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Modeling duckweed growth in wastewater treatment systems

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Abstract

Species of the genera *Lemnaceae*, or duckweeds, are floating aquatic plants that show great promise for both wastewater treatment and livestock feed production. Research conducted in the Southern High Plains of Texas has shown that *Lemna obscura* grew well in cattle feedlot runoff water and produced leaf tissue with a high protein content. A model or mathematical expression derived from duckweed growth data was used to fit data from experiments conducted in a greenhouse in Lubbock, Texas.

The relationship between duckweed growth and the total nitrogen concentration in the mediium follows the Mitscherlich Function and is similar to that of other plants. Empirically derived model equations have successfully predicted the growth response of *Lemna obscura*.

Keywords: Duckweed, *Lemna*, *Lemnaceae*, model, total nitrogen, wastewater

Introduction

Duckweeds belong to the monocotyledon family *Lemnaceae*, a family of floating aquatic plants. Duckweeds are among the smallest and simplest flowering plants, consisting of an ovoid frond a few millimeters in diameter and a short root usually less than 1 cm long. The frond represents a fusion of leaves and stems. Their minute flowers are rarely found in most species. Under adverse conditions such as low temperature or desiccation, modified fronds called turions appear which sink to the bottom of the water body. These turions can resurface at the onset of favorable conditions of light, moisture and temperature to start new generations of duckweed plants (Hillman 1961).

Because flowering in *Lemnaceae* is rare, reproduction normally occurs by budding from mature fronds. A population of duckweed is actually a population of individual fronds (leaves) that can reproduce and divide to produce daughter fronds. The rate at which this occurs is affected by temperature, availability of nutrients in the medium, and light intensity. Each duckweed species will behave differently under the same conditions of light, temperature and nutrient availability and each species will grow best under different amounts of these same three factors. The growth behavior of individual species can be studied under controlled conditions (such as those present in a growth chamber) and mathematical relationships derived from this behavior can then be applied to the more natural conditions present in a greenhouse or field environment such as a wastewater treatment plant.

The major objective of this study was to develop a mathematical model of duckweed population dynamics when duckweed was grown on a wastewater medium of cattle feedlot runoff water. The research was carried out at Texas Tech University to utilize duckweed species as part of a system for recycling cattle wastes from feedlots. Large feedlots where thousands of cattle are fattened before slaughter produce huge quantities of cattle manure and other wastes. Rain falling onto the feedlot drains into low-lying feedlot runoff ponds and carries with it large quantities of organic material derived from exposed cattle manure present in the feedlot. These runoff ponds function as facultative wastewater treatment lagoons where both aerobic and anaerobic decomposition takes place. The resulting cattle feedlot runoff water is a liquid which can be pumped into tanks and stored for later use. Its composition is shown in Table 1. This feedlot runoff water was used to grow duckweed in these greenhouse growth trials.

Table 1. Water quality parameters of cattle feedlot runoff water (from Gebriel 1994)

Parameter	**mg/litre**
Total Solids	25.9
Total Volatile Solids	20.0
Total N	329
Ammonia (NH_3-N)	264
Nitrate (NO_3-N)	<0.03
SO_4	640
PO_4	231
Cl	693
K	909
Ca	682
Mg	208
Na	33
Fe	16
Zn	4

Materials and methods

A system for growing duckweed in vinyl troughs (length 3.04m) was constructed in a greenhouse on the Texas Tech campus in Lubbock, Texas (101W51, 33N35). Each trough was 9 cm deep. These troughs were arranged in groups of four with pipe connections between each trough. Each trough was supplied sequentially with runoff water diluted with tap water from a large fiberglass head tank. One head container supplied each of the four troughs with four mixtures of diluted runoff water (Figure 1). Approximately 17.5 $\pm$ 2 g of fresh, wet *Lemna obscura* plants were stocked into each of the four troughs. Small standpipes prevented the duckweed from passing between troughs. The flow rates in each system were 1 litre/hour in the first four troughs of the system [head tank #1 containing pure tap water at 0 mg/litre total nitrogen (TN)], 0.36 litres/hour in the second four troughs of the system (head tank #2 containing 8.3 mg/litre TN), 0.44 litres/hour in the third series of troughs in the system (head tank #3 containing a 30 mg/litre TN), and 0.19 litres/hour for the last four series of troughs in the system (head tank #4 containing 60 mg/litre TN) (Table 2).

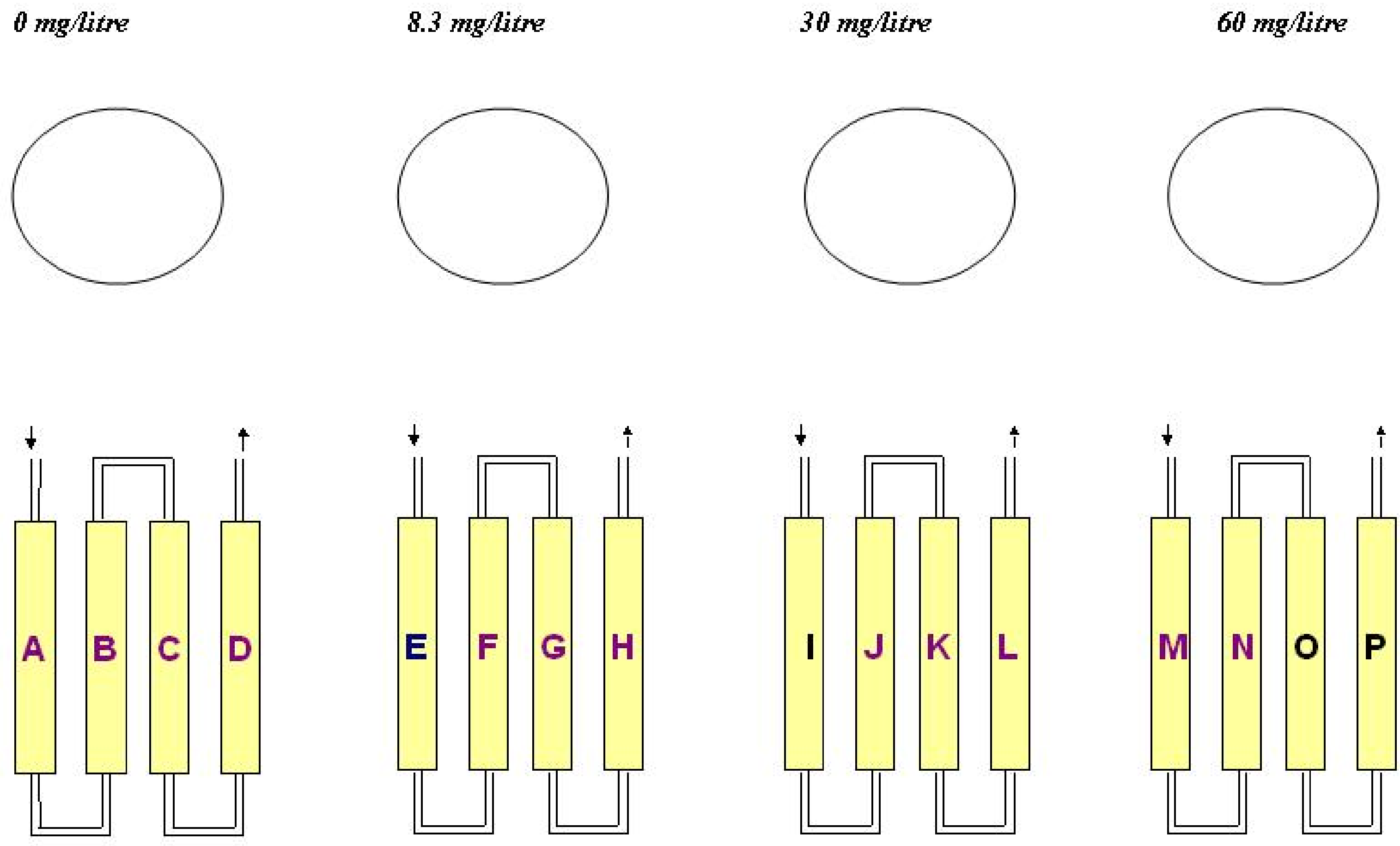

Figure 1. Arrangement of the 16 linear troughs in groups of four with one head tank (circular tank) per series of 4 troughs. The concentration of total N (mg/litre) measured in the wastewater is given above each series. Arrows indicate the direction of flow.

These flow rates resulted in a media replacement time of 2 days for the first four troughs, 5.4 days for the second four troughs, 4.4 days for the third series of troughs and 10.2 days for the last (fourth) series of troughs.

Table 2. Flow of N in diluted wastewater during 24 h

N in diluted wastewater, mg/litre	Flow rate, litres/h	Total N delivered each day, mg
0	1	0.0
3.9	0.36	33.7
8.9	0.36	76.9
13.9	0.44	120.1
28.5	0.19	130.0
54.6	0.19	249.0

After 6 or 7 days duckweed was harvested from each trough, weighed and dried for N analysis (AOAC 1990). This system was used to test the effect of six N delivery rates (0, 33.7, 76.9, 120, 130 and 249 mg N/day) on the growth of *L. obscura* and its protein content. The 0, 76.9, 120, and 130 mg N/day delivery rates were tested in August through September, while the 0, 33.7, 120 and 249 mg N/day delivery rates were tested in October through November. Duckweed fronds from each trough were collected at the end of each trial run, dried and analyzed for their N content (AOAC 1990).

The growth responses of these duckweed species were determined by using two measures:

- relative growth rate (RGR or $\log_e$ Final Wt - $\log_e$ Initial Wt) / days of growth)
- percentage weight gain (PWG or wet weight increase divided by the initial wet weight / days of growth).

Together these two parameters compare growth rates in a way that corrects for the difference in scale between a 250ml beaker in a growth chamber and a 3m long tank in a greenhouse (South 1995).

Results

In the four growth trials carried out in August and September the maximum daily gain per m^2 as well as the relative growth rate (RGR) values for fresh *L. obscura* biomass appeared to occur at a total N concentration of approximately 8.9 mg/litre, followed closely by 13.9 mg/litre (Figure 2). In the four growth trials carried out during October and November the greatest daily gain per m^2 appeared to occur in *L. obscura* grown at a medium N concentration of 13.9 mg/litre (Figure 2).

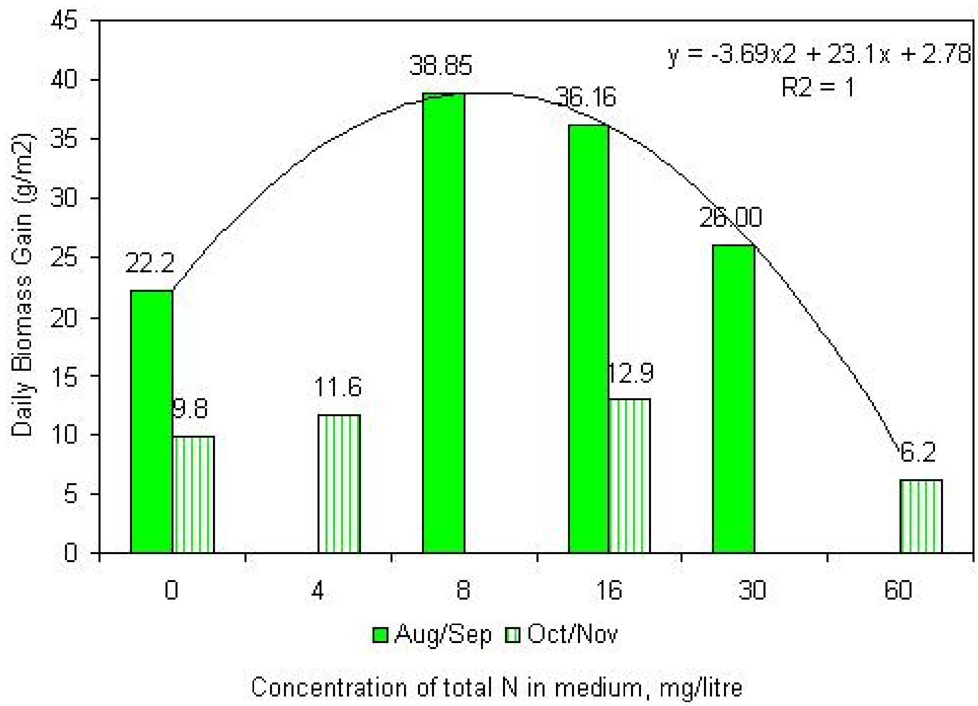

Figure 2. Daily fresh biomass gain of *Lemna obscura* cultured on N concentrations varying from 0 to 54.6 mg/litre between August and November ($y = -3.69x^2 + 23.1x + 2.78$; $R^2 = 1$).

Although the total amount of N delivered by each of the 6 runoff water dilutions increased along with the TN concentration of each mixture, the corresponding daily mean *Lemna* production rates supported the observations just discussed (Tables 3 and 4). Therefore from these results it appears that the instantaneous nitrogen concentration is more important than the total amount of nitrogen delivered during each 6-day growth trial.

Table 3. Mean daily *Lemna* production (g/m^2) on feedlot wastewater medium at different N loading rates

mg/day total N	21-Aug	27- Aug	3-Sep	Sep-16	Mean ± SE
0	17.9	20.9	17.7	9.9	16.6± 2.4
77	82.4	45.7	42.5	62.6	58.3 ± 9.2
130	74.5	75.7	28.4	45.2	56.0 ± 11.6
147	57.8	54.6	20.0	40.5	43.2 ± 8.6
	8-Oct	**16-Oct**	**25-Oct**	**8-Nov**	
0	13.6	25.1	10.2	2.5	12.8 ± 4.7
34	6.4	18.5	3.7	3.5	8.0 ± 3.5
130	36.8	21.8	8.1	4.4	17.8 ± 7.4
249	15.7	6.3	1.0	-0.7	5.6 ± 3.7

Table 4. Mean daily weight gain, RGR and PWG of Lemna grown on feedlot wastewater medium at different N loading rates

N supplied, mg/ litre	Daily gain in DW, g/m^2	RGR#	PWG##

0	16.6±2.4	0.16±0.01	0.31±0.03
77	58.3±9.2	0.22±0.02	0.57±0.09
130	56.0±11.6	0.21±0.02	0.51±0.05
147	43.2±8.6	0.18±0.02	0.38±0.07
0	12.8±4.7	0.11±0.04	0.19±0.07
34	8.0 ±3.5	0.12±0.04	0.22±0.08
130	17.8±7.4	0.13±0.05	0.26±0.12
249	5.6±3.7	0.07±0.05	0.13±0.08

 # relative growth rate; ## percentage weight gain

In the conditions under which these experiments were conducted in the Texas Tech greenhouse (mean daily indoor temperatures of 29 °C in August and 16.6 °C in November, mean light intensities of 300 W/m^2 in August and 145 W/m^2 in November) the maximum production of wet *L. obscura* biomass occurred between 9 and 14 mg/litre N. We define duckweed production as the final harvested biomass minus the initial stocked biomass (both figures measured in wet weight). Mean daily production of *L. obscura* expressed as g/m^2 was greatest at the 8.9-mg/litre N level. After an initial period of increasing growth, *L. obscura* growth decreased with further increases in N concentration. The inflection point occurred at a N concentration of approximately 11 mg/litre (Figure 3).

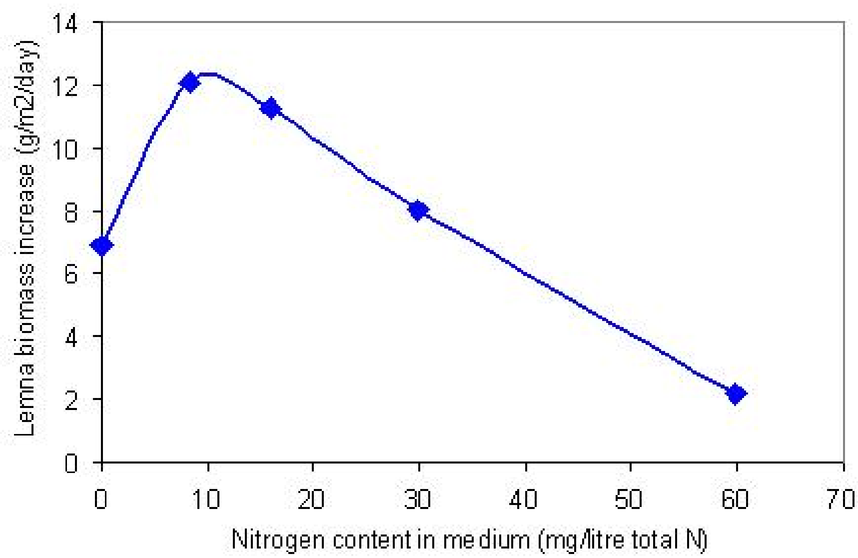

Figure 3. The model (line) and data (points) indicating the daily fresh biomass increase of *Lemna obscura* as a function of the total nitrogen concentration of the medium

The duckweed grown on the highest wastewater concentration (54.6 mg/litre TN) had the highest protein level. There was a positive correlation between the TN content of the medium and the protein content of the *L. obscura* grown on the medium (Figure 4). A highly significant linear relationship was found between the concentration of TN in the wastewater medium and the protein content of the *Lemna* grown on this wastewater (R^2= 0.95). The data used in the above analyses were based on *Lemna* biomass harvested from the first trough in each series (A, E, I and M Figure 1) that received the wastewater at one of these six concentrations (0, 3.9, 8.9, 13.9, 28.5 and 54.6 mg/litre TN).

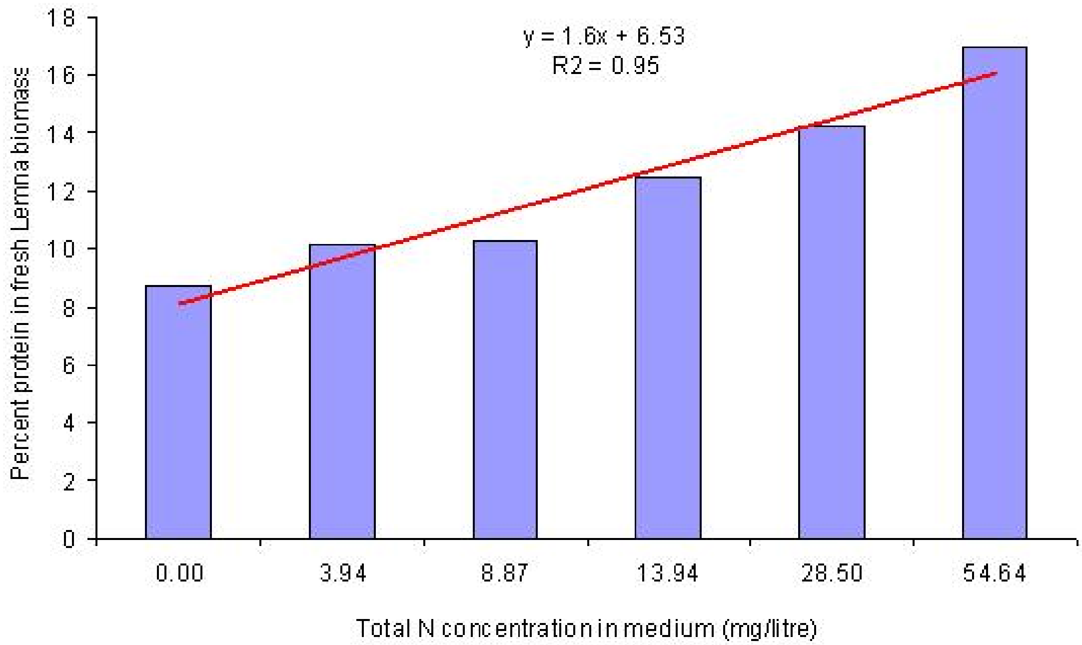

Figure 4. Relationship between protein content (% in DM) of *Lemna obscura* and the total nitrogen concentration of the medium

Discussion

Model development

The main objective of developing this model was to separate the effects of N, temperature, and solar radiation on the growth and protein content of duckweed. Initially, only the effect of total N on the growth rate of duckweed was considered. This was done to study the growth response of duckweed to varying N concentrations. It was found that there was a certain minimum growth even with zero N concentration in the growth medium. This indicates that duckweed fronds may utilize nitrogen from other sources including the atmosphere through nitrogen fixation by bacteria living on the trough walls and cyanobacteria living in the duckweed fronds (Tran and Tiedje 1985). The growth rate of duckweed initially increased exponentially with N concentration and then reached a maximum value, before decreasing linearly (Figure 3). This is a typical biological growth response for plants. Mitscherlich had already developed the basic model of this relationship (Briggs 1925). Based on Mitscherlich's earlier equation, the following model was developed:

$$Y = [(1 - e^{-A_1 X}) * (A_2 - A_3 X) + A_4] \qquad (1)$$

Where,

Y = Growth rate (wet weight, g/day)
X = Total nitrogen concentration (mg/litre as N)
A_1 = Change in growth rate.
A_2, A_3 = Model coefficients
A_4 = Increase in wet weight of duckweed at zero N concentration

The first part of the equation (**$1 - e^{-A_1 X}$**) explains the increase in the growth rate of duckweed with increase in N concentration until the growth reaches a maximum value. Then the growth rate decreases gradually in a linear form explained by the expression (**$A_2 - A_3X$**). The data were analyzed using the program MERV (Gregory and Fedler 1986) to evaluate the coefficients and find the R-square value and the significance level. When the data were plotted using the above model the resulting curve increased sharply from 0 to about 11 mg/litre N and then decayed linearly as N reached 54.6 mg/litre (Figure 3).

Based on the growth response data from August to September, the optimum temperature was 26 °C and the optimum solar radiation 138 W/ m^2 (11.9 MJ/day). To apply the temperature and solar radiation effect on the growth rate on any particular day, the model equation was multiplied by the temperature factor and the solar radiation factor. This factor is the temperature or solar radiation on any particular day divided by the respective optimal temperature or solar radiation. Since temperatures and light intensities higher than the optimum also decrease duckweed growth, the temperature and solar radiation factors were adjusted by subtracting the absolute value of the observed temperature or solar radiation from the optimum value. This predicted a decrease in duckweed growth estimates when the optimum values were exceeded (Zaki 1978). The final model for the growth of duckweed considering the effects of N, temperature and solar radiation was,

$$Y = [(1 - e^{-A_1 X}) * (A_2 - A_3 X) + A_4] * (1 - |1 - T_{obs}/ T_{opt} |) * (1 - |1 - SR_{obs}/SR_{opt} |) \quad (2)$$

Where,

Y = Growth rate (wet weight g/day)
X = Total nitrogen concentration (mg/l as N)
A_1 = Change in growth rate
A_2, A_3 = Model coefficients
A_4 = Increase in wet weight of duckweed at zero TN concentration
T_{obs} = Observed temperature (^{0}C)
T_{opt} = Optimal temperature for duckweed growth (26 ^{0}C)
SR_{obs} = Observed solar radiation (W/m^2)
SR_{opt} = Optimal solar radiation for duckweed growth (138 W/m^2)

Model verification

The program MERV was used for finding the coefficients of the duckweed growth model. The coefficients derived from applying this model to the August and September data were found to be A1= 0.308, A2 = 7.18, A3 = 0.201 and A4 = 7.01. This growth model has an R-square value of 0.99 and a probability level of 95% (alpha = 0.05), which is very high for a biological model (Gregory and Fedler 1986). This model was used to predict the growth rate of *L. obscura* for the four experiments conducted during October and November. The predicted values were compared with the measured values from the October and November experiments using the program MERV. A goodness of fit (R^2 value) of 0.93 was obtained, which is also very high for a biological model. The duckweed growth model had a goodness of fit probability level of 95% (alpha =0.05). This model can be used in predicting the growth rate of duckweed by knowing the N concentration, temperature and solar radiation (equation 2).

The relationship of the percent protein in *L. obscura* frond tissue and the total N concentration of the medium in which it grew can be described as a linear regression equation with a goodness of fit R^2 value of 0.95 (Figure 4). This regression equation applies to wastewater media with a range of total N values from 0 to 55 mg/litre. Researchers working in Australia (Leng 1999) and in Vietnam (Rodriguez and Preston 1996) found a similar relationship between *Lemna* species and the N content of its medium However, my results showed higher much concentrations of protein in fresh *Lemna obscura* biomass than these authors observed. This may be due to the greater availability of nitrogen to the growing duckweed fronds in the flow-through systems I used compared to the static systems they used. Furthermore the *Lemna obscura* strain I used was originally collected in Louisiana from domestic wastewater treatment ponds. Bergman et al (2000) found large differences among inter-specific and intra-specific strains of *Spirodela* and *Lemna* duckweed species in growth rates and protein contents of dry biomass. Their observations can also help explain the differences in our results. Finally, other researchers failed to observe a sharp decline in duckweed production with an increased levels of nitrogen in duckweed growth medium (Leng 1999). The decline in duckweed growth rate we observed (Figure 3) may have been the result of a buildup of sulphates and chlorides in the growth medium (Table 1) as the ratio of runoff water to tap water increased. This runoff water also was very alkaline (pH 8 or more) so unionized ammonia may also have stressed growing duckweed fronds, reducing growth at higher runoff water concentrations.

Using these regression model equations will provide duckweed producers and researchers a tool to predict relative growth rates under different nitrogen concentrations in the growth medium. However separate equations to represent duckweed growth must be derived from data obtained from each set of environmental conditions used.

Conclusions

- Duckweeds of the family *Lemnaceae* are small, floating, aquatic plants with a worldwide distribution. They are one of the fastest growing angiosperms and can double their biomass within 2 days under optimal conditions.

- They have a high protein content (10 to 40% protein on a dry weight basis) although the moisture content (95%) of fresh duckweed biomass is quite high as well.

- Potentially members of the *Lemnaceae* (of the genera *Lemna, Spirodela*, *Landoltia* and *Wolffia*) can produce edible protein six to ten times as fast as an equivalent area planted with soybeans.

- Species of *Lemnaceae* may have a great value in agriculture and wastewater treatment.

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