$; $NO_3$-N = 10.5 mg $l^{-1}$; $PO_4$-P = 3.0 mg $l^{-1}$; K = 23.0 mg $l^{-1}$; Ca = 20.0 mg $l^{-1}$; Mg = 5.0 mg $l^{-1}$; Fe-EDTA = 0.6 mg $l^{-1}$ and micronutrients. *Azolla* was cultured in N-free nutrient medium. Micronutrients were applied through commercially-available liquid fertilizer (Nutrispray-Sunniland, Chase & Co., Sanford, FL) to obtain a final concentration of 4 mg Fe $l^{-1}$; 0.2 mg Cu $l^{-1}$; 1.5 mg Mn $l^{-1}$; 0.04 mg B $l^{-1}$; 0.02 mg Mo $l^{-1}$; and 3 mg S $l^{-1}$. The nutrient medium was mixed by submersible pumps that operated on a 12 h/day cycle. Once a week, water in each tank was replaced with fresh medium containing the above described chemical composition.

At the end of each week, plants from the PVC frames were removed and wet weights were obtained after draining excess water. Plant samples were obtained at the end of each growth cycle. Throughout the study period, ambient air temperatures and solar radiation were recorded. Plant samples were dried at 70°C for 48 h and dry weights determined. At the beginning and end of each week, water samples were obtained and analyzed for ammonium N, nitrate N, ortho-P, and volatile solids using standard methods (A.P.H.A., 1980). Plant samples were digested using standard methods (Jackson, 1958), and analyzed on an autoanalyzer (A.P.H.A., 1980).

## RESULTS AND DISCUSSION

### *Growth curve*

The *Azolla* growth curve was significantly influenced by seasonal changes in temperature and solar radiation (Fig. 1). Minimal initial lag phase in the growth curve was observed in all seasons. Growth rates were highest in the linear phase

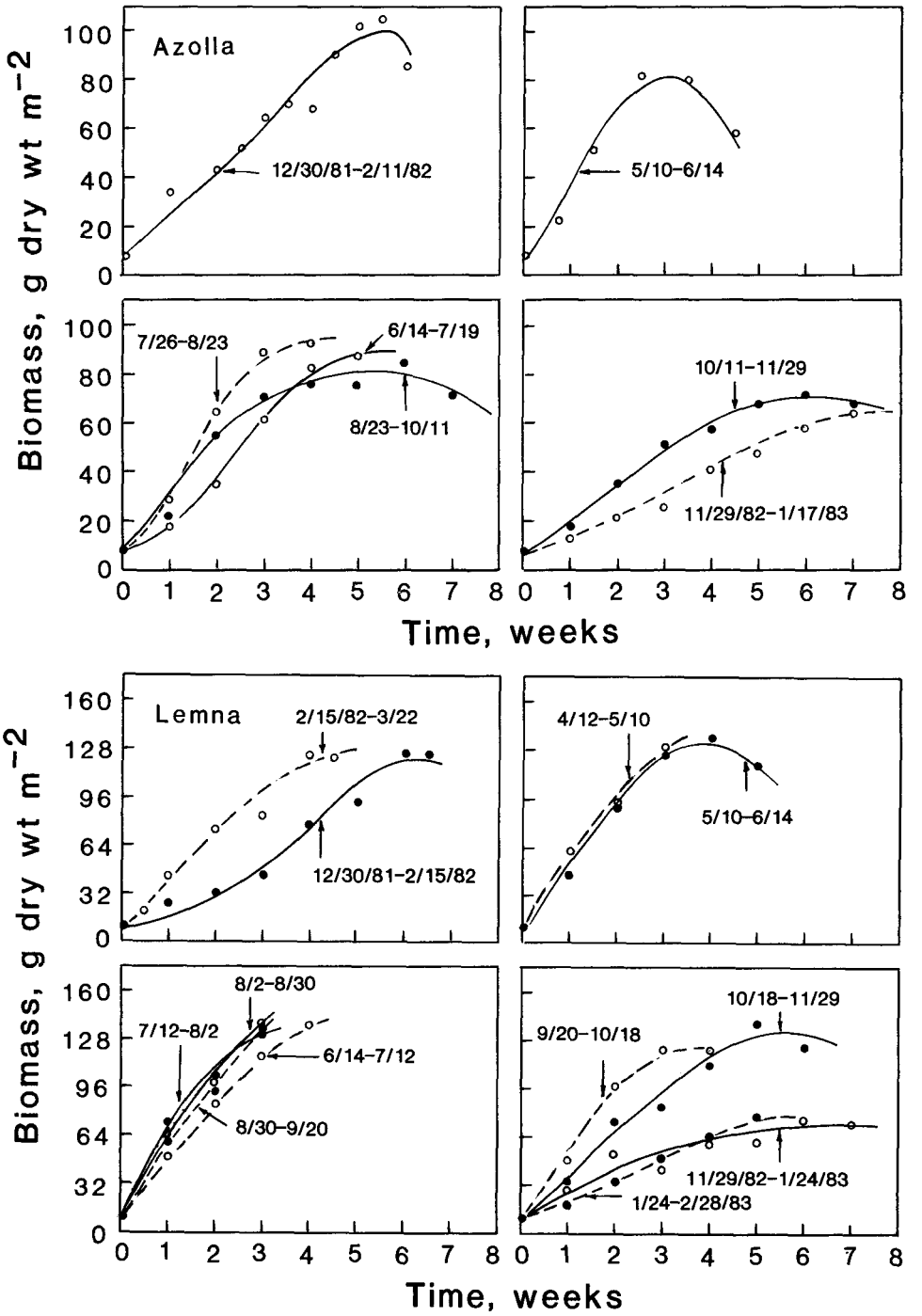


**Fig. 1-2.** Fig. 1. Growth curves of *Azolla caroliniana* at various times during the growing season. Fig. 2. Growth curves of *Lemna minor* at various times during the growing season.

TABLE 1. MAXIMUM GROWTH RATE (ANNUAL AVERAGE OF THE LINEAR PHASE OF ALL GROWTH CURVES) AND OPERATIONAL PLANT DENSITY OF SELECTED SMALL-LEAF, FLOATING PLANTS.

| Plant type | Growth rate g dry wt $m^{-2}$ $day^{-1}$ | Operational plant density g dry wt $m^{-2}$ |
|---|---|---|
| *Azolla* | 2.9 ± 1.0 | 10–80 |
| *Lemna* | 4.4 ± 2.7 | 10–120 |
| *Spirodela* | 3.1 ± 1.3 | 10–88 |
| *Salvinia* | 8.8 ± 3.4 | 35–240 |

of the growth curve. For *Azolla,* the linear growth phase occurred within the plant density range of 10–80 g dry wt $m^{-2}$, which was considered optimum plant density or operational plant density (Reddy et al., 1983). To achieve maximum biomass yield of *Azolla,* production systems should be operated in this density range. Tran and Dao (1973) and Talley et al. (1977) observed maximum yields of *Azolla pinnata, A. mexicana* and *A. filiculoides* when cultured at a density of about 0.5 kg fresh wt $m^{-2}$ ($\simeq$25 g dry wt $m^{-2}$). Maximum plant density of 105 g dry wt $m^{-2}$ was observed during the winter season, while during the remainder of the season, maximum density was about 90 g dry wt $m^{-2}$. During warmer months, growth ceased at relatively lower densities than in cooler months.

Potential maximum growth rates of *Azolla* were calculated using the least square fit of data points in the linear phase of the growth curve (Table 1). During the months of May through September, growth rates were in the range of 3.2–4.3 g dry wt $m^{-2}$ $day^{-1}$ (average = 3.7 ± 0.5 g dry wt $m^2$ $day^{-1}$), while during October through February, growth rates ranged from 1.2–2.4 g dry wt $m^{-2}$ $day^{-1}$ (average = 1.8 ± 5 g dry wt $m^{-2}$ $day^{-1}$). Plant growth during winter months was affected by the low incoming solar radiation and low temperature.

Growth curves of *Lemna* are shown in Fig. 2. During warmer months, *Lemna* reached a maximum density of about 125–140 g dry wt $m^{-2}$. Growth rates decreased at higher plant densities. Slopes of the linear phase of the growth curve indicated growth rates (4.7–6.0 g dry wt $m^{-2}$ $day^{-1}$) were approximately the same during the months of April through October (average = 5.4 ± 0.9 g dry wt $m^{-2}$ $day^{-1}$). During cooler months, growth rates were in the range of 1.5–3.7 g dry wt $m^{-2}$ $day^{-1}$ (average = 2.8 ± 0.9 g dry wt $m^{-2}$ $day^{-1}$). Reported growth rates are frequently in the range of 3–7 g dry wt $m^{-2}$ $day^{-1}$ (Harvey and Fox, 1973; Sutton and Ornes, 1975; DeBusk et al., 1981). These estimates are based on short-term growth studies. The average annual growth rate observed in our study was found to be 4.4 g dry wt $m^{-2}$ $day^{-1}$ (15.9 mt $ha^{-1}$ $yr^{-1}$). The linear growth phase occurred in a plant density range of 10–120 g dry wt $m^{-2}$ $day^{-1}$ (Table 1). This wide range in operational plant density allows a greater flexibility in scheduling harvests. However, the linear phase appears to occur only for a maximum period of 3–4 wk, indicating that *Lemna* should be harvested much more frequently than many of the large-leaf floating plants such as water hyacinth.

*Spirodela* growth curves were also influenced by season (Fig. 3). A lag phase in the growth curve was observed only during winter months. Maximum growth rate (slope of the linear phase of the growth curve) was found to be in the range

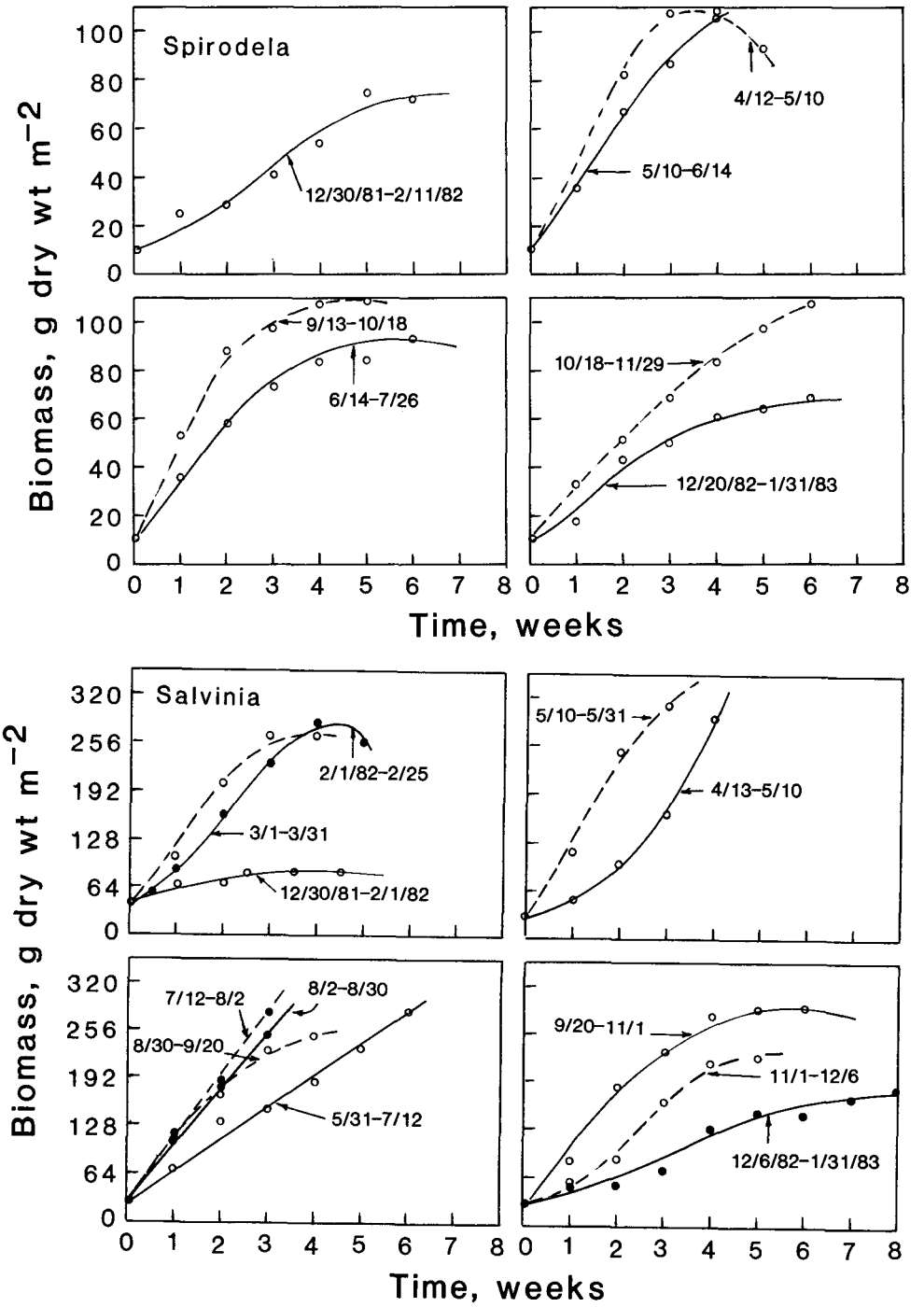


**Fig. 3-4.** Fig. 3. Growth curves of *Spirodela polyrhiza* at various times during the growing season. Fig. 4. Growth curves of *Salvinia rotundifolia* at various times during the growing season.

of 1.8–5.6 g dry wt $m^{-2}$ $day^{-1}$, with an annual average of 3.1 g dry wt $m^{-2}$ $day^{-1}$ (11.3 mt $ha^{-1}$ $yr^{-1}$). Operational plant density for *Spirodela* was in the range of 10–88 g dry wt $m^{-2}$ (Table 1). Sutton and Ornes (1977) reported much lower growth rates (1.9 g dry wt $m^{-2}$ $day^{-1}$) for *Spirodela polyrhiza* cultured in static sewage effluent.

*Salvinia* appeared to be very sensitive to winter temperatures as evidenced by poor growth (Fig. 4). Low growth rates (1.6–3.4 g dry wt $m^{-2}$ $day^{-1}$) were observed during the months of December, January, and February, while during the remainder of the year growth rate was found to range from 5.8–13.9 g dry wt $m^{-2}$ $day^{-1}$. Average annual growth rate was 8.8 g dry wt $m^{-2}$ (32 mt $ha^{-1}$ $yr^{-1}$). Linear growth phase was observed within the plant density range of 35–240 g dry wt $m^{-2}$ and lasted for a period of 3–4 wk (Table 1).

### *Specific growth rate*

Specific growth rates (SGR) were calculated using the equation presented by Jackson (1980). Specific growth rates of all the plants evaluated were found to be significantly influenced by the plant density. Maximum SGR was observed at the lowest plant density. At low plant density (15 g dry wt $m^{-2}$) SGR of *Azolla* was found to range from 0.125–0.22 $day^{-1}$ (doubling time = 3.2–5.5 days), while at high plant density (80 g dry wt $m^{-2}$) SGR was found to be 0.005–0.01 $day^{-1}$ (doubling time = 69–138 days).

At low plant density (30 g dry wt $m^{-2}$) SGR of *Lemna* was in the range of 0.118–0.273 $day^{-1}$ (doubling time = 2.5–5.9 days). At high plant density (130 g dry wt $m^{-2}$) SGR decreased significantly to 0.01–0.05 $day^{-1}$ (doubling time = 13.9–69.3 days). Similar results were observed for *Spirodela* with SGR of 0.081–0.237 $day^{-1}$ (doubling time = 2.9–8.7 days) at low plant density (20 g dry wt $m^{-2}$), and 0.002–0.01 $day^{-1}$ (doubling time = 69.3–346.5 days) at high plant density of about 100 g dry wt $m^{-2}$.

*Salvinia* SGR was in the range of 0.065–0.196 $day^{-1}$ (doubling time = 3.5–10.7 days) at a plant density of about 60 g dry wt $m^{-2}$. At high plant density (250 g dry wt $m^{-2}$) SGR decreased to 0.003–0.03 $day^{-1}$ (doubling time = 23.1–231 days).

### *Relationship between growth rates and growth-controlling factors*

Two growth measuring methods were evaluated: 1) specific growth rate (SGR), which is defined as the fractional increase per day and is independent of biomass or the units in which biomass is measured (e.g., dry wt), 2) growth rate (GR) expressed as the biomass increase per unit area and time. These 2 growth measures were correlated with various growth-controlling factors such as plant density, and environmental variables such as solar radiation and temperature (Table 2). Specific growth rate (SGR) was significantly correlated with plant density. Decrease in SGR at high plant densities was probably due to self shading, nutrient depletion or changes in other processes needed to maintain optimal growth (Jackson, 1980). In our study, nutrient nonlimiting conditions were created by replenishing the nutrients once every 7 days. Growth rate (GR), which is the product of SGR × density, was poorly correlated with the plant density. However, highly significant

TABLE 2. CORRELATION COEFFICIENTS BETWEEN GROWTH RATES AND GROWTH-CONTROLLING FACTORS.

| Plant type | Growth rate | Plant density | Solar radiation | Air temperature | | |
|---|---|---|---|---|---|---|
| | | | | Mean | Min. | Max. |
| *Azolla* | SGR | 0.74** | 0.48** | 0.27 (NS) | 0.23 (NS) | 0.31 (NS) |
| | GR | 0.09 (NS) | 0.43** | 0.15 (NS) | 0.11 (NS) | 0.19 (NS) |
| *Lemna* | SGR | 0.66** | 0.30 (NS) | 0.19 (NS) | 0.16 (NS) | 0.21 (NS) |
| | GR | 0.12 (NS) | 0.59** | 0.46** | 0.41** | 0.49** |
| *Spirodela* | SGR | 0.75** | 0.16 (NS) | 0.17 (NS) | 0.14 (NS) | 0.20 (NS) |
| | GR | 0.36* | 0.27 (NS) | 0.22 (NS) | 0.19 (NS) | 0.25 (NS) |
| *Salvinia* | SGR | 0.66** | 0.35* | 0.19 (NS) | 0.17 (NS) | 0.20 (NS) |
| | GR | 0.02 (NS) | 0.52** | 0.33* | 0.29 (NS) | 0.37* |

SGR = Specific growth rate, $day^{-1}$.
GR = Growth rate, g $m^{-2}$ $day^{-1}$.
* = Significant at 0.05 level of probability.
** = Significant at 0.01 level of probability.
NS = Not significant.

correlation was observed between GR and solar radiation. Poor relationship of growth rates with plant density was due to the similar growth rates observed throughout the wide operational density range. Since plant density showed the best relationship with SGR, predictive equations for SGR were calculated only as a function of plant density (Table 3). Adding environmental variables such as solar radiation or ambient air temperature to the regression equation did not result in a significant increase in correlation. According to the equations in Table 3, SGR approaches 0 at plant densities of 93, 139, 101, and 309 g dry wt $m^{-2}$ for *Azolla, Lemna, Spirodela,* and *Salvinia,* respectively. These values are in close agreement with the experimental values presented in Fig. 1–4.

Based on the results of this study, among the small-leaf, floating plants evaluated, potential biomass yields were in the order of *Salvinia* > *Lemna* > *Azolla* > *Spirodela.* Annual biomass yields of these plants were significantly lower than yields of the large-leaf, floating plants such as water hyacinth, water lettuce, and pennywort (DeBusk et al., 1981; Reddy and DeBusk, 1984b). Although small-leaf, floating plants may not be suitable for monoculture systems requiring high

TABLE 3. LINEAR REGRESSION EQUATIONS FOR PREDICTING SPECIFIC GROWTH RATE OF SELECTED AQUATIC PLANTS.

| Plant type | Equation | R | n |
|---|---|---|---|
| *Azolla* | SGR = 0.141–0.00152PD | 0.74** | 44 |
| *Lemna* | SGR = 0.182–0.00131PD | 0.66** | 48 |
| *Spirodela* | SGR = 0.159–0.00157PD | 0.75** | 39 |
| *Salvinia* | SGR = 0.136–0.00044PD | 0.66** | 48 |

SGR = Specific growth rate, $day^{-1}$.
PD = Plant density, g dry wt $m^{-2}$.
** = Significant at 0.01 level of probability.
R = Regression coefficient.
n = Number of observations.

TABLE 4. SELECTED CHARACTERISTICS OF SMALL-LEAF, FLOATING PLANTS CULTURED IN THE NUTRIENT MEDIUM.

| Plant type | Dry matter | Volatile solids | Tissue composition | | N/P ratio |
|---|---|---|---|---|---|
| | | | N | P | |
| | % | | | | |
| *Azolla* | 4.08 | 86.1 | 3.41 | 1.47 | 2.3 |
| *Lemna* | 5.72 | 86.8 | 5.36 | 1.41 | 3.8 |
| *Spirodela* | 4.95 | 83.7 | 5.16 | 1.29 | 4.0 |
| *Salvinia* | 3.70 | 84.2 | 3.24 | 1.03 | 3.1 |

biomass yields, they may be suitable for inclusion in polyculture systems with larger aquatic plants.

Data on the chemical composition of the plants evaluated are shown in Table 4. Total N content of *Azolla* cultured in N-free medium was 3.41%. This N was primarily derived from biological fixation of $N_2$ through its symbiotic relationship with the alga, *Anabena.* Estimated $N_2$ fixation rates by *Azolla* cultured in N-free medium were found to be in the range of 1.4–2.7 kg N $ha^{-2}$ $day^{-1}$ (Talley et al., 1977; Watanabe et al., 1977; Lumpkin and Plucknett, 1980). In our study, *Azolla* fixed 0.99 ± 0.34 kg N $ha^{-1}$ $day^{-1}$ as a result of biological $N_2$ fixation. The high N content of small-leaf, floating plants (crude protein = 20–33%) suggests that these plants can be used as animal feed. Nitrogen removal rates by *Lemna, Spirodela,* and *Salvinia* were 236 ± 145, 160 ± 67, and 285 ± 110 mg N $m^{-2}$ $day^{-1}$, respectively. Phosphorus removal rate due to plant uptake was 43 ± 15, 62 ± 38, 40 ± 17, and 91 ± 35 mg P $m^{-2}$ $day^{-1}$, for *Azolla, Lemna, Spirodela,* and *Salvinia,* respectively. Although overall N and P removal by these plants is low compared to many large-leaf, floating plants, the narrow N/P ratio (Table 4) suggests that these plants are efficient in removing P from wastewaters. The high P removal capacity (Sutton and Ornes, 1975; Reddy and DeBusk, 1984a) and low light requirements (Wedge and Burris, 1982) of *Azolla, Lemna,* and *Spirodela* suggest that these plants can be ideally integrated into wastewater treatment systems based on water hyacinth or cattail to improve overall nutrient removal efficiency.

## ACKNOWLEDGMENTS

This paper reports results from a project that contributes to a cooperative program between the Institute of Food and Agricultural Sciences (IFAS) of the University of Florida and the Gas Research Institute (GRI), entitled, "Methane from Biomass and Waste."

## LITERATURE CITED

A.P.H.A. 1980. Standard methods for the examination of water and wastewater. 15th ed, p. 1134. American Public Health Association. Washington, DC.

Boyd, C. E. 1970. Vascular aquatic plants for mineral nutrient removal from polluted waters. Econ. Bot. 24: 95–103.

Cornwell, D. A., J. Zoltek, Jr., C. D. Patrinely, T. des Furman, and J. I. Kim. 1977. Nutrient removal by water hyacinths. J. Water Pollut. Control Fed. 49: 57–65.

Culley, D. D., Jr., and A. E. Epps. 1973. Use of duckweed for waste treatment and animal feed. J. Water Pollut. Control Fed. 45: 337–347.

DeBusk, T. A., J. H. Ryther, M. D. Hanisak, and L. D. Williams. 1981. Effects of seasonality and plant density on the productivity of some freshwater macrophytes. Aquatic Bot. 10: 133–142.

Harvey, R. M., and J. L. Fox. 1973. Nutrient removal using *Lemna minor*. J. Water Pollut. Control Fed. 45: 1928–1938.

Jackson, G. A. 1980. Marine biomass production through seaweed aquaculture. *In* A. San Pietro, ed, Biochemical and Photosynthetic Aspects of Energy Production, p. 31–58. Academic, New York.

Jackson, M. L. 1958. Soil Chemical Analysis, p. 498. Prentice-Hall, London.

Lakshman, G. 1979. An ecosystem approach to the treatment of wastewaters. J. Environ. Qual. 8: 353–361.

Lumpkin, T. A., and D. L. Plucknett. 1980. *Azolla*: botany, physiology, and use as a green manure. Econ. Bot. 34: 111–153.

Reddy, K. R., and W. F. DeBusk. 1984a. (in press). Phosphorus removal potential of *Azolla caroliniana* cultured in nutrient-enriched waters. *In* W. S. Silver and B. C. Schroder, ed, Symp. Proc. Practical Applications of Azolla in Rice Production. Nov. 17–19, 1982, Mayaguez, Puerto Rico. Martinus Nijhoff/Dr. W. Junk, The Hague.

———, and ———. 1984b. Growth characteristics of aquatic macrophytes cultured in nutrient-enriched water: I. Water hyacinth, water lettuce, and pennywort. Econ. Bot. 38: 229–239.

———, D. L. Sutton, and G. E. Bowes. 1983. Biomass production of freshwater aquatic plants in Florida. Proc. Soil Soc. Florida 42: 28–40.

———, K. L. Campbell, D. A. Graetz, and K. M. Portier. 1982. Use of biological filters for agricultural drainage water treatment. J. Environ. Qual. 11: 591–595.

Sutton, D. L., and W. H. Ornes. 1975. Phosphorus removal from static sewage effluent using duckweed. J. Environ. Qual. 4: 367–370.

———, and ———. 1977. Growth of *Spirodela polyrhiza* in static sewage effluent. Aquatic Bot. 3: 231–237.

Talley, S. N., B. J. Talley, and D. W. Rains. 1977. Nitrogen fixation by *Azolla* in rice fields. *In* A. Hollaender, ed, Genetic Engineering for Nitrogen Fixation, p. 259–281. Plenum, New York.

Tran, Q. T., and T. T. Dao. 1973. *Azolla*: A green compost. Vietnamese Studies 38, Agric. Problems, Agron. Data 4: 119–127 (cited by Lumpkin, T. A., and D. L. Plucknett, 1980, Econ. Bot. 34: 111–153).

Watanabe, I., C. R. Espinas, N. C. Berja, and V. B. Alimagno. 1977. Utilization of the *Azolla-Anabaena* complex as a nitrogen fertilizer for rice. Int. Rice Res. Inst. Phillipines. Res. Paper Ser. No. 11, p. 1–15.

Wedge, R. M., and J. E. Burris. 1982. Effects of light and temperature on duckweed photosynthesis. Aquat. Bot. 12: 133–140.

Wolverton, B. C. 1982. Hybrid wastewater treatment system using anaerobic microorganisms and reed (*Phragmites communis*). Econ. Bot. 36: 373–380.

———, and R. C. McDonald. 1979. Upgrading facultative wastewater lagoons with vascular aquatic plants. J. Water Pollut. Control Fed. 51: 305–313.