Interim Report For The
Upper Mississippi River - Illinois Waterway
System Navigation Study

Effects of Sediment Resuspension and Deposition on Plant Growth and Reproduction
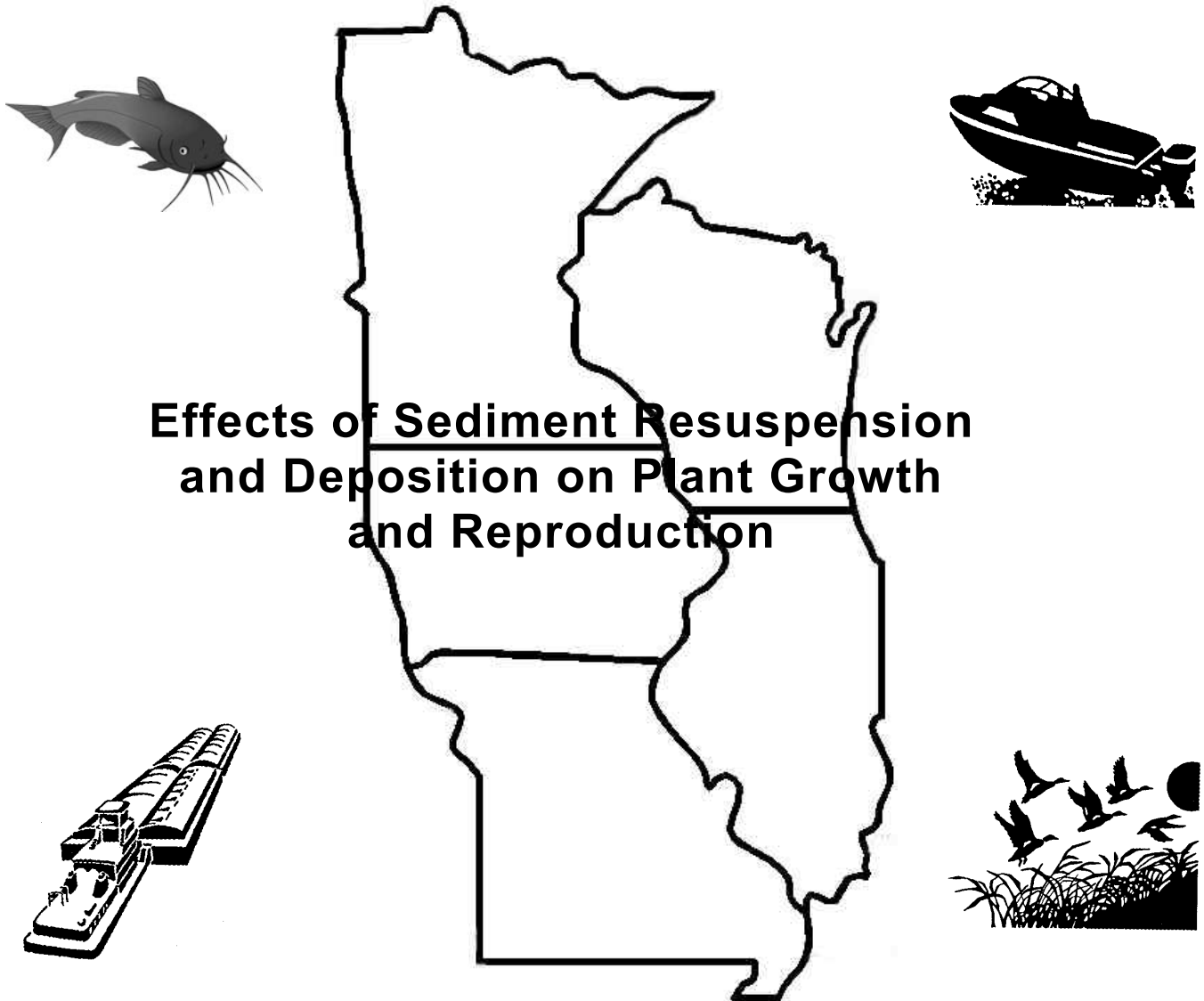
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**US Army Corps
of Engineers**

Effects of Sediment Resuspension and Deposition on Plant Growth and Reproduction

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Interim report

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Preface

The work reported herein was conducted as part of the Upper Mississippi River - Illinois Waterway (UMR-IWW) System Navigation Study. The information generated for this interim effort will be considered as part of the plan formulation process for the System Navigation Study.

The UMR-IWW System Navigation Study is being conducted by the U.S. Army Engineer Districts of Rock Island, St. Louis, and St. Paul under the authority of Section 216 of the Flood Control Act of 1970. Commercial navigation traffic is increasing and, in consideration of existing system lock constraints, will result in traffic delays that will continue to grow in the future. The system navigation study scope is to examine the feasibility of navigation improvements to the Upper Mississippi River and Illinois Waterway to reduce delays to commercial navigation traffic. The study will determine the location and appropriate sequencing of potential navigation improvements on the system, prioritizing the improvements for the 50-year planning horizon from 2000 through 2050. The final product of the System Navigation Study is a Feasibility Report which is the decision document for processing to Congress.

The Principal Investigator for this research was Dr. Robert Doyle, Ecosystem Processes and Effects Branch (EPEB), Environmental Processes and Effects Division (EPED), Environmental Laboratory (EL), U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS. Dr. Doyle prepared this report and was responsible for experimental design, data analysis, and interpretation. The study was conducted at the ERDC Lewisville Aquatic Ecosystem Research Facility in Lewisville, TX. Dr. Michael Smart (ERDC) and Dr. Gary Dick (University of North Texas) contributed to the experimental design and mesocosm setup for the experiments. Mr. Kenny Banks and Mr. David Honnell (AScI) provided technical assistance. Dr. John W. Keeley was Acting Director, EL, ERDC.

Mr. Robert C. Gunkel, Jr., EL, ERDC, was responsible for coordinating the necessary activities leading to publication. At the time of publication of this report, Director of ERDC was Dr. James R. Houston, and Commander of ERDC was COL James S. Weller, EN.

1   Introduction

Importance of Light Attenuation on Submersed Aquatic Macrophytes

Reduced light availability may impact aquatic plant communities in two major ways. First, deterioration in the light climate may impact existing plant communities to the point that they can no longer survive at a given site. Second, poor light conditions may place constraints on the spring regrowth of aquatic plants from overwintering progagules and effectively prevent them from regrowing in areas subject to high turbidity.

Numerous communities of established aquatic macrophytes (sea grasses or freshwater species) have experienced dramatic losses during the past two decades (e.g., Bulthuis 1983, Orth and Moore 1983). In some cases the declines in aquatic macrophytes appear to be related to episodic diseases affecting key species (e.g., den Hartog 1987). However, more commonly the decline is attributed to changes in light climate related to anthropogenic influences. These influences are usually either related to increased nutrient inputs to the system which stimulate algal growth, or modifications resulting in increased amounts of resuspended particles to the water column.

Increases in nutrients available within the water column may limit light available to macrophytes by promoting the growth of phytoplankton (e.g., Jupp and Spence 1977) or periphyton growing on the leaf surfaces (Sand-Jensen and Sondergaard 1981). Under conditions of moderately increased nutrient fertility in the water column, the species composition of the aquatic plant community may begin to change. Commonly, a shift away from slow-growing, long-lived species toward those of more rapidly growing plants is observed (Duarte 1995), or toward those showing an erect or canopy-producing growth form (Chambers 1987) is seen. Under conditions of higher nutrient availability, phytoplankton dominate and most submersed aquatic macrophytes are lost (Duarte 1995). The specific mechanism for macrophyte loss may be primarily related to increased growth of epiphytes and blanketing filamentous algae over the plant surfaces and only indirectly related to direct shading by phytoplankton (Phillips et al. 1978). Under levels of intermediate fertility, shallow lakes often exhibit alternating states and exist either in a clear state dominated by aquatic vegetation, or in a

turbid state characterized by high algal biomass (Scheffer et al. 1993, Blindow et al. 1993).

A second important determinant of underwater light climate which may impact existing plant communities is the level of suspended non-algal particles in the water column. In this case the particles of primary concern are those with slow fall velocities which are generally either very small particles (clays) or organic particles (Blom et al. 1994). In some cases increased non-algal turbidity appears to be related to dredging activities within the watershed which are then washed into the areas where submersed plants are growing (e.g., Giesen et al. 1990) resulting in significant declines in the plant populations. In addition to reducing the total quantity of light, both algal and clay particles in the water also change the underwater light quality by absorbing specific wavelengths (Wetzel 1983).

Controlled experiments and field observations have confirmed the central role of light climate on the growth and development of individual plants. Plants grown under reduced light conditions attempt to compensate for the light deficit by both morphological changes in growth patterns and adjustment in the photosynthetic apparatus (Barko and Smart 1981, Barko and Filbin 1983, Goldsborough and Kemp 1988, Dennison and Alberte 1982, Tanner et al. 1993). Field experiments with light manipulations have shown that severe shading may result in death of the plants, while more moderate shading resulted in significantly decreased growth relative to unshaded control plants (Bulthuis 1983). Similarly, *Egeria densa* plants growing under controlled conditions showed declines in relative growth rates and biomass accumulation as levels of suspended clays in the water column increased (Tanner et al. 1993).

Transplant experiments have also demonstrated that due to the constraints of turbidity, plant survival is often limited to very shallow depths or sites where light transparency is high (Zimmerman et al. 1995, Carter and Rybicki 1985). Over longer time periods, the levels of light penetration into the water determine the maximum depths to which submersed plants can grow (Duarte 1991, Vant et al. 1986, Dennison 1987, and Gallegos 1994).

In some cases the decline of a macrophyte community is not directly related to changes in the light climate but to unusual disturbances of the community such as droughts or disease. However, once the plants are lost from a given locale, the conditions change so that re-establishment of plants becomes difficult. In some situations, unconsolidated, shifting sediments prevent aquatic plant establishment by preventing plants from rooting effectively. Also, increased sediment resuspension may place significant constraints on plant regrowth at that site due to reductions in light. For example, Engel and Nichols (1994) report that in the early 1970's the macrophyte community of Rice Lake (Wisconsin, USA) was impacted by periods of unusually high water resulting in poor light conditions. Once water levels returned to normal conditions, the plant community was mostly gone and wind resuspension of sediments resulted in unusually turbid conditions. These turbid conditions appear to have prevented the subsequent regrowth of the plant community. More than a decade later, the

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lake continues to have high levels of resuspended sediment turbidity and a very sparse macrophyte community.

Recent declines in vallisneria populations in the Upper Mississippi River System (UMRS) have also been attributed to poor light conditions. Various hypotheses have been presented, but all focus on reduced light conditions within the system. Fischer and Claflin (1995) hypothesize that physical changes and disturbances within the UMRS have resulted in increased sediment resuspension and poor light conditions. Kimber et al. (1995a) suggest that high algal turbidity during a drought year when the hydraulic residence time of the system was high may have also contributed to the decline of vallisneria.

Although the exact cause of the decline along the UMRS is not known, there is little disagreement that increased sediment resuspension and turbidity within the water column is now making regrowth of vallisneria populations very difficult. Field and laboratory data have recently demonstrated the magnitude of the problem. When tubers were planted at three depths within backwater areas of the river, only those at the shallowest depth (highest light availability) survived (Kimber et al. 1995a). Given the high light extinction coefficients common in these backwater areas, the authors calculate that overwintering tubers would survive at a depth of only 0.8 m or less. Similarly, seed collected within the river system germinated under a wide range of light conditions, but survival and subsequent tuber production was limited to light regimes found only at depths less than or equal to 0.5 m in the field (Kimber et al. 1995b). Recently, experimental data have demonstrated that total biomass of tubers was reduced in plants grown under poor light conditions (Korshgen et al. 1997).

Overview of ERDC Experiments

A series of controlled experiments were designed to investigate the impacts of suspended inorganic turbidity on two native submersed aquatic plants important to the UMRS. Species selected were vallisneria (*Vallisneria americana*) and sago pondweed (*Potamogeton pectinatus*). The experiments were conducted at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX, between 1995 and 1997. Four experiments were conducted as summarized in Table 1.

Turbidity in all experiments was maintained by addition of natural clay sediments to the water column. Because sediment characteristics along the river are quite variable, it was not considered essential to utilize a sediment from the UMRS for this test. A local sediment consisting of 80% clay and 20% silt was chosen. A large quantity of dry sediment was pulverized and sieved through a 1-mm sieve prior to the beginning of the experiment. A sediment slurry was prepared for addition to the tanks by adding 100 g of dry clay to 500 ml of DI water and then sonicating the mixture for 2 hours in a large capacity ultrasonic cleaner (Fisher Scientific model FS28). The sonication procedure dispersed the clay particles and was essential to ensure that the clay particles would not clump and sink to the bottom of the tanks upon addition to the water.

Table 1
Summary of Turbidity Experiments Conducted at LAERF Between July 1995 and December 1997

Experiment	Primary Study Objective	Experimental Design/Analysis
1. Turbidity Effects on Mature Plant Populations-I (Greenhouse 1995)	Pilot experiment to determine the level of turbidity needed to demonstrate impacts on mature, well-established populations of plants.	Dose-response experiment utilizing 3 turbidity levels. Regression analysis and ANOVA were used for statistical analysis.
2. Turbidity Effects on Mature Plant Populations-II (Deep Tank 1996)	Full-scale implementation of effects of turbidity on mature, well-established plant populations.	Factorial Design (2 levels of turbidity and 2 pulsing regimes). ANOVA followed by means comparison test used to determine differences among treatment means.
3. Turbidity Effects on Tubers (Greenhouse 1996)	Investigate impacts of turbid water on initial regrowth of plants from tubers.	Dose-response experiment utilizing 3 turbidity levels. Regression analysis and ANOVA were used for statistical analysis.
4. Turbidity Effects on Seedling Growth (Laboratory 1997)	Investigate impacts of turbid water on initial regrowth of vallisneria seedlings.	Seedlings grown under 2 turbidity regimes (clear and turbid). *t*-test used.

There was an excellent linear relationship between total suspended solids (TSS) and water column turbidity (Figure 1). The amount of suspended sediments in the water column of each tank was routinely monitored by measuring turbidity. Turbidity was analyzed with a turbidimeter (Hach Corp., Loveland, CO) calibrated each day with secondary standards and each month with primary standards and reported as Nephelometer Turbidity Units (NTU). TSS could not be measured with the standard glass fiber filter filtration method because a significant portion of the clays would go through all glass fiber filters tested. Instead, TSS was determined as the difference between total solids and total dissolved solids. Total solids were measured gravimetrically as the increase in weight of a tared aluminum boat after evaporation of a known volume of raw water. Total dissolved solids were determined by evaporation of a water sample sequentially filtered through a glass fiber filter (Gelman AE) followed by filtration through a 0.2μ Nucleopore filter.

Use of clay turbidity for these experiments is superior to use of neutral density shade cloth to reduce light because it more closely mimics natural conditions in the field. Turbidity in the water column results in very steep light gradients (light is exponentially attenuated with depth) and shifts in the spectral quality of the light (some wave lengths are absorbed more strongly than others by the suspended sediments). These real-world conditions are likely to be ecologically important to the plant communities but are not mimicked by use of shade cloth. All experiments described in this report utilized clay turbidity to achieve light reductions.
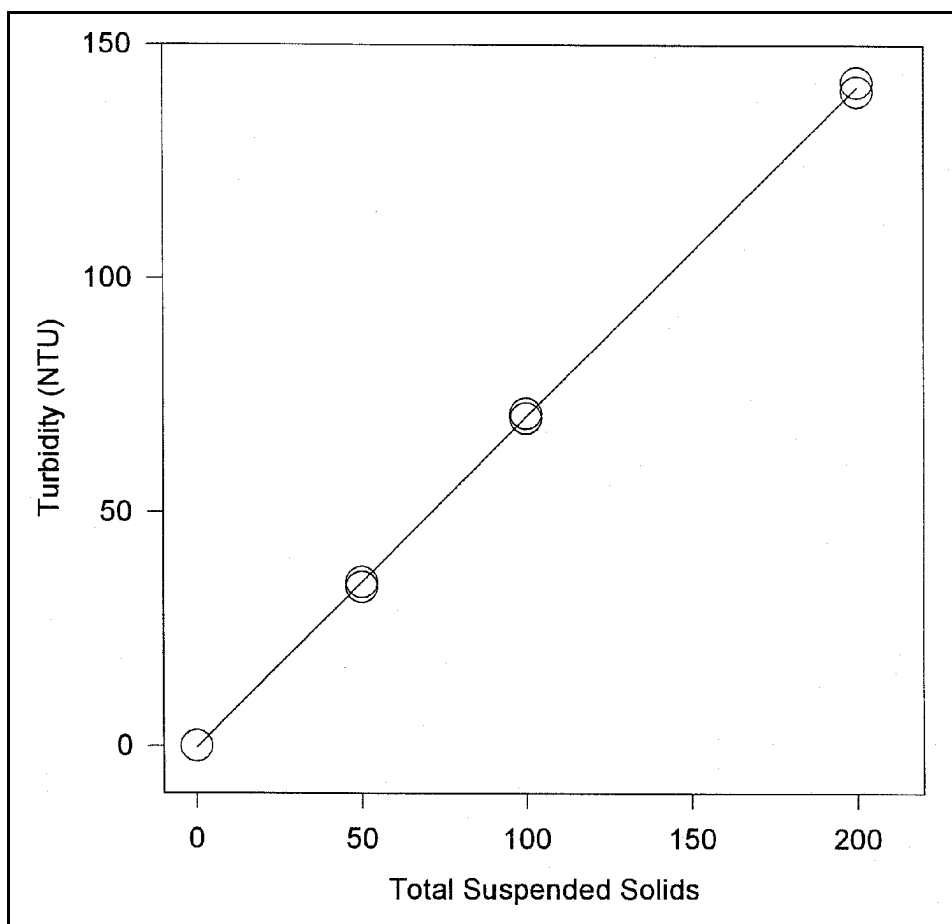
Figure 1.   Relationship between water column turbidity and Total Suspended
              Solids (TSS) added as inorganic clay sediments

Statistical Analysis

Several of the experiments shown in Table 1 utilize a dose-response
experimental design. Dose-response experiments are those whose treatments are
simply varying levels of a single factor (in this case different levels of clay
turbidity). Analysis of dose-response experiments utilizing three or more levels
of a factor may utilize regression analysis rather than the more commonly used
ANOVA (analysis of variance). The null hypothesis for a dose-response
experiment is that the slope of a regression line across all treatment levels is not
significantly different from zero (Figure 2). Experiments utilizing only three
levels of a factor can only test for linear differences. That is, does the response
variable (e.g., plant mass, leaf length, etc) increase, decrease, or stay the same as
the level of turbidity increases. The r^2 of the regression equation is a measure of
how much of the variability in the response variable is controlled by the
experimental factor. For example, a regression with a significant negative slope
and a high r^2 (Figure 2, left panel) indicates that as the level of the experimental
factor increases, the response variable decreases and that most of the variability
in the response variable is controlled by changes in the levels of the experimental
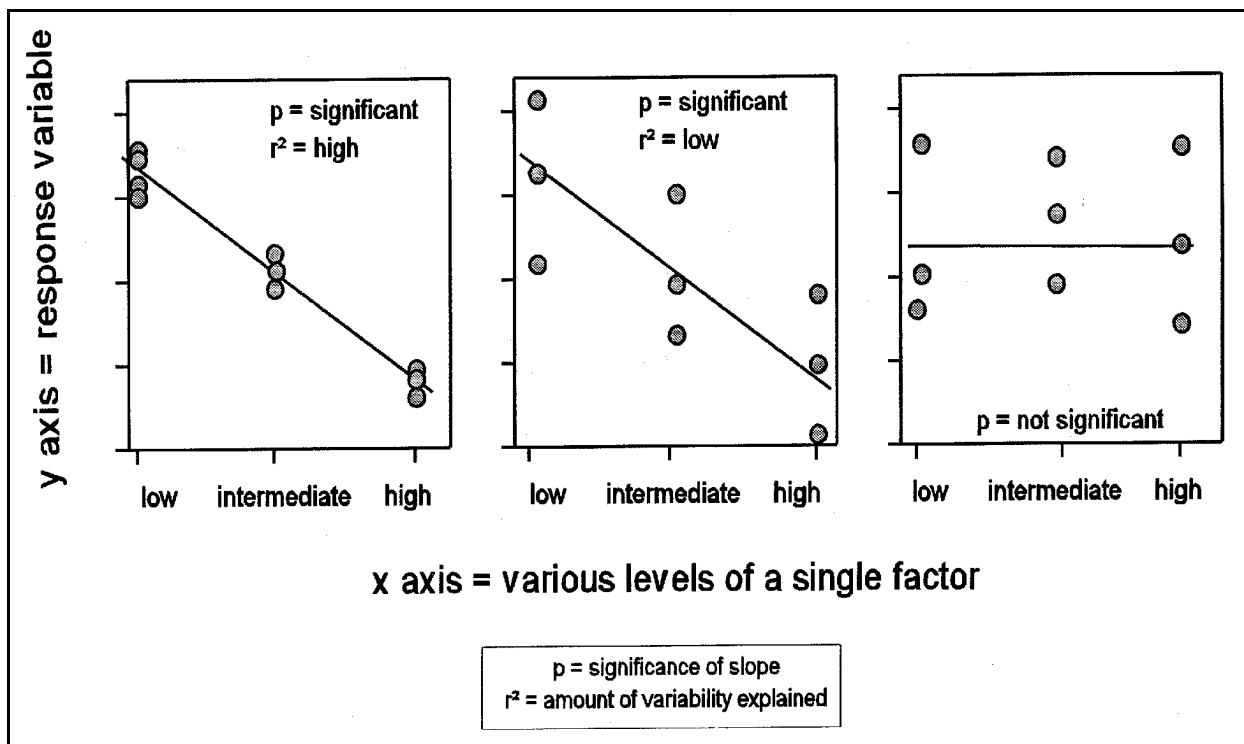
Figure 2.   Dose-response experiments test the null hypothesis that the slope of the regression line is not significantly different from zero. The "p" value indicates whether or not the slope differs significantly from zero while the r^2 value indicates how well the chosen regression fits the data

factor. Alternatively, a significant slope and a low r^2 value (Figure 2, middle panel) indicate that the response variable is significantly negatively related to the experimental factor, but that other (and likely unmeasured) factors also affect the level of the response variable. Finally, a relationship where the slope is not significantly different from zero (Figure 2, right panel) indicates that the response variable is unaffected by the level of the experimental factor. Experiments utilizing four or more levels of a factor can also test for nonlinear responses to increasing levels of the experimental factor.

Analysis of variance (ANOVA) tests were utilized for some experiments to test for differences among treatments. Data were tested to ensure that they were normally distributed and that variances were homogeneous. Significant ANOVAs were followed by a mean comparison test (Tukey HSD) to determine significant differences among individual treatment means. Experiments having only two treatments were analyzed with a *t*-statistic. P-level for significance was 0.05 unless noted. All statistical analyses were performed using *Statgraphics Plus* (Manugistics, Inc., Rockville, MD).

2 Effects of Turbidity on Mature Plants: I. Greenhouse Pilot Experiment

The first experiment was designed as a pilot-scale experiment to determine the turbidity levels required to impact well-developed, mature submersed plants. Plants were initially grown under clear water conditions and subsequently exposed to 9 weeks of growth under one of three turbidity regimes.

Materials and Methods for Greenhouse Pilot Experiment

Greenhouse system

The experiment was conducted in a system of 1,200-L white fiberglass tanks housed in a greenhouse facility at LAERF. The tanks measure 150 cm long by 90 cm wide by 90 cm deep. The greenhouse is constructed of translucent acrylic panels and consequently allows about 45% ($\sim 900 \mu E \ m^{-2} s^{-1}$ midday maximum) of full sunlight to reach the water surface within the tanks. Water temperature was maintained at 25 ± 1 °C with thermostatically controlled liquid circulators (Remcor Corp., Chicago, IL), which recirculated the water in the tank approximately six times per day. To ensure that the tanks remained well-mixed and to facilitate gas exchange, compressed air was continuously pumped through two air lifts in each tank. To minimize CO_2 depletion related to conducting the experiments in static tanks, the airstream was amended with CO_2 to an approximate 10 X enrichment (~ 3500 ppm CO_2). CO_2 was provided from reagent-grade compressed gas cylinders. The tanks were filled with pond water treated with alum (aluminum sulfate) to remove soluble phosphorus (P).

Plant material

To obtain well-established plants, four or five tubers of vallisneria or sago pondweed were planted in 5-L plastic pots (0.062 m^2 surface area per pot) of

fertile, fine-textured pond sediment and cultured in the greenhouse in clear water for 12 weeks. Physical and chemical characteristics of these sediments have been described previously (Smart et al. 1995). Tubers of both species were collected in Wisconsin and shipped to Texas, where they were maintained under refrigeration until used for the experiments.

Prior to planting the tubers, the pond sediment was heat-treated at 90 °C for >6 hours to kill any propagules of other aquatic plant species. The sediment was then homogenized in a concrete mixer to a desirable consistency. The pots were filled to about 80% of their capacity with the heat-treated sediment and then covered with a 2-cm layer of coarse sand to facilitate maintenance of an oxidized zone over the sediments, thereby minimizing nutrient (especially P) losses from the sediment to the water column. During the establishment phase, the planted tubers grew into mature plants. Plants were long (70-80 cm), pot-bound, and their leaves were at or near the water surface at the beginning of the experiment.

Experimental setup

After this establishment period, the pots of plants were visually culled to select plants in a similar stage of development; pots filled with unusually vigorous or unusually sparse plants were eliminated. Plants of each species were then randomly assigned into one of four groups, each containing four pots of plants. One group was immediately harvested to document the initial conditions of the plants. The other three groups of each species were then assigned to one of three turbidity treatments (Clear, 50 NTU, and 100 NTU) in this dose-response design experiment. A broad turbidity range from 0 to 100 NTU was selected for this first pilot-scale experiment. Along the upper portions of the UMRS, the water is clear and turbidities <15 NTUs are common; within the lower reaches of the river, however, the turbidities are much higher and commonly in the 50-100 NTU range.

Plants were placed into appropriate tanks and allowed to grow for 9 weeks (July 2- September 6, 1995) under experimental conditions. The water column within the tanks was 85 cm high (65 cm above sediment surface of the pots). The 9-week experimental growth period represents approximately 30% of the annual growing season along the UMRS. Turbidity in each tank was adjusted to the target level every 5 to 7 days by addition of clay sediment slurry. The incremental and total amounts of sediment added to each tank were recorded.

The light climate in each tank was monitored. Photosynthetically active radiation (PAR, 400-700 nm) measured as light quanta ($\mu E\ m^{-2}\ s^{-1}$) incident upon the tanks was measured in the air just above the water surface with a flat quantum sensor (Li-Cor model SR sensor). A flat sensor measures only downwelling light which can penetrate into the water. However, once light is in the water, the direction of light travel is not important, since leaves can utilize downwelling as well as reflected light. Therefore, light attenuation in the tanks was determined by measuring PAR just below the water surface and near the sediment surface (65-cm depth) utilizing a spherical quantum sensor (Li-Cor

model LI-193SA sensor). The measurements were taken between 10 AM and 2 PM.

Plant harvest

Plants were harvested after the experimental growth period in such a way that the amount of accumulated sediments on their leaves could be quantified. Each planted pot was enclosed in a plastic bag while still underwater. The bags with their contents were then removed to a separate basin. Shoots were clipped at the sediment surface, and attached sediments were removed by rinsing and manual washing. Total volume of rinse water for each pot was recorded and subsamples were collected for TSS analysis. Corrections were made for background suspended solid levels in the tanks. Sediments remaining in the tanks after all plants had been removed were homogenized and subsamples were collected for TSS determination. A mass balance was calculated for the sediments in each tank to follow the fate of the added sediments.

Plant biomass variables were also measured. The numbers of stems (sago pondweed) or rosettes (vallisneria) were counted. Flowers and seed pods from both species were counted and separated from the remainder of the above-ground biomass. Below-ground tissues were washed over a 1-mm sieve to remove sediment and debris. Tubers, if present, were counted and separated from the remainder of the below-ground tissues. Plant tissues were then bagged and dried at 60 °C in a forced draft oven to constant weight. After weighing, the samples were ground in a Wiley mill to pass through a 40-mesh sieve. Subsamples of the ground material were then combusted at 550 °C in a muffle furnace to determine ash content. Ash content for each tissue type for each turbidity treatment was determined separately. All biomass data for all experiments were corrected for ash content and expressed as ash-free dry weight (AFDW).

Statistical analysis

Statistical analysis consisted first of a one-way ANOVA followed by means testing on the per pot data. For this analysis, each pot is considered as a single experimental unit. In addition, regression analysis of tank totals (all pots of a given species summed to provide a population response) was also used. The target NTU value was used as the independent variable for the regression analysis (treatments), and measured plant parameters constituted the dependent variables. Because all pots of a given treatment were exposed to experimental conditions within the same tank, these replicates are technically pseudo-replicates. Although the statistical validity of pseudo-replicates can be questioned, the test was considered suitable for an initial scaling experiment. Larger scale tests to be described later (Chapters 3 and 4) all utilize multiple tanks for each treatment, providing true replication and avoiding possible statistical confusion.

Tank totals for each of the three treatments were also utilized for a linear regression analysis. Due to the low number of replicates, p values < 0.1 were considered significant.

Results of Greenhouse Pilot Experiment

Turbidity and light climate

Turbidity over the range selected resulted in a wide range of light attenuations (Figure 3). Control tanks had 80-90% (650-750 μE m^{-2} s^{-1} midday maximum) of light penetrating to the surface of the pots (65-cm depth), while intermediate and high turbidity treatments had about 10% (midday maximum $\approx$ 70 μE m^{-2} s^{-1}) and 2% (midday maximum $\approx$ 15 μE m^{-2} s^{-1}) of light at that depth at turbidities of 50 and 100 NTU, respectively.
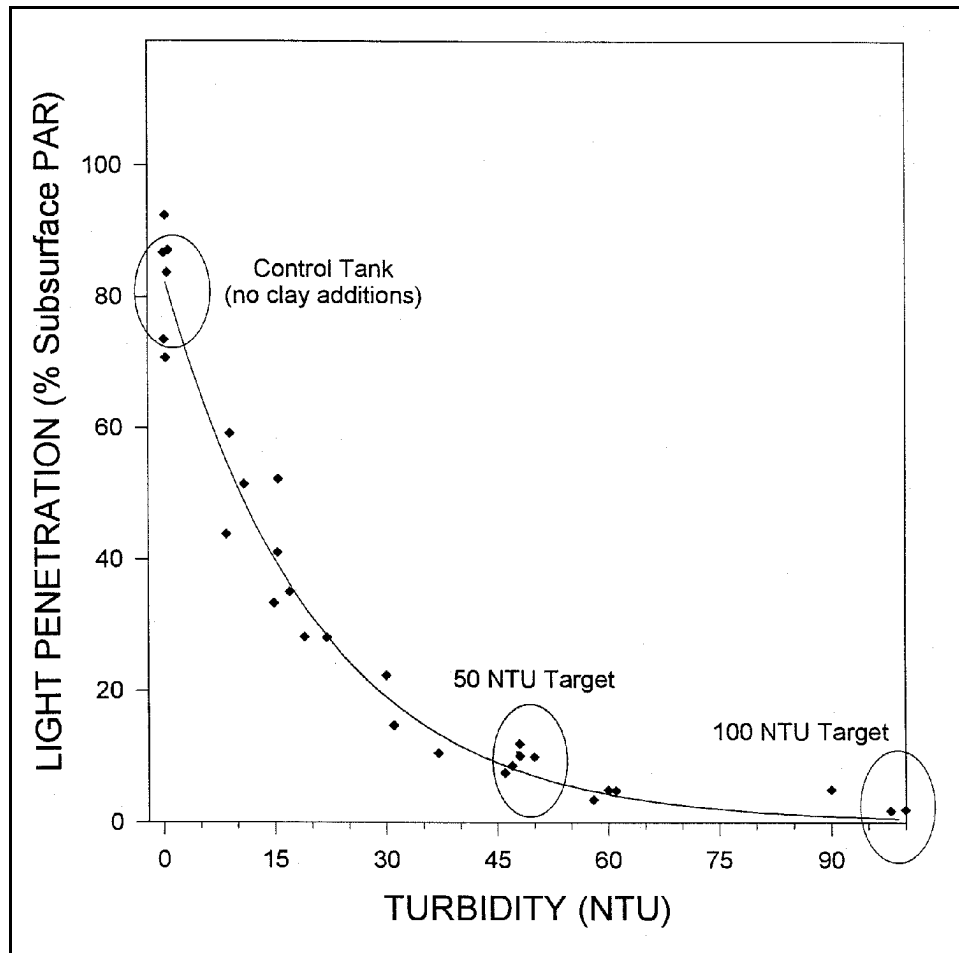
Figure 3. Relationship between percent of subsurface light penetrating to a depth of 65 cm and turbidity in the greenhouse tanks. Circles indicate levels for the three turbidity treatments

Turbidities in treated tanks were at their target levels at each sediment addition, but declined slowly over the ensuing 5- to 7-day period before additional sediments were added. Consequently, the light climate within the tanks was somewhat variable. Turbidity ranged from 50 to 20 NTU in the intermediate turbidity tank, between 100 and 50 NTU in the high turbidity tank, and remained constant in the clear tank (Figure 4). Consequently, the actual light quantity reading at the pot surface within the tanks varied between 10 and 30% (midday max. $\approx$ 90-270 μE m^{-2} s^{-1}) and 2 and 10% (midday max. $\approx$ 20-90 μE m^{-2} s^{-1}) of subsurface light for the intermediate and high turbidity tanks, respectively. However, the light levels actually experienced by the leaves were much higher than those values since the plants had well-developed leaves when the experiment began (leaves extending to very near the water surface).
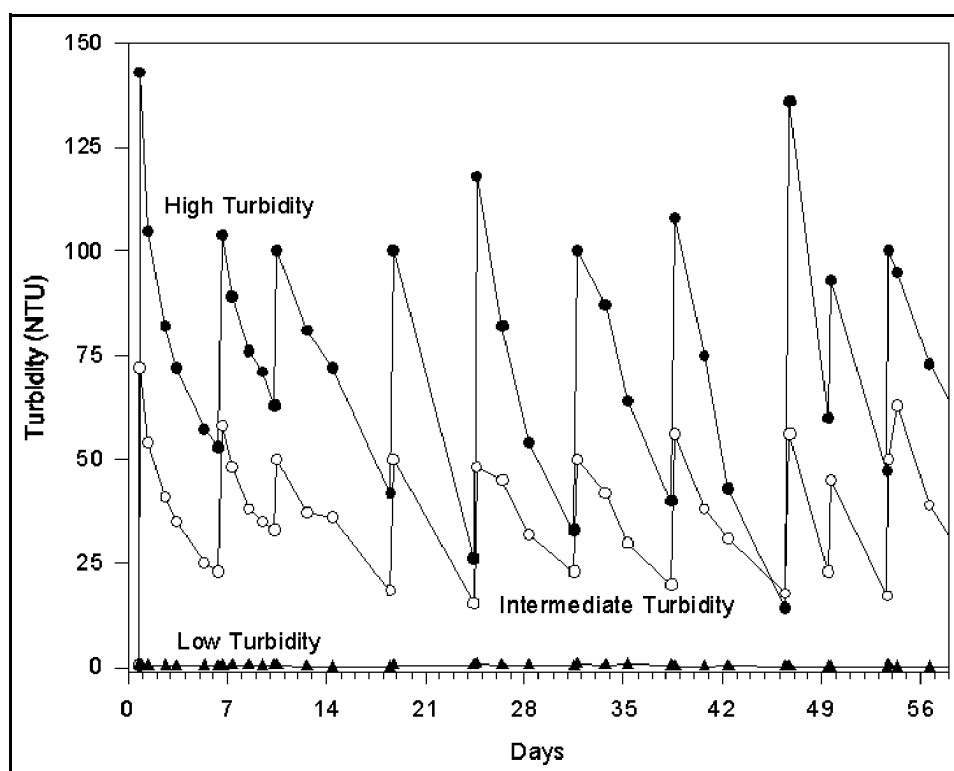
Figure 4.   Turbidity levels through the study period for the three treatment levels. Turbidity was adjusted to target levels every 5-7 days in the high turbidity and intermediate turbidity tanks

Fate of added sediments

At the end of the experimental period, most of the leaves of vallisneria and sago pondweed were coated with a fine layer of sediment. Quantification, however, showed that relatively little of the total sediment added to the tanks over the course of the experiments was actually adhered to the leaf surfaces of the plants (Table 2). Most of the added sediments had simply settled on the bottom of the tank. Settling experiments showed that the amount of sediments

Table 2
Sediment Mass Balance

Treatment	Total Sediment Added (g)	Sediments on Plants (g)	Sediments in Water Column (g)	Settled to Bottom of Tank (g)	Recovery (%)
50 NTU	860.4	39.9	45.9	829.1	106.3
100 NTU	1753.0	40.5	103.0	1592.0	99.0

Note: Sediments were added every 5-7 days during the experimental growth period. At the end of the experiment the distribution of sediments in the water column, on the plant leaves, and settled to the bottom of the tank was determined.

found on the leaves might have reduced the amount of light actually reaching the leaf surface by 10-25%.

Plant growth

Plant mass values are all expressed on an ash-free basis. Dry weights for each tissue type of each species for each treatment were corrected for ash content according to the data in Table 3. At the beginning of the experimental period plants were growing well (Table 4). Vallisneria pots contained an average of over 10 rosettes and sago pondweed pots had almost 30 rooted stems. Neither species had yet begin to flower. Vallisneria had not yet initiated tuber production, but sago pondweed had already produced some tubers.

Table 3
Biomass, Excluding Ash, in Percent of Total Dry Weight

Species and Treatment	Above-ground Biomass	Below-ground Biomass	Inflorescence	Tubers
vallisneria Control	78.8 ± 1.0	50.6 ± 18.7	77.1 ± 0.4	84.5 ± 8.2
Intermediate Turbidity	75.7 ± 8.0	65.0 ± 4.5	76.8 ± 1.2	89.7 ± 4.0
High Turbidity	52.4 ± 3.4	59.2 ± 9.3	74.7 ± 0.9	93.2 ± 1.0
sago pondweed Control	85.1 ± 0.4	59.4 ± 15.8	93.2 ± 0.5	81.4 ± 3.8
Intermediate Turbidity	85.4 ± 7.0	65.0 ± 4.5	92.9 ± 0.5	91.5 ± 1.9
High Turbidity	81.9 ± 1.1	42.5 ± 8.0	88.0 ± 2.9	80.3 ± 5.0

Note: Means ± se (n=4).

Table 4
Initial Values per Pot for Plant Parameters at Beginning of Experimental Period

Parameter Measured	vallisneria	sago pondweed
Rosettes (val) or stems (sago) (number pot^{-1})	10.4 ± 1.25	29.2 ± 5.94
Above-ground biomass (g AFDW pot^{-1})	2.97 ± 0.28	5.79 ± 0.58
Below-ground biomass (g AFDW pot^{-1})	0.39 ± 0.05	0.51 ± 0.06
Tuber number (number pot^{-1})	0	17.6
Tuber mass (g AFDW)	0	0.88 ± 0.16
Inflorescence (g AFDW pot^{-1})	0	0
Total biomass (g AFDW pot^{-1})	3.35 ± 0.28	7.18 ± 0.54
Note: (mean ± se, n=4 pots). Pots represent 0.062 m^2 surface area.		

Vallisneria growth over the nine-week experimental growth period was strongly influenced by the turbidity of the tanks (Figure 5). Whether expressed as an increase in number of rosettes or increase in total mass, vegetative growth was strongly negatively affected by water column turbidity. ANOVA's for both variables were significant, and mean separation tests (Tukeys HSD) revealed that the highest turbidity had means significantly lower than the other two treatments ($p<0.05$, Figure 5 A,C). Mean of the 50 NTU treatment was lower than that of the control. However, the difference was not significant due to the low number of replicates and the high pot-to-pot variability within treatments.

Population totals, however, show a clear pattern of declining number of rosettes and plant mass with increasing turbidity (Figure 5 B,D). Under the highest turbidity treatment, the vallisneria plants virtually stopped forming new daughter plants (change in number of rosettes over experimental period ≈ 0, Figure 5 B) and showed only a 2-3 X increase in biomass over the nine-week growth period relative to the initial biomass. In contrasts, the population in the control tank increased the number of daughter plants by 2-4 X and had a 4-7 X increase in total mass.

Vallisneria reproduction, expressed as either the formation of tubers (asexual reproduction) or formation of flowers (sexual reproduction) was also strongly negatively impacted by turbidity (Figure 6). ANOVAs were again significant for both parameters ($p<0.05$) and the highest turbidity treatment had means significantly lower than the others. Tuber numbers and flower/seed mass were both 2.5-3X higher in the control tank than in the highest turbidity tank. The population totals again showed a clear pattern of declining production in tubers or flower/seed mass with increasing turbidity (Figure 6 B,D).

Sago pondweed growth was less sensitive than vallisneria to turbidity in this experiment (Figure 7). ANOVA analysis demonstrated that the number of stems per pot was not significantly affected by turbidity (Figure 7 A), although total
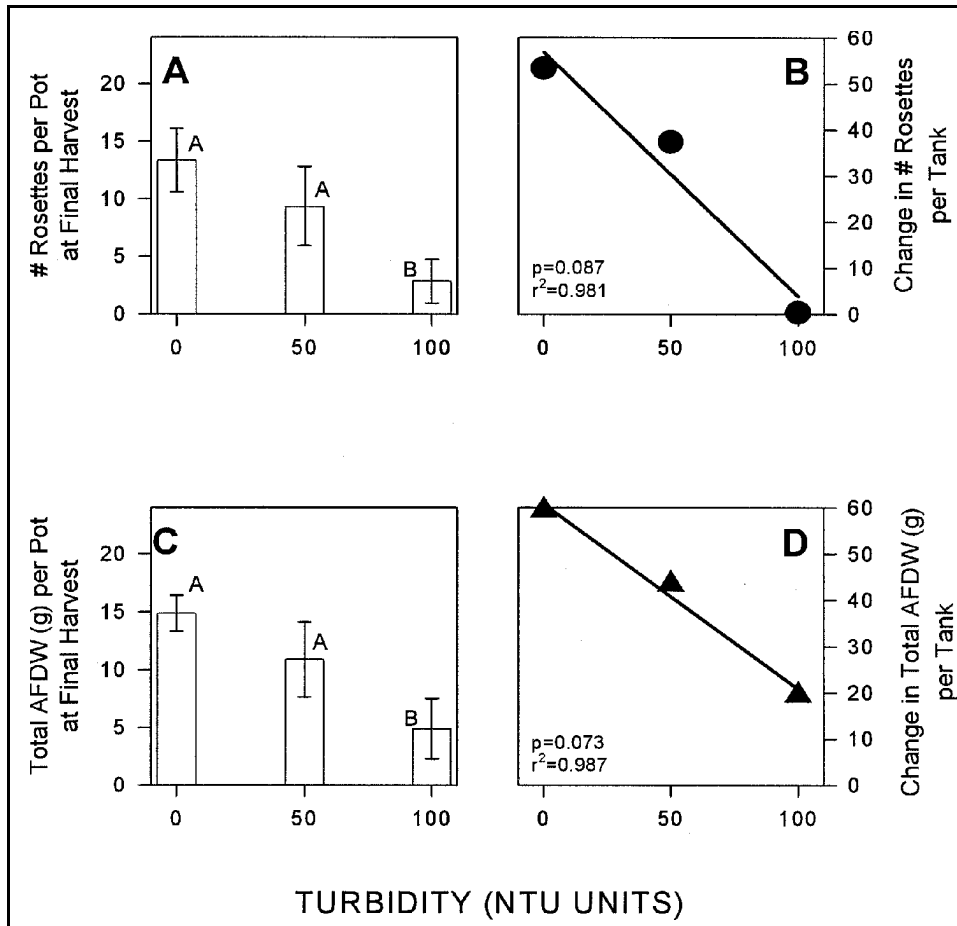
Figure 5. Relationship between turbidity and vallisneria growth. A and C show mean growth per pot ± se (n=4) for plants grown under different turbidities. ANOVAs were significant for both response variables and letters indicate significant differences among treatment means. B and D show regression analysis of changes in tank totals (sum of all pots at time of final harvest - initial tank totals) versus turbidity
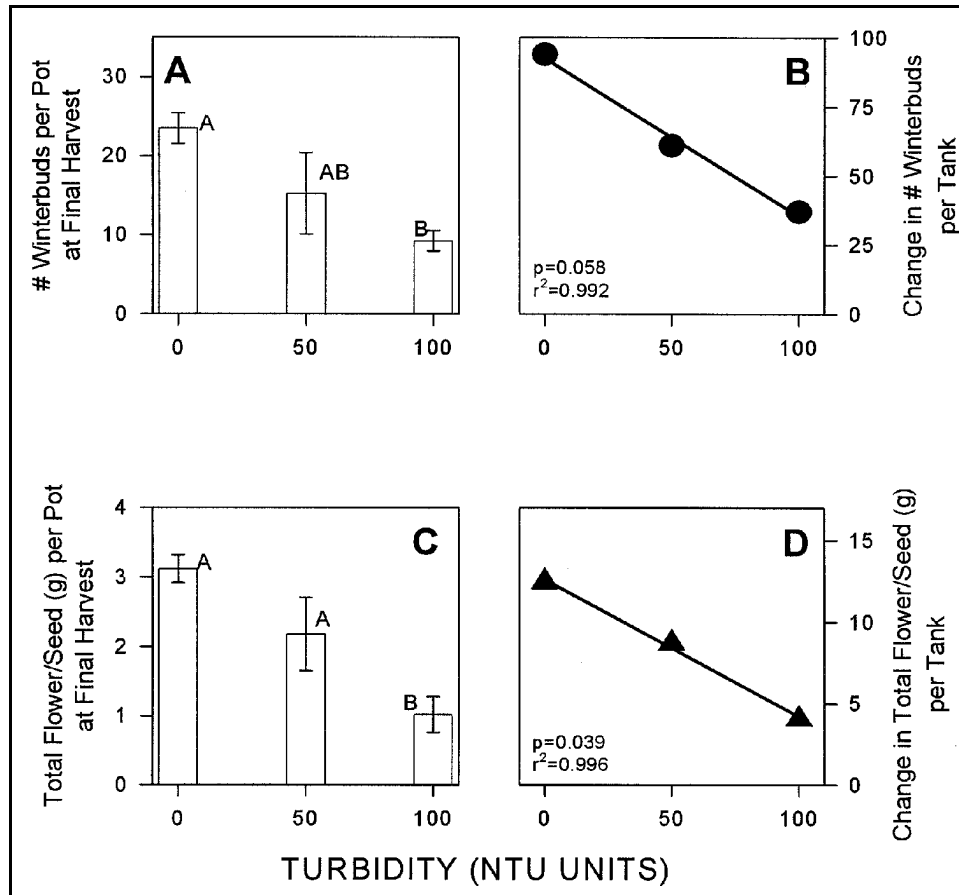
Figure 6.   Relationship between turbidity and vallisneria reproduction. A and
C show mean growth (± se, n=4) for plants grown under different
turbidities. ANOVAs were significant for both response variables
and letters indicate significant differences among treatment means.
B and D show regression analysis of change in tank totals (sum of
all pots at time of final harvest - initial tank totals) versus turbidity
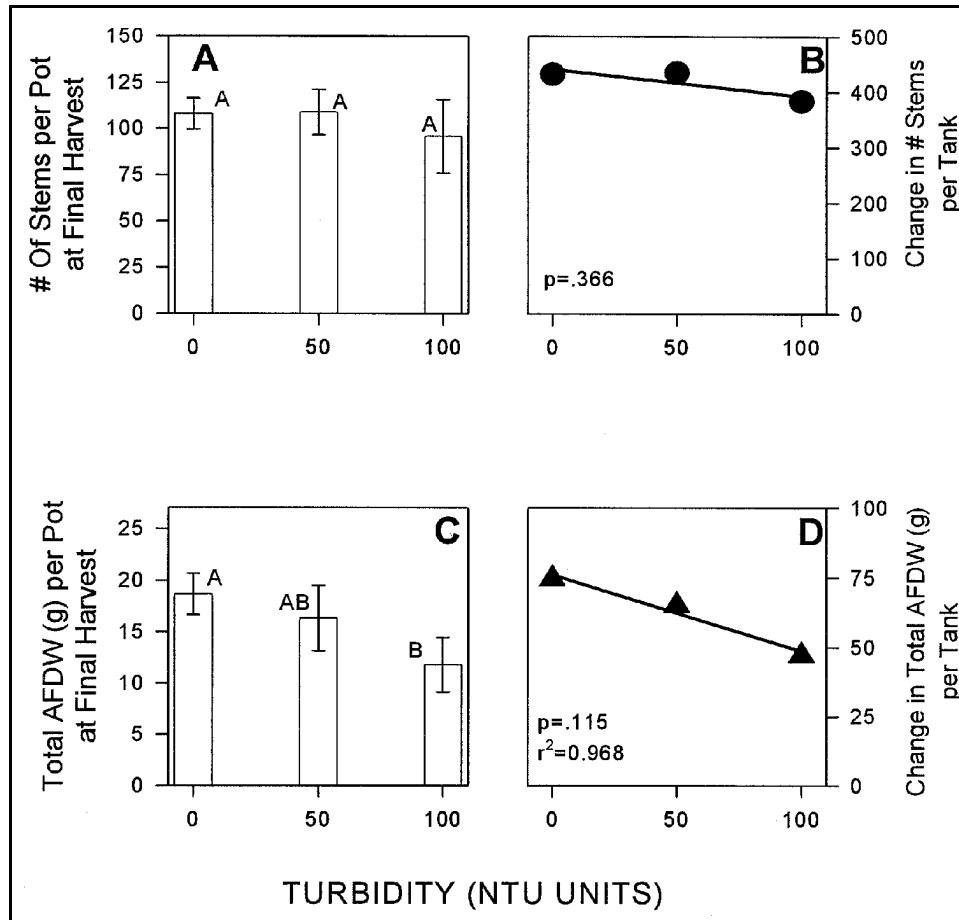
Figure 7.   Relationship between turbidity and sago pondweed growth. A and
             C show mean growth (± se, n=4) for plants grown under different
             turbidities. ANOVA was significant for total mass (C), but not for
             number of stems per pot (A). Letters indicate significant differences
             among treatment means. B and D show regression analysis of
             change in tank totals (sum of all pots at time of final harvest - initial
             tank totals) versus trubidity

mass was lower in the highest turbidity tank and intermediate in the intermediate
turbidity tank (Figure 7 C). Population totals for the tanks reflect similar
patterns (Figure 7 B,D).

    Sago pondweed reproduction via tuber or seed production was significantly
impacted by turbidity, but only at the highest turbidity level tested (Figure 8
A,C). Population totals showed a declining trend for tuber production with
increasing turbidity, but there was no difference between the control and
intermediate turbidity tanks in flower/seed production (Figure 8 B,D).
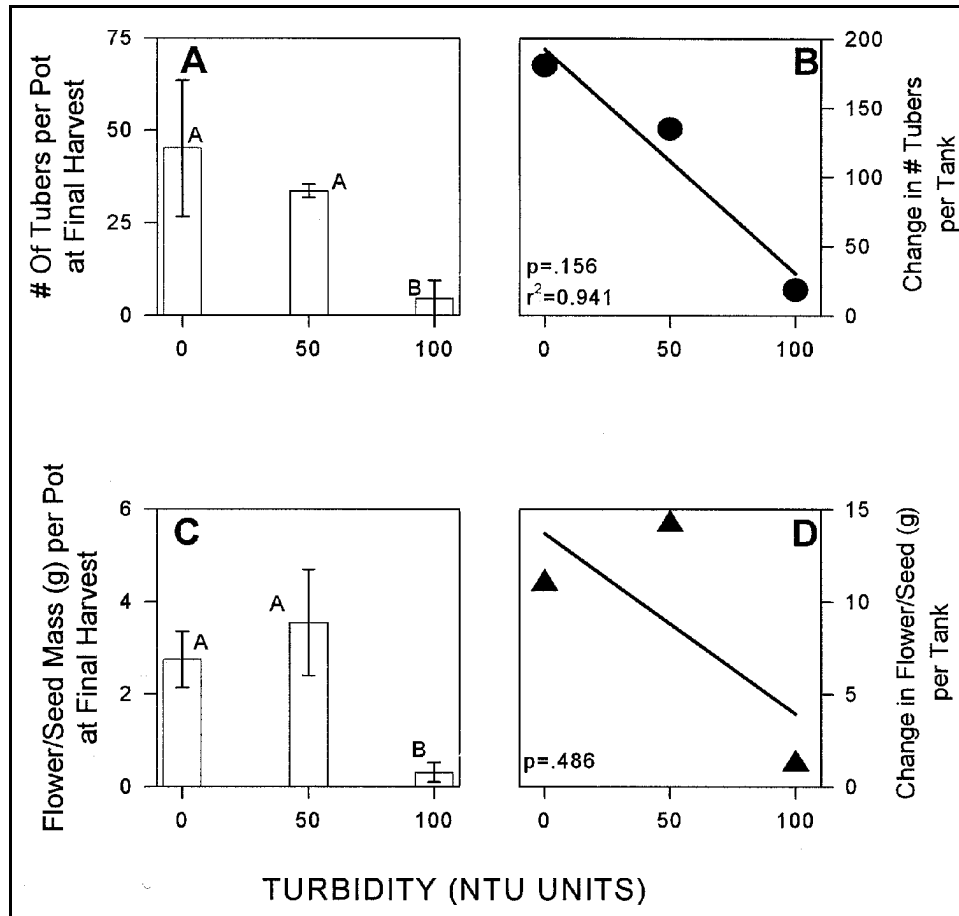
Figure 8.   Relationship between turbidity and sago pondweed reproduction. A and C show mean growth (± se, n=4) for plants grown under different turbidities. ANOVAs were significant for both response variables and letters indicate significant differences among treatment means. B and D show regression analysis of change in tank totals (sum of all pots at time of final harvest - initial tank totals) versus turbidity

Conclusions and Recommendations Based on Greenhouse Pilot Experiment

    Water column turbidity significantly impacted growth and reproductive potential of the submersed plants tested, even though the experimental systems were very shallow (65 cm) and the exposure time was short relative to the growing season (~30%). Vallisneria appears to be particularly vulnerable to turbidity effects. Mature vallisneria plants exposed to the most turbid conditions tested (~100 NTU) ceased to form new rosettes and produced significantly less biomass, fewer tubers, and less flower/seed than plants growing in control tanks.

Vegetative growth was far less affected by turbidity in sago pondweed than that in vallisneria. Turbidity did not affect the number of new stems produced by the plants and had only limited effect on biomass production. Sago pondweed reproduction was significantly impacted by turbidity, but only at the highest level tested. Although there was no difference between number of tubers or flower mass produced between the control and 50 NTU treatments, the 100 NTU tanks produced virtually no tubers or flowers. This result is likely due to the differences in architecture and location of the growth meristem in the two species. Vallisneria has a rosette growth form with the growth meristem located at the base of the leaf and the plant's biomass relatively homogeneously distributed throughout the water column. In contrast sago pondweed has a terminal growth meristem and concentrates leaf biomass very near the surface. As a result, if the initial propagule of sago pondweed has sufficient energy for the apical meristem to reach the water surface, the major growth tissues of the plant are then located in very good light conditions despite relatively low light at the sediment surface.

These results indicate that reduced light conditions at even the highest turbidity level tested in this experiment are not sufficient to cause death of mature plants in shallow water. However, although plants survived, they grew more slowly and produced fewer tubers and seed, and, thus, the long-term survival of the population under such conditions is questionable. This limitation in formation of reproductive propagules may in fact be one of the primary mechanisms by which turbidity impacts well-established plant communities. If the vegetative growth is slowed and formation of reproductive propagules declines, while losses (natural mortality, grazing, hydraulic disturbances, etc.) remain constant, the population would be expected to decline over time.

Based on the experience gained in this pilot experiment, the following suggestions were made for the subsequent phases of the research:

a. Use deeper tanks to investigate the impacts of turbidity on mature plant populations. Shallow tanks as utilized for the pilot experiment minimize the impacts of turbidity because the plants can easily grow to the water surface, where light levels are high. A water depth of 1.25-1.5 m above the sediment surface is recommended.

b. Keep turbidity levels more constant or incorporate turbidity "pulsing" into the experimental design. The pulsed nature of this experiment made the actual light climate fairly variable and made determination of specific breakpoints where tubidity begins to impact submersed plants more difficult to interpret.

3 Effects of Turbidity on Mature Plants: II. Deep Tank Factorial Experiment

This experiment was designed to investigate the effects of water column turbidity and frequency of sediment resuspension on the growth and reproductive potential of established macrophyte communities. The experiment was designed as a 2 X 2 factorial with two levels of turbidity and two sediment resuspension frequencies and incorporated the recommendations from the pilot experiment described in Chapter 2.

Materials and Methods for Deep Tank Experiment

Deep Tank Mesocosm Facility

This experiment was conducted in a system of 14,000-L fiberglass tanks at LAERF (Deep Tank Facility, Figure 9). These round tanks measured 2.6 m in diameter and 3.0 m in depth and were filled with pond water filtered with a swimming pool sand filter. Because of the high temperatures characteristic of summers in Texas, the tanks were cooled during part of the 8-week experiment by evaporative cooling supplemented with thermostatically controlled liquid circulators (Remcor Corp., Chicago, IL). Evaporative cooling for each tank was provided by wrapping the tanks with geotextile cloth and then keeping the wrapping continuously moist. The water column in each tank was continuously aerated with ambient air utilizing five air lifts (see Figure 9) and evaporative losses (2-3 cm per week) were made up with additional filtered pond water.

Although the outdoor tanks utilized had no means of precisely regulating temperatures, it was agreed that the tank temperatures would be maintained below 30 °C so that they would not exceed extreme maximum temperatures in shallow waters along the UMRS.
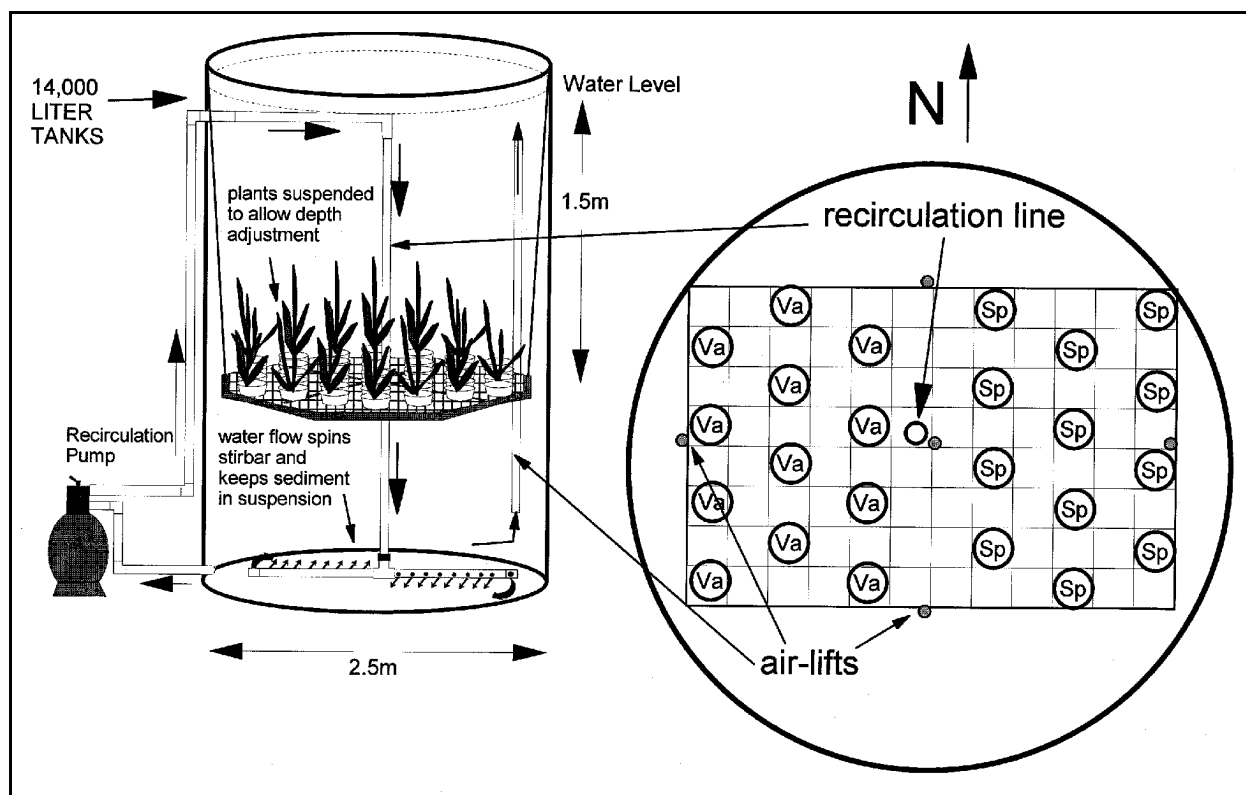
Figure 9. Schematic of one of the deep tanks at LAERF utilized for the turbidity experiment. The rack was positioned 150 cm below the water surface (sediment surface of pots at 133-cm depth). Water in the "continuous" tanks was constantly recirculated through the tank. Water was forced down through the recirculation line and out through the stir bar, which kept sediments constantly in suspension. "Pulsed" tanks were recirculated twice a week and allowed to settle during the interim

Plant material

Well-established pots of vallisneria and sago pondweed were obtained by planting five vallisneria tubers or four sago pondweed tubers into 2.4-L plastic pots (15 cm in diameter or 0.0182 m² in surface area per pot) of heat-sterilized sediments. Tubers of both species were collected in Wisconsin and sent to Texas. Tubers were maintained under refrigeration until they were planted on April 24, 1996. After planting, they were cultured under greenhouse conditions for 7 weeks prior to the beginning of the experiment on June 6, 1996.

Twelve pots of each plant species were then randomly assigned to each of 15 groups. Thirteen groups were then placed into each of the tanks utilized in the experimental design (see below) and the remaining two groups (24 pots) were harvested to document the initial conditions of the plants. A rectangular rack was constructed to fit within the tanks and was suspended at a depth of 1.5 m below the water surface (Figure 9). Since the 17-cm-tall pots were fastened to this rack, the sediment surface of the pots was located at a depth of approximately 1.33 m. The rack was oriented directly north/south and the

vallisneria pots occupied the west side of the tank while the sago pondweed pots were on the east side. Since the wall of the tank exerted some shading effect in the mornings and evenings, this systematic orientation allowed similar light characteristics for plant populations in different tanks.

Experimental setup

The experimental design was a 2 X 2 factorial experiment with two levels of turbidity (Low, High) and two sediment resuspension regimes (Continuous, Pulsed) for a total of four experimental treatment combinations (Low:Pulsed, Low:Continuous, High:Pulsed, High:Continuous). Each of the four treatment combinations was replicated in three tanks (12 pots of each species in each of three tanks) except for the Low:Pulsed treatment combination which was replicated in four tanks (12 pots of each species in each of four tanks). This additional replicate was added because the variability among tanks for this treatment was expected to be higher than for the other treatments.

The low turbidity tanks had a target turbidity of 10 NTU, while the high turbidity tanks had a target of 30 NTU. The tanks were operated on a pulsed or continuous mixed regime. A pulsed regime was obtained by mixing the tanks two times per week. Although initial discussions on pulsing frequency focused on adjusting mixing frequency to that of barge traffic, it was later decided that the major impacts of barge traffic on plants due to sediment resuspension would likely occur over longer time periods. Since most of the plant beds are apparently located out of the direct impact of barge waves, the concern focused on a gradual "ratcheting up" of suspended sediments within the system due to increased navigation. The frequency with which this turbid water would be moved over plant beds was unknown, but a pulsing frequency of two times per week was considered sufficient to answer questions about continuously turbid water versus water which is stirred up periodically but is allowed periods of settling.

The tanks were mixed for 1.5 hours, although complete homogenization of the water column was obtained after 15 minutes of mixing. After the 1.5-hour mixing period the turbidity in the tank was checked and adjusted as necessary by adding more sediment slurry to return the turbidity to the target level. Continuous turbidity was maintained by constant stirring of the tanks. However, even with constant recirculation, the turbidity in the tanks slowly dropped as sediments were deposited on the plant surfaces or on the sides of the tanks. To maintain turbidity as constant as possible, turbidity was checked in each of the continuously mixed tanks two or three times each day and adjusted as necessary with sediment slurry.

The light climate in the tanks was again monitored. Photosynthetically active radiation (PAR, 400-700 nm) measured as light quanta (μE m^{-2} s^{-1}) incident upon the tanks was measured in the air just above the water surface with a flat quantum sensor (Li-Cor model SR sensor). A flat sensor measures only downwelling light which can penetrate into the water. However, once light is in the water, the direction of light travel is not important, since leaves can utilize

downwelling as well as reflected light. Therefore, light attenuation in the tanks was determined by measuring PAR just below the water surface and at various depth within the tank utilizing a spherical quantum sensor (Li-Cor model LI-193SA sensor). Underwater light quality was also measured utilizing an underwater spectral radiometer (LiCor LI-1800UW). Measurements were first made in air above the water surface of the tanks and then at various depths within the tanks. Measurements were taken in 2-nm wavelength increments over the PAR range of 400-700 nm. Spectral light quality was measured in clear, low turbidity, and high turbidity tanks. Shifts in light quality recorded are assumed to be primarily due to suspended sediment differences, although algae within the tanks may have also contributed to light absorption. All light measurements were taken between 10 AM and 2 PM under clear skies. PAR measurements were made at approximately two-week intervals throughout the study.

Mixing to resuspend the settled sediments was accomplished by pumping water into the tanks with a swimming pool sand filter (operated in "recirculate" not in "filter" mode). The water was routed to the bottom of the tank through a 1.5-in. PVC pipe. Near the bottom of the tank was placed a swivel T coupling and attached capped PVC pipes to form a "stir-bar" (Figure 9). One-centimeter holes were drilled along the axis of each arm. The holes were oriented in opposite directions, and the thrust from the water flow served to spin the "stir-bar."

The relationship between turbidity, total suspended solids (TSS), and attenuation of photosynthetically active radiation (PAR) was investigated by measuring these variables simultaneously in the tanks. PAR was measured at various depths within the tanks under differing turbidities. PAR incident upon the tanks was measured with a flat PAR quantum sensor, while light within the tanks was measured with a spherical underwater PAR quantum sensor. Since the insides of the tanks were painted black, there was no light reflectance off the sides of the tanks.

Water chemistry in the tanks was monitored four times over the 8-week experimental growth period. Parameters analyzed included: Alkalinity, total phosphorus, potassium, and plankton chlorophyll-a. These parameters were analyzed according to standard methods (American Public Health Association (APHA) 1992), and replicate samples and sample splits were routinely analyzed to validate the data.

Plant harvest

All plants were harvested at the end of the 8-week experimental growth period. Three pots of each plant species in each tank were harvested so that the amount of accumulated sediments and epiphytes on their leaves could be quantified. These plants were enclosed in a plastic bag while still underwater. The planted pot was then transferred into a separate basin. The plants were clipped at the sediment surface, and the leaves rinsed carefully to remove attached sediments and epiphytes. The volume of the rinse water was measured and subsamples were collected for TSS and chlorophyll-a analysis. Corrections

were made for background TSS and chlorophyll-a levels in the tanks. The remainder of the pots were then harvested from each tank.

Various biomass variables were measured in the harvested plant material. Numbers of vallisneria rosettes and numbers of female flowers and/or seed pods for each species were counted. The female flower/seed pod tissue of vallisneria (but not of sago pondweed) was separated from the remainder of the above-ground biomass. Above- and below-ground tissues were separated by cutting the plant stems at the sediment surface. Above-ground tissues were washed to remove accumulated sediments and epiphytes. Below-ground tissues were washed over a 1-mm sieve to remove sediment and debris. Tubers, if present, were counted and separated from the remainder of the below-ground tissues. For vallisneria, each individual growing tip was considered a tuber. Plant tissue samples were then bagged and oven-dried to constant weight at 60 °C. After weighing, the samples were ground in a Wiley mill to pass through a 40-mesh sieve. Subsamples of the ground material were then combusted at 550 °C in a muffle furnace to determine ash content. All biomass data were corrected for ash content and expressed as ash-free dry mass. Leaf chlorophyll-a content in a subsample from each pot was determined after extraction in dimethyl sulfoxide (DMSO) (Hiscox and Israelstam 1979).

Statistical Analysis

The data below were analyzed separately for each plant species on both a per pot basis and on a population basis.

Per pot analysis. In the first case (per pot basis) the mean mass per pot in each tank is compared with the mass at the beginning of the experiment utilizing a t-statistic (N=12 for tank mean of each species, N=24 for initials of each species). This analysis asks the question is the mass of material in the pots at the end of the experimental growth period significantly different than that at the beginning of the growth period. This analysis clarifies the changes which took place at the individual pot level (did the plant mass per pot increase, decrease, or stay the same). In addition, the average value of all pots of a given treatment is computed to illustrate the magnitude of change among different treatments. However, no statistical comparisons are made because pots growing within the same tank are not completely independent (i.e., they are pseudo-replicates and not true replicates). Statistical treatment comparisons are all made at the population level of analysis. For such analysis, each tank equals one experimental unit (see below).

Population level analysis. For the population analyses, total mass of the population in each tank was calculated by summing the mass of each pot of that species within the tank. For this analysis, the total population of each species within each tank is considered a single experimental unit (N=3 for each treatment except for Low:Pulsed where N=4). Data were tested to ensure that they were normally distributed and variances were homogeneous.

Three population-level analyses were performed. First, because the experimental design was a 2 X 2 factorial, the effects of the two treatment variables (turbidity and mixing) on the plant population of each species within the tanks was analyzed using a two-way ANOVA. Interaction between the two main factors proved significant for only one variable of vallisneria (number of tubers produced) as described below. In all other cases, interactions were not significant and main effects were considered significant at a confidence level of $\alpha \leq 0.10$ due to the relatively low number of experimental units.

Second, because the two factors utilized (turbidity level and mixing regime) both influenced light availability, the data were then analyzed as a one factor experiment (four discrete levels of total light availability) using a one-way ANOVA followed by multiple range testing.

Finally, because light availability is a continuous variable, the experiment was also analyzed as a dose-response design using linear regression techniques. The mean proportion of available light penetrating to a depth of 0.5 m was used as the independent variable and the measured plant responses as the dependent variables. This analysis ignores the periodicity of the light fields in the pulsed tanks, and looks only at the total PAR available over the entire experimental growth period.

Results of Deep Tank Experiment

Water quality within tanks

Water quality in the tanks varied relatively little over the experimental period (Table 5). Alkalinity was sufficient to sustain plant growth; it declined moderately (~15%) in the low-turbidity tanks, while remaining virtually constant in the high-turbidity tanks. Total phosphorus, potassium, and plankton chlorophyll levels showed no consistent pattern of change. Although total phosphorus levels in the High:Continuous tanks averaged about twice those of the Low:Pulsed tank (~30 μL^{-1} vs. ~15 μL^{-1}) the plankton levels were very similar and remained below 5 μL^{-1} in both treatments.

Light and temperature regimes

Temperature remained between 25 and 28 °C most of the experimental period, but exceeded this range during a particularly hot week in July when temperatures soared to over 30 °C (Figure 10). At the beginning of the experiment, tank temperatures averaged 25.8 °C but rose steadily during the remainder of June. During the first week of July, unusually hot temperatures in North Texas caused tank temperatures to rise to over 30 °C. At that time, cooling of the tanks was initiated utilizing evaporative cooling and recirculating chillers. These efforts succeeded in lowering the tank temperatures back to 25-27 °C where they remained for the remainder of the experiment.

Table 5
Water Quality Parameters Measured in Deep Tanks

Parameter/Date	Treatment			
	Low Pulsed	Low Continuous	High Pulsed	High Continuous
Alkalinity (mg/L)				
6/10/96	74.3	73.7	72.7	72.7
6/25/96	70.5	70.7	73.3	73.7
7/8/96	68.3	72.7	72.7	77.3
7/22/96	62.8	68.3	70.3	75.0
8/5/96	59.3	63.7	67.0	72.7
Total Phosphorus (μg/L)	11.8	11.7	15.7	14.3
6/10/96	15.0	13.7	19.3	31.7
6/25/96	22.3	17.7	13.7	32.7
7/8/96	18.7	15.0	25.0	31.0
8/5/96				
Potassium (mg/L)				
6/10/96	3.78	3.73	3.90	3.93
6/25/96	5.73	4.87	4.97	4.97
7/8/96	3.90	4.03	4.20	4.33
7/22/96	3.53	3.40	3.53	3.40
8/5/96	3.35	3.63	3.93	3.97
Chlorophyll-a (μg/L)				
6/10/96	3.2	3.3	2.9	3.4
6/25/96	2.0	2.4	1.8	3.5
7/8/96	0.6	2.5	0.6	4.7
7/22/96	2.4	3.0	0.7	3.7
8/5/96	1.0	2.9	1.9	2.6

Note: Values shown are means of replicated tanks for each of the four treatments. N=3, except for Low Pulsed where n=4 tanks. Individual tanks varied by less than 15% from the mean.

These temperatures are likely higher than those along the UMRS and may have influenced the response observed. Howerver, it is believed that the relative effect of the turbidity treatments are valid.

Preliminary work in tanks without plants had indicated that continuous stir-bar mixing was sufficient to keep the turbidity in the "continuously turbid tanks" constant, and that turbidities would decrease by less than 5% per day. However, in the current experiment the turbidity in all tanks dropped rapidly during the first 2-3 days after plants were added to the tanks (Figure 11 B and D). Apparently, plants added substantially to the surface area on which sediments could be deposited and they may also have dampened the mixing effect. Consequently, the tanks were monitored and turbidities adjusted 2-3 times each day in the continuously turbid tanks (both low and high turbidity). In this way the turbidity in the continuous tanks was maintained relatively constant through the remainder of the study (Figure 11). Turbidity in the pulsed tanks was more variable through time since a brief mixing period was followed by two days of no mixing. However, replicate pulsed tanks behaved very similarly and varied by less than 10% from one another during the experiment (Figure 11 A and C).
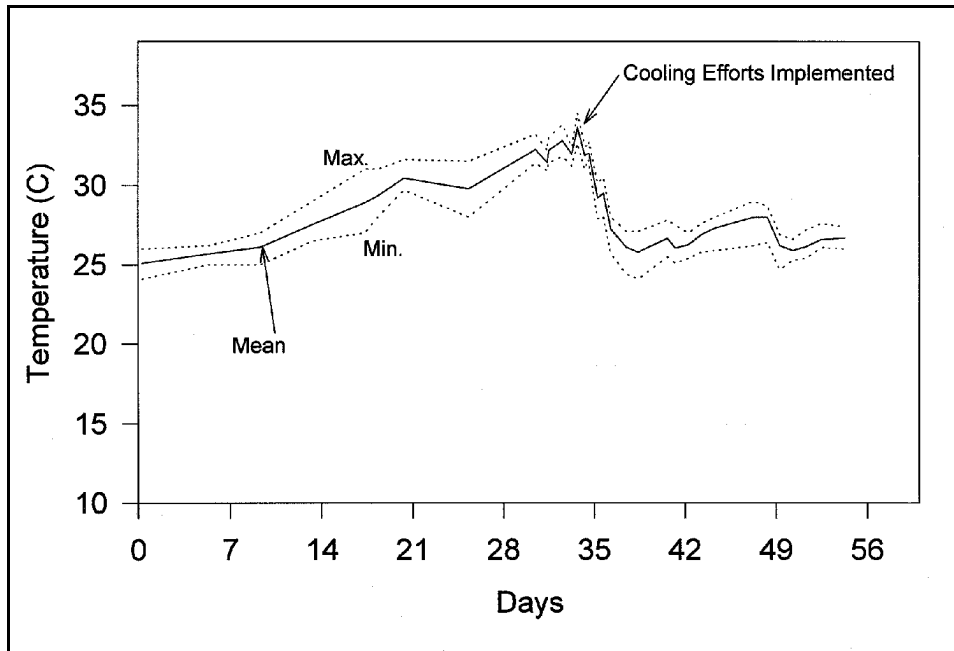
Figure 10. Mean, maximum, and minimum temperatures in the deep tanks
used for the turbidity experiment. When temperatures rose above
30 °C, cooling was implemented to bring tank temperatures back
below 28 °C

Light attenuation was strongly and linearly related to turbidity (Figure 12).
The 30 NTU corresponded to a light extinction coefficient (Kd) of 2.8-3.2 and
allowed 20-35% of subsurface light to reach 0.5-m depth and about 5% to reach
1-m depth; 10 NTU corresponded to a Kd of 1.3-1.8 and allowed about 40% of
subsurface light to reach 0.5-m depth and about 20% to reach 1-m depth
(Figure 13). Light penetration to 1.0 m corresponded to ~20% and ~5% in the
10 and 30 NTU tanks, respectively. Plant leaves experienced far higher
irradiance near the water surface than at greater depth.

Based on the light-turbidity relationships (Figures 12 and 13) and the detailed
record of turbidity through time (Figure 11), the mean proportion of total
available PAR (100% = subsurface PAR) which penetrated to depths of 0.25,
0.5, and 1.0 m was computed (Table 6).
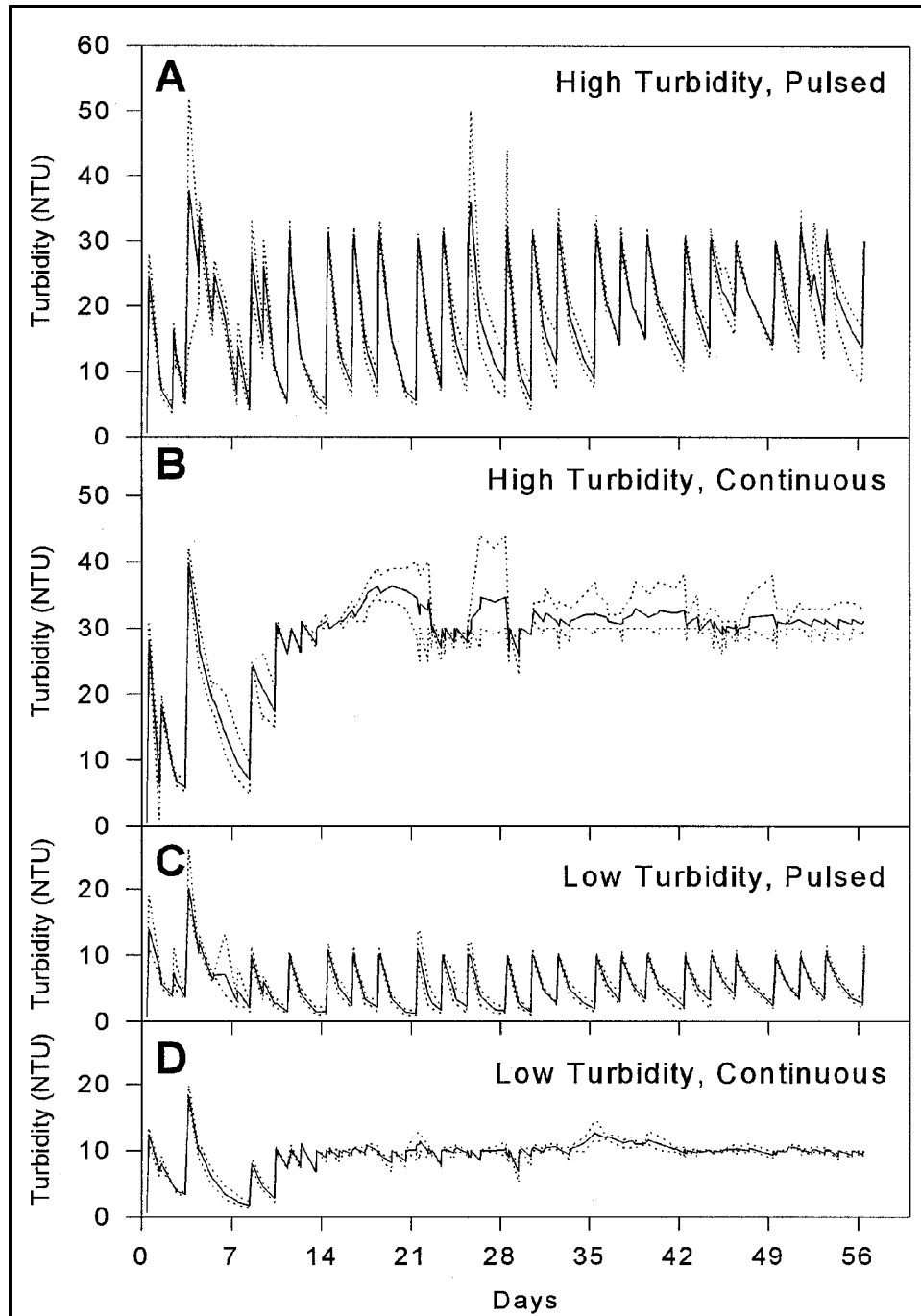
Figure 11.   Average, maximum, and minimum turbidities in tanks of each experimental treatment
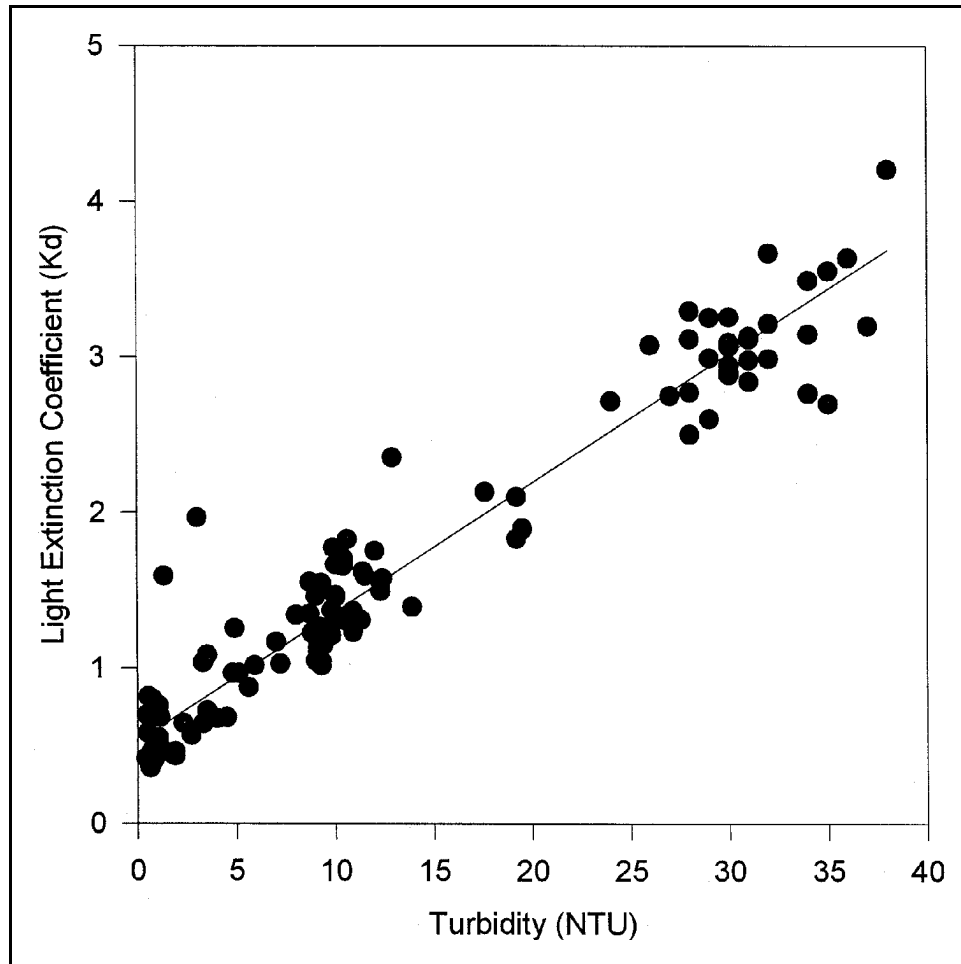
Figure 12. Relationship between light extinction coefficient (Kd) and turbidity. Line represents least square linear regression through points. Kd=0.53+(0.0831*NTU), r^2=0.933
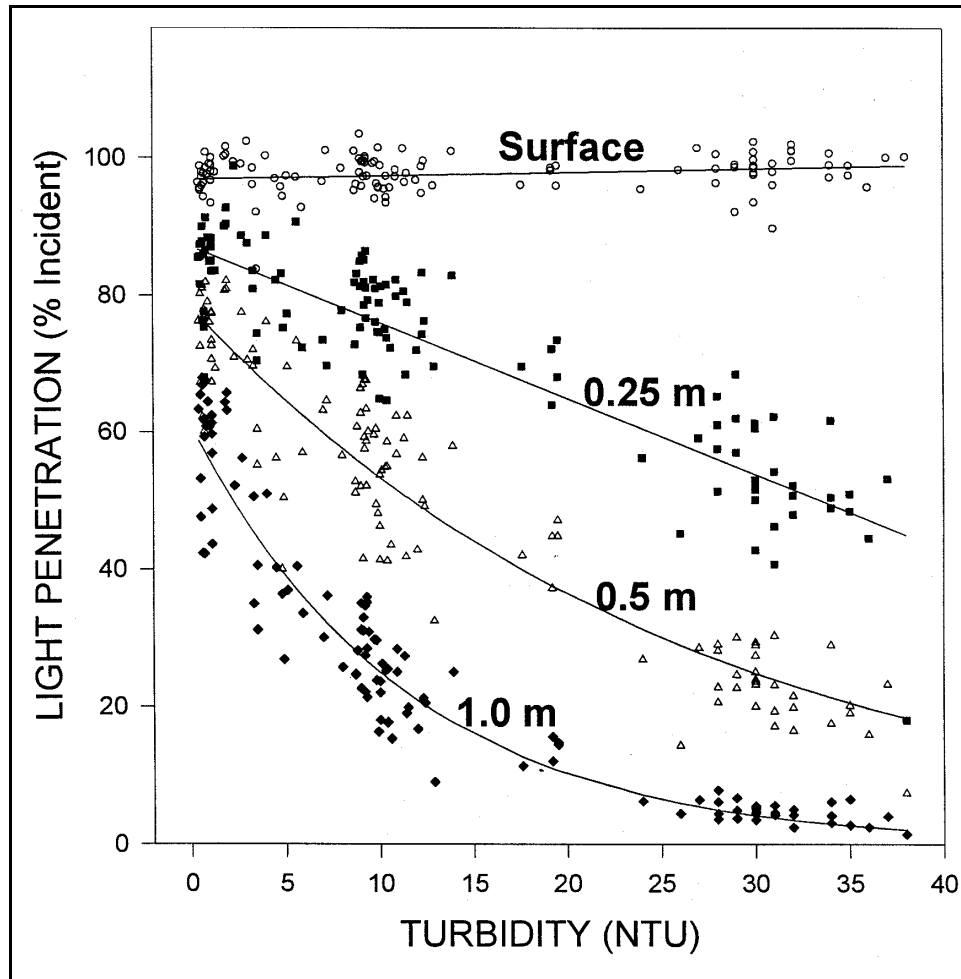
Figure 13. Relationship between light penetration to various depths within the experimental deep tanks and the water column turbidity. Lines show least squares regression of linear (surface and 0.25 m) or exponential (0.5 and 1.0 m) functions

Table 6
Average Turbidity in Tanks of Each Treatment and Associated Estimated Percent Subsurface Light Penetrating to Various Depths Within Tanks (mean ± SD, n= 160)

Treatment (Turbidity / Mixing Regime)	Average Turbidity (NTU)	Average Percent Subsurface Light at Depth		
		0.25 m	0.50 m	1.0 m
Low Turbidity / Pulsed (L/P)	6.7 ± 3.3	80.4 ± 3.2	62.8 ± 7.1	37.2 ± 9.3
Low Turbidity / Continuous (L/C)	9.4 ± 2.2	76.8 ± 2.3	54.9 ± 5.0	27.0 ± 6.4
High Turbidity / Pulsed (H/P)	21.9 ± 8.8	66.5 ± 8.2	39.1 ± 11.8	13.8 ± 9.6
High Turbidity / Continuous (H/C)	29.1 ± 6.6	56.7 ± 6.8	27.0 ± 9.1	6.0 ± 1.8

Turbidity regimes also caused changes in the spectral composition of the light (Figure 14). Clay turbidity preferentially blocked light in the 400- to 550-nm range compared to filtered pond water. Thus, although light quantity was lower in turbid tanks, the light which did penetrate was relatively enriched in the wavelengths between 550 and 700 (yellow - red) and depleted in light in the 400- to 550-nm range (blue - green).

Plant biomass

Plant biomass values were corrected for ash content according to the results for each tissue type within each experimental treatment (Table 7).

Table 7
Contribution of Organic Dry Weight Expressed as Percent of Total Dry Weight (mean ± se, n=4)

| Species and Treatment	Above-ground Mass (Leaves and Stems)	Below-ground Mass (Roots and Rhizomes)	Inflorescence	Tubers
vallisneria				
Low Turb (cont)	63.9 ± 1.3	71.7 ± 3.3	22.3 ± 1.8	93.7 ± 0.3
Low Turb (pulsed)	62.0 ± 2.2	72.3 ± 2.4	33.9 ± 3.3	92.1 ± 1.7
High Turb (cont)	62.2 ± 1.9	56.3 ± 2.8	25.4 ± 3.4	91.9 ± 1.0
High Turb (pulsed)	65.3 ± 1.6	64.4 ± 5.6	no data	92.1 ± 0.6
sago pondweed				
Low Turb (cont)	83.8 ± 0.6	79.2 ± 2.3		90.8 ± 2.3
Low Turb (pulsed)	80.4 ± 0.6	78.4 ± 2.8	not determined	81.7 ± 2.6
High Turb (cont)	79.0 ± 0.8	79.1 ± 2.4		79.1 ± 2.6
High Turb (pulsed)	75.9 ± 1.3	82.6 ± 2.5		89.4 ± 3.1

Individual pot response. All pots contained well-established plants at the beginning of the experiment (time=t_o) (Table 8, Figure 15). Vallisneria pots contained an average of almost eleven rosettes, while sago pondweed pots had an average of over 30 stems. Total plant biomass per pot was similar for the two species with an average of 2.7 and 3.1 g of ash-free dry mass per pot for vallisneria and sago pondweed, respectively. At the beginning of the experiment, neither species had begun flowering or tuber development.
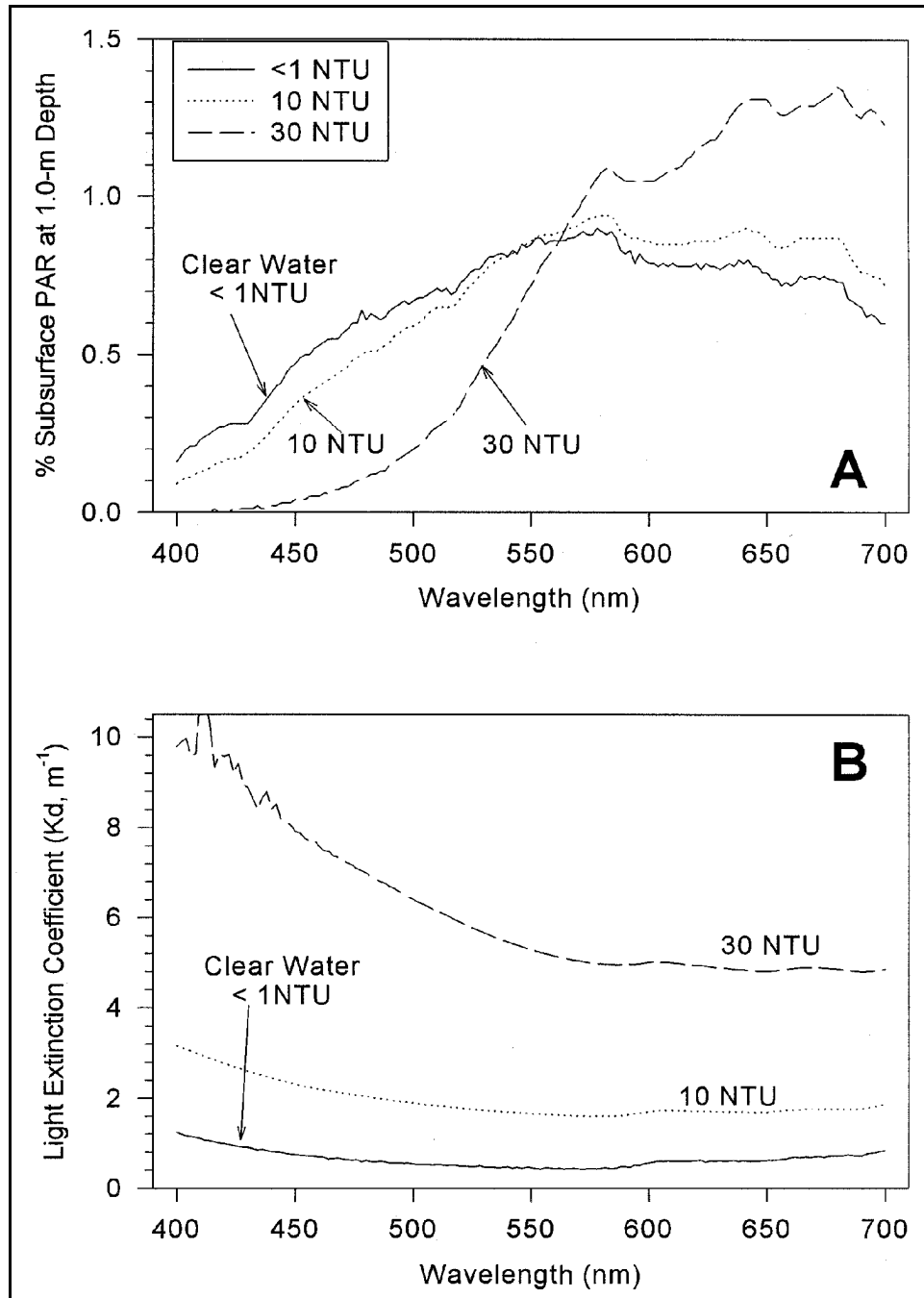
Figure 14.   Changes in spectral composition of light at three turbidity levels.
(A) Percent of subsurface light penetrating to a depth of 1.0 m for
different wavelengths across the PAR spectrum. Measurements
were taken in 2-nm band widths. (B) Light extinction coefficient of
different wavelengths

Table 8
Plant Parameters Measured at Beginning (t_o) and End of Experimental Period (t_f)

Variable Measured	Initial (t_o)	t_f High/Cont (17.2)	t_f High/Pulsed (39.1)	t_f Low/Cont. (54.9)	t_f Low/Pulsed (62.8)
		Treatment (Turbidity/Mixing Regime, $\bar{x}$ PAR at 0.5-m Depth)			
vallisneria					
Maximum length (cm)	68.4 ± 2.3	ND			
Rosette number (n)	10.8 ± 0.8	16.6 ± 1.5	15.3 ± 0.9	24.0 ± 1.4	22.1 ± 1.1
Leaf mass (g)	2.36 ± 0.13	0.9 ± 0.1	3.2 ± 0.4	3.4 ± 0.3	4.8 ± 0.3
Root mass (g)	0.37 ± 0.04	0.3 ± 0.04	0.8 ± 0.1	1.4 ± 0.1	1.8 ± 0.1
Tuber number (n)	0	18.0 ± 1.5	40.0 ± 2.6	63.8 ± 2.6	62.2 ± 3.0
Tuber mass (g)	0	0.8 ± 0.1	2.0 ± 0.1	3.6 ± 0.2	3.8 ± 0.2
Inflorescence mass (g)	0	0.003 ± 0.001	0.11 ± 0.04	0.06 ± 0.02	0.13 ± 0.03
Total biomass (g)	2.73 ± 0.15	2.1 ± 0.2	5.7 ± 0.4	8.5 ± 0.5	10.5 ± 0.4
Leaf chlor-a (mg/g dw)	ND	11.2 ± 0.7	11.6 ± 0.6	8.7 ± 0.6	8.6 ± 0.6
sago pondweed					
Maximum length (cm)	91.8 ± 1.4	122.5 ± 6.2	131.5 ± 4.2	122.7 ± 3.1	124.5 ± 3.0
Above-ground mass (g)	2.51 ± 0.11	4.6 ± 0.6	6.2 ± 0.7	9.5 ± 0.6	11.1 ± 0.5
Root mass (g)	0.61 ± 0.03	1.3 ± 0.1	1.9 ± 0.2	3.5 ± 0.3	4.9 ± 0.4
Tuber number (n)	0	6.8 ± 1.2	22.3 ± 4.9	56.2 ± 5.9	72.4 ± 5.6
Tuber mass (g)	0	0.08 ± 0.02	0.4 ± 0.1	1.0 ± 0.1	1.6 ± 0.2
Inflorescence number (n)	0	17.7 ± 3.4	20.3 ± 4.0	18.9 ± 3.5	37.4 ± 6.0
Total biomass (g)	3.12 ± 0.14	6.0 ± 0.7	8.5 ± 1.0	14.0 ± 1.0	17.5 ± 1.0
Leaf chlor-a (mg/g)	ND	5.8 ± 0.3	5.8 ± 0.3	6.3 ± 0.2	5.2 ± 0.2

Note: Values shown are means ± se of all pots harvested (for biomass values, t_o N=24, for t_f N=36 pots harvested for all treatments except Low/Pulsed, where N=48). All biomass values are expressed as ash-free dry mass. Vallisneria chlorophyll-a values are for leaves > 35 cm long, (N= 38, 55, 36, and 63 for the four treatments, respectively). ND= not determined

The above-ground tissues for both species were also well-developed. Vallisneria maximum length averaged about 68 cm while that of sago pondweed averaged over 90 cm. Since the pots were placed within the tanks such that the sediment surface was ~133 cm below the surface (see Figure 9), the leaves of vallisneria and sago pondweed reached 50 and 70% of the way up through the water column, respectively.

Plant growth within a pot appears to be influenced by the turbidity/mixing regime under which it was grown (Table 8, Figure 15). Vallisneria plants developed the highest mass per pot under the lowest turbidity regime (Low:Pulsed). Under the most turbid conditions (High/Cont), vallisneria did
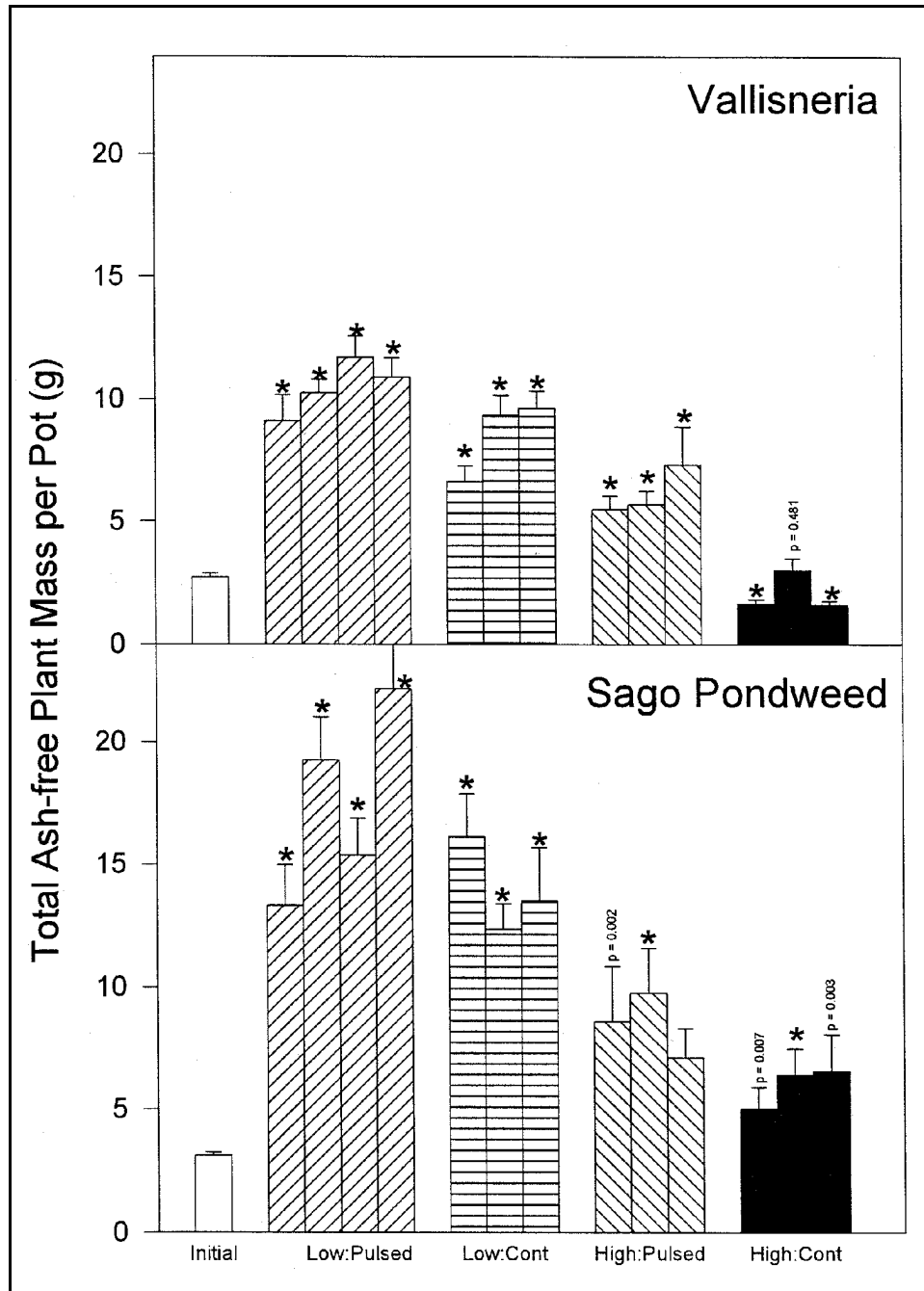
Figure 15. Mean and standard error of total plant mass per pot at beginning of experiment (initial) and in each tank of each treatment at end of experiment. Mean mass per pot in each tank at the end of the experiment is compared to the initial mass utilizing a t-test (N=12, except for initials where N=24). Asterisk indicates tank means which differ from initials at $\alpha < 0.001$

very poorly and the plants in two of the tanks were actually significantly smaller at the end of the experiment than at the beginning while the average mass per pot in the third tank was not significantly different from the initials (Figure 15). The decline in mass appears to be concentrated in the leaf tissues, since the leaf mass for this treatment appears to have declined by more than 50% while root mass per pot was similar to the initial values (Table 8). Although the mean number of rosettes seems to have increased from 11 to about 17, these were generally very small rosettes and many were found along stolons growing out of the pots and not rooted in the sediment. As turbidity level dropped, the performance of vallisneria increased, and, under the best light conditions, the total plant mass increased by a factor of 3.8 relative to initial mass.

Vallisneria reproduction also appears to be influenced by the experimental regimes. Although plants produced flowers and tubers in all treatments, the magnitude of production appears related to the turbidity level. Tuber number and mass were low under the most turbid conditions (High/Cont), intermediate under the High/Pulsed treatment, and higher for both Low Turbidity treatments (Table 8). Unlike all other parameters measured, vallisneria flower/seed production showed a pattern related to the frequency of pulsing. Flower/seed production was low for both continuously turbid tanks (Low/Cont, High/Cont) while it was much higher for the two pulsed regimes.

Vallisneria leaf chlorophyll-a content was related to both leaf length (Figure 16) and turbidity level (Table 8). Leaf chlorophyll-a concentration (mg chlorophyll-a per g dw leaf tissue) was very variable for short leaves (<20 cm) but on average was far higher than in longer leaves (Figure 16 B). There was a significant (p<.05) log-linear relationship between leaf chlorophyll-a content and leaf length (Figure 16 A) although the r^2 for the relationship was relatively low (r^2=0.32). The chlorophyll-a content of mature leaves (>35 cm) appears higher in both high turbidity treatments than in the two low-turbidity treatments (Table 8).

Unlike vallisneria, sago pondweed showed positive growth under all experimental treatments (Table 8, Figure 15). Plants in all treatments elongated to the water surface, and there was no difference among treatments as to the maximum length of the plants. Above-ground mass (leaves+flower/seed) appears lower in the high turbidity treatments than in the low turbidity treatments. Total biomass increase followed a similar pattern. Sago pondweed plants in the Low/Pulsed treatment had higher root mass and tuber production than in the Low/Cont treatment. Under the least turbid conditions, sago pondweed increased by a factor of 5.6 relative to initials, and at the end of the experiment had about 1.7 X higher mass than the vallisneria pots. Both tubers and flowers were produced under all experimental treatments (Table 8). Tuber production increased with decreasing turbidity, while flower production was about 2 X higher for the Low/Pulsed treatment than for any of the other treatments.
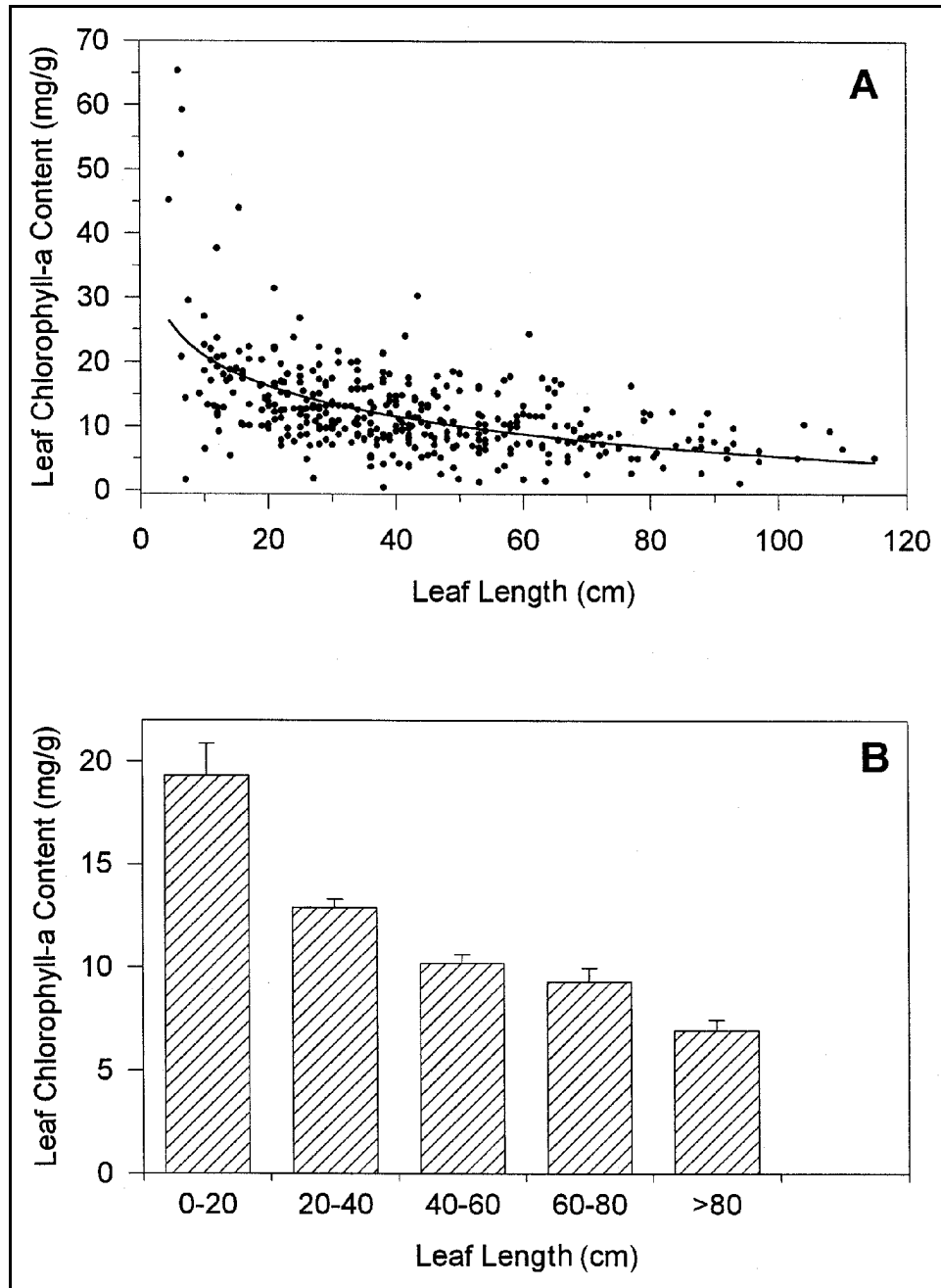
Figure 16.   Vallisneria leaf chlorophyll-a content (mg chlorophyll-a per g AFDW leaf tissue) for leaves of various lengths. A) Values for individual leaves. Regression line is the log-linear relationship (r^2=0.32). B) Mean ± se of all leaves within given size class, n= 60, 142, 107, 48, and 25 for the five size classes, respectively

    Chlorophyll-a content of sago pondweed appears to be almost identical for the two high-turbidity treatments, while that of the Low:Continuous tanks may be higher than that of the Low:Pulsed tanks (Table 8).

Population response: two factor analysis. Most measured parameters for vallisneria were significantly influenced by both treatment factors (turbidity level and mixing regime) and there was usually no significant interaction between the treatment factors (Table 9). Changes in total mass, calculated as the difference between final and initial (t_f-t_o), were strongly influenced by both factors, with population means in the low turbidity tanks being about 6 X higher than in the high turbidity tanks. Total vallisneria mass in pulsed tanks was also significantly higher than in continuous tanks. Similar patterns were observed for above-ground mass, root mass, and tuber number, but not for number of rosettes produced (Table 9). The latter parameter was strongly influenced by turbidity regime, being >7 X higher in low rather than high turbidity, but was not influenced by the mixing regime.

Table 9
Two-way ANOVA for Plant Parameters Measured in vallisneria Populations in Tanks

PARAMETER Factor	DF	P-value	Factor Mean ± se		
CHANGE IN TOTAL MASS (g)			TURB	(low)	79.7 ± 5.3
turbidity	1	0.0000		(high)	13.6 ± 5.7
mixing	1	0.0011	MIXING	(pulsed)	65.2 ± 5.3
interaction	1	0.1408		(cont)	28.2 ± 5.7
CHANGE IN NUMBER OF ROSETTES			TURB	(low)	135.5 ± 15.7
turbidity	1	0.0006		(high)	18.6 ± 16.7
mixing	1	0.6851	MIXING	(pulsed)	81.8 ± 15.7
interaction	1	0.2684		(cont)	72.2 ± 16.7
T_f Above-ground MASS (g)			TURB	(low)	50.1 ± 3.7
turbidity	1	0.0014		(high)	25.6 ± 3.9
mixing	1	0.0020	MIXING	(pulsed)	49.4 ± 3.7
interaction	1	0.3040		(cont)	26.3 ± 3.9
T_f ROOT MASS (g)			TURB	(low)	19.6 ± 1.3
turbidity	1	0.0001		(high)	7.1 ± 1.4
mixing	1	0.0207	MIXING	(pulsed)	16.0 ± 1.3
interaction	1	0.7540		(cont)	10.7 ± 1.4
T_f TUBER MASS (g)			TURB	(low)	44.4 ± 2.8
turbidity	1	0.0001		(high)	16.7 ± 3.0
mixing	1	0.0868	MIXING	(pulsed)	34.5 ± 2.8
interaction	1	0.1568		(cont)	26.6 ± 3.0
T_f TOTAL # Tubers			TURB	(low)	756 ± 32
turbidity	1	0.0000		(high)	348 ± 34
mixing	1	0.0264	MIXING	(pulsed)	613 ± 32
interaction	1	0.0134		(cont)	491 ± 34
T_f FLOWER/SEED MASS (g)			TURB	(low)	1.2 ± 0.3
turbidity	1	0.8005		(high)	1.1 ± 0.3
mixing	1	0.0137	MIXING	(pulsed)	1.8 ± 0.3
interaction	1	0.2301		(cont)	0.4 ± 0.3
T_f AG:BG RATIO			TURB	(low)	0.79 ± 0.10
turbidity	1	0.2314		(high)	0.99 ± 0.11
mixing	1	0.0633	MIXING	(pulsed)	1.05 ± 0.10
interaction	1	0.5202		(cont)	0.73 ± 0.11

The flower/seed mass and the AG:BG (above-ground mass: below-ground mass) ratio were unaffected by turbidity, but were significantly related to the mixing regime. Flower/seed production was 4.5 X higher in pulsed tanks than in continuous tanks. Likewise, the AG:BG ratio was also higher in pulsed tanks.

The significant interaction effect in tuber production was caused by the fact that pulsing frequency significantly affected tuber production in the high turbidity tanks but not in the low turbidity tanks. In the low turbidity tanks, tuber production averaged 62.2 ± 3.0 and 63.8 ± 2.6 per pot in the pulsed and continuous tanks, respectively (means not significantly different, $p>0.1$). Under high turbidity conditions, tuber production averaged 40.0 ± 2.6 in the pulsed tanks, but dropped to 18.0 ± 1.5 in the continuous tanks (means significantly different, $p< 0.01$).

Sago pondweed was also responsive to both treatment factors and the interaction between the factors was never significant (Table 10). Total mass, above-ground mass, and root mass were all significantly influenced by both factors, with values being higher in the low turbidity and in the pulsed tanks. Leaf:root mass ratio and tuber mass were influenced by turbidity, but not by the mixing regime. Apparently, when grown in darker conditions, the plants are forced to invest relatively more energy in producing above-ground tissue to capture sufficient light to survive and are unable to invest that energy to produce tubers. Number of flowers produced by sago pondweed populations was not related to either of the factors.

Population response: one factor analysis: Because both treatment factors influenced the amount of light available to the plants, the four treatment combinations of the two actually resulted in four levels of light available to the plants. Therefore, the data have also been analyzed as a one-factor experiment. For this analysis, the proportion of subsurface irradiance penetrating to a depth of 0.5 m was taken as the criteria (see Table 6). Accordingly, treatments represent the following values: High/Continuous=27.0, High/Pulsed=39.1, Low/Continuous=54.9, and Low/Pulsed=62.8 percent of subsurface irradiance.

Treatment effects on the population level were virtually identical to those on the individual pot level. Total vallisneria mass per tank again increased with increasing light (Figure 17 A). The populations growing under the lowest light level (High/Continuous) showed significant losses in total mass relative to initials and showed no increase in number of rosettes over the experimental period. Populations growing under the two higher light regimes had similar numbers of rosettes (Figure 17 B), although the highest light treatment produced significantly more mass than any other treatment (Figure 17 A). Above- and below-ground mass likewise showed an increase in mass with increasing light (Figure 17 C, D).

Vallisneria population reproductive potential was also influenced by the experimental regimes. High turbidity resulted in fewer tubers produced than low turbidity treatments (Figure 17 E). Flower production was more related to the mixing regime than to the level of turbidity. The pulsed tanks showed higher

Table 10
Two-way ANOVA for Plant Parameters Measured on sago pondweed Populations in Tanks

PARAMETER factors	DF	P-value	Factor Mean ± se		
CHANGE IN TOTAL MASS (g)			TURB	(low)	149.2 ± 11.9
turbidity	1	0.0002		(high)	47.2 ± 12.7
mixing	1	0.0688	MIXING	(pulsed)	116.1 ± 11.9
interaction	1	0.6663		(cont)	80.2 ± 12.7
T_f Above-ground MASS (g)			TURB	(low)	123.2 ± 6.6
turbidity	1	0.0002		(high)	64.8 ± 7.1
mixing	1	0.0767	MIXING	(pulsed)	103.7 ± 606
interaction	1	0.9815		(cont)	84.3 ± 7.1
T_f ROOT MASS (g)			TURB	(low)	50.7 ± 3.4
turbidity	1	0.0001		(high)	19.3 ± 3.7
mixing	1	0.0438	MIXING	(pulsed)	40.9 ± 3.4
interaction	1	0.3664		(cont)	29.1 ± 3.7
T_f TUBER MASS (g)			TURB	(low)	15.3 ± 2.2
turbidity	1	0.0040		(high)	2.7 ± 2.4
mixing	1	0.1559	MIXING	(pulsed)	11.5 ± 2.2
interaction	1	0.6094		(cont)	6.4 ± 2.4
T_f TOTAL # FLOWERS			TURB	(low)	338 ± 75
turbidity	1	0.3450		(high)	228 ± 80
mixing	1	0.2807	MIXING	(pulsed)	346 ± 75
interaction	1	0.4135		(cont)	220 ± 80
LEAF:ROOT MASS RATIO			TURB	(low)	2.49 ± 0.14
turbidity	1	0.0021		(high)	3.38 ± 0.15
mixing	1	0.1554	MIXING	(pulsed)	2.77 ± 0.14
interaction	1	0.6026		(cont)	3.10 ± 0.15

production than the continuously turbid tanks, and the highest total produced was in the High/Pulsed treatment rather than in the treatment receiving the most light (Figure 17 F).

The sago pondweed populations all showed positive growth over the experimental period, although the amount of growth was significantly higher in the lower turbidity tanks (Figure 18 A). While both above-ground mass and root mass increased with increasing light (Figure 18 C, D), the leaf mass:root mass ratio increased with declining light (Figure 18 B). This indicates that the plants had to invest relatively more energy into leaf tissue when underwater light levels decreased to maintain below-ground biomass. Tuber mass was also low in the high turbidity treatments (Figure 18 E) and there were no significant differences among treatments in number of flowers produced (Figure 18 F).

Regression analysis was used to search for patterns in response with changing light levels. For vallisneria, significant, strong linear relationships were found between light levels and the population totals of all parameters except flower/seed mass (Figure 19). Although turbidity clearly influences flower production, since the lowest light treatment produced virtually no flowers, the relationship is not linear and is very variable. Variations of a factor

Chapter 3   Effects of Turbidity on Mature Plants: II. Deep Tank Factorial Experiment
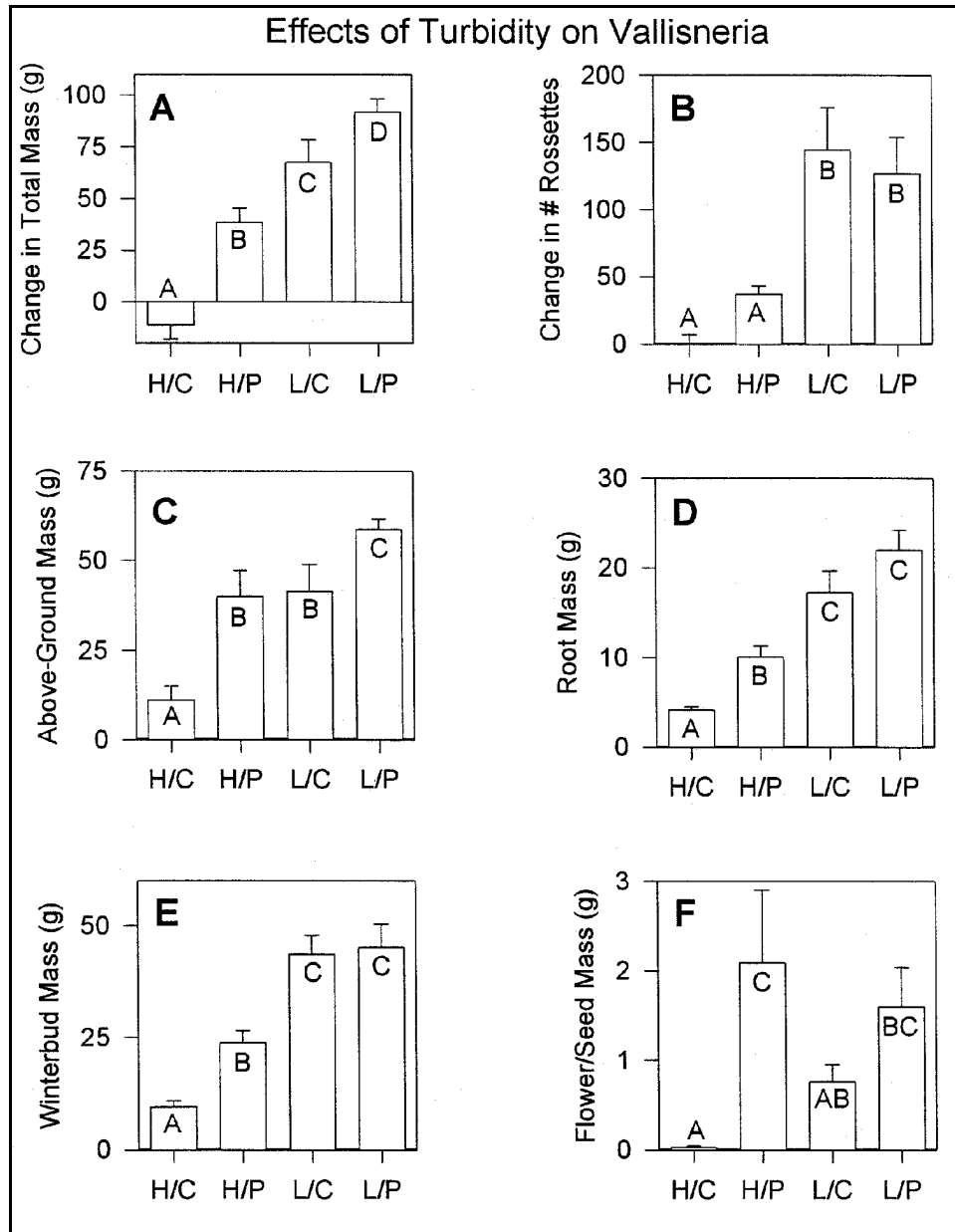
Figure 17. Effect of turbidity on various parameters of vallisneria population growth within a tank. All biomass values given as ash-free dry mass. Values shown are tank total means ± se (N=3 for all treatments except H/P where N=4). One way ANOVA analyses were significant for all parameters. Letters indicate significant differences among treatment means (p<0.1)
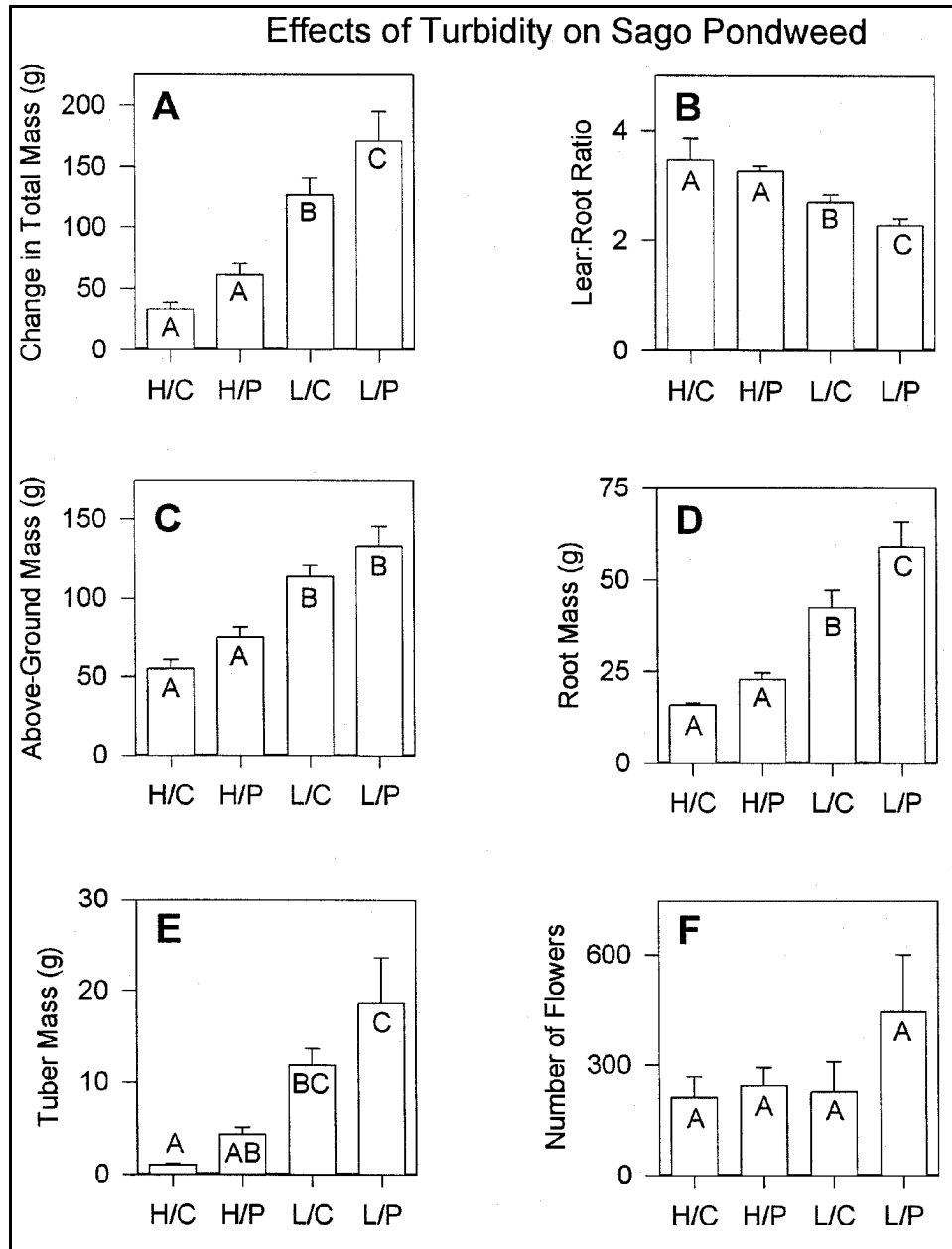
Figure 18. Effect of turbidity on various parameters of sago pondweed population growth within a tank. All biomass values given as ash-free dry mass. Values shown are tank total means ± se (N=3 for all treatments except H/P where N=4). One way ANOVA analyses were significant for all parameters except number of flowers (F). Letters indicate significant differences among treatment means (p<0.1)

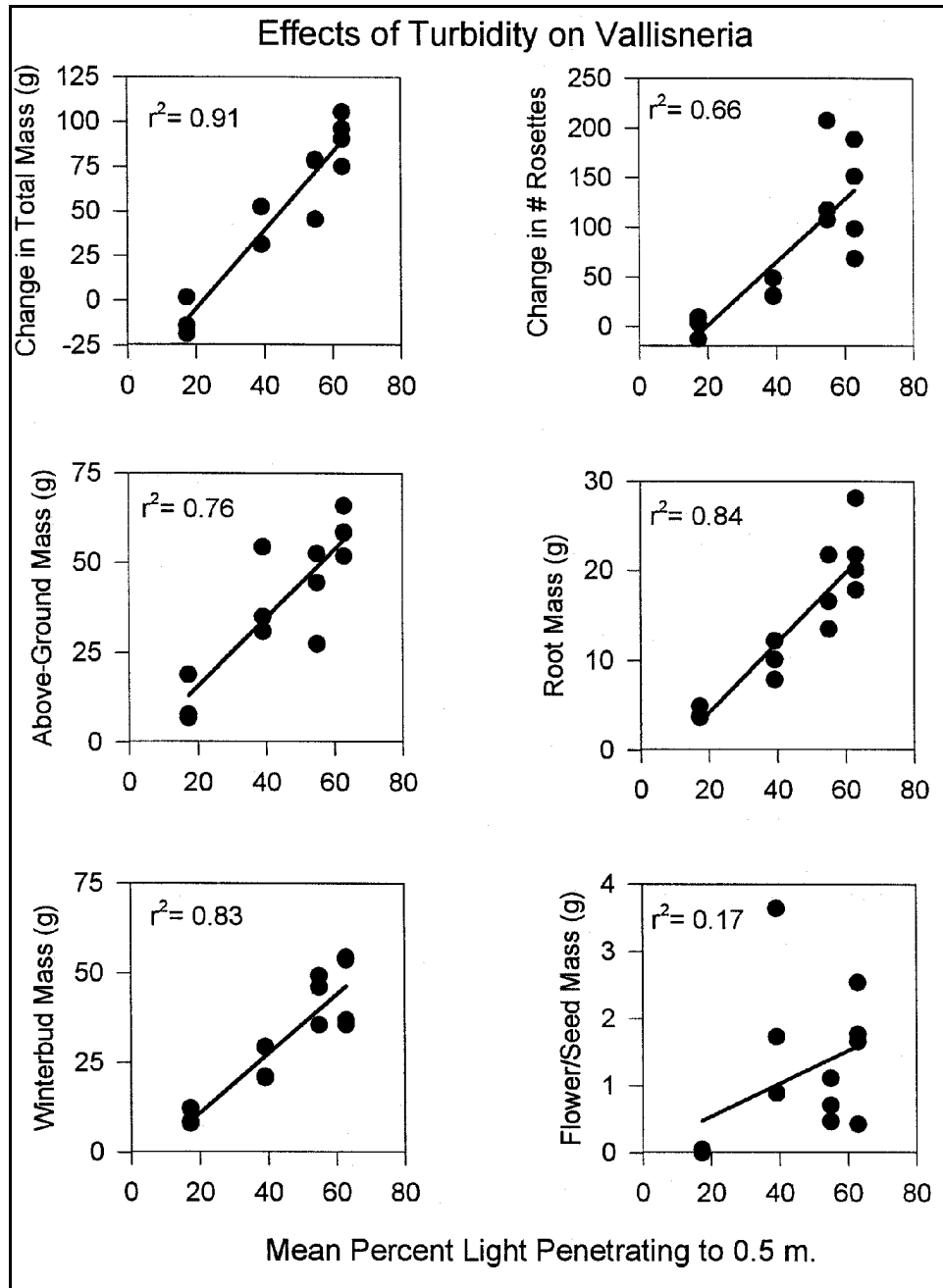Figure 19.   Least squares linear regression analysis of vallisneria tank totals
versus average light conditions. Slopes of all regressions were
significantly different from zero (p<0.05)

of 3 to 5 were observed in the mass of flowers produced among replicate tanks.
For the other variables, the relationship appears to be truly linear, since the
scatter was more-or-less symmetrical around the fitted line.

Significant, although more variable (lower r^2) linear relationships were also seen between light availability and most parameters for sago pondweed (Figure 20). The greater apparent tolerance of sago pondweed to turbid conditions is likely related to the plant's growth characteristics. Unlike vallisneria, sago pondweed concentrates most of its photosynthetic tissues at the water surface where light conditions are relatively good, even under quite turbid conditions. The relationship between plant growth and light may not be truly linear for sago pondweed, since the scatter was not symmetrically distributed around the fitted line (e.g. 40% light values consistently fall below the fitted line). No significant linear relationship existed between light and number of flowers produced (Figure 20).

Periphyton and sediments attached to plant leaves

Visual observation of the plants throughout the study period revealed that no heavy periphyton development occurred on the leaves. Although the leaves in all tanks were covered to some degree with deposited sediments, no exuberant growth of algae was observed in any of the treatments. Never-the-less, total solids (TS) and periphyton accumulated on the leaves to some degree, although there was no consistent pattern related to the treatments when expressed on a whole-plant basis (Figure 21 A-D). Total solids or total chlorophyll-a attached to the plants showed no significant differences among treatments for vallisneria (Figure 21 A, B). However, for sago pondweed plants, there was typically less TS and chlorophyll-a accumulation at the highest light level (Low/Pulsed) than on any others. However, when expressed on a biomass-specific basis (mg TS or chlorophyll-a per g above-ground plant tissue), a clearer pattern emerges (Figure 21 E-H). For vallisneria, biomass-specific epiphytic TS and chlorophyll-a increases with increasing turbidity.
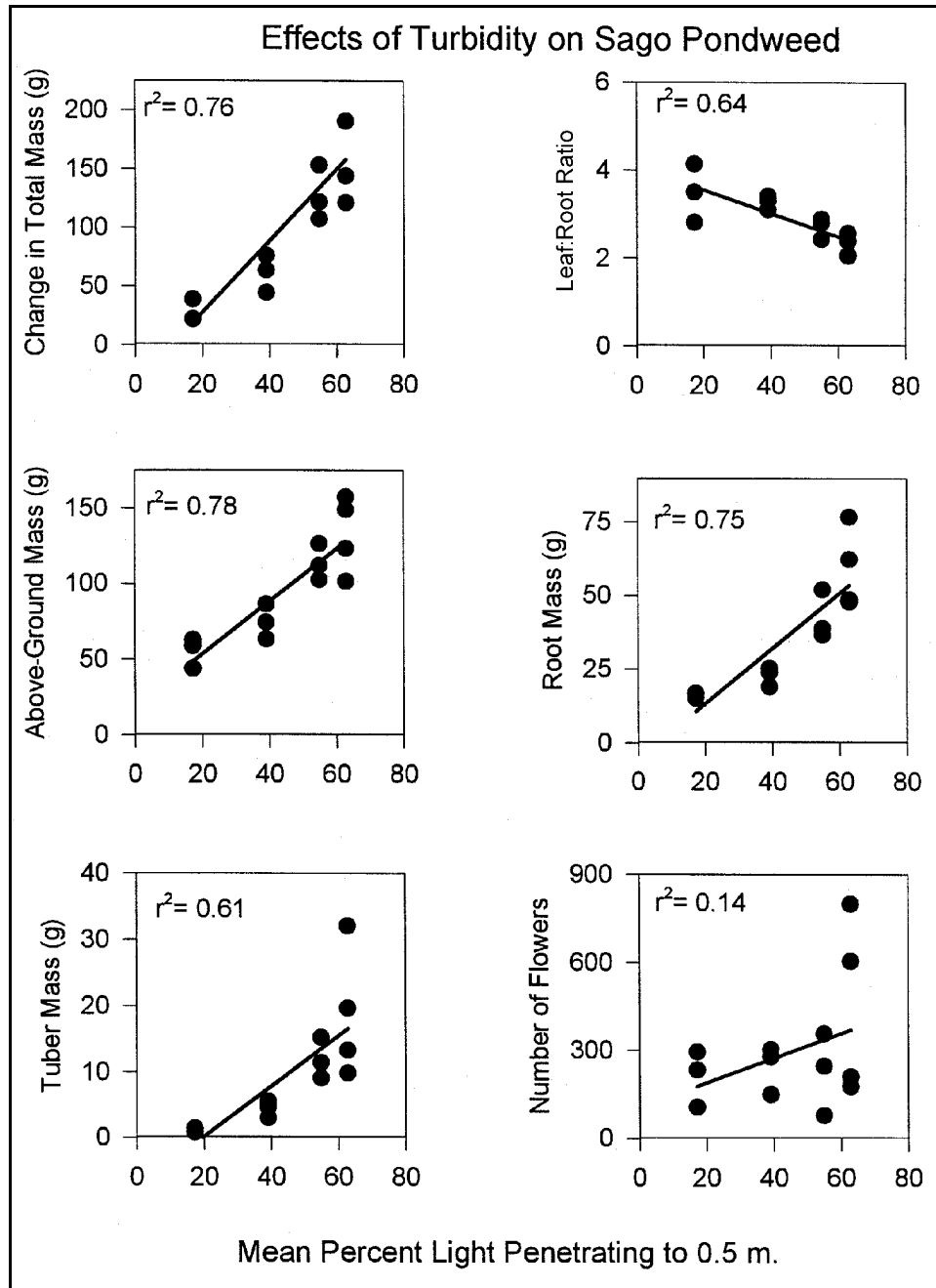
Figure 20. Least squares linear regression analysis of sago pondweed tank totals vs. average light conditions. Slope of all regressions were significantly different from zero (p<0.01) except for numbers of flowers produced where p=0.20
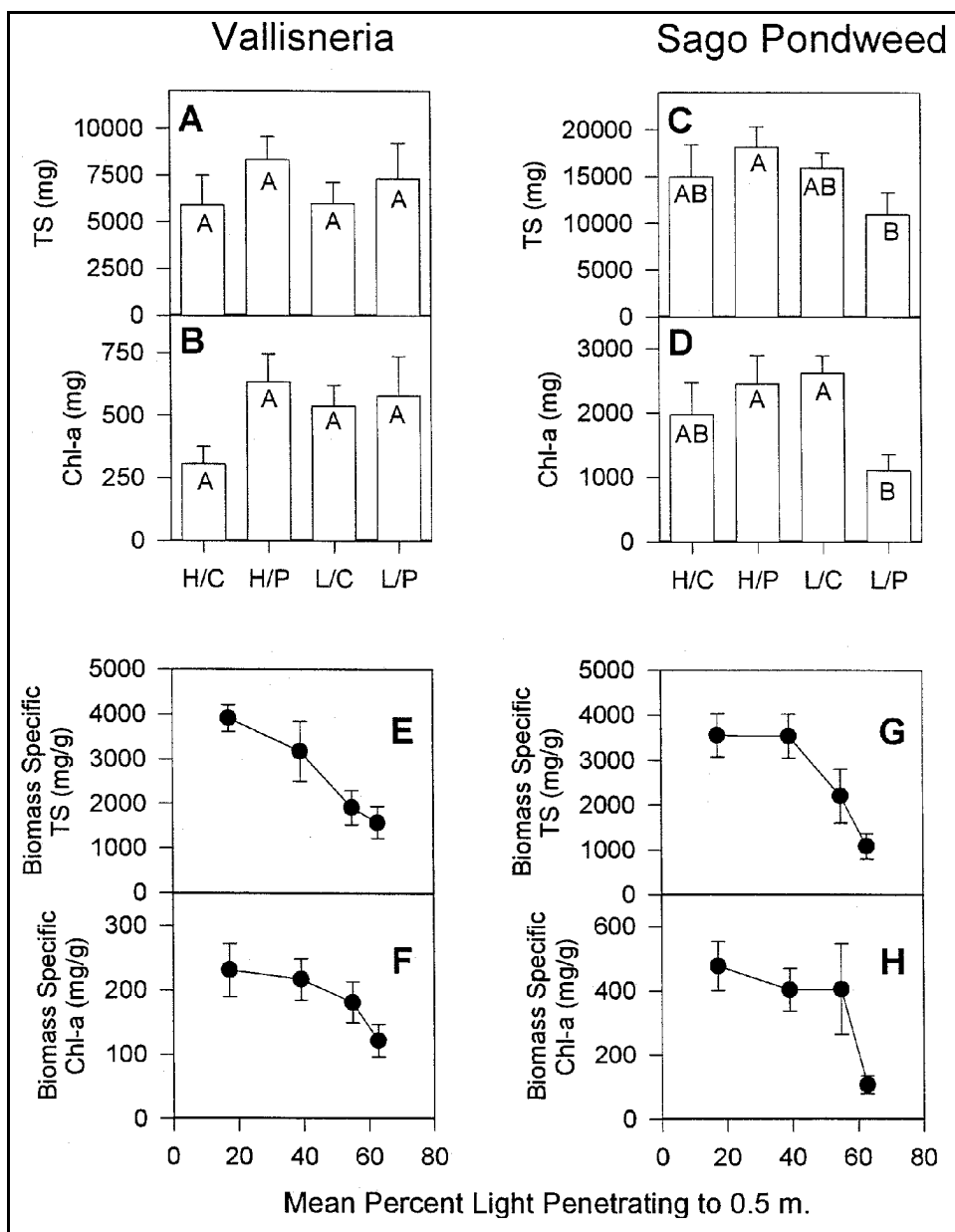
Figure 21. Chlorophyll and total solids (TS) coating plant leaves at time of final harvest. A-D. Values shown are mean (±se) total mass of chlorophyll or TS attached to individual plants for the four experimental treatments (N=3 for each tank). ANOVA analysis was not significant for either parameter on vallisneria leaves, but was significant for both parameters on sago pondweed leaves. Letters indicate significant differences in treatment means ($\alpha < 0.05$). E-H. Biomass-specific values for TS or chlorophyll attached to plants (total mass attached/AFDW of plant). Values are means ± se for all plants harvested for each treatment. Lines connect means to show declining trend as light levels increase

4   Effects of Turbidity on Tubers

This experiment was designed to investigate the impacts of turbidity on survival and short-term growth of vallisneria and sago pondweed tubers.

Materials and Methods

This experiment was conducted in the greenhouse facility at LAERF. Recently sprouted tubers were grown for 8 weeks under one of three turbidity levels in this dose-response experiment. Three groups of tubers were utilized. Tubers of sago pondweed were purchased from Wildlife Nurseries, Inc. (Oskosh, WI) where they were field-collected in early Spring (April) of 1996. Tubers of vallisneria were provided by two sources: one batch came from Wildlife Nurseries, and the other one was provided by the Environmental Management and Technology Center (EMTC) in Onalaska, WI. The Wildlife tubers were collected from a Wisconsin lake in April of 1996, while the EMTC tubers were collected from Lake Onalaska, Wisconsin, by EMTC personnel in early May 1996. Because of a size difference (about a factor of six) between the Wildlife and EMTC tubers of vallisneria, these were kept separate throughout the experiment. Tubers were shipped to LAERF on ice and were kept refrigerated until mid June 1996.

The experiment began on June 17, 1996, when the tubers were removed from the refrigerator and allowed to warm up in the greenhouse in shallow containers of water. After 10 days, tubers which were beginning to sprout were separated for the experiment. Sixty-four tubers were selected from each of the three groups. The tubers were randomly divided into nine groups of six tubers each for the experiment, and the remaining 10 tubers were harvested to document initial conditions. Each tuber was blotted dry and weighed to determine fresh weight. Fresh weight: dry weight ratios and percent ash content were determined for the 10 remaining tubers and used to compute the ash-free dry-weight equivalents for each tuber.

Sprouted tubers were individually planted in 1.0-L plastic pots filled with pond sediment. One group (six pots) of each of the three categories were randomly assigned to one of nine greenhouse tanks. According to this

experimental design, six pots of each tuber category were placed in each of the nine tanks. To prevent interaction among the categories as the tubers sprouted and began to grow, the pots from the three categories were placed in separate areas of the tanks. The nine greenhouse tanks were then assigned to one of three turbidity levels. The experimental setup consisted of three replicate tanks of each of three turbidity levels. Each tank contained six pots of each of three tuber categories.

Water levels and water temperatures were monitored daily and evaporative losses of water were made up for by addition of more alum-treated pond water. To ensure that the tanks remained well-mixed and to facilitate gas exchange, compressed air was continuously pumped through two air lifts in each tank. To minimize CO_2 depletion related to conducting the experiments in static tanks, the airstream was amended with CO_2 to an approximate 10 X enrichment ($\sim$3500 ppm CO_2). The tanks were filled with pond water treated with alum to remove soluble P. Water depth in the tanks was maintained at 85 cm, but since the pots were 12 cm tall, this corresponded to a water column depth of 73 cm above the sediment surface.

The turbidity target levels selected were Clear (no clay additions), 15 NTU, and 45 NTU. These turbidities are somewhat higher than those used in the deep tank factorial experiment (Chapter 3) because of the shallower nature of the greenhouse tanks. Turbidity in each tank was measured and adjusted daily during the experiment utilizing clay sediment slurries (100 g dry clay per 500 mL deionized water). Light levels at 65-cm depth within the tanks averaged about 82%, 40%, and 10% for the Clear, 15 NTU, and 45 NTU tanks, respectively.

Plants were harvested at the end of the experimental growth period. Survival was determined for the tuber in each pot. Rosette number (vallisneria) or stem number (sago pondweed) in each pot was counted and plant mass was harvested. The number of vallisneria leaves in 20-cm length categories was determined for each surviving pot. Plant material was washed to remove debris and dried to constant weight at 60°C. Ash content was determined for each plant category and used to correct mass measurements. All plant mass values were expressed as ash-free dry weight.

Data were analyzed on a population basis, with the sum of the six pots of each category in each tank representing the population for that tank. Regression analysis was used to analyze for treatment effects in this dose-response experiment. The light climate in which the tubers were grown was used as the independent variable, and the plant response (% survival, number of plants, and leaf length) were the dependent variables. The null hypothesis tested is that the slope of the regression line is not significantly different from zero. Comparisons between the performance of large and small vallisneria tubers were made using a two-sample comparison test (t-test).

Results of Tuber Experiment

Water temperature in the tanks over the course of the experiment averaged about 27 °C (Figure 22). These temperatures are similar to maximum temperatures during the summer and likely somewhat higher than the temperatures under which tubers would normally germinate in late spring.
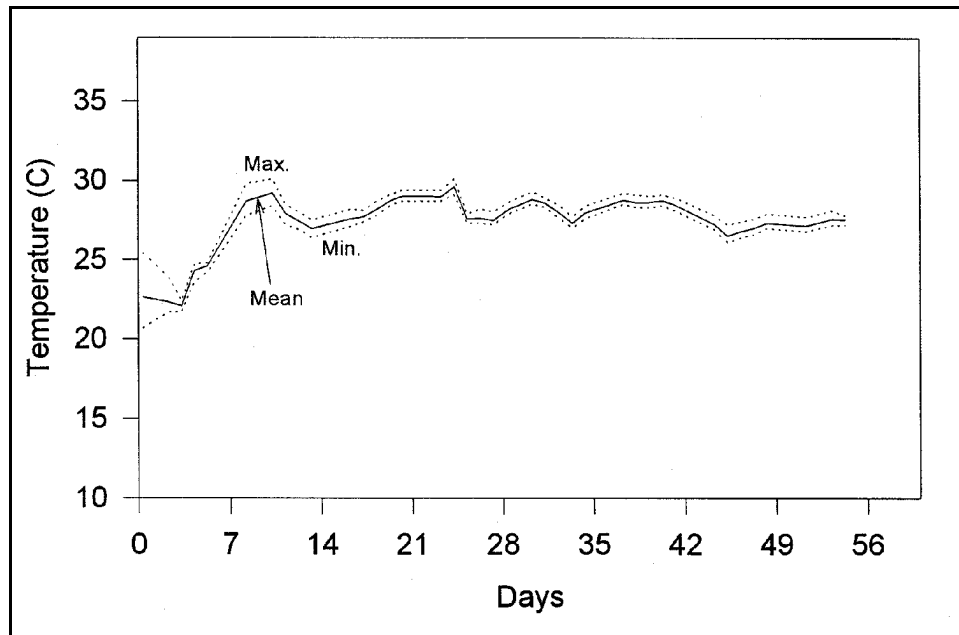

Figure 22.   Greenhouse tank temperatures during the 8-week experimental growth period

The turbidities selected for the three experimental treatments resulted in significantly different light climates at a depth of 65 cm within the tank (~8 to 10 cm above sediment surface, Figure 23). PAR extinction coefficients (Kd) corresponded to about 0.2, 1.5, and 3.8 m^{-1} (Figure 24) at NTU values of 0.2, 15, and 45, respectively. Although turbidity was adjusted daily, there was still some fluctuation in turbidity and the values ranged from 35-45 NTU and 10-15 NTU for the 45 and 15 NTU tanks, respectively, while the Clear tanks always had NTU values of <1 (Figure 25). Average percent subsurface PARs penetrating to a depth of 65 cm over the course of the experiment were 82.4%, 44.5%, and 11.9% for the Clear, 15 NTU, and 45 NTU tanks, respectively.

The initial tuber weights for the three tuber categories (Table 11) show the size difference between the vallisneria tubers obtained from the two sources. For vallisneria, a single growing tip was considered a single tuber. The Lake Onalaska tubers were about 18% of the mass of the Wildlife Nursery tubers. The sago pondweed tubers were the largest tubers used in this experiment.

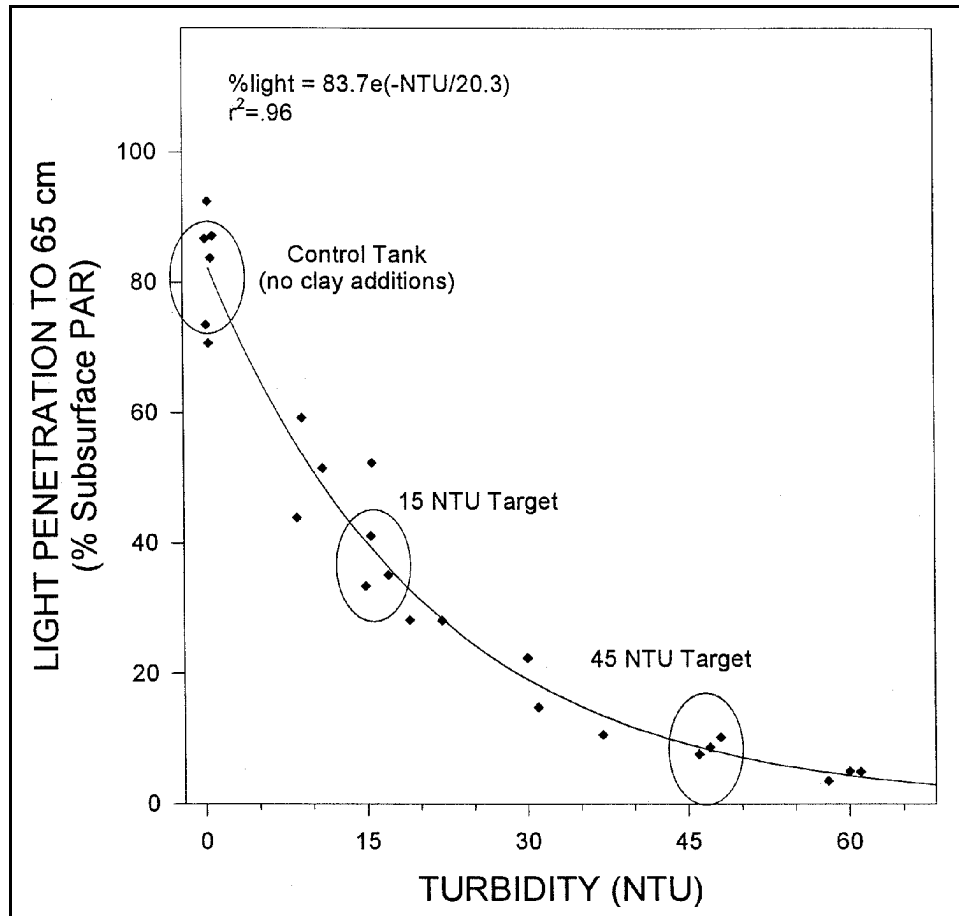$$\%light = 83.7e(-NTU/20.3)$$
$$r^2 = .96$$

Figure 23. Light penetration within greenhouse tanks as a function of water column turbidity. Circles indicate target turbidity conditions of the three experimental treatments utilized
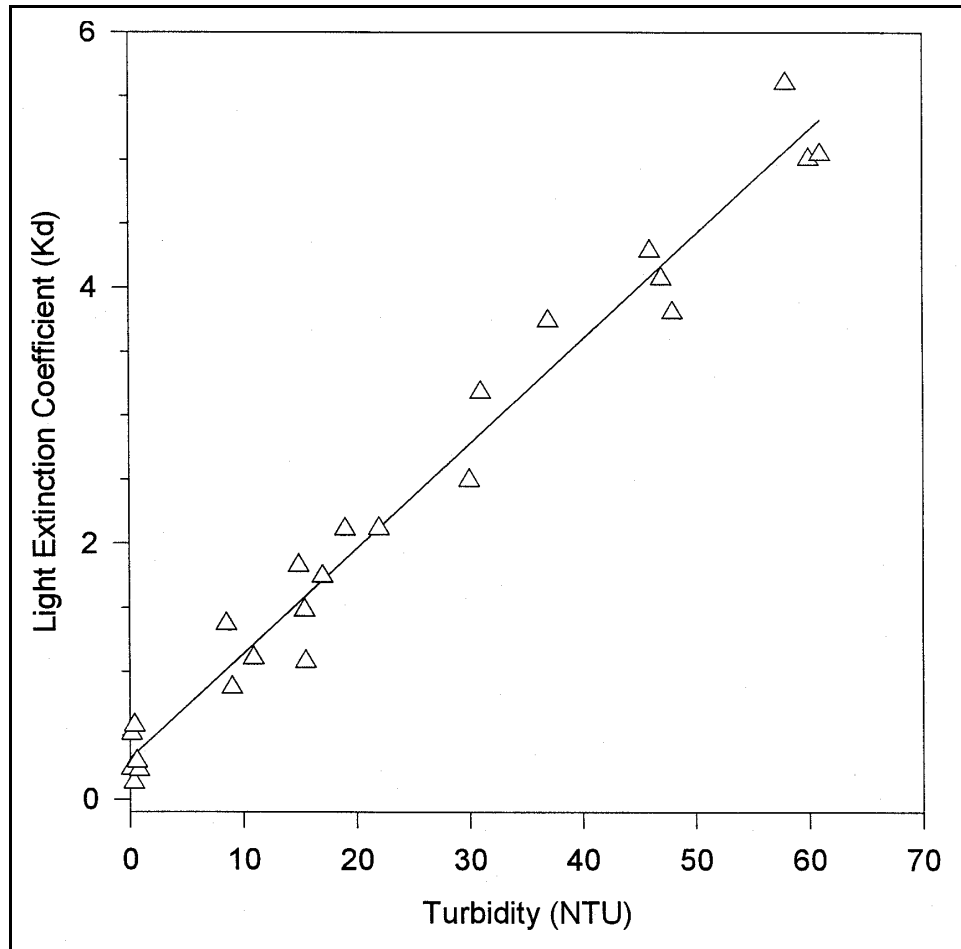
Figure 24.   Relationship of light extinction coefficients to water column turbidity in greenhouse tanks
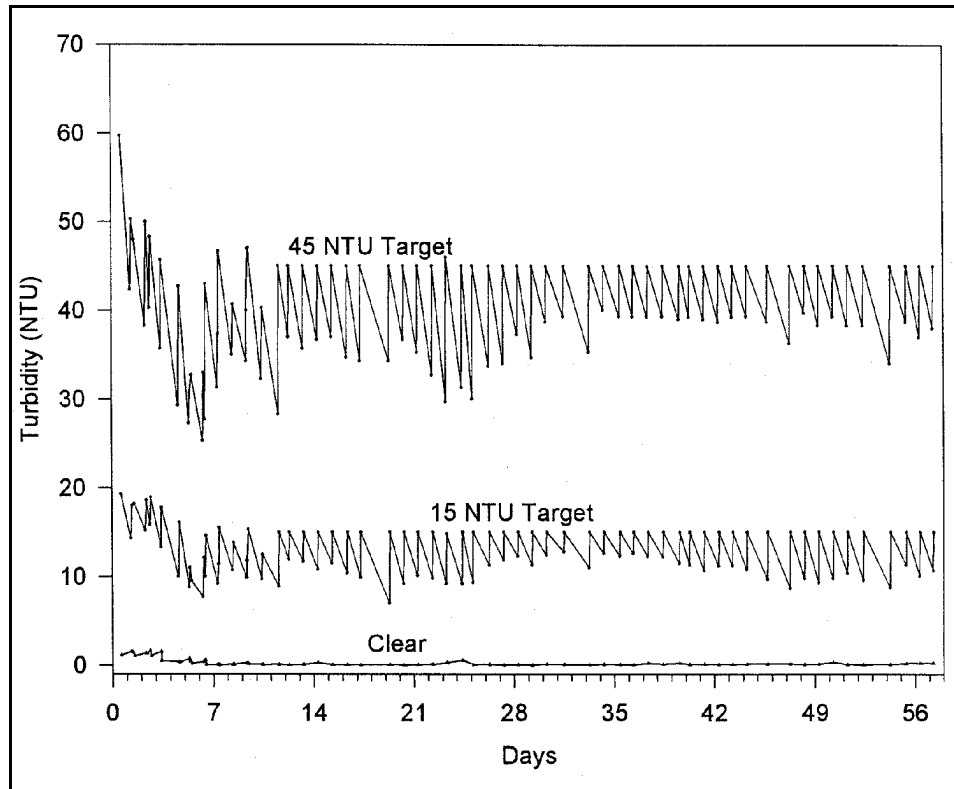
Figure 25. Mean water column turbidity of greenhouse tanks for each experimental treatment. Individual tank values are not shown for simplicity, but all replicate tanks of a given treatment were always within 15% of the plotted mean

Table11
Ash-free Dry Weights of Tubers at Beginning of Experiment (g, n=54)

Category	Mean	SE	Minimum	Maximum
vallisneria (large)	0.264	0.009	0.17	0.43
vallisneria (small)	0.047	0.002	0.02	0.09
sago pondweed	0.321	0.002	0.18	0.47

Vallisneria tuber survival was significantly affected by the turbidity treatment, but sago pondweed tubers showed 100% survival at all turbidity levels (Figure 26). Survival of both large and small vallisneria tubers increased as light levels increased, but the effect was much larger for the smaller tubers. Survival of small tubers under the most turbid conditions was very poor (0-17%) but increased to >50% at the highest light conditions. Large vallisneria tubers survived well at both of the lower turbidities, but had more variable survival in
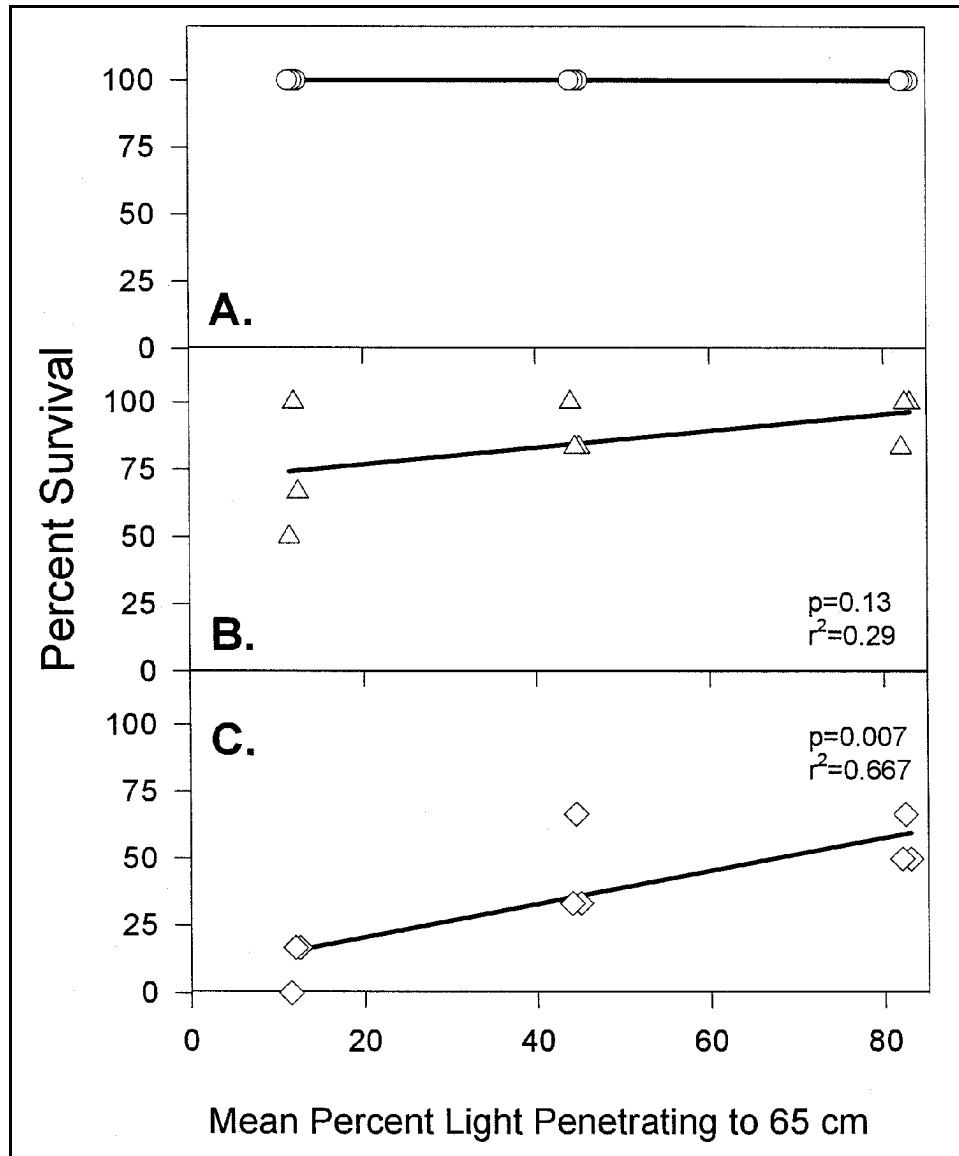
Figure 26. Regression of percent tuber survival as a function of light conditions. All tubers of sago pondweed survived (A), and the regression of survival versus light was not significant for large vallisneria tubers (p>0.1) (B). Light conditions significantly impacted survival of small vallisneria tubers (C)

the three replicate tanks at the highest turbidity where survival ranged from 50-100%.

The number of sago pondweed stems per tank (total of 6 pots) was not influenced by turbidity and averaged 60-80 stems per tank for all treatments (Figure 27). The number of vallisneria rosettes produced by large tubers or small tubers was significantly related to light (Figure 27). Similarly, under the lowest light levels, small tubers averaged only about 1 rosette per tank (total of
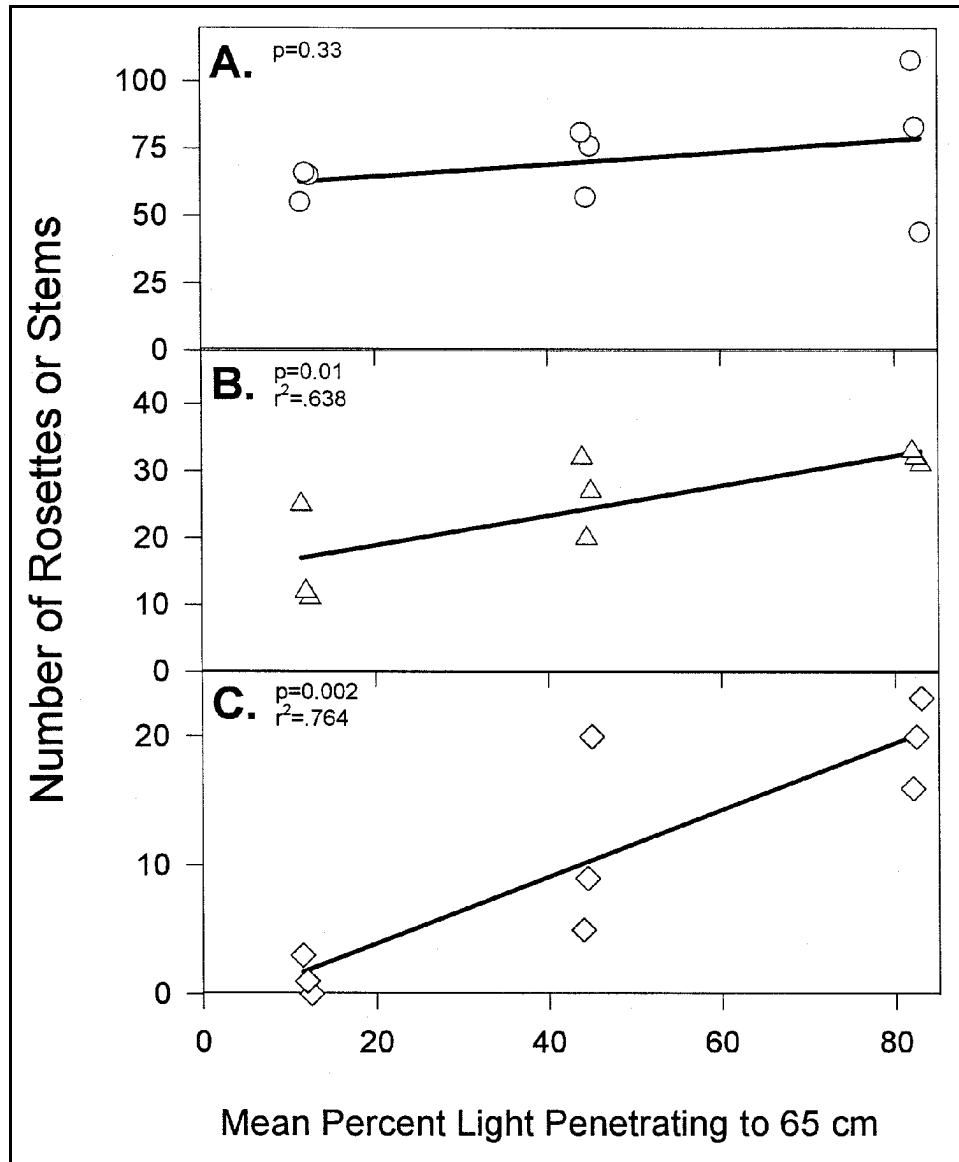
Figure 27. Total number of stems (sago pondweed) or rosettes (vallisneria) in each tank at the end of the experimental growth period as a function of light. Number of sago pondweed stems was not significantly related to light (A), but number of rosettes produced by large (B) and small (C) tubers was impacted by light availability

6 pots) while the total increased to about 20 at the highest light level. Under the lowest light conditions the larger tubers averaged about 16 rosettes per tank and increased to about 32 rosettes per tank at the highest light level.

Number and length of vallisneria leaves was also influenced by turbidity (Figure 28). At the lowest light level the larger tubers produced an average of about 200 leaves per tank and 55% of these were longer than 60 cm in length.
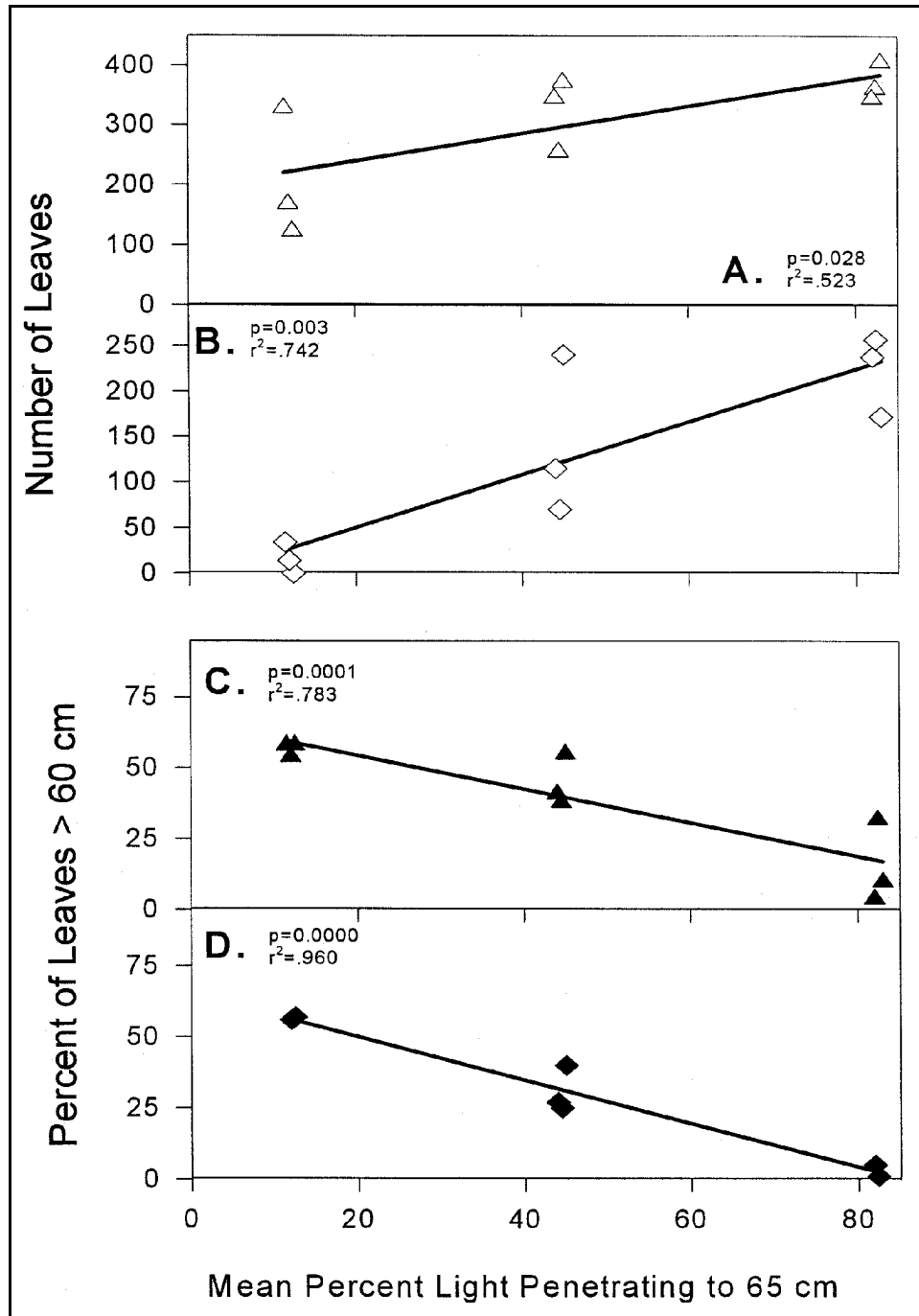
Figure 28.   Impacts of light conditions on vallisneria leaves. Total number of leaves produced in each tank was significantly related to light conditions for both tuber size classes (A,B). In addition, the frequency distribution of leaf length was impacted. Under lower light conditions, plants produced proportionally more long leaves (>60 cm length, C,D)

Under the highest light conditions, the average number of leaves produced per tank increased to almost 400, while the percentage longer than 60 cm dropped to 19% (Figure 28 A,C). The smaller vallisneria tubers produced fewer leaves per tank. Under the lowest light conditions, the average was only 16 leaves per tank. The total increased to over 200 leaves per tank at the highest light (Figure 28 B). Percentage of leaves longer than 60 cm fell from 56.5% to 3.2% as the light level increased (Figure 28 D).

The larger vallisneria tubers performed significantly better than the smaller tubers for all measured parameters at all turbidity levels (Figure 29). However, as light levels increased, the difference between small and large tubers decreased. For example, at the lowest light level, the small tubers produced only 8% of the number of plants produced by the large ones (average of 1.3 vs. 16.0 plants per tank, Figure 29). However, at the highest light level, the small tubers produced about 62% of the number produced by the larger tubers (average of 19.7 vs. 32.0 plants per tank). Similar patterns were observed for plant survival and total mass produced (Figure 29 A and C).
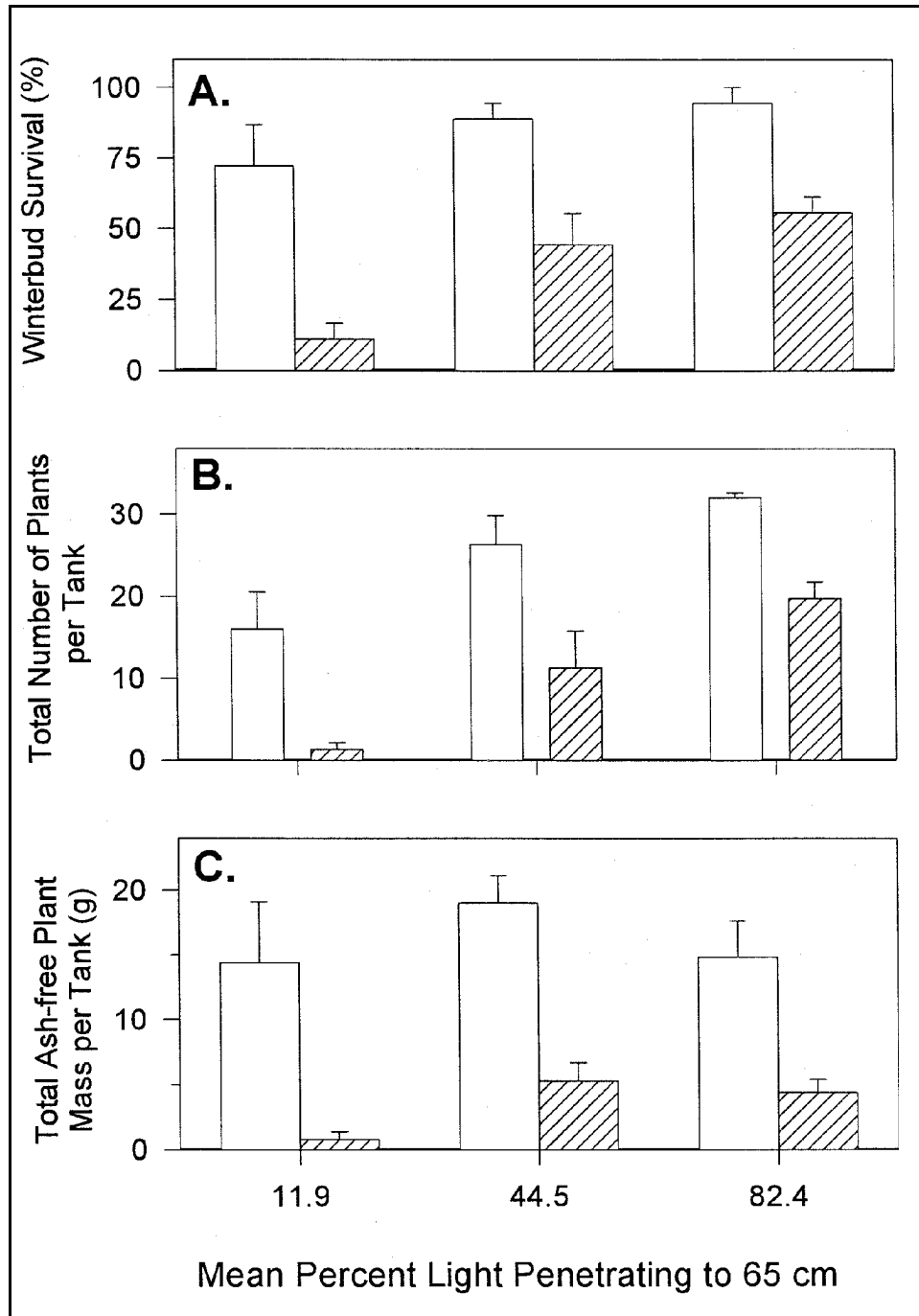
Figure 29. Large vallisneria tubers performed significantly better (p<0.05, t-test)
for all measured plant variables at all turbidity levels tested.
However, the difference between large and small tuber performance
declined as light levels improved. Values shown are means of tank
totals ± se (N=3 tanks)

5   Effects of Turbidity on Vallisneria Seedlings

This experiment was designed to investigate the impacts of turbidity on the survival and short-term growth of vallisneria seedlings under controlled laboratory conditions

Material and Methods for Seedling Experiment

This experiment was conducted in the laboratory at LAERF under artificial light conditions. Seeds were collected from culture stocks at LAERF in November 1996 and maintained under refrigeration until January 1997. These seeds were from populations of vallisneria initially collected in Texas and were not from the UMRS. The seed pods were removed from refrigeration and opened to expose the seeds. The seeds were rinsed and placed in petri dishes containing moist sterile toweling to germinate. After germination, recently germinated seeds of vallisneria were planted into small pots (125 mL) filled with heat-sterilized pond sediment. Three seedlings were planted into each pot. The pots were placed in a 300-L tank filled with alum-treated pond water and held for 7 days prior to initiation of the experiment to ensure that the seedlings did not die or become uprooted.

After the initial week, the pots were visually inspected to ensure that all seedlings were viable and were then randomly assigned to one of two experimental tanks. One tank was then selected as the "Turbid" tank while the other was the "Clear" tank. Water depth in the tanks was 40 cm which corresponded to 30 cm above the pot surface. The turbidity in the turbid tank was then adjusted to 50 NTU and was corrected daily as needed.

Light was provided by bulbs especially designed to replicate the spectral composition of sunlight (Ultramarine Ultralux 6500K bulbs) and was set on a 14h:10h light:dark photoperiod. Light levels in the turbid tank corresponded to about 75-95 $\mu E\ m^{-2}\ s^{-1}$ at the sediment level, while those in the clear tank ranged from 450-490 $\mu E\ m^{-2}\ s^{-1}$. Assuming an average summer incident light of about 2000 $\mu E\ m^{-2}\ s^{-1}$, these values correspond to about 4-5% and 22-25% of incident

PAR for the turbid and clear tanks, respectively. Temperature in both tanks averaged about 22 °C (ambient lab temperature).

After eight weeks of growth, the pots were harvested. Survival was determined for each of the pots by counting the number of non-clonal plants in the pot (multiple rosettes attached via stolen = 1 non-clonal plant). Total numbers of rosettes were enumerated, and the plant tissue was harvested. Tissues were separated into above- and below-ground tissues, washed, and dried to constant weight at 60 °C.

Statistical comparison between the tanks was performed utilizing a t-statistic. Each pot is assumed to be an independent experimental unit (N=8 for each treatment). Due to the relatively low number of experimental units, $\alpha < 0.1$ is used for statistical significance.

Results of Seedling Experiment

Vallisneria seedling survival was significantly higher in the clear tank than in the turbid tank (66.6 vs. 45.8%, Figure 30 A). Total number of plants present in the pots was also much higher (~4 X) in the clear tank than in the turbid tank (Figure 30 B). In addition to having higher survival of the planted seedling, one (and only one!) seedling in each of the pots in the clear tank had produced 1-3 daughter plants. No seedling in the turbid tank produced any daughter plants.

The difference between the tanks was even greater for total plant mass produced. The plant mass in the pots within the clear tank exceeded that of the pots in the turbid tank by about 7 X (Figure 30 C). Finally, the allocation of biomass in the developing seedling differed between the two treatments: above-ground to below-ground ratios in the clear tank averaged only 1.86 but soared to 2.85 in the turbid tank (Figure 30 D). Seedlings in the turbid tank invested proportionally more energy in developing leaf tissues than those in the clear tank.

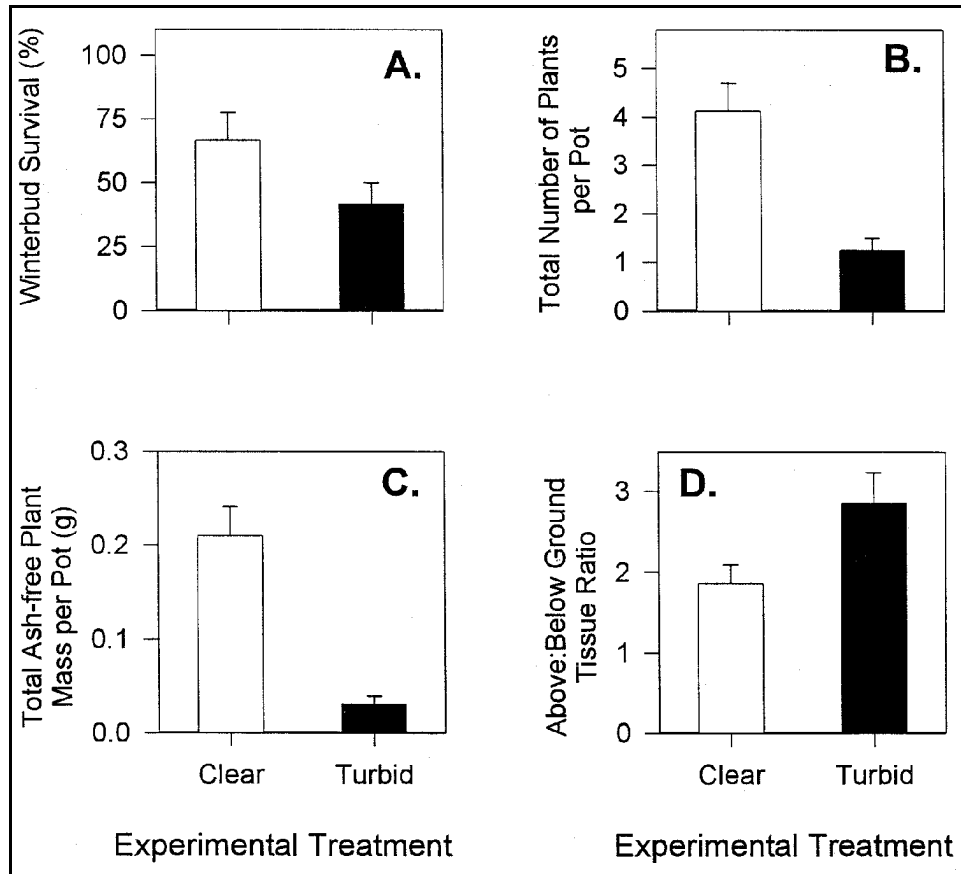Figure 30. Comparison of vallisneria seedling growth in clear (white bars) and
turbid (black bars) tanks. Mean ± se of eight pots grown under each
experimental condition. Under clear water conditions, seedlings has
higher survival (A), produced more rosettes (B) and had more plant
mass (C). In addition, the seedlings in clear tanks produced
proportionally more root mass (D). All p values <0.05, except A,
where p=0.09

6   Summary of Effects of Turbidity on Vallisneria and Sago Pondweed

The turbidity introduced into the experimental tanks changed both the quantity and quality of the light available to the plants. The clay turbidity showed a strong absorption of light energy in the 400- to 550-nm range (Figure 14 A). Thus, clay turbidity may be preferentially removing a very significant portion of the PAR spectra, since the chlorophyll-a and b absorption spectra both show strong absorption in this range. However, the effect, if any, of this shift in spectral composition could not be specifically determined in this series of experiments.

What is clear from the research presented here is that the reduction in total PAR quantity has significant effects on various stages of the life history of vallisneria and sago pondweed. Although significant effects are seen for both species, the magnitude of the effect appears to be greater for vallisneria. Following is a summary of effects:

Effects of Turbidity on Mature Plants

The effect of turbidity on mature plants was the primary focus of the research presented. The greenhouse pilot experiment and the deep tank experiment (Chapters 2-3) both focused on the impacts of turbidity when mature plants, previously growing under good light conditions, were exposed to significant periods under turbid conditions.

Turbidity significantly depressed the continued vegetative growth of vallisneria. As turbidity increased, vallisneria plants produced fewer daughter plants and accumulated less biomass (Figures 5, 16, and 18). In fact, the most turbid conditions prevented the plants from producing new daughter plants and decreased total plant mass.

In addition to impacting continued vegetative growth, turbidity impeded the ability of vallisneria to reproduce, i.e. to form tubers and flower. Tuber

production was reduced under turbid conditions (Figures 6, 16, and 18). In the greenhouse experiment plants growing in turbid tanks produced less flower/seed mass than those growing in clear tanks (Figure 6). In the deep tank experiment, flower production was related to frequency of sediment resuspension and was significantly higher for those populations experiencing a "pulsed" regime, where periods of clear water occurred (Figure 16).

Turbidity also impacted the vegetative growth and reproductive capacity of sago pondweed, although the magnitude of the effect appeared to be less than for vallisneria. Total mass produced declined as light levels dropped (Figures 17, 19). As turbidity increased, so did the leaf:root mass ratio of the plants. Apparently, under turbid conditions, the plants had to invest proportionally greater amounts of energy in light-acquiring tissues, and produced fewer roots.

In terms of reproductive potential, in the deep tank experiment turbidity negatively affected tuber production (Figures 17, 19) but not flower production. A similar pattern was seen in the greenhouse pilot experiment (Figure 8) although flower production was virtually zero under the highest turbidity treatment.

Effects of Turbidity on Tubers

Turbidity had a much stronger effect on vallisneria tubers than on sago pondweed tubers. Sago pondweed tubers showed 100% survival under all turbidity treatments (Figure 26), and produced similar numbers of stems under all treatments (Figure 27). Total mass per tank was also not significantly different among turbidity treatments.

Turbidity did influence vallisneria tuber survival and growth, especially for the smaller category of tubers. Small tubers showed very low survival (<20%) under the highest tubidity but survival increased with light levels (Figure 26). The number of rosettes produced by a single tuber during the experimental growth period was significantly affected by turbidity for both size classes of vallisneria tubers (Figure 27).

Turbidity also influenced the number and length of vallisneria leaves produced. Under turbid conditions, vallisneria produced fewer and longer leaves than under better light conditions (Figure 28).

Finally, and perhaps not surprisingly, larger tubers of vallisneria performed significantly better for all measured variables than the smaller tubers (Figure 29).

Effects of Turbidity on Vallisneria Seedlings

Vallisneria seedlings were profoundly influenced by turbidity. Under turbid conditions, seedlings had higher mortality, produced fewer daughter plants, and accumulated much less biomass than seedlings in clear tanks (Figure 30). In

additions, the seedlings in the turbid tanks had to invest proportionally more energy into above-ground tissues in an effort to compensate for the lower light conditions.

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13. ABSTRACT *(Maximum 200 words)*

This report summarizes a series of controlled experiments designed to investigate the impacts of suspended inorganic turbidity on the growth and reproductive potential of two submersed macrophytes of importance to the Upper Mississippi River System. Experiments were conducted on vallisneria (*Vallisneria americana*) and sago pondweed (*Potamogeton pectinatus*). Separate controlled experiments addressed the impacts of turbidity on mature plants, recently sprouted tuberlings, and recently germinated seedlings (vallisneria only).

Turbidity significantly depressed the continued vegetative growth of mature vallisneria. As turbidity increased, vallisneria plants produced fewer daughter plants and accumulated less biomass. In fact, the most turbid conditions (continuous exposure to 30 NTU) prevented the plants from producing new daughter plants, and decreased total plant mass. In addition, vallisneria tuber and flower production were reduced under turbid conditions.

Turbidity also impacted the vegetative growth and reproductive capacity of sago pondweed, although the magnitude of the effect was less than for vallisneria. Total mass produced declined as light levels dropped due to increasing turbidity. As turbidity increased, so did the shoot:root mass ratio of the plants. Apparently, under turbid

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conditions, the plants had to invest proportionally greater amounts of energy in light-acquiring tissues, and produced fewer roots. In terms of reproductive potential, turbidity negatively affected tuber production but did not impact flower production.

Turbidity had a much stronger effect on recently sprouted tuberlings of vallisneria than of sago pondweed. Sago pondweed tubers showed 100% survival under all turbidity treatments, and produced similar numbers of stems under all treatments. Total mass per tank was also not significantly different among turbidity treatments. Turbidity did influence vallisneria tuber survival and growth, especially for the smaller tubers. Small tubers showed very low survival (<20%) under the highest turbidity but survival increased to > 60% with decreased turbidity. The number of rosettes produced by a single vallisneria tuber was negatively affected by turbidity.

Finally, vallisneria seedlings were profoundly negatively influenced by turbidity. Under turbid conditions, seedlings had higher mortality, produced fewer daughter plants, and accumulated less biomass than seedlings in low turbidity conditions. In additions, the seedlings in the turbid tanks had to invest proportionally more energy into above-ground tissues in an effort to compensate for the lower light conditions.