FINAL REPORT

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THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. E-3

Endangered and Threatened Species Conservation

Project No. 38: Continued Maintenance, Reintroduction and Research on Texas Wildrice (*Zizania texana*)

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TEXAS
PARKS &
WILDLIFE

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February 16, 2001

FINAL REPORT

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PROJECT TITLE: Continued Maintenance, Reintroduction and Research on Texas Wildrice (*Zizania texana)*

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APPROVED BY: Neil E. Carter 5/18/01

Federal Aid Coordinator
Texas Parks & Wildlife Department

Project No. 38

CONTINUED MAINTENANCE, REINTRODUCTION AND RESEARCH ON *ZIZANIA TEXANA* (TEXAS WILDRICE)

Final Report

Submitted to Texas Parks and Wildlife Department

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February 2001

EXECUTIVE SUMMARY

This project was a 5-year project beginning in September 1991 and ending in August 1996 with a separate contract for each year. The purpose of the project was three-fold. First, the project developed and implemented methodology for maintaining a seed-producing Texas wildrice population in an outdoor cement raceway at Southwest Texas State University. Methods were developed to reliably store (short-term <1 year) and germinate Texas wildrice seed. A partnership was established with the National Seed Storage Laboratory in Ft. Collins, Colorado, to develop protocols for long-term (20-100 years) seed storage. This work represented the first comprehensive attempt to manage a seed bank for Texas wildrice. The study recommends storing seeds in cold (3 °C) water for a maximum of 1 year. After 1 year, seed viability and seedling vigor are reduced. Storage > 1 year requires cryopreservation. The study also recommends growing Texas wildrice in water flowing ≥0.08 m/sec to extend its life span. Plants grown in water flowing about 0.015 m/sec live approximately 1 year.

Second, Texas wildrice was reintroduced into five sites in Spring Lake. This work resulted in the first long-term (>5 years) establishment of Texas wildrice in the San Marcos River ecosystem. Current velocity, water depth, and sediment were factors examined at each site. Of those three factors, current velocity appeared to be the most critical environmental factor associated with successful transplantation. This was the first study to identify herbivory as a contributor to sexual reproductive failure (See reprint: "Reintroduction of Texas wildrice (*Zizania texana*) in Spring Lake: Some important environmental and biotic considerations"). The study recommends selecting reintroduction sites in Spring Lake with current velocity ≥0.08 m/sec and caging plants if seed production is a goal.

Third, the project carried out a number of laboratory and field research projects addressing factors influencing growth and threats to the species. Significant factors influencing growth were current velocity, inorganic carbon source for photosynthesis, and sediment type. Two studies showed a positive relationship between current velocity and productivity (See reprint: "Effects of current velocity and substrate composition on growth of Texas wildrice (*Zizania texana*)"). The results from these studies lead to an investigation of carbon use by Texas wildrice in cooperation with Dr. Robert Doyle, Lewisville Aquatic Ecosystem Research Facility. Carbon dioxide (rather than bicarbonate) was identified as the inorganic carbon source for photosynthesis. Submersed leaves in flowing water experience CO_2 enrichment and productivity increases. Submersed leaves in slower flowing water are carbon stressed and productivity decreases (Manuscript entitled "Effects of current velocity and carbon use on growth in the endangered Texas wildrice (*Zizania texana*)" submitted to Journal of Ecology January 2001).

Resource allocation patterns also were influenced by current velocity. After 1 year of growth, plants grown in higher current velocities (0.250-0.369 m/sec) had total biomass 10X greater than plants grown in slower current velocities (0.0-0.010 m/sec). Given the previous work on carbon utilization, submersed leaves in slower current velocities in this study were likely carbon stressed, and proportionally more biomass was allocated to reproductive structures, which readily take up carbon from the atmosphere.

Two studies examined the effects of sediments on plant growth. In the first study plants were significantly more productive when grown in sandy clay loam compared with clay or gravel (See reprint: "Growth of Texas wildrice (*Zizania texana*) in three sediments from the San Marcos

River"). In a second study, sediments collected from locations above and below dams in the San Marcos River were analyzed and a growth study conducted. Plants showed the greatest increase in biomass when grown on sediments of intermediate density and organic matter content. Because of the overriding importance of current velocity on Texas wildrice, the study recommends analyzing density and organic matter content of sediments coupled with current velocity at the site before assessing potential Texas wildrice habitat.

The final study examined a potential threat to the species: floating vegetation mats entangled in Texas wildrice stands. The results of the study indicated floating mats slow current velocity, block light, and shred Texas wildrice leaves. The negative effects of vegetation mats on Texas wildrice is evident after 6 weeks. This was the first study to positively identify floating vegetation mats as a serious threat to Texas wildrice (See reprint: "Direct and indirect effects of floating vegetation mats on Texas wildrice (*Zizania texana*)"). The study recommends that the source of floating mats be identified and reduced, and floating mats not be allowed to accumulate over Texas wildrice stands.

SIGNIFICANT DEVIATIONS

This report contains data on reintroduction of Texas wildrice into Spring Lake. The initial reintroduction design, as outlined in the contract, was followed, but shortly after planting, a flood washed away many of the experimental plants and some exclosures. It was necessary to reassess the experimental design because there was not enough plant material available to repeat the entire experiment. The changes were outlined in a draft interim report submitted to TPWD and USFWS. The changes included: 1) one age-class (4-6 months) for transplants instead of three age-classes; 2) add a growth experiment and; 3) two replicates for each exclosure treatment instead of three replicates. This work was carried out and ultimately published in Southwestern Rare and Endangered Plants: Proceedings of the Second Conference, September 11-14, 1995; Flagstaff, Arizona. Gen. Tech. Rep. RM-GTR-283. Between 1991 and 1996, Texas wildrice plants were transplanted into five sites. Plants persisted in two sites and, as of February 2001, are still present in those sites. This is the only recorded successful Texas wildrice reintroduction effort.

The stated objective for contract #335-0198 and #336-0225 was to "initiate and/or continue and complete organic matter additions and plant growth experiments." There were no stated procedures for this objective. Between 1994 and 1996, six sediments from above and below dams on the San Marcos River were analyzed for organic matter content rather than incrementally adding organic matter to a single sediment. The collected sediments then were used in a growth experiment. Plants were most productive on sediments of intermediate density and organic matter content. In a second growth experiment, nitrogen content was manipulated. This was done because information on nitrogen would have broader management potential than organic matter alone. Plant growth was limited by nitrogen on sediments of intermediate density and organic matter content; and limited by nitrogen and other factors on sediments of high and low density and organic matter content.

Publication in refereed journals was not a stipulation in any of the contracts; however, in an effort to facilitate the acquisition of information about the endangered Texas wildrice by the scientific community, three articles were published in refereed scientific journals (one international, one national, and one regional journal), two articles appeared in proceedings from scientific meetings, and one article currently is in review by an international journal. Reprints are included in the appendix, a copy of the manuscript in review is available upon request.

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February 16, 2001

Mr. Gareth Rowell
Texas Parks and Wildlife
3000 IH 35 South
Suite #100
Austin, Texas 78704

Dear Gareth,

Please find enclosed an Executive Summary and Significant Deviations for Project No. 38, "Continued Maintenance, Reintroduction, and Research on *Zizania texana* (Texas wildrice)." I reviewed comments on the draft report and responded to those which I felt appropriate. I appreciate your attention, help, and cooperation on this report and hope that it satisfies the project requirements.

Sincerely,

Paula Power

Paula Power

cc: Alisa Shull, U.S. Fish and Wildlife Service

ACKNOWLEDGMENTS

This research would not have been possible without the advice, support and encouragement of many individuals. I would like to thank Dr. K. Kennedy and Ms. A. Shull, U.S. Fish and Wildlife Service, Ms G. Janssen and Ms J. Poole, Texas Parks and Wildlife Department, and Dr. M. Smart and Dr. R. Doyle, Lewisville Aquatic Ecosystem Research Facility. I would also like to thank Mr. R. Richter, Mr. R. Perry, Mr. M. Apodakis, Mr. J. Miller, Mr. C. Wood and Mr. D. Solanik for assistance with field work. Special thanks to Stephen McClintik for technical support on the pH drift experiment. I would like to thank Ms R. Daniel, Mr. S. Gilmer, Mr. C. Wood, and Mr. R. Cobb, San Marcos Parks and Recreation Department for their help and cooperation. I would like to thank Dr. T. Brandt for his support.

This research was supported by grants from U.S. Fish and Wildlife Service , Texas Parks and Wildlife Department and Texas Organization for Endangered Species.

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1-2. Draft Interim Report, Contract # 331-0253.

1-3 Letter from Raymond Neck, Texas Parks and Wildlife Department

REPRINTS

Effects of current velocity and substrate composition on growth of Texas wildrice (*Zizania texana*). Aquatic Botany, 55:199-204.

Reintroduction of Texas wildrice (*Zizania texana*) in Spring Lake: Some important environmental and biotic considerations. *In*: Proceedings from Second Southwestern Rare and Endangered Plant Conference: Rocky Mountain Forest & Range Experimental Station.

Growth of Texas wildrice (*Zizania texana*) in three sediments from the San Marcos River. Journal of Aquatic Plant Management, 34:21-23.

Direct and indirect effects of floating vegetation mats on Texas wildrice (*Zizania texana*). Southwestern Naturalist, 41(4):462-464.

Moisture, seeds, and reproductive failure in Texas wildrice (*Zizania texana*). Southwestern Naturalist, 42(4):435-440.

Effects of oxygen concentration and substrate on seed germination and seedling growth of Texas wildrice (*Zizania texana*). Southwestern Naturalist, 40(1): 1-4.

SUMMARY OF CONTRACTS

Project 38: Continued Maintenance, Research and Reintroduction of Texas wildrice
Contract # 332-0094 - September, 1991- August, 1992

I. Segment Objectives:

a) Maintain cultivated population and collect and store seeds

b) Establish a population of wildrice in Spring Lake

c) Determine the extent and frequency of nutria damage

Procedures:

1) monitor the growth and health of the cultivated wildrice, remove excess algal growth, inspect for detrimental insects or disease, examine portable nutria exclosures to assess their effectiveness, and check water level flow in raceway at least three times a week;

2) check for mature seeds on cultivated Texas wildrice plants at least three times a week, and collect mature and store the hulled seeds in deionized water at 3°C;

3) after initial collection check stored seeds on a daily basis for a week for germination, after one week examine on a weekly basis and test 10% of ungerminated seeds for viability via a tetrazolium test;

4) plant 50 individuals each of three different age classes (4 week-old, 4 month-old and 1 year-old) of cultivated wildrice in five treatments sites consisting of three different flow regimes and two different exclosure treatments (no detectable flow/caged, no detectable flow/uncaged, low flow/cage, low flow/uncaged, and rapid flow/uncaged) in Spring Lake and monitor on a weekly basis for nine months for survivorship to determine which age class transplants most successfully;

5) use three exclosures to cover half of the plants at each of the no and low flow treatments to insure statistically meaningful data and monitor plants weekly for nine months to examine survivorship and the effects of herbivory;

6) provide copies of all data acquired during the study to the Receiving Agency as well as written progress report.

II. Summary of Progress:

Segment Objectives a, b, and c were completed on schedule.

III. Significant Deviations:

None

IV. Findings

a) Plants were maintained in the raceways at SWT. Water depth ranged from 0.5-0.75 m, current velocity was approximately 0.015 m/s. Plants grown under these conditions are primarily emergent and reproductive. Most plants set seed and die after 1 growing season.

During FY91-92, 6,699 seeds were collected and stored in aerated water at 3°C. Using germination and tetrazolium testing, seed viability ranged from 70% to 100% when stored for 0-36 weeks.

Germinated seeds followed a predictable developmental pattern, the shoot emerged first followed by the primary root. Chlorophyll and secondary roots develop after exposure to oxygen. Occasionally seedlings do not develop either roots or chlorophyll. Without chlorophyll or roots the seedling dies.

Seeds were used 1) for germination trials, 2) to generate new plants for transplanting into Spring Lake or, 3) for maintaining the cultivated population.

During FY91-92 approximately 1,000 seeds were sent to Dr. Ervin Oelke, University of Minnesota and approximately 100 seeds were sent to Dr. Robin Probert, Royal Botanical Gardens, Kew, England.

Problems associated with the cultivated population included algal growth, herbivory by crawfish, and vandals removing the boards which control water level in the raceway.

b) Texas wildrice plants were established in Spring Lake in three locations, eachwith different environmental conditions. Plants protected with exclosures were more likely to survive than those plants outside exclosures. Plants in flowing water (0.038 - 0.079 m/s) were more likely to live longer than those in slower flowing water (0 - 0.027 m/s).

c) Nutria (*Myocaster coypus*) chewed leaves and culms at the base of the plant. Nutria were most active during late summer and early fall. Plants kept in captivity should be protected from Nutria.

Contract #33-0201 September, 1992 - August, 1993

I. Segment Objectives:

a) maintain a cultivated population of wildrice and collect and store seeds from these plants

b) establish a population of wildrice in Spring Lake

Procedures

1) monitor the growth and health of the cultivated wildrice, remove excess algal growth, inspect for detrimental insects or disease, examine portable nutria exclosures to assess their effectiveness, and check water level flow in raceway at least three times a week

2) check for mature seeds on cultivated Texas wildrice plants at least three times a week, and collect mature and store the hulled seeds in deionized water at 3°C

3) after initial collection check stored seeds on a daily basis for a week for germination, after one week examine on a weekly basis and test 10% of ungerminated seeds for viability via a tetrazolium test

4) establish at least a 20 square meter plot of wildrice in Spring Lake at a density of 6 plants per square meter to total at least 120 plants

5) develop and utilize methods to protect reintroduced plants from herbivory

6) submit a progress report

II. Summary of Progress:

Segment Objectives a, and b were completed on schedule.

III. Significant Deviations:

None

IV. Findings

a) Plants were maintained in the raceways at SWT. Water depth ranged from 0.5-0.75 m, current velocity was approximately 0.015 m/s. Plants grown under these conditions were primarily emergent and reproductive. Most plants set seed and died after 1 growing season.

During FY92-93, 11,778 seeds were collected from the cultivated population. Germination (viability) ranged from 38%-94% when stored from 28-52 days.

Seeds exhibited the same developmental pattern as in 1992. Preliminary data suggest there is an inverse relationship between proper seedling development and length of storage. This area requires further study.

Seeds were used 1) for germination trials, 2) to generate new plants for transplanting into Spring Lake or, 3) for maintaining the cultivated population.

Problems associated with maintaining the cultivated population included, crawfish, nutria, and vandals removing boards which control water level and shutting off the water to the raceway.

b) Texas wildrice plants were transplanted into Spring Lake at the Dam site. Plants in this site are more likely to survive than the Wetland or Intermediate site. The most important environmental factor contributing to the success of the plants at the Dam site compared with other sites was current velocity (0.038 - 0.079 m/s at Dam site, 0 m/s at Wetland site, and 0.03 m/s at Intermediate site).

Contract #434-0198 September, 1993 - August, 1994

I. Segment Objective

a) investigate to what extent velocity and substrate influence productivity and resource allocation patterns in wildrice when grown at a constant depth

b) collect and store seeds from the protected population on the SWT campus

c.) reintroduce an additional plot of Texas wildrice in Spring Lake

d) determine to what extent wildrice uses bicarbonate ion as a carbon source or if it is strictly a CO_2 user.

II. Summary of Progress

Segment Objectives a, b, and d were completed on schedule.

III. Significant Deviations

Segment Objective c) an additional plot of wildrice was planted in Spring Lake during fall, 1995.

IV. Findings

a) To determine the effects of substrate composition and current velocity on plant growth, plants were grown in pots containing either fine or coarse sediment at three sites in Spring Lake. Each site was similar in depth but differed in current velocity. Plants were allowed to grow for 6 weeks and then harvested. Growth was greater on fine sediments. There was significant interaction between flow and sediment with respect to aboveground biomass and stem density and, there was significant flow effect on leaf length and stem density. Stem density was greater in fast flowing water (0.40-0.49 m/s) than either moderate (0.12-0.24 m/s) or slow flowing (0.05-0.12 m/s) water.

b) Plants were maintained in the raceways at SWT. Water depth ranged from 0.5-0.75 m, current velocity was approximately 0.015 m/s. Plants grown under these conditions were primarily emergent and reproductive. Most plants set seed and died after 1 growing season.

During FY93-94 approximately 24,665 seeds were collected from the cultivated population and stored in aerated water at 3°C. Seeds were used 1) for germination trials, 2) to generate new plants for transplanting into Spring Lake, 3) for maintaining the cultivated population.

A plant growth experiment was conducted to determine suitable soil for the cultivated population. Results indicated sediment texture combined with nutrient content were important sediment characteristics. The results of the study were published in the Journal of Aquatic Plant Management (see Attached Reprint, Power, 1996).

Funding was allocated by the SWT Biology Department for a chain link fence around the raceway to secure the area.

c) Texas wildrice produces long, submerged ribbon-like leaves and short, broad aerial leaves in response to environmental conditions. To determine the carbon source for submerged leaves, dissolved inorganic carbon was manipulated in a closed chamber using pH drift techniques. Photosynthesis slowed to detection limits as pH approached 8.7 (CO_2 <0.02 mM, HCO_3 >4.5 mM), suggesting that the submerged leaves of Texas wildrice cannot utilize HCO_3 and are obligate CO_2 users. We suspect obligate CO_2 use limits productivity in submerged leaves under certain environmental conditions.

Contract #335-0198 September, 1994 - August, 1995

I. Segment Objectives

a) complete investigations on the extent velocity and substrate influence productivity and resource allocation patterns in wildrice when grown at a constant depth

b) continue the collection and storage of seeds from the protected population on the SWT campus

c) continue the seasonal monitoring and data compilation on the reintroduction plots of wildrice in Spring Lake

d) initiate and/or continue organic matter additions and plant growth experiments

e) continue the management of the conservation populations in the raceways.

II. Summary of Progress

Segment Objective b, c, e were completed on schedule. Segment Objective a in progress. Segment Objective d in progress.

III. Significant Deviations

d) I analyzed six sediments for organic content followed by a growth experiment. In addition, I manipulated nitrogen content in a growth experiment, to establish baseline information on the nitrogen requirements for Texas wildrice. I felt the added information on nitrogen would have broader management potential than organic matter alone.

IV. Findings

a) Texas wildrice plants were grown in pots and transplanted to three sites with different current velocities in Spring Lake and on the Southwest Texas State University campus. Plants were harvested on five occasions between May and September 1995. Plants growing in the Slow Flow site (0 - 0.010 m/s) had the lowest root, leaf, culm, and total biomass and allocated more biomass to reproductive parts (65%), suggesting plants are growing under environmentally stressful conditions. Plants in the Fast Flow site (0.250 -0.369 m/s) had the greatest root, leaf, culm, and total biomass and allocated less biomass to reproductive parts (30%). Mechanical stimulation of the meristem and physiological enrichment (CO_2) due to flowing water may play a role in increased productivity in the Fast site.

b) and e) Plants were maintained in the raceways at SWT. Water depth ranged from 0.5-0.75 m, current velocity was approximately 0.015 m/s. Plants grown under these conditions were primarily emergent and reproductive. Most plants set seed and died after 1 growing season.

During FY94-95 approximately 36,625 seeds were stored in aerated water at 3-5°C. Seeds were used 1) for research purposes, 2) to generate new plants for transplanting into Spring Lake, 3) for maintaining the cultivated population.

The only problem associated with the cultivated population was algal growth. Removing algae required about 1.5-2 hours/week.

c) 160 plants were planted in Spring Lake during winter, 1995. Plants were transplanted at a density of 10 plants/m^2. Compilation of monitoring data on reintroduced plants has identified an initial increase in plant size followed by high mortality in three of five sites. Data suggest stem density is a good indicator of future transplant success and that current velocity is an important environmental factor associated with transplant success. Herbivores continually clipped reproductive culms just below the water surface. This limits transplants to clonal reproduction.

d) in progress.

Contract #336-0225 September, 1995 - August, 1996

I. Segment Objectives

a) Continue the collection and storage of seeds from the protected population on the SWT campus.

b) Continue seasonal monitoring and data compilation on the reintroduction plots of Texas wildrice in Spring Lake.

c) Initiate and/or continue and complete organic matter additions and plant growth experiments.

d) Continue the management of the conservation population in the raceways.

e) Initiate and/or continue and complete the quantification of the effect of floating vegetation mats on productivity and survivorship of Texas wildrice.

f). Continue and complete (with increased sample size) the study of flow vs. productivity and reproductive effort/success of Texas wildrice.

g) Prepare to submit a progress report to the Receiving Agency on or before October 1, 1996.

II. Summary of Progress

Segments Objectives a-g were accomplished on schedule.

III. Significant Deviations:

c) I analyzed six sediments for organic content followed by a growth experiment. In addition, I manipulated nitrogen content in a growth experiment to establish baseline information on the nitrogen requirements for Texas wildrice. I felt the added information on nitrogen would have broader management potential than organic matter alone.

IV. Findings:

a) and d) Plants were maintained in the raceways at SWT. Water depth ranged from 0.5-0.75 m, current velocity was approximately 0.015 m/s. Plants grown under these conditions were primarily emergent and reproductive. Most plants set seed and died after 1 growing season.

During FY95-96 approximately 70,641 seeds were stored in aerated water at 3-5°C. Seeds were used 1) for research purposes and, 2) for maintaining the cultivated population.

Seed were shipped to Dr. Christina Walters, National Seed Storage Laboratory, Ft. Collins, Colorado.

The only problem associated with the cultivated population was algal growth. Removing algae required about 1.5-2 hours/week.

b) A reintroduction program was initiated in 1992. Plants were transplanted into five microhabitats at a density of 10 plants/m^2. Seasonal monitoring identified an initial increase in plant size followed by high mortality in three of five sites. Data suggest stem density is a good indicator of future transplant success and that current velocity is an important environmental factor associated with transplant success. Herbivores continually clipped reproductive culms just below the water surface. This limits transplants to clonal reproduction.

c) Texas wildrice was grown on six sediments collected above and below three dams on the San Marcos River in Hays Co., Texas. Sediments could be divided into above and below dam groups based on sediment density. Water content and organic matter content were correlated to sediment density (r^2=0.997; $p<0.05$ and r^2=0.87; $p<0.05$ respectively). Total biomass was greatest on intermediate density sediments (above dam sediment). Growth was diminished on high and low density sediments (above and below dam sediments). Location of sediments relative to dams was not a good indicator of potential plant growth, rather a better indicator was sediment density and organic matter content. Nitrogen was added to sediments at five levels 0 mg N (control), 50 mg N, 100 mg N, 200 mg N and 400mg N. Prior to N additions high root/shoot ratios suggested nutrient limitation on all sediments. After N additions, plants increased in size on intermediate density sediments. However root/shoot ratios dropped on all sediments suggesting growth was limited by nitrogen availability but growth on low and high density sediments was further limited by other factors, possibly multiple nutrient limitation. Tissue nutrient concentration was not a good indicator of nutrient limitation.

Current velocity is also an important factor in Texas wildrice growth (Power, 1996b), therefore when considering site potential for plant growth in the wild both sediment density and current velocity should be taken into account.

e) Floating vegetation mats have negative direct and indirect effects on Texas wildrice. Direct negative effects include shredding Texas wildrice leaves and interference with emergence of reproductive culms. Indirect negative effects include blocking sunlight, which interferes with photosynthesis, and slowing current velocity, which may reduce nutrient uptake from the open water (Smith and Walker, 1980; Boeger, 1992; Rose and Power, 1994).

f) Texas wildrice plants were grown in pots and transplanted to three sites with different current velocities in Spring Lake and on the SWT campus. Plants were harvested on seven occasions between May 1995 and February 1996. There was a significant difference and interaction between factors in roots, leaf, reproductive culm, and total biomass over time (repeated measures ANOVA). Plants grown in fast flowing water (0.250 m/s - 0.369 m/s) had the greatest increase in size. Plants had significantly different biomass allocation patterns over time (repeated measures ANOVA). There appeared to be a trade off between biomass allocated to roots and biomass allocated to reproductive parts. Plants in the fast site allocated biomass to roots at the expense of reproductive parts while the opposite occurred in the slow site. There was no significant difference in the proportion of biomass allocated to submerged leaves among sites. Current velocity appears to be an important factor influencing growth and allocation patterns. Plants in slow flowing water tend toward a shorter life span.

CHAPTER 1.

CULTIVATED POPULATION OF *Zizania texana*

I. CULTIVATED POPULATION PRIOR TO SECTION 6 PROJECT NO. 38

Prior to 1986 a number of articles were published on *Zizania texana.* They included species descriptions (Silveus 1932, 1933), threats to the species (Emery 1967, 1977), and observations on *Z. texana* in its habitat (Terrell, et al.1978). In addition, Z. texana was listed in the Federal Register (U.S. Fish and Wildlife Service, 1985) and a recovery plan was written (US Fish and Wildlife Service, 1984). Prior to 1986, there had been no manipulative studies on *Z. texana* or detailed written accounts on cultivation techniques.

The San Marcos River Recovery Plan (U. S. Fish and Wildlife Service, 1984) outlined the processes by which the endangered species within the San Marcos River could be recovered to nonthreatened status. One process was to "establish and maintain captive stock" (U. S. Fish and Wildlife Service, 1984 p. 63). Presumably captive stock would protect *Z. texana* from "the ever present possibility of accidental pollution by runoff of locally applied herbicides (U. S. Fish and Wildlife Service, 1984 p. 58). Federal Contract14-16-0002-8-222 was initiated in response to a proposal submitted by Fonteyn and Power in which they proposed to "establish a population at the Aquatic Station at Southwest Texas State University (SWT) to create a seed bank and nursery for future experiments and reintroduction to historic native habitat" (Attachment 1-1).

Establishing a seed bank and a nursery required having the expertise to 1) harvest seed, 2) germinate seed, 3) store seed and, 4) grow plants which produce seed, in a predictable, efficient manner. What appears in the following paragraphs is how the *Z. texana* seed bank and nursery were eventually established.

Early references to cultivating *Z. texana* were by Terrell et al. (1978) and Emery and Guy (1979). Terrell et al. (1978) noted that "in cultivation *Z. texana* changed from a prostrate, immersed plant to an erect, emergent one." Terrell et al. (1978) attempted cultivating *Z. texana* at a USDA facility in Beltsville, Maryland. They placed three clumps of *Z. texana* in "house potting soil" in a 1x3 m tank containing circulating water "a few centimeters over the soil surface of the pots." Two clumps died within months, one clump survived for about 1.5 years. From this clump, 80 seeds were collected and germinated, but seedlings failed to reach maturity. They concluded cultivation of *Z. texana* "needed special requirements not adequately met at Beltsville."

Emery removed 4 clones of *Z. texana* from the San Marcos River in 1975. The clones were placed in an outdoor cement raceway at SWT (1 m wide and 50 m long). The water source was an artesian well fed by the Edwards Aquifer. Water level was manipulated with removable boards and flow was manipulated with an adjustable valve. During summer 1975, 1,500 seeds were produced. Seeds were placed in water at 3°C (Terrell et al., 1978). Terrell et al. (1978) suggested (erroneously) that *Z. texana* had an "extended dormancy" and that 105 days was adequate to break dormancy. Seeds were placed in petri dishes with tap water for germination (Terrell et al., 1978).

Emery retired in the late 1970's and by 1986 *Z. texana* was not in cultivation at any facility, nor was there a seed bank.

In 1986 Fonteyn and Power collected 7 clumps of *Z. texana* from a population immediately upstream from the IH35 bridge. The clumps were transported to SWT and placed in the outdoor cement raceway used by Emery about 10 years earlier. Water level varied between about 0.5-0.75 m. Flow was about 0.015 m/s. The 7 clumps were divided into many smaller clumps and transplanted into 10 cm peat pots filled with sediments collected from the San Marcos River. Most of the small plants in peat pots were transplanted into the San Marcos River for a competition experiment. The competition experiment is described and discussed in Chapter 2 of this report.

Once brought from the wild to the raceway at SWT, plants changed from prostrate to emergent within 2-3 weeks. Emergent plants were reproductive and set seed. Mature seeds were collected by gently dragging the hand along the inflorescence. Ripe seeds would fall from the panicle, immature seeds would not. Seeds were placed in vials filled with water and stored at 3°C as per Terrell, et al. (1978).

Identifying conditions which resulted in consistently high germination in the lab proved problematic. First, simply placing seeds "in petri dishes with tap water" as suggested by Terrell et al. (1978) did not initiate germination. Second, seed supply was very limited and it was imperative to find a satisfactory method of germinating seeds without using many seeds in the process. Our approach was to conduct the best studies possible with limited seeds which would identify a predictable, repeatable method for obtaining high percent germination.

Seeds which were stored for 4 months were placed in open petri dishes in an environmental chamber at various light, dark and temperature regimes. Germination varied between 0% and 25% (Table 1-1, Appendix 1-1). However when seeds were placed in closed vials under similar environmental conditions germination rose to 50%-100% (Table 1-1, Appendix 1-1). A literature search identified low oxygen as an important germination cue in many wetland and aquatic plant species (Fenner, 1985; Kardan, 1974; Kennedy et al., 1980; Ponnamperuma, 1972; Pons and Schroder, 1986; Simpson, 1966). Evidently, respiring seeds in closed vials took up oxygen. When oxygen was lowered to a critical concentration, seeds germinated.

Power conducted a series of experiments which identified low oxygen concentration in water as a germination cue for *Z. texana* (Table 1-2, Appendix 1-2). This work was published in Southwestern Naturalist (Power and Fonteyn, 1995, attached).

For a seed bank, seeds must be stored for as long as possible without germinating or losing viability. Because low oxygen was a germination cue, Power compared storing seeds in water with different oxygen levels. Storage conditions was no more than 20 seeds placed in a vial. Vials were either capped (anaerobic) or covered with cheese cloth and placed in a beaker with an aerator (aerobic). Some seeds were placed in vials with water which had been boiled then cooled. Boiling water drives off oxygen and lowers oxygen concentration. These vials were capped. Preliminary results did not show a

Table 1-1. Results from preliminary germination trials to determine conditions which would obtain consistently high percent germination. Trials included variable light and temperature regimes. Seeds were germinated either in open petri dishes or closed vials. *Zizania texana* seeds were stored 4 months at 3°C under anaerobic and aerobic conditions. Storage conditions were thus: no more than 20 seeds were placed in a vial. Vials were either capped (anaerobic) or covered with cheese cloth and placed in a beaker with an aerator (aerobic). For each trial the number of seeds varied from 4 to 15.

			Storage Conditions	
			Treatments	
			Aerobic	Anaerobic
Petri dish				
	24h L	21°C	0%	0%
	24h L	15/25°C	0%	25%
	12/12h L	21°C	0%	0%
	12/12h L	15/35°C	0%	0%
	24h Dk	25°C	10%	0%
Closed Vial				
	24h L	21°C	71%	86%
	12/12h L	21°C	100%	85%
	12/12h L	21°C	78%	67%
	24h Dk	21°C	100%	50%

pronounced difference between treatments (Table 1-3, Appendix 1-3). After these preliminary experiments, seeds were stored in vials, placed in an aquarium with an aerator. The aquarium was placed in a refrigerator set at 3-5°C.

Seeds were tested for viability using percent germination followed by a tetrazolium chloride test. The tetrazolium test is based on the visual reduction (development of red staining) of 2,3,5-triphenyl-2H-tetrazolium chloride. Tetrazolium testing is used in agriculture to supplement germination tests and to test viability of dormant seeds (Grabe, 1970).

Seeds stored 0-42 weeks were tested for viability by placing twenty seeds in a watch dish filled with deionized water. The dish was covered for 7 days. Temperature was about 21°C with ambient light. After 7 days the number of germinated seeds were counted. Ungerminated seeds were subjected to the tetrazolium test (Kearns and Inouye, 1993). Seeds which stained dark pink or red were considered viable.

Seeds did not germinate readily for the first three weeks after collection, although seed viability was between 80-100%. Viability remained above 80% until the 32nd week in storage after which viability dropped below 50% (Figure 1-1, Appendix 1-4).

A standard method of germinating seeds to grow new plants was developed. One hundred seeds were placed in a 10 cm diameter glass dish filled with 300 ml deionized water.

Table 1-2. Percent germination of *Z. texana* at oxygen concentration in the water between 0.1 ppm and 5.0 ppm. Numbers are the average of 8 replicates with 20 seeds/replicate. SD in parenthesis.

Oxygen (ppm)	Germination (%)	
0.2	71.9	(11.933)
1.0	77.8	(7.559)
2.0	60.0	(14.638)
3.0	16.2	(17.269)
4.0	2.5	(2.673)
5.0	2.5	(5.345)

Table 1-3. Percent germination of *Z. texana* seeds in 1989. Seeds were stored, not more than 20 in per vial, in water, in a refrigerator. Water was boiled to drive off oxygen. There were 4 vials for each treatment, SD in parenthesis.

	Germination (%)		
Time in storage	Open Vial (Aerobic)	Closed Vial (Anaerobic)	Boiled Water
4 Months	81.25 (8.539)	63.75 (7.500)	97.5 (5.000)
6 Months	75 (7.071)	81.25 (6.291)	

The dish was covered and placed on the lab counter. Seeds germinated within 7 days. The shoot emerged first and was achlorotic. The cover was removed after the first week for another 7 days; during the second 7 days, the seedling was exposed to oxygen. Upon exposure to oxygen, the seedling developed chlorophyll and a primary root emerged followed by adventitious roots. After the second week, seedlings were transplanted to 10 cm diameter peat pots filled with sediments collected from ponds on the SWT campus.

In addition to germinating seeds in the lab, seeds were also germinated in different sediments in the raceway to test the hypothesis that oxygen concentration was an important germination cue. Sediments were selected which had either very low or very high oxygen content. SWT pond sediments had organic matter and bacteria present. Oxygen would be low in this sediment due to aerobic and anaerobic respiration by bacteria and very little space between soil particles for oxygenated water to pass through. In contrast, sand which is very porous with high flow through capability would have a higher oxygen content. Germination was highest on sand and lowest on SWT pond sediments. This work was included in Power's

Table 1-4. Seedling growth by cultured *Z. texana* in the raceway at SWT. Sand was purchased, other sediments were collected from the San Marcos River upstream from the IH35 bridge, or collected from ponds on the SWT campus. n=5 for sand and pond sediments, n=4 for San Marcos River sediments. Sd in parenthesis.

	Leaf Area (cm^2)		
Time (weeks)	Sand	San Marcos River Sediment	SWT Pond Sediment
2.4		5.90 (3.576)	
3	2.40 (0.765)		3.14 (2.605)
4		12.77 (6.407)	
5.4		19.72 (8.040)	
6	13.6 (7.195)		47.4 (42.040)
12	86.6 (34.001)		195.9 (93.294)

thesis (1990) and published in Southwestern Naturalist (Power and Fonteyn, 1995, attached).

Seeds which germinated in sand, SWT pond sediments, and San Marcos River sediments were monitored for about 3 months to compare growth on different sediments. This was done to test which sediments resulted in the most robust plants. Seedlings were most productive in SWT pond sediments (Table 1-4; Appendix 1-5). Some of these data were included in an article published in Southwestern Naturalist (Power and Fonteyn, 1995, attached).

In addition to leaf area, the basal area and reproductive parts of ten seedlings which had been planted in April 1989 were measured on 13 March 1990. The basal circumference ranged from 29 cm^2 to 448 cm^2. The average basal circumference was 136 cm^2 (sd=124.0). The number of emergent culms per plant ranged from 0 to 20. The average number of emergent culms was 12.1 (sd=5.6) (Appendix 1-6).

Ten plants were tagged and the number of seeds produced was recorded. The number of seeds collected ranged from 0 to 1,252 seeds/plant. The average number of seeds/plant was 606 (sd=426.8). These data were included as an appendix to Power's Master's thesis (1990). See Appendix 1-7.

By the end of 1990 it was possible to grow plants from seed, and collect, store and germinate seed in a predictable, repeatable fashion. Thus it was now possible to maintain the cultivated population of *Z. texana* for conservancy and research purposes without disturbing the wild population. Research material was available for researchers from other parts of the country. Seeds were sent to Dr. Ervin Oelke (Appendix 1-8).

Francis Rose and Paula Power obtained funding to maintain the cultivated population of *Z. texana* at SWT and to begin a reintroduction project in Spring Lake. The duration of the contract (TPWD contract #331-0253) was 28 June 1991 - 31 August 1991. Between mid-June 1991 and 15 August 1991 Gena Janssen was employed as a field assistant. During this time she was responsible for collecting seeds and maintaining the cultivated *Z. texana* population. A draft interim report was submitted September 16, 1991 (Attachment 1-2). This contract was a prelude to the Section 6 contracts covered in this report.

II. CONTRACT #332-0094

TPWD contract # 332-0094 began on 1 September 1991 and ended on 31 August 1992. The stated contract segment objective was: Maintain cultivated population and collect and store seeds. Procedures included, 1) monitor the growth and health of the cultivated wildrice, remove excess algal growth, inspect for detrimental insects or disease, examine portable nutria exclosures to assess their effectiveness (see Chapter 2), and check water level flow in raceway at least three times a week, 2) check for mature seeds on cultivated Texas wildrice plants at least three times a week, and collect mature and store the hulled seeds in deionized water at 3 C; 3) after initial collection check stored seeds on a daily basis for a week for germination, after one week examine on a weekly basis and test 10% of ungerminated seeds for viability via a tetrazolium test;

Seeds from the seed bank were germinated and potted seedlings were placed in the raceway throughout the year. Plants were observed at least 3 times per week for health, vigor and mature seeds. Mature seeds were collected between 1 September 1991 and 12 December 1991. Immature inflorescences were first noted on 4 March 1992. Seed harvest began again on 28 April 1992 and ended on 12 November (Appendix 1-9 and 1-10). Mature seeds were immediately returned to the lab, hulled, counted and placed in vials in aerated water at ~3°C. Approximately 100 seeds were shipped to Robin Probert, Royal Botanical Gardens, Kew England (Appendix 1-8). During FY91-92 6,699 seeds were collected from cultivated *Z. texana*.

A second germination/viability test on seeds in storage up to 36 weeks was carried out as per methods described in the previous section. Percent germination was 0 and percent positive tetrazolium test was 100 immediately after collection. Germination increased to 60% after 2 weeks and 84.2% after 4 weeks (Figure 1-2). Germination tests coupled with tetrazolium tests indicated total viability was between 70% and 100% (ave.=91%;sd=9.291; Figure 1-2; Appendix 1-11).

Seeds fall into 1 of 2 broad categories, orthodox or recalcitrant. Orthodox seeds go through a period of desiccation until a critical moisture level is reached. At a low critical moisture level, metabolic activity is suspended and embryos are dormant. Recalcitrant seeds go through a period of desiccation during development but do not reach the moisture level at which metabolic activity is suspended. As a result, embryos of recalcitrant seeds do not go dormant but continue to grow (at a very slow rate) until germination occurs. Low percent germination of seeds in storage less than 3 weeks was most likely because seeds are recalcitant and maturation continues after the seed has abscised from the parent plant (Vertucci, et al. 1994). This suggests that embryos must mature for another 2-3 weeks after abscission before germination is possible.

Fungi are a problem associated with the storage of recalcitrant seeds. Recalcitrant seeds must be stored in water or moist paper towels, an environment conducive to fungal infection. Fungi infect seeds and can give a false positive on a tetrazolium test (pers. comm. Christina Walters). This is a problem not faced by orthodox seeds which are stored in dry conditions. Tetrazolium chloride is also considered a hazardous chemical with special disposal requirements. Germination tests alone provide an accurate representation of viability and the adequacy of the storage technique without false positive results and the environmental cost of using hazardous chemicals. It is recommended that germination tests are used for routine viability testing without follow-up tetrazolium tests.

After germination, seeds followed a consistent developmental pattern. The first structure to emerge from the seed coat was the shoot. The shoot was achlorotic in low oxygen conditions. When the cover was removed from the germination dish and seeds were exposed to oxygen, chlorophyll and roots developed. Occasionally, however, seedlings did not develop chlorophyll or roots. These seedlings did not develop further. To quantify the incidence of improperly developed seedlings, seedling development was observed in 18 trials. Seeds in these trials were stored 88 - 210 days. Germination was 68.5% (sd=23.9). Proper seedling development occurred in 45.9% (sd=33.8) of seeds which germinated, chlorophyll was present but roots were absent in 9.4% (sd=8.9) of seeds which germinated and chlorophyll was absent in 44.6% (sd=34.3) of seeds which germinated (Figure 1-3; Appendix 1-12).

Seedlings were transferred to peat pots filled with sediments and placed in the outdoor cement raceway. They were transplanted into Spring Lake (see Chapter 2) or maintained for seed production.

Problems associated with maintaining the cultivated population included algal growth, herbivory by crawfish, the presence of snails, and vandals. Green filamentous algae grew continuously among *Z. texana* leaves and on the water surface. Algae was removed about once a week by entering the raceway, walking among the plants, lifting the algae up from around the leaves and out of the raceway. The algal growth, although unsightly, did not appear to interfere with seed production when managed in this way but did require about 1.5-2 hours per week when maintaining 50 or more plants.

Crawfish were first noted in April 1991. Young seedlings "appeared as if some organism chewed the seedling in half at the root". Between 15 June 1992 and 1 July 1992, 33 crawfish were removed from the raceway and seedling loss stopped.

On 8 July 1992 hundreds of snails were found in the raceway. They caused no damage and most died by fall. The snails were probably *Helisoma* sp. (Tom Arsuffi, pers. comm.).

Finally, vandals removed the boards controlling water level in the raceway. No mortality was observed due to changing water levels.

III. CONTRACT #33-0201

TPWD contract #33-0201 began on 1 September 1992. The contract segment objective stated: Maintain cultivated population and collect and store seeds. Procedures included, 1) monitor the growth and health of the cultivated wildrice, remove excess algal growth, inspect for detrimental insects or disease, examine portable nutria exclosures to assess their effectiveness (see Chapter 2), and check water level flow in raceway at least three times a week, 2) check for mature seeds on cultivated Texas wildrice plants at least three times a week, and collect mature and store the hulled seeds in deionized water at 3° C, 3) after initial collection check stored seeds on a daily basis for a week for germination, after one week examine on a weekly basis and test 10% of ungerminated seeds for viability via a tetrazolium test.

Seeds from the seed bank were germinated and seedlings were placed in the raceway throughout the year. Plants were observed for health, vigor and mature seeds. Mature seeds were collected between 16 September 1992 and 4 January 1993. The 1993 seed harvest began again on 15 April 1993 and ended on 29 November 1993 (Appendix 1-10 and 1-13). Mature seeds were immediately returned to the lab, hulled and placed in vials in aerated water at ~3°C. During FY92-93 11,778 seeds were collected from cultivated *Z. texana*. During June the number of seeds collected became so great, it was impractical to count them all. As an alternative to counting the number of seeds, the harvest was weighed. The number of seeds could be estimated by determining the average weight of a seed (Table 1-5).

Table 1-5. Weight of *Z. texana* seeds collected from the cultivated population at SWT. Sd in parenthesis.

N	Weight(g)	Ave. Weight/seed (g)
100	1.16	0.0116
10	0.08	0.008
25	0.24	0.0096
25	0.29	0.0116
Mean		0.01(0.002)

Viability of seeds was determined using germination tests. Germination ranged from 38% -94% (the number of seeds in each test ranged from 12% to 100%; n=11; mean=74.8%; sd=18.8%). Seeds that were stored for 28-52 days were germinated and seedlings were observed for developmental patterns. Proper seedling development occurred in 78.1% (sd=16.5) of seeds which germinated, chlorophyll was present but roots were absent in 2.8% (sd=3.0) of seeds which germinated, and chlorophyll was absent in 17.3% (sd=12.2) of seeds which germinated (Figure 1-3; Appendix 1-14). A higher percentage of seeds developed properly in the 1993 trials when compared with trials in the previous year (see Appendix 1-12). This may be due to shorter time in storage for the 1993 trials (28-52 days in 1993 vs. 88-210 days in 1992).

Seedlings were transferred to peat pots filled with sediments collected from the SWT ponds and placed in the outdoor cement raceway at SWT. They were eventually transplanted into Spring Lake (see Chapter 3) or maintained for seed production.

Problems associated with maintaining the cultivated population were algae, vandals, crawfish, and nutria (*Myocaster coypus*, an introduced rodent). On 19 October 1992 boards which maintain water level had been removed. On 2 November 1992 the valve which controls water flow into the raceway was shut off. On 10 November 1992 crawfish appeared in the raceway. They were removed promptly.

In April 1993, 2 nutria appeared in the raceway. They damaged at least 25 plants at the base, separating the leaves from the roots. They were removed, then reappeared 2 days later. They were removed a second time and did not reappear.

IV. CONTRACT #434-0198

TPWD contract #434-0198 began on 1 September 1993 and ended on 31 August 1994. The contract segment objective stated: collect and store seeds from the protected population on the SWT campus. There were no written procedures for this objective.

Plants, raised from seed were grown in the raceway and were observed regularly for health, vigor and mature seeds. Mature seeds were collected between 2 September 1993 and 29 November 1993. The 1994 seed harvest began again on 30 March 1994 and ended on 26 July 1994. (Appendix 1-15). Mature seeds were immediately returned to the lab, weighed and placed in vials in aerated water at ~3°C. During FY93-94, 24,665 (estimated by weight) seeds were collected from the cultivated population.

Seeds were germinated as per standard method (p.15). Germinated seeds were placed in peat pots filled with sediments. Sediments were collected from the San Marcos River, ponds on campus, and purchased "black gumbo" from the San Marcos River flood plain. From a previous study (Table 1-4; Power and Fonteyn, 1995), we observed that seedling productivity varied with respect to sediments; but, it was unknown what role nutrients played in sediment/plant growth interactions.

A plant growth experiment was conducted using different soils to determine a suitable planting medium for the cultivated population of *Z. texana*. This was not specifically outlined in the contract but the information gained facilitated maintenance of the cultivated population. The soils used in the growth study were clay, sandy clay loam and gravel. Sediments were shipped to A&M Soil Testing Laboratory and analyzed for Kjeldahl nitrogen, nitrates, phosphorus, potassium, calcium, magnesium, zinc, iron, manganese, copper, sodium, sulfur, pH, texture, and organic matter (Table 1-6). Plants were grown on different sediments, then harvested and weighed. Plants grown on sandy clay loam had the highest total biomass (0.790 g) followed by plants grown on gravel (0.138 g), then clay (0.013 g; one-way ANOVA; $p<0.05$ and Tukey Multiple Comparison; Table 1-7).

Root to shoot ratios from plants grown on each sediment type were significantly different. Root to shoot ratio for plants grown in clay was 0.74; gravel, 0.50 and sandy clay loam, 0.29 (one-way ANOVA; $p<0.05$ and Tukey Multiple Comparison; data were arc transformed). High root to shoot ratios coupled with low total biomass on clay and gravel sediments suggested plants were nutrient stressed. Note that nutrients were present in clay sediments at concentrations similar to sandy clay loam sediments. Nutrients were present in clay sediments but evidently plants were unable to take up nutrients possibly due to textural consideration.

When sediments were selected for cultivation of *Z. texana*, gravel and clay were avoided. Sediment selection focused on sandy clay loam. This work was eventually published in the Journal of Aquatic Plant Management (Power, 1996, reprint attached).

Problems associated with maintaining the cultivated population included algae, Nutria during July and August, and the presence of the introduced Ram's horn snail (*Marisa).*

Table 1-6. Nutrient content (mg/kg dry soil) of three sediments; clay, sandy clay loam and gravel, collected in and around the San Marcos River. *Zizania texana* seedlings were planted in each sediment

Nutrient	Clay	Sandy clay loam	Gravel
*NO3	1	1.3	0.3
*Kjeldahl N	776	1517	71
*Phosphorus	55	47	15
Potassium	335	55	15
Calcium	18893	18413	4830
Magnesium	322	840	225
*Zinc	0.4	2.2	0.6
*Iron	19	83	5
*Manganese	5.6	5.2	1.2
Copper	1.6	0.8	0.1
Sodium	25	54	17
Sulfur	304	1750	428
pH	8.2	7.8	8.5

*Nurtients which coprecipitate; their source is sediment. All other nutrients are salts which dissolve in water and are most likely taken up by shoots (Barko et al., 1991).

Table 1-7. Shoot biomass, root biomass, total biomass (g/dry weight) and root to shoot ratio of *Z. texana* grown in three sediments. Letter following mean indicates significant difference using Tukey's multiple comparison procedure at the 0.05 level of significance. Sd in parenthesis.

Biomass	Clay	Sandy clay loam	Gravel
Shoot	0.014a	0.616b	0.094a
	(0.006)	(0.205)	(0.043)
Root	0.011a	0.174b	0.044a
	(0.007)	(0.051)	(0.015)
Total	0.025a	0.790b	0.138a
	(0.013)	(0.254)	(0.057)
R:S	0.74a	0.29b	0.50c

cornuarietis) in the raceway. A single Marissa was noted on 27 September 1993 and 2 were noted on 27 January 1994. They were immediately removed.

During summer 1994, the Biology Department at SWT appropriated funds for the construction of a chain link fence around the raceway in an attempt to secure the area.

V. CONTRACT #335-0198

TPWD contract #335-0198 began on 1 September 1994 and ended on 31 August 1995. The contract segment objective stated: continue the collection and storage of seeds from the protected population on the SWT campus and continue the management of the conservation populations in the raceways. There were no written procedures for this objective.

Plants, raised from seed, were grown in the raceway and observed regularly for health, vigor and mature seeds. Mature seeds were collected between 7 July 1995 and 7 November 1995 (Appendix 1-16). Mature seeds were immediately returned to the lab, weighed and placed in vials in aerated water at ~3°C. During FY94-95 36,625 seeds (estimated by weight) were collected from the cultivated population.

On 2 June 1995, 10 plants were transported to the Endangered Species exhibit at Aquarena Center, San Marcos, Texas. They did not flourish and eventually were eaten by fish (Ron Coley, director, Aquarena Center thought Talapia ate the young plants, pers. comm.).

Problems associated with the cultivated population included algal growth and nutria. In June 1995, the gate was opened to the raceway and nutria climbed in and destroyed many plants. The nutria were removed but the seed harvest was delayed as a result. A more secure lock was than placed on the gate to the chain link fence.

VI. CONTRACT #336-0225

TPWD contract #336-0225 began on 1 September 1995 and ended on 31 August 1996. The contract segment objective stated: Continue the collection and storage of seeds from the protected population on the SWT campus. Continue the management of the conservation population in the raceways.

Plants, raised from seed, were grown in the raceway and observed regularly for health, vigor and mature seeds. Mature seeds were collected between 18 September 1995 and 7 November 1995. The 1996 seed harvest began on 29 April 1996 and continued through August 30 1996. Mature seeds were immediately returned to the lab, weighed and placed in vials in aerated water at ~3°C (Appendix 1-16 and 1-17).

Between 10 May 1996 and 15 May 1996 *Z. texana* plants were collected from the wild as part of U.S. Fish and Wildlife Service Contingency Plan. The Plan was implemented to preserve genetic diversity of the wild population in the event the spring flow from the San Marcos Springs dropped below a critical level. Plants were taken to San Marcos National Fish Hatchery and Technology Center, Uvalde National Fish Hatchery, and SWT. SWT potted 26 plants which had been collected from the wild. The plants were A, B2, B14d, B15f, C2, E14, F11, J6b, J10, J12, J20, K10c, K37a, and K41 (TPWD naming system). Some plants were divided and placed in more than one pot. These plants changed from prostrate to emergent and on 12 July 1996 the first seeds were collected from wild plants. Seeds from wild plants were combined with seeds from the cultivated population in the seed bank. During FY95-96 70,641 seeds were collected from the cultivated population and 406 seeds were collected from wild plants in the raceway (Appendix 1-16 and 1-17).

On 5 December 1995 about 50 nodes from the cultivated population were shipped to Christina (Vertucci) Walters at the National Seed Storage Lab in Ft. Collins, Colorado (Appendix 1-8).

The only problem associated with the cultivated population during FY95-96 was algae growing in and among *Z. texana* leaves. Removing algae required about 1.5-2 hours/week.

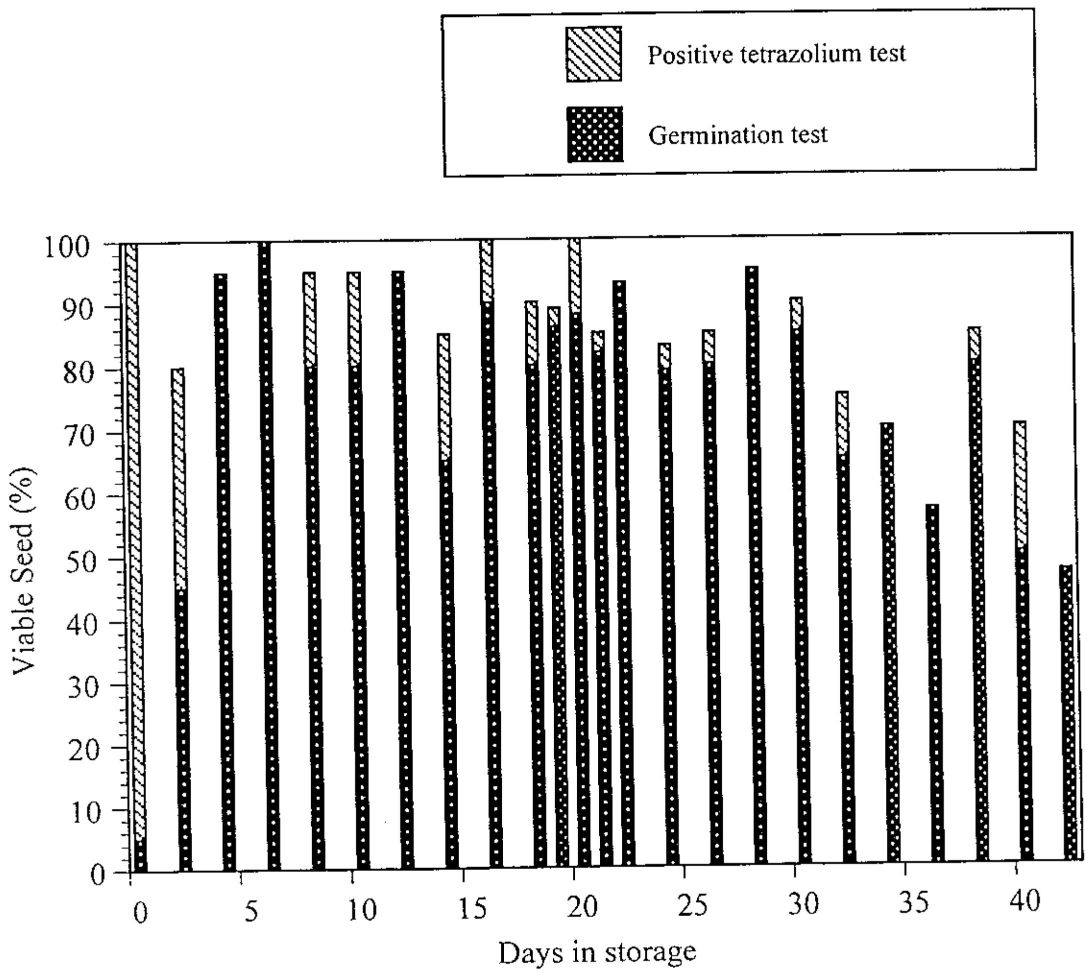

Figure 1-1. Viability of *Z. texana* seeds in storage between 0 and 42 weeks. Viability is measured as percent germination and percent seeds with positive tetrazolium chloride. All seeds were stored at 3°C in aerated water. (data for TPWD contract #331-0253; 28 June 1991-31 August 1991.

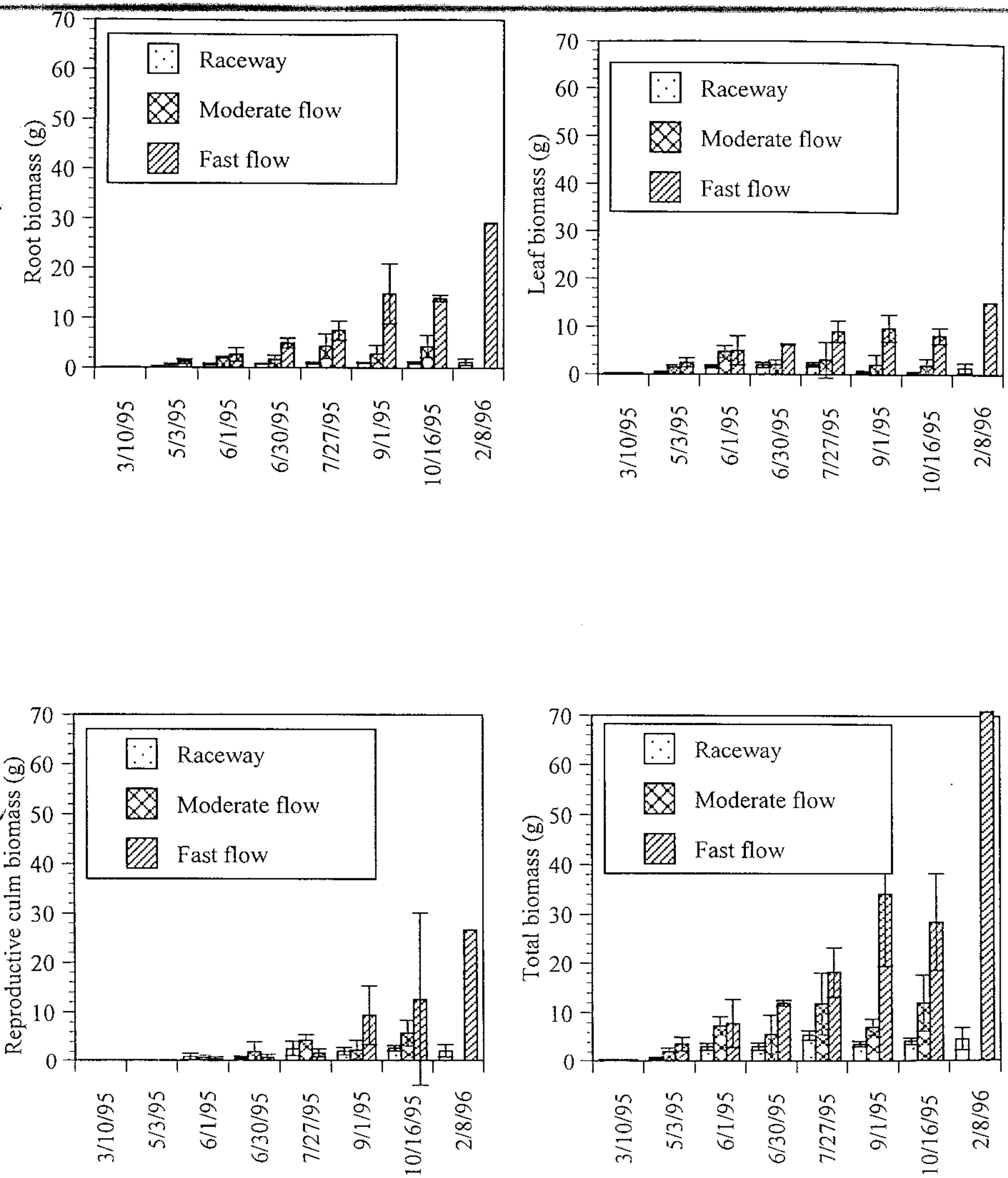

Figure 5-1. Root, leaf, reproductive culm and total biomass for *Zizania texana* grown in three current ·elocities over one year.

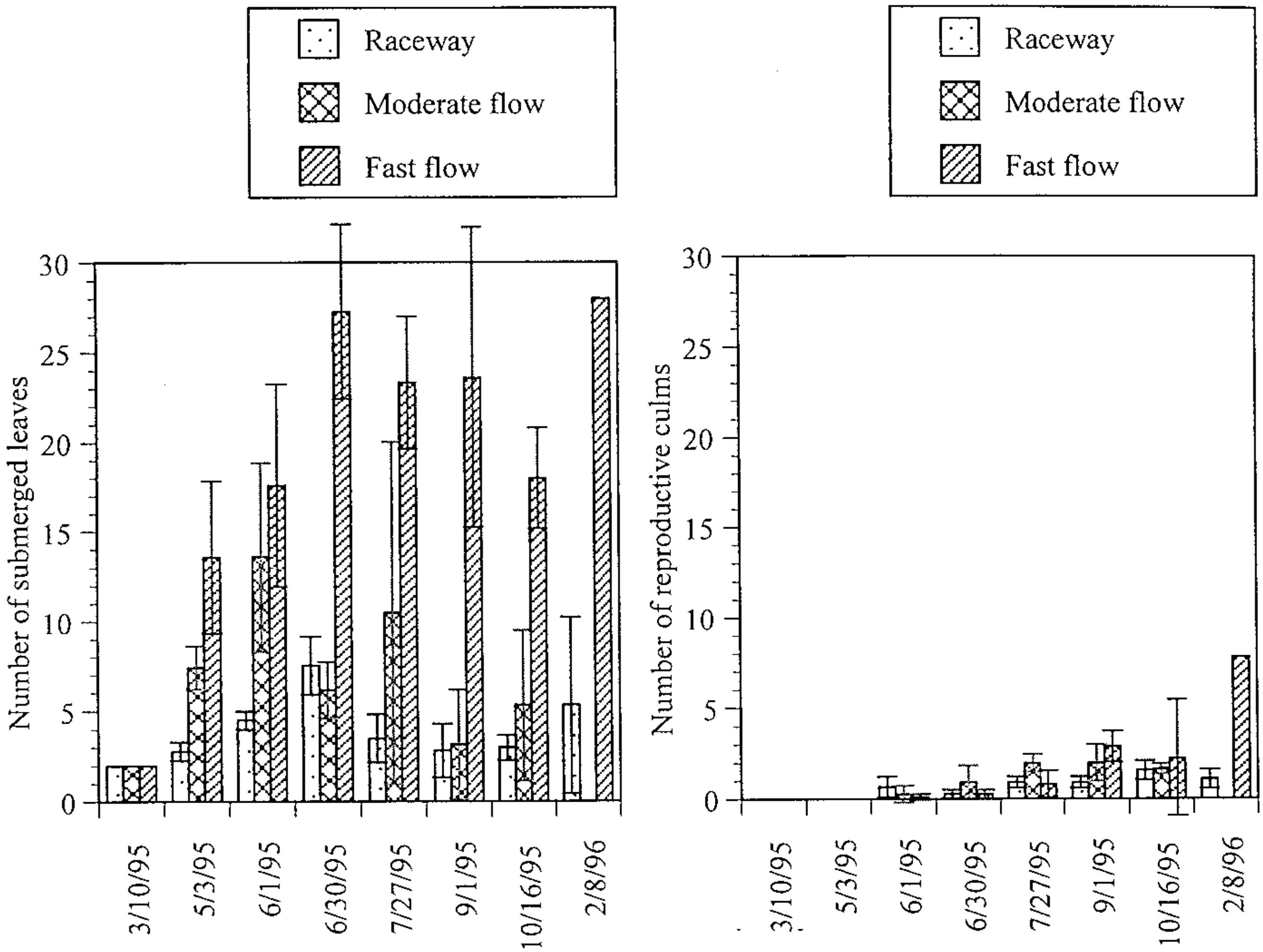

Figure 5-2. Number of leaves and number of reproductive culms for *Zizania texana* grown in three current velocities over one year.

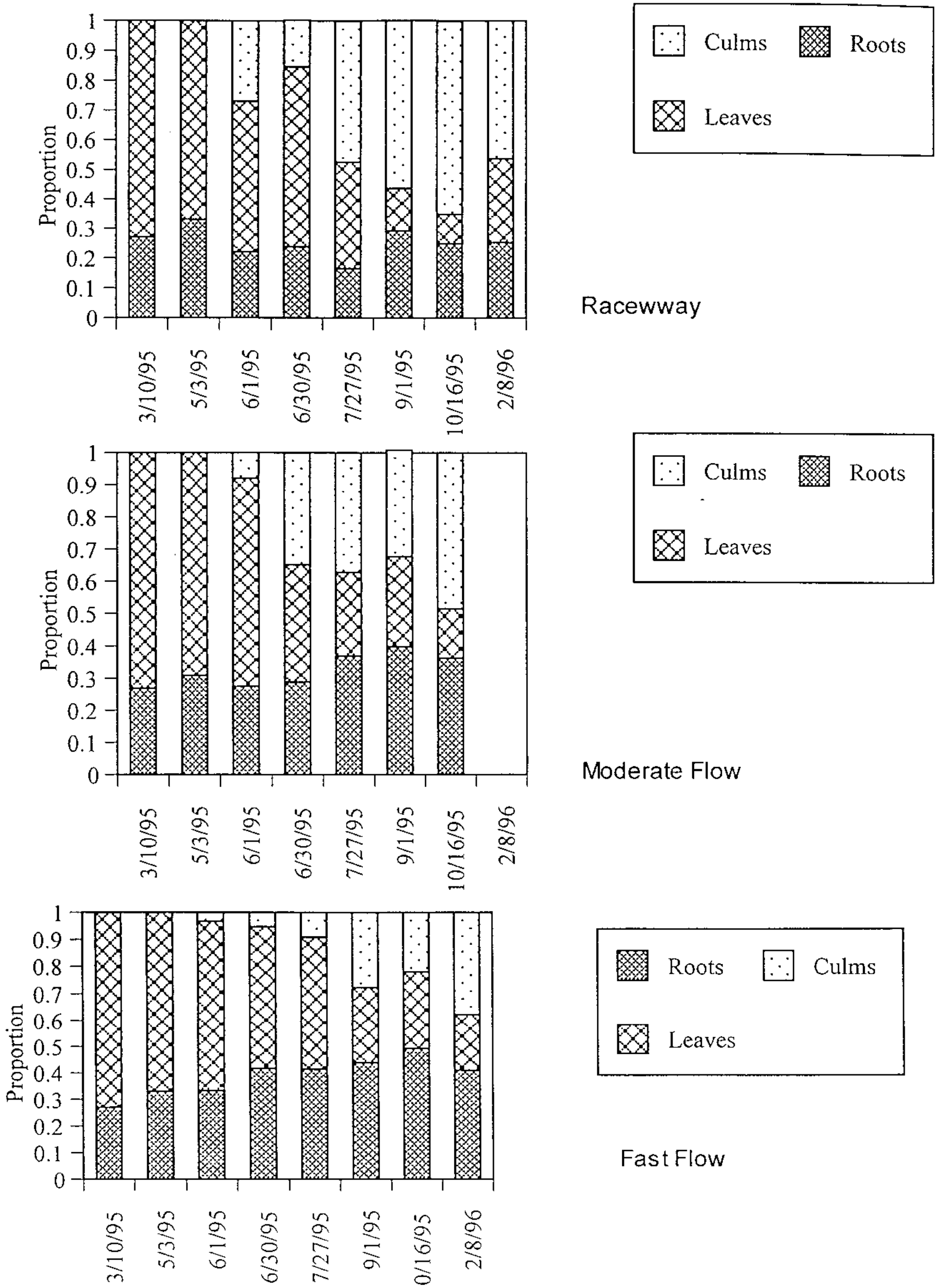

Figure 5-3. Proportion of biomass allocated to roots, leaves and culms for *Zizania texana* grown in three current velocities over one year.

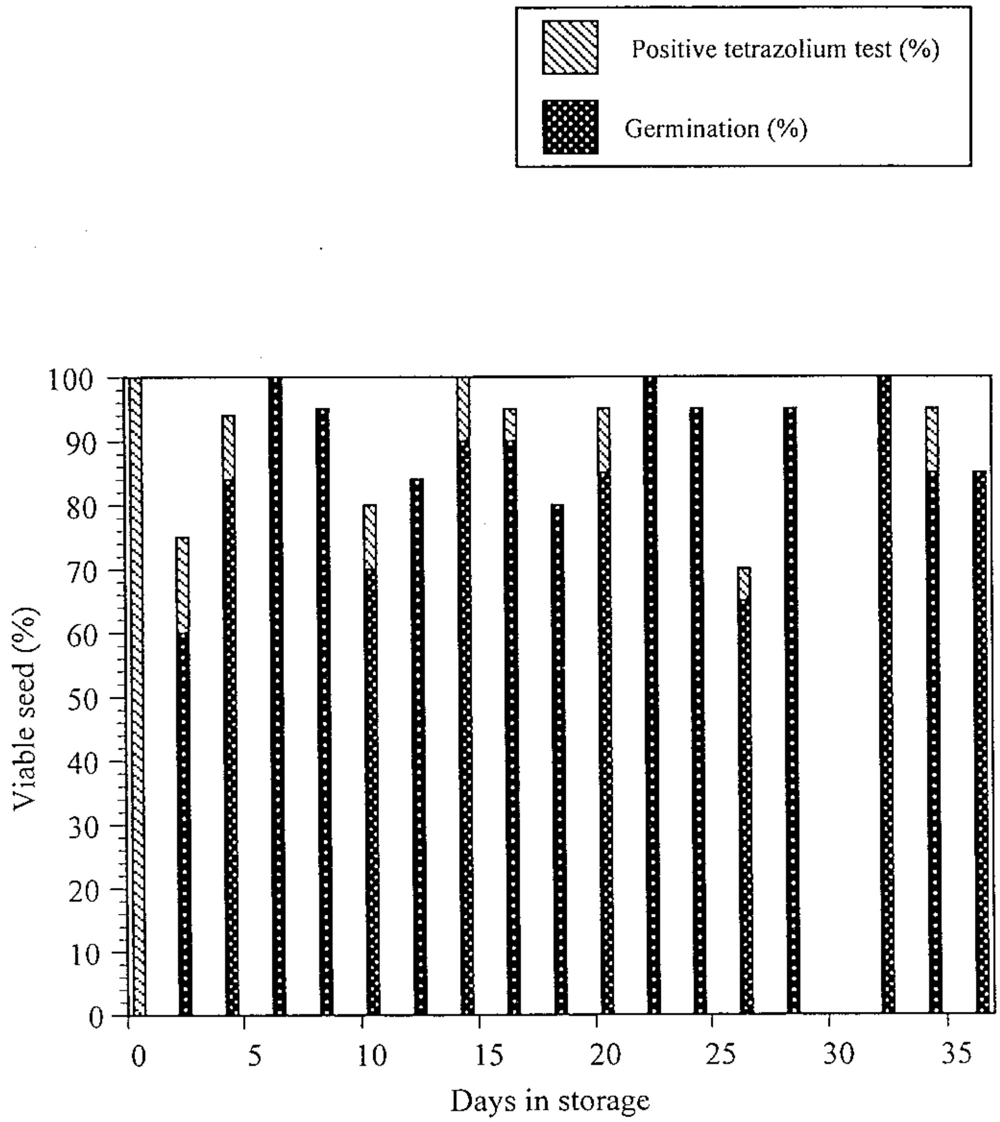

Figure 1-2. Viability of *Z. texana* seeds in storage between 0 and 38 weeks. Viability is determined by positive tetrazolium chloride test and positive germination test. All seeds were stored at 3°C in aerated water.

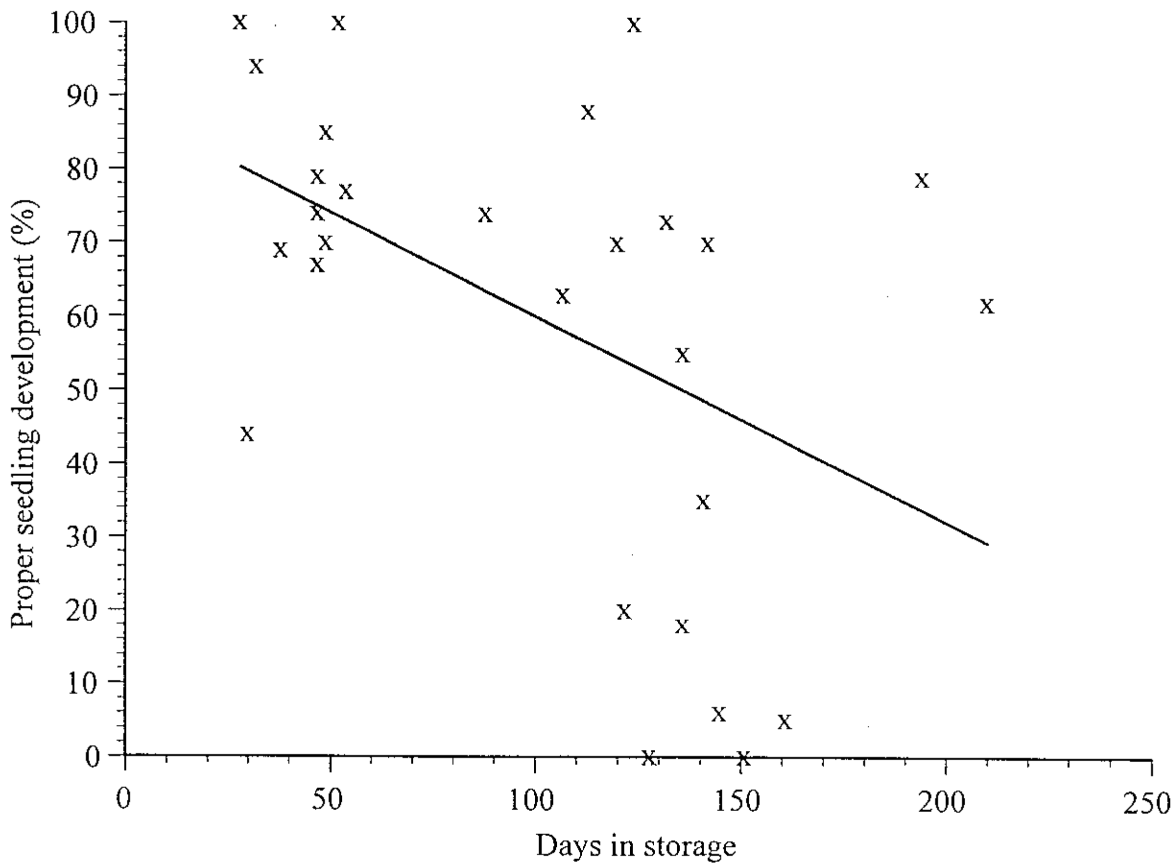

Fig. 1-3. Seedlings with chlorophyll and adventitious roots developed properly. Seeds were stored in aerated deionized water at 3°C. Seeds from 1993 collection were germinated after 28-52 days in storage. Seeds from 1992 collection were germinated after 88-210 days in storage.

CHAPTER 2.

Reintroduction of Texas wildrice in Spring Lake

I. REINTRODUCTION EFFORTS PRIOR TO SECTION 6 PROJECT NO. 38, July 1986-August 1991

Early accounts of the distribution of *Z. texana* were by Watkins (1930), Silveus (1933), and Duval (1940). Watkins (1930) mapped the distribution of *Z. texana* (misidentified as *Z. aquatica*) in Spring Lake. According to Watkin's map, *Z. texana* extended from Spring Lake dam northward up the old river channel approximately 200 m (Figure. 2-1).

Silveus (1933) stated that *Z. texana* "seems to be confined to the artificial lake at the head of the San Marcos River and the irrigation ditches and river some distance below the town of San Marcos." He continued "...the growth is so luxuriant that the irrigation company has great difficulty in keeping the artificial lake and ditches clean."

Emery (1967) was the first to note a population decline. There was "a substantial reduction in its area of dominance and its population size. There is but one plant in Spring Lake... and no specimens of the grass were observed in the first half mile of the river." Furthermore, *Z. texana* "reproduces itself primarily by vegetative means. This method, however, appears unable to maintain the population, and unless the trend can be reversed this species will become extinct." *Zizania texana* was subsequently listed as endangered (U. S. Fish and Wildlife Service, 1985).

Interest in reestablishing *Z. texana* in its native habitat was initiated by Emery (1977). He stated that "repeated attempts to culture the Texas Wild Rice (*sic*) outside its natural habitat have been tried without success. He continued "initial plantings have been successfully established and the planning for a large scale effort during the spring and summer of 1976 is well advanced." In a letter dated September 18, 1978 from Emery to Heber Taylor, Emery concurs that he attempted to grow *Z. texana* in "spring-fed sites in other parts of Texas" and that "he has been successful at New Braunfels, Del Rio, and Saledo." However... "relocated plants are eventually lost."(Attachment 2-1). According to the San Marcos Recovery Plan (U. S. Fish and Wildlife Service, 1984) Emery transplanted more than 100 clones between 1976 and 1982 to other spring-fed sites in central Texas. "Transplanting efforts were unsuccessful and transplantings in Spring Lake were eaten by Nutria." In spite of these set backs, interest in reintroduction persisted.

Paul Fonteyn obtained funding from U. S. Fish and Wildlife to "develop recovery management techniques" (James E. Johnson, Attachment 2-2). This work focused on a *Colocasia esculenta* (Elephant Ear) removal/competition study. During 1987, *Z. texana* grown in peat pots, (but originally collected from a stand immediately upstream from the IH35 bridge) were transplanted into 8 plots along the banks of the San Marcos River adjacent to A. E. Wood State Fish Hatchery. Each plot was divided into three treatment areas. Treatments included 1) removal of *C. esculenta* by hand, 2) removal of *C. esculenta* by herbicide and, 3) an exclosure treatment. Exclosures would allow for the comparison of herbivory on plants outside exclosures with plants inside exclosures. Ten *Z. texana* plants were transplanted in each treatment area. The number of transplanted *Z. texana* totaled 240. Within weeks the San Marcos River flooded and many transplants were lost. Two hundred and seventeen *Z. texana* were planted in October, 1987 to replace those lost. By May 1988 only one plants survived. The surviving plant was protected by an exclosure. During summer and fall of 1988 the single surviving plant was emergent, set seed, then died.

It was nearly impossible to pinpoint what precipitated the loss of 457 *Z. texana* plants in the *C. esculenta* removal experiment. One possibility was allelopathy by *C. esculenta* (release of obnoxious chemical(s) by *C. esculenta* into the sediment which would then inhibit the growth of other plant species).

To test the allelopathy hypothesis, a reciprocal transplant experiment was designed using sediments in which *C. esculenta* had grown. Sediments were collected 1) among *C. esculenta* plants, 2) where *C. esculenta* had been removed by herbicide and 3) where *C. esculenta* had never grown. *Zizania texana* seedlings were placed in the pots containing one of three sediment types and transplanted to 1) the raceway at SWT, 2) where *C. esculenta* had been manually removed and 3) where *C. esculenta* had been removed with herbicide. All plants in the San Marcos River were within exclosures. After 6 weeks, leaf area was recorded. The data showed a site effect but no sediment effect. Average leaf area of plants growing in the raceway in all soil types was 215.2 cm^2. Leaf area of plants growing in all soil types where *C. esculenta* had been manually removed was 6.9 cm^2. Leaf area of plants growing in all soil types where *C. esculenta* had been removed with herbicide was 17.3 cm^2. This eliminated allelopathy by *C. esculenta* as a factor in mortality in the original experiment. It was still unclear however, what techniques and microhabitats were required for reintroduction of *Z. texana* into the San Marcos River. It became evident that a series of exploratory studies in the wild would be necessary before another reintroduction was attempted.

On 9 January 1990, 11 2 month-old seedlings were planted in Spring Lake by a stand of cattails in the "slough" area of the lake. The seedlings were covered with a small hardware cloth cage to protect the plants from crawfish. The top of the cage was about 5 cm below the water surface.

On 20 February 1990, a large exclosure constructed of polyvinyl chloride (PVC) pipe and 2.5 cm wire mesh was placed over the young transplants.

By 12 March 1990, one plant was alive and emergent. Another plant was "chewed in half. The axis was ragged." Power speculated in her field notes: "Could it be that there is herbivory of the soft tissue but once it produces a certain amount of tough tissue, herbivores (crawfish?) won't eat it any more? I need to transplant different age classes to find the survivorship of different age classes 4 wks, 6 months, 1 year."

By 29 May 1990, there was a single terminal inflorescence on a culm approximately 1 m tall on the surviving plant in Spring Lake.

On 19 July 1990 Fonteyn and Power submitted a proposal to TPWD for reintroduction of *Z. texana* into Spring Lake. The proposal called for transplanting *Z. texana* into three microhabitats using transplants of three different ages. Half of the transplants would be in exclosures, half would be outside exclosures. Exclosures would allow for the quantification of herbivory by *Myocaster coypus* and other herbivores. Fonteyn and Power stressed the need for a sexually reproducing population in Spring Lake. "Replenishing Spring Lake and the San

Marcos River with seeds will increase the probability of plants establishing naturally within the lake and river." The proposal was discussed with Jackie Poole. Fonteyn and Power were encouraged to resubmit the proposal with minor revisions the following spring. A revised proposal was submitted and funded.

II. CONTRACT #332-0094

TPWD contract # 332-0094 began on 1 September 1991 and ended on 31 August 1992. The stated contract segment objective was: Establish a population of wildrice in Spring Lake and determine the extent and frequency of nutria damage. Procedures included, 1) plant 50 individuals each of three different age classes (4 week-old, 4 month-old and 1 year-old) of cultivated wildrice in five treatments consisting of three different flow regimes and two different exclosure treatments (no detectable flow/caged, no detectable flow/uncaged, low flow/cage, low flow/uncaged, and rapid flow/uncaged), in Spring Lake and monitor on a weekly basis for nine months for survivorship to determine which age class transplants most successfully; 2) use three exclosures to cover half of the plants at each of the no and low flow treatments to insure statistically meaningful data and monitor plants weekly for 9 months to examine survivorship and the effects of herbivory.

First Reintroduction

SITE DESCRIPTION

The San Marcos River arises from springs emerging from the Edwards Aquifer. The San Marcos springs are one of a series of springs located along the Balcones Fault Line separating the Edwards Plateau to the west and the Blackland Prairie to the east. Approximately 500 m downstream from the San Marcos Springs a dam was built in 1849 and the original river channel was flooded along with its intermittent tributary, Sink Creek. Today, the impounded water in the old river channel and a portion of Sink Creek form Spring Lake (Figure 2-1).

Depth of Spring Lake is controlled by removable boards in the dam. Near spring openings, depth is 6-9.5 m. The old river channel is commonly 3-4.6 m deep. Current velocity is a function of spring flow which is directly related to discharge from the Edwards Aquifer. Spring flow averages 165 cfs with a low of 45 cfs in 1956 (Buckner and Shelbey, 1991) and a high of approximately 400 cfs in 1992.

METHODS AND MATERIALS

Plants were started from seed in the raceway at SWT during March, September and October of 1991 in order to have seedlings in three age classes: 6 weeks, 4 months, and 9 months. Nine month old transplants had gone through a complete growth and flowering cycle and were considered 1 year-old plants. It was necessary to plant many more seedlings than was required by contract because of the reoccurring problem with crawfish destroying *Z. texana* seedlings in the raceway (See Chapter 1).

Plants were transplanted into three locations in Spring Lake. In two sites, half the plants were protected with exclosures. As per contract, there were no exclosures at the dam site because of difficulty securing an exclosure in the current, (Figure 2-1, Table 2-1). There were three replicates for each treatment at each site.

Table 2-1. First reintroduction of *Z. texana* into Spring Lake. The following data are the number, age, and location of transplants before and after 20 December 1991 flood.

	Number of Individuals						
		December 91			January 92		
		Age of transplant			Age of transplant		
	Replicate	6 wks	4 mon.	9 mon.	6 wks	4 mon.	9 mon.
Wetland							
Exclosure	1	10	10	1	7	10	1
	2	10	10	1	0	0	0
	3	10	10	1	0	0	0
No exclosure	1	10	10	1	0	0	0
	2	10	10	1	0	0	0
	3	10	10	1	0	0	0
Intermediate							
Exclosure	1	-	10	3	-	10	3
	2	-	10	3	-	0	0
	3	-	10	3	-	0	0
No exclosure	1	-	10	3	-	5	2
	2	-	10	3	-	10	1
	3	-	10	3	-	0	0
Dam							
No exclosure	1	-	10	3	-	10	0
	2	-	10	3	-	8	0
	3	-	10	3	-	6	0

Exclosures were constructed of 2 cm diameter PVC pipe with 2.5 cm wire mesh. The dimension of the exclosure was 1 m^3. A hinged lid allowed access to plants for observation and measurements. Replicate quadrats without exclosures were marked on four corners with stakes.

At the time of transplanting, ribbon-like submerged *Z. texana* leaves floated on the water surface at the Wetland site. Within 6 days of transplanting all leaves outside exclosures at the wetland site were clipped by herbivores. Submerged leaves were either clipped at the water surface or clipped about 15 cm above the sediment. There was no damage to leaves inside exclosures.

On 20 December 1991, there was flooding in Spring Lake and the San Marcos River. Four of six exclosures washed away. One hundred and twelve of 126 *Z. texana* transplants in the wetland site were lost, 60 of 78 transplants in the intermediate site were lost and 15 of 39 transplants in the dam site were lost (Table 2-1). At this point it was necessary to reassess the experimental design because there were not enough plants available to repeat the entire experiment.

We discussed possible changes to the project with U. S. FWS and TPWD personnel and came to an agreement. On 15 March 1992 a draft interim report was submitted to TPWD and U.S. FWS. The report outlined changes in the experimental design due to 1) flood damage and 2) limited availability of plant material in different age classes (Attachment 2-1). The changes included:

1) one age class (4-6 months)
2) include a growth experiment
3) two replicates for each exclosure treatment

Terrell et al. (1978), Emery (1967, 1977) and Power and Fonteyn noted that San Marcos River populations were primarily submerged with vegetative growth while raceway populations were primarily emergent with reproductive growth but there was no documentation or quantification of this observation. A growth experiment, with three replicates, was added to quantify the relationship between growth pattern and environmental conditions.

It was agreed that two replicates for the exclosure treatment was acceptable. Although two replicates would limit the statistical value of the study, we were limited by available plant material. We felt this was the best possible study given the circumstances. The work undertaken as per the draft interim report provided a basis for future studies which were published in peer review journals (See Chapter 3 and 5 for studies on *Z. texana* in Spring Lake).

Table 2-2. Environmental factors at three sites in Spring Lake where *Z. texana* was transplanted. Data collected in July 1992.

Site	Depth (m)	Velocity (m/s)	D.O. (mg/l)	Temperature (C)	pH	Alkalinity (mg/l)
Wetland n=12	0.31	0	2.19	26.0	7.6	254*
Intermediate n=15	0.76	0.027	5.30	22.7	7.3*	260.6*
Dam n=12	1.61	0.061	5.92	22.5	7.3*	261.2*

*n=3

Second Reintroduction

The relationship between macrohabitat and survivorship, reproduction, productivity, and biomass allocation patterns in *Z. texana* was examined. Three sites were selected in Spring Lake each representing a different habitat type: a wetland habitat, a deep, flowing water habitat, and a habitat intermediate between the two (Figure 2-1) At each site, the depth was recorded, velocity was measured with a Marsh McBirney Model 201 portable water current meter, dissolved oxygen and temperature were measured with an Orion Dissolved Oxygen meter model 840, and alkalinity was determined by titration (Clesceri, et al., 1989). These data were collected in July 1992 (Table 2-2). The wetland habitat was 0.3 m in depth with a velocity of 0 m/s. Dominant vegetation was primarily emergent or floating and included *Typha latifolia* and *Eichornia crassipes*. The intermediate habitat was located in deeper water (0.76m) with a velocity of 0.027 m/s. Vegetation was primarily submerged and included *Cabomba caroliniana*, *Ludwegia repens*, *Myriophyllum spicatum*, *Ceratophyllum demersum* and *Sagittaria platyphylla*. The dam site was 1.6 m in depth, and the velocity was 0.06 m/s. *Hydrilla verticillata* and *S. platyphylla* were the only macrophyte species present.

Table 2-3. Experimental design for second reintroduction of *Z. texana* in Spring Lake. Plants were transplanted between 16 January 1992 and 29 January 1992.

	Replicate	Survivorship and reproduction experiment	Growth Experiment
Wetland			
Exclosure	1	10	30
	2	10	
No exclosure	1	10	
	2	10	
Intermediate			
Exclosure	1	10	30
	2	10	
No exclosure	1	10	
	2	10	
Dam			
No exclosure	1	10	30
	2	8	
	3	6	

METHODS AND MATERIALS

Survivorship and reproduction. Plants were grown from seed collected from the SWT conservation population. Seedlings were potted in September 1991. In January 1992 seedlings were transported to three study sites in Spring lake. At each site, a 1m^3 exclosure constructed of 2.5 cm wire mesh and PVC pipe, was used as a control. Treatment plots, 1 m^2, were marked but left exposed to herbivores. There were two control replicates and two treatment replicates at the Wetland and Intermediate sites. Control and replicate plots each had 10 *Z. texana* transplants. There were three treatment replicates at the Dam site (Table 2-3).

During the study period plants were counted monthly. Terminal inflorescences on emergent culms were tagged weekly using cotton string.

Productivity and Biomass Allocation. In January 1992, 30 seedlings were planted in each site. Each seedling had 6 - 12 leaves and leaves was approximately 40 cm in length. The initial average dry weight of leaves was 1.55 g (Appendix 2-1). Three plants were harvested from each site on 17 March 1992, 27 April 1992 and 8 June 1992 (6 week intervals). The number of nodes with adventitious roots, inflorescences, clipped leaves, chewed leaves, and emergent leaves were recorded. Length of the longest leaf and internode length were measured. Then all plant parts were dried at 40°C and weighed.

Data were analyzed by one-way ANOVA and repeated measures ANOVA (Zar, 1984). Proportional data were arcsine transformed before analysis.

RESULTS

Survivorship and reproduction. At the wetland site, survivorship was 0 % outside exclosures within one month of transplanting (Appendix 2-2). Plants in the wetland site were either lost due to herbivory by crawfish, *M. coypus* or waterfowl; or uprooted by turtles. See Appendix 2-3 for description of herbivory in field notes. Survivorship inside exclosures was 55% (11 of 20 plants) after 4 months and dropped to 0 % after 1 year in the wetland site (Table 2-4). Mortality within exclosures was likely due to failure to thrive from transplant shock, environmental conditions, or crawfish chewing young leaves.

In the intermediate site, survivorship inside exclosures was 70 % (14 of 20 plants) after four months. Outside exclosures survivorship was 40% (8 of 20 plants). In one replicate quadrate ~~but~~ all plants were lost within one month of transplanting. Plants were transplanted into a slightly different substrate (sandy) than the other quadrats (soft mud) and mortality most likely was due to edaphic factors (Appendix 2-2).

Survivorship at the dam site was 92% after 3 months. Five years after transplanting plants were present in this site (Table 2-4).

Between 21 February and 7 July 1992 plants within exclosures in the wetland site produced 222 terminal inflorescences. During the same time period 14 plants in exclosures in the intermediate produced 104 inflorescences (Appendix 2-4). Flowers on these inflorescences were pollinated and set seed (estimated >1,250 seeds in the wetland site and >470 seeds in the intermediate site). This was the first recorded observation of sexually reproducing *Z. texana* in the wild since Duvall reported briefly on *Z. texana* in his thesis (1940).

One inflorescence was observed outside exclosures in the intermediate site on 26 May 92, however the inflorescence was clipped before seed set. Five emergent culms were observed in the intermediate site (clipped on 1 June 1992 before seed set) and 3 emergent culms were observed in the dam site (clipped 20 May 1992 before seed set).

Productivity and Biomass Allocation

Plants in the Wetland, Intermediate and Dam site increased in size during the experiment between January 1992 and June 1992, but differences were not significant in any of the characteristics measured (Table 2-5, 2-6). Resource allocation patterns was examined using plants from the final harvest. There was a significant difference in allocation patterns among sites (Figure 2-2). Biomass allocated to reproductive culms ($p<0.0001$), emergent leaves ($p<0.0001$), and submerged leaves ($p<0.0004$) was significantly different. There was no

Table 2-4. *Zizania texana* was transplanted into five sites in Spring Lake between 1991 and 1995. Plant density decreased each year after transplanting. E=exclosure, OE=outside exclosure; final=last monitoring day under contract.

	Plants/m^2						
Time after planting (months)	Wetland E	Wetland OE	Intermediate E	Intermediate OE	Dam	Springs	Spillway
0	10	10	10	10	8	12	10
1	9.5	0	9	5	8	-	-
2	9.5	0	9	4	8	-	-
3	6	0	7	4	7	-	-
4	5	0	7	4	-	-	-
6	-		-	-	-	1.4	-
8	-		-	-	-	2	-
11	0			-	-	-	9.2
14				-	-	3.2	-
15				-	-	-	3.1
17				-	-	4.2	-
18				-	-	-	1.7
20				-	-	0.4	-
21				-	-	-	2.1(final)
23				-	-	1.6	
24				-	-	0	
37				0.4	-		
38				-	1.2		
41				0.4	-		
42				0	1		
44					4.8		
47					2.4		
55					2.1		
58					0.5		
61					0.1 (final)		

difference in seeds, inflorescences, tiller roots, or roots.

Plants concentrated biomass in either reproductive or vegetative parts. Plants in the Wetland site allocated more biomass to reproductive biomass than the other sites, while plants in the Dam site allocated more biomass to submerged leaves.

Table 2-5. Productivity and reproductive activity by *Z. texana* in three sites in Spring Lake. Plants were harvested on three dates, 17 March 1992, 27 April 1992 and 8 June 1992.

Site	Date	#Submerged leaves	#Culms	#Nodes/ culm (tillers)	#Rooting nodes	#Emergent leaves	Internode length (cm)	#Inflorescences	Longest leaf length (m)	#Clipped leaves	#Chewed leaves
Wetland											
	17 March 1992										
Mean		14.3	6.7	-	0	-	-	0	-	10.7	6.7
sd		(4.9)	(3.1)							(6.1)	(3.1)
	27 April 1992										
Mean		15.3	6.3	3.5	1.5	-	6.0	0	0.75	13	1.7
sd		(9.1)	(2.5)	(3.5)	(0.7)		(0.9)		(0.27)	(7.8)	(0.6)
	8 June 1992										
Mean		3	4	3.7	0.8	5	10.1	1.3	-	6.7	1.7
sd		(2.6)	(3)	(2.3)	(1.2)	(1.7)	(4.1)	(1.5)		(5.5)	(0.6)
Intermediate											
	17 March 1992										
Mean		20	8	-	0	-	-	0	-	5.7	2
sd		(2.0)	(1.7)							(1.1)	(1)
	27 April 1992										
Mean		56.7	21.3	1.3	0.3	-	13.1	0	1.4	2.5	0.5
sd		(20.1)	(7.5)	(1.1)	(0.6)		(8.1)		(0.2)	(0.7)	(0.7)
	9 June 1992										
Mean		78	16.3	4.0	0.8	35.7	14.8	2.3	1.56	15.0	8.3
sd		(48)	(13.6)	(1.7)	(0.8)	(21.1)	(5.6)	(1.1)	(0.2)	(16.8)	(13.6)
Dam											
	17 March 1992										
Mean		53	22.7	-	0	-	-	0	-	4.3	2
sd		(28)	(9.9)							(2.1)	(1.7)
	28 April 1992										
Mean		95.7	39.3	1.5	0	-	17.5	0	1.91	-	-
sd		(47.4)	(22.1)	(0.7)			(6.1)		(0.12)		
	9 June 1992										
Mean		166	21.7	3.5	0.7	0	19.2	0	3.25	51.3	2
sd		(186.5)	(20.6)	(2.0)	(0.8)		(7.7)		(0.1)	(47.1)	(2)

Table 2-6. Productivity and biomass allocation by *Z. texana* grown in three sites in Spring Lake. Plants were harvested on three separate occasions.

Site	Date		Roots (g)	Tiller roots (g)	Submerged leaves and reproductive culms (g)	Inflorescences (g)	Seeds (g)	Total (g)
Wetland								
	17 March 1992							
		Mean	0.34	0	2.48	0	0	2.82
		sd	(0.20)		(2.00)			(2.17)
	4 May 1992							
		Mean	0.41	0.21	2.38	0	0	3.01
		sd	(0.02)	(0.37)	(1.69)			(2.03)
	12 June		0.77	0.38	11.48			
Intermediate			(0.54)	(0.44)				
	17 March 1992							
		Mean	0.44	0	2.28	0	0	2.72
		sd	(0.02)		(0.72)			(0.71)
	4 May 1992							
		Mean	1.60	0.01	10.99	0	0	12.59
		sd	(0.83)	(0.01)	(4.11)			(4.59)
Dam								
	17 March 1992							
		Mean	1.21	0	9.69	0	0	10.9
		sd	(0.37)		(2.68)			(2.5)
	4 May 1992							
		Mean	3.07	0.001	14.41	0	0	28.38
		sd	(1.93)	(0.001)	(14.61)			(16.29)

continued next page

Table 2-6. Continued.

Date	Site		Roots (g)	Tiller roots (g)	Submerged leaves (g)	Emergent leaves (g)	Culms (g)	Inflorescences (g)	Seeds (g)	Total (g)
12 June 1992										
	Wetland									
		Mean	0.77	0.38	0.56	2.86	8.07	0.17	0.12	12.94
		sd	(0.54)	(0.44)	(0.65)	(0.48)	(0.47)	(0.25)	(0.21)	(3.41)
	Intermediate									
		Mean	5.37	0.65	13.14	5.31	23.85	0.85	0.14	49.31
		sd	(0.09)	(0.43)	(0.06)	(2.55)	(13.02)	(0.32)	(0.15)	(27.12)
	Dam									
		Mean	5.68	1.99	58.27	0	41.73	0	0	107.34
		sd	(7.97)	(2.40)	(55.59)		(42.32)			(108.50)

DISCUSSION

Survivorship and Reproduction. Plants outside exclosures at the wetland site did not survive. Herbivory was the primary cause of mortality.

Survivorship outside exclosures was 40% at the intermediate site and 92% at the dam site (Table 2-4). Because water depth, current velocity and surrounding vegetation were greater in the intermediate and dam site than the wetland site, submerged leaves were less accessible to waterfowl and nutria, thereby gaining some protection from herbivores. Mortality at the intermediate site was due to edaphic considerations. Plants transplanted into sandy sediments did not become established; those transplanted into fine (muddy) sediments survived 41 months.

Surviving plants outside exclosures produced emergent culms but culms were clipped before floral development occurred Swans, muscovy ducks, mallards and nutria were observed at the intermediate and dam site. Nutria tended to damage many leaves in a single visit by chewing the leaf, separating it from the body of the plant and leaving most of the damaged leaf floating in the water. This pattern was observed a number of times in the raceway at SWT (see Chapter 1) and in the San Marcos River in 1989. Waterfowl clipped individual culms and leaves immediately below the water line.

Plants inside exclosures at the wetland site and intermediate site had a higher chance of survival than plants outside exclosures. Those which survived inside exclosures produced terminal emergent inflorescences and set seed (Appendix 2-4). Seeds were dispersed into Spring Lake. No seedling were observed at the wetland site, however at least one seedling was observed at the intermediate site.

Productivity and Biomass Allocation

Plants in all sites increased in size but the change was not significant. I feel this was an artifact of a small sample size. In a later experiment, I increased sample size and harvested plants over a longer period of time. In the later experiment, there were significant differences in plant size and resource allocation patterns between sites and over time. See Chapter 5 for a description and discussion of the later experiment.

Plants had significantly different resource allocation patterns (Figure 2-2). Wetland plants lost most of their submerged leaves by the final harvest and over 70% of biomass was allocated to reproductive culms and emergent leaves. *Zizania texana* is a CO_2 user and it is likely submerged leaves are CO_2 stressed in a wetland environment while emergent plant parts can readily take up CO_2 from the atmosphere. The shift in resources from vegetative to reproductive may very well be a stress response. In contrast, plants in the Dam site allocated about 50% of resources to submerged leaves. Flowing water in the dam site delivered a continuous supply of CO_2 to submerged leaves. For a detailed description and discussion of CO_2 use by *Z. texana* see Chapter 4.

III. CONTRACT #33-0201

The stated objective for contract #33-0201 was to establish a population of wildrice in Spring Lake. The procedures included, 1) establish at least a 20 square meter plot of wildrice in Spring Lake at a density of six plants per square meter for a total of at least 120 plants and, 2) develop and utilize methods to protect reintroduced plants from herbivory.

During FY92-93 the dam site was selected for establishment of an additional plot of *Z. texana*. Plants, grown from seed in peat pots in the raceway at SWT, were transplanted into the dam site at a density of nine plants/m^2 by gently pressing the transplants, including peat pot, by hand, into the sediments. Transplants were approximately 3 months old. Leaf length was approximately 0.75 m (n=8). Transplanting took place on three dates, 15 December 1992, 31 March 1993 and 14 July 1993. Field assistants included two students certified in the use of SCUBA gear, and Gena Janssen and David Hernandez of Texas Parks and Wildlife Department.

During FY92-93, a total of 183 young *Z. texana* plants were transplanted into Spring Lake. The plants occupied an area approximately 27 m in length. Width varied from 0.5m to 12m. Areal coverage was approximately 30m^2. The Spring Lake population was equivalent to 2% of the estimated wild population (Texas Parks and Wildlife Department, 1990).

A floating exclosure, constructed of PVC pipe and 2.5 cm wire mesh with styrofoam blocks attached at the base, was designed to protect emergent inflorescences from herbivory without interfering with current velocity. Two exclosures were placed directly over *Z. texana* and attached to trees along the bank with rope. Exclosures were in place during spring and summer 1993.

Aquarena Center (formerly Aquarena Springs) cut aquatic vegetation regularly (about weekly; daily during summer months). Although cutter/harvester equipment was used, floating and drifting vegetation was released downstream. The vegetation became entangled in stationary objects, forming large mats. The floating exclosure protected emerging culms from herbivores, but drifting vegetation became entangled in *Z. texana* and pushed emerging culms under water, impeding floral development and preventing pollination (Power, 1997). It became apparent from this study that drifting mats interfered with sexual reproduction in *Z. texana*. The effects of vegetation mats on *Z. texana* was studied during FY95-96 (see Chapter 7). This work was published in Southwestern Naturalist (reprint attached).

Another problem associated with the reintroduction effort was *Hydrilla* / *Z. texana* interactions. Power noted on 14 July 1993 that "plants planted in March were not very successful. They were overtaken by *Hydrilla*. Plants must be tended regularly (competitors removed and cut vegetation cleared" (Appendix 2-3). Hydrilla / *Z. texana* interactions require further study.

On 27 May 1993, Gena Janssen (TPWD), Gene Sultanfus and Keith Wall (Aquarena Springs Resort) and Paula Power met at Aquarena Center and agreed to plant additional *Z. texana* plants near spring openings by the ferry dock on the northwest side of the lake.

IV. CONTRACT #434-0198 FY93-94

The stated objective for contract # 434-0198 was to reintroduce an additional plot of Texas wildrice in Spring Lake.

INTRODUCTION

During FY 93-94 *Z. texana* was transplanted into two sites, one on the northwest side of the lake near spring openings where CO_2 content was greatest (Appendix 2-7)(see Chapter 4 for a discussion of carbon use by *Z. texana*) and a second site was located near the spillway where flow was greatest (see Chapter 3, 5 and Power, 1996a reprint attached).

Field assistants equipped with SCUBA gear prepared the spring site for transplanting by hand-pulling vegetation on 14 September 1993. Pulled vegetation included *Ceratophyllum demersum* and *Hydrilla verticellata*. On six separate occasions field assistants assisted in planting a total of 410 *Z. texana* transplants at the spring site as per methods from the previous year. A 1 m^2 quadrat constructed of PVC pipe was used as a guide for transplanting *Z. texana* at a density of 10 plants/m^2. Water depth ranged from 2.3m - 4.3m. Current velocity ranged from 0.030 m/s - 0.079 m/s (Table 2-7).

Table 2-7. Ranges for light, soil characteristics, water depth, and current velocity found in *Z. texana* reintroduction sites in Spring Lake. Wt = wetland, In = intermediate, Dm = dam, Sp = spring, Sw = spillway. Observations and measurements were collected between 1992 and 1995; + = presence; O = absence.

	Wt	In	Dm	Sp	Sw
Light	Full sun	Full sun	Partial sun	Full sun	Partial sun
Sediment	Soft mud	Soft mud	Soft mud	Soft mud	Mud/sand/gravel
Water depth (m)	0.31	0.76	0.91 - 2.04	2.3 - 4.3	0.70 - 1.13
Current velocity (m/s)	0	0.03	0.03 - 0.08	0.03 - 0.04	0.02 - 0.34
Presence/absence	O	O	+	O	+

On 22 July 1994, 100 transplants were planted in the spillway site (Figure 2-1). Water depth ranged from 0.70 m - 1.13 m. Current velocity ranged from 0.088 m/s - 0.345 m/s.

Although not specifically outlined in the contract, plants were monitored seasonally in the wetland, intermediate, dam, and spring site. Two belt transects, 0.25m x 10m, were placed parallel to the current in each site. The number of plants intersecting the transect was recorded. From these data, plant density was calculated. The first four plants intersecting the transect were measured The following characteristics were indicators of health and vigor: 1) basal area, 2) stem density, 3) leaf length. Basal area was determined as follows: basal length and width were measured, then basal area was calculated using the formula for the area of a circle, r^2 or the area of an ellipse (minor *r*)(major *r*). Stem density was determined by slipping a three sided 10cm x 10cm frame around or into the base of the plant. Stems within the frame were counted (#stems/100cm^2). Leaf length was determined by calculating the average length of six randomly selected leaves/individual. Presence of culms was recorded as a measure of sexual reproductive effort. Presence of tillers was recorded as a measure of asexual reproductive effort.

Monitoring data are discussed in Section VI Contract #336-0225 FY95-96

Problems associated with transplants at the dam site and spring site included 1) *Hydrilla* and *C. demersum* growing among plants and apparently competing for light, nutrients and space, and 2) drifting vegetation entangled in *Z. texana* leaves.

V. CONTRACT #335-0198

The stated objective for contract #335-0198 was to continue the seasonal monitoring and data compilation on the reintroduction plots of wildrice in Spring Lake. No specific procedures were outlined.

During FY94-95, 160 *Z. texana* plants were transplanted into Spring Lake at the spillway site. Planting density was 9 plants/m^2.

Plants in all sites were monitored quarterly as per previous description. Monitoring data is discussed in Section VI Contract #336-0225 FY95-96

Problems associated with transplants at the spring site included silt on leaves and *C. demersum* entangled in submerged leaves.

VI. CONTRACT #336-0225 FY95-96

The stated objective for contract #336-0225 was to continue seasonal monitoring and data compilation on the reintroduction plots of Texas wildrice in Spring Lake. There were no stated procedures.

During FY 95-96, 70 *Z. texana* plants were added to the spillway site.

The following is results and discussion of monitoring data collected between 1993 and 1996 with data on transplant efforts between 1991 and 1993 included.

Zizania texana was transplanted into five sites in Spring Lake between 1991 and 1995. Transplants survived no more than 11 months in the wetland site, 42 months in the intermediate site and 23 months in the spring site. As of the date of this report, plants survived eight years in the dam site and six years in the spillway site.

Plant density decreased in all sites over time (Table 2-4). A number of factors may have been responsible for the loss of plants including transplant shock, drifting vegetation, herbivory, or poor environmental conditions (current, sediment). It is unlikely plants were lost due to intraspecific competition.

Stem density increased in all sites after transplanting indicating plant growth, then dropped to 0 in the intermediate and spring sites (Figure 2-3). In the dam site stem density ranged from 13 - 29 stems/100cm^2 during the monitoring period. In the spillway site, stem density ranged from 15 to 29 stems/100cm^2. Stem density in wild stands has reached 69.7 stems/100cm^2 (Power, 1996b). Current velocity appears to a critical factor influencing stem density both in this reintroduction effort (Table 2-5 and 2-7) and wild stands (Power, 1996b). In study after study, there is a positive relationship between current velocity and productivity (measured as photosynthesis, see Chapter 4; biomass, see Chapter 3and 5; or stem density, see Chapter 7). Of all characteristics measured, stem density was the character which most accurately represented the health of the plant.

Leaf length was not a measure of future success of the plant. Leaves tended to be longer in deeper sites and shorter in shallower site (Figure 2-4). Plants often sustained leaf damage. Damage to leaves and stems was due, in part, to floating and drifting mats of aquatic vegetation, which was mechanically cut upstream by Aquarena Center (formerly Aqurena Springs). Despite attempts to harvest cuttings, some cuttings drifted downstream. The cuttings became entangled in *Z. texana* leaves forming large floating mats. The mats slowed current velocity, blocked sunlight and shredded *Z. texana* leaves (See Chapter 7).

During 1995 and 1996, culms from each plant were counted and then followed both by hand and visually to the terminal end. Many culms were still in early stages of development and were submerged. Pooling data from three monitoring dates, 36% of culms were clipped at the dam site and 13% of culms were clipped at the spillway site. I believe the culms were clipped after emergence and the unclipped culms were still developing and had yet to reach the surface. It was very rare to see emergent culms at either site unless protected by an exclosure (See Contract #33-0201, p. 38-39). Based on the clipping pattern and observation, I believe resident waterfowl were the herbivore responsible for clipping *Z. texana* culms.

Reproductive failure has been noted many times as a serious threat to the survival of Z. texana, but there has been no documentation of the cause of reproductive failure (Emery, 1967, 1977, Terrell et al., 1978, U.S. Fish and Wildlife Service, 1984). This is the first study to identify herbivory as a contributor to reproductive failure. With the loss of reproductive culms to herbivory, *Z. texana* transplants are limited to clonal reproduction.

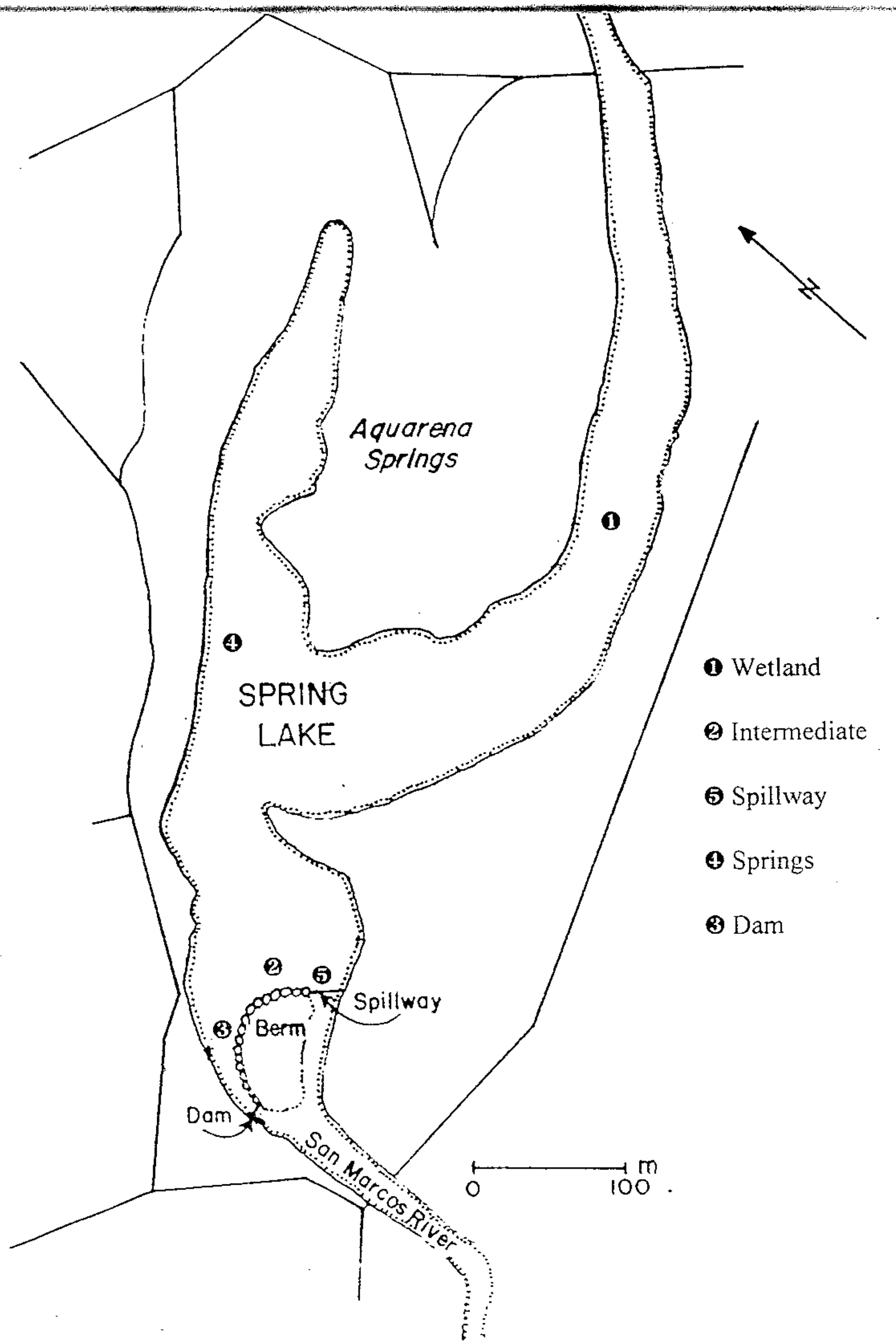

Figure 2-1. Spring Lake, San Marcos, Hays County, Texas. *Zizania texana* was reintroduced into Spring Lake in five sites between 1991 and 1995. Sites were Wetland, Intermediate, Springs, Spillway and Dam sites. Transplants have persisted in Dam site and Spillway site.

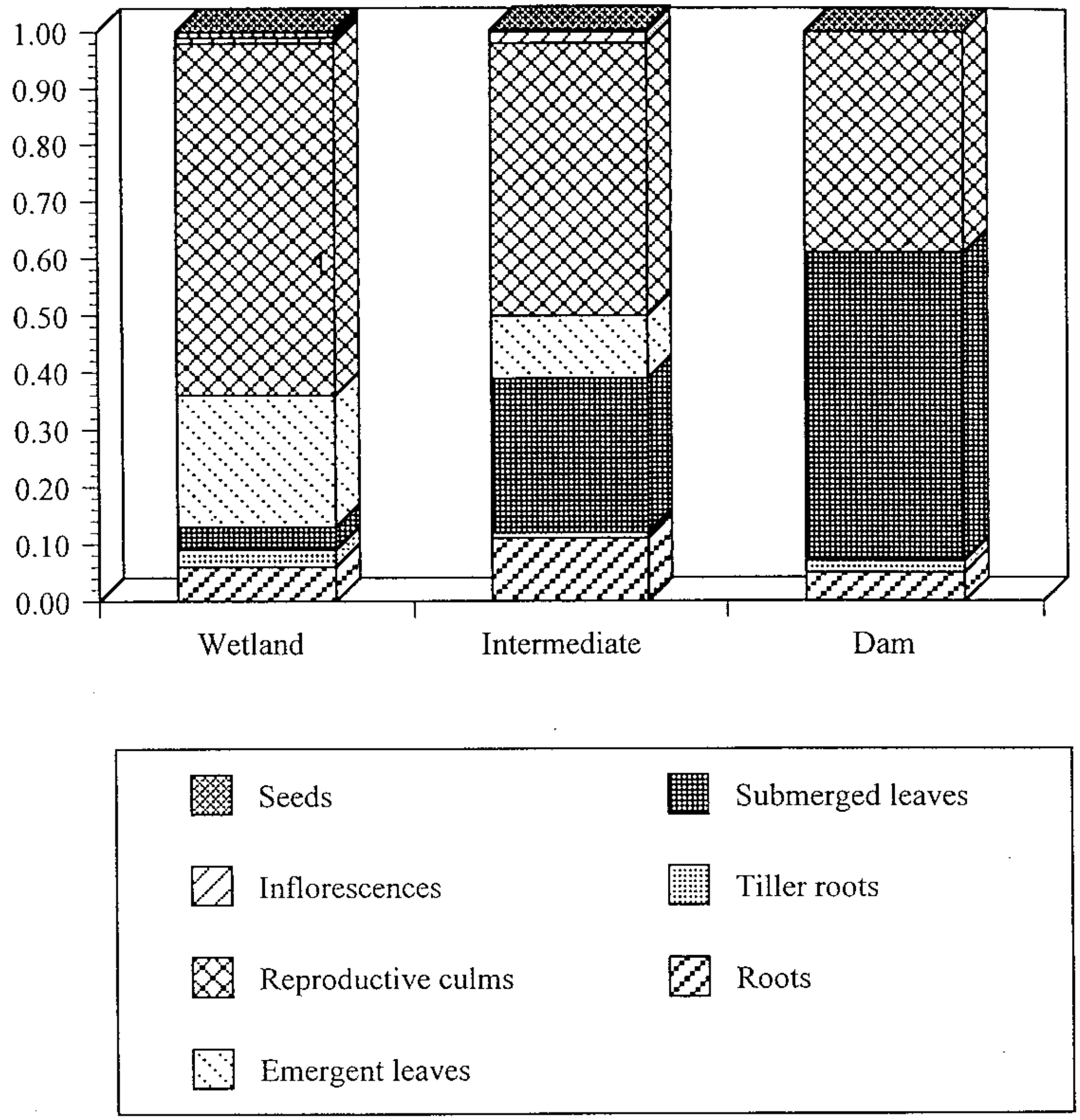

Figure 2-2. Biomass allocation in *Zizania texana* when grown in three current velocities in Spring Lake. Plants were grown for 6 weeks. There was a signifcant difference in biomass allocated to reproductive culms and submerged leaves among sites ($p<0.0001$) and significantly less biomass allocated to emergent leaves in the Dam site when compared wtih the Wetland and Intermediate site ($p=0.004$).

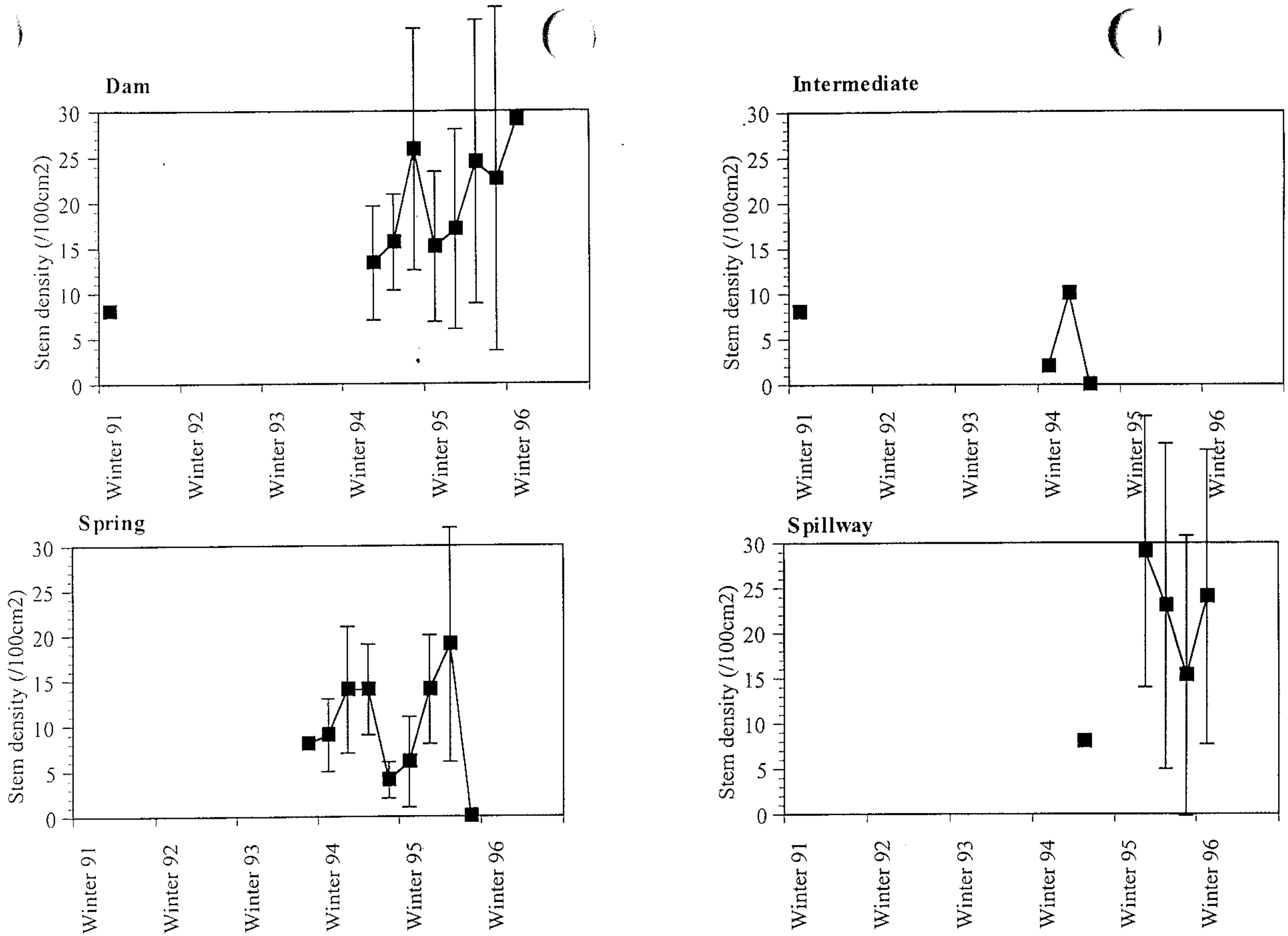

Figure 2-3. Stem density of *Zizania texana* grown in four sites in Spring Lake. Plants in the Spillway and Dam site increased in stem density and persisted until the present (February 2000). Plants in Spring and Intermediate site eventually died.

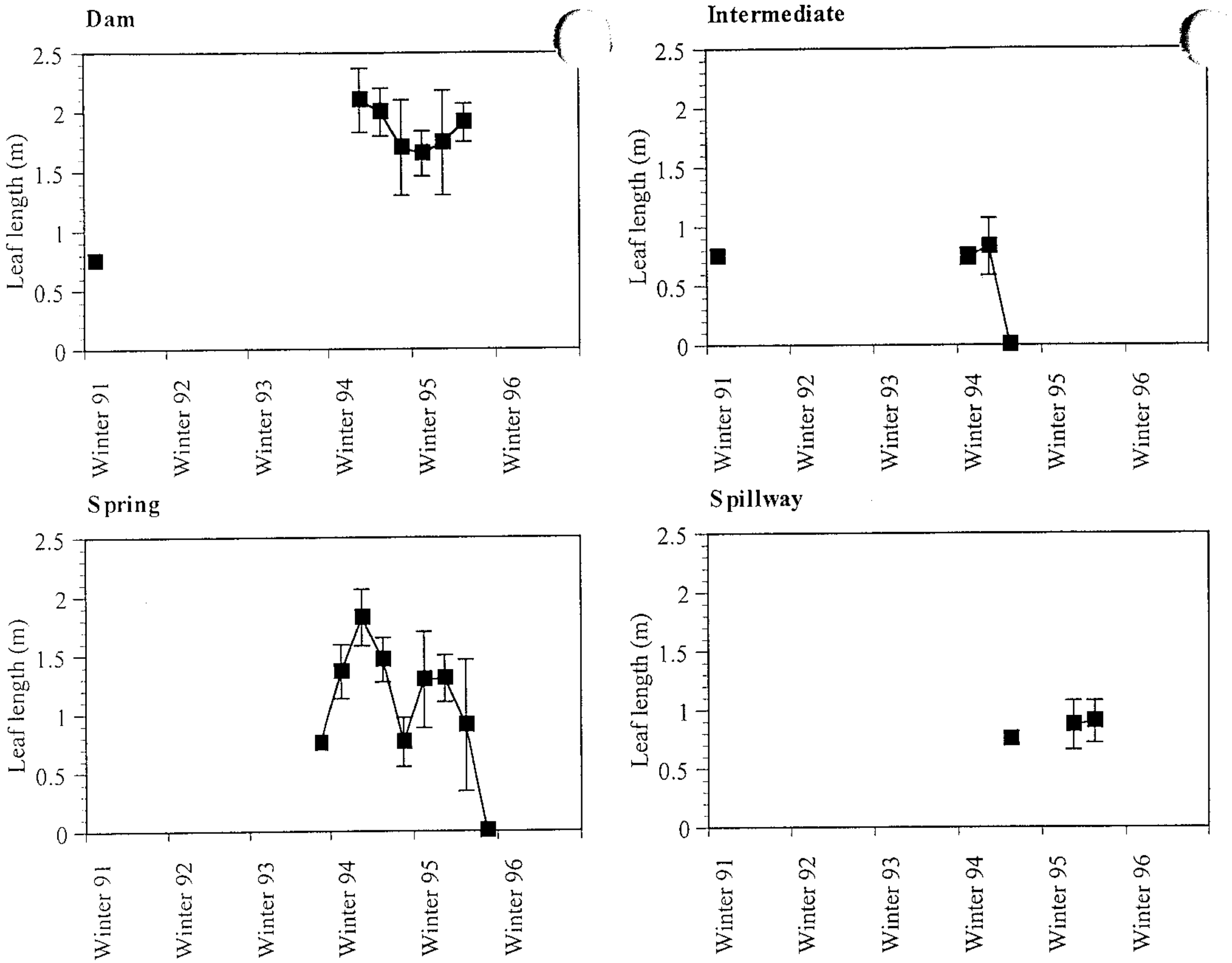

Figure 2-4. Leaf length of *Zizania texana* when grown in four sites in Spring Lake. There appeared to be an inverse relationship between leaf length and water depth. Survivorship of plants was not a function of water depth.

CHAPTER 3.

Effects of Flow and Substrate on Productivity

I. CONTRACT #434-0198

The stated objective for contract #434-0198 was to investigate to what extent velocity and substrate influence productivity and resource allocation patterns in wildrice when grown at a constant depth. There were no state procedures.

INTRODUCTION

Macrophyte growth in streams is influenced by the surrounding environment and the presence of macrophpytes can alter the habitat in which the macrophytes occurr. For example low to moderate current velocities have a positive effect on metabolism by delivering nutrients, including CO_2, to the boundary layer surrounding submerged leaves (Westlake, 1967; Smith and Walker, 1980). In addition to the water in which macrophytes occur, sediments also are a source of nutrients for rooted macrophytes (Chambers, et al., 1989). Fine sediments tend to be more fertile than coarse sediments and plants grown on fine sediments tend to be more productive than those grown on coarse sediments (Barko and Smart, 1978, 1979).

Rooted macrophytes can alter stream microhabitat characteristics. Macrophyte beds alter flow characteristics, provide more substrate surface area and have more fine particle sediments when compared with unvegetated areas (Gregg and Rose, 1982; Losee and Wetzel, 1993).

Zizania texana , an endangered emergent macrophyte in the family Poaceae, has long ribbon-like submerged leaves and emergent reproductive culms (Silveus, 1933). Plants typically form dense stands in fast flowing water and only occur in the first 2.4 km of the San Marcos River, Hays Co., Texas (Terrell et al., 1978). The upper reaches of the San Marcos River are characterized by numerous riffles and pools; its headwaters are springs arising from the Edwards Aquifer. *Zizania texana* is endangered, in part, because of its limited distribution and a significant population decline that occurred between 1940 and 1967 (Devall, 1940; Emery, 1967 and US fish and Wildlife Service, 1994). At present, there have been few ecological studies on *Z. texana.* Terrell et al. (1978) provide descriptive observations on the ecology of *Z. texana* and Power and Fonteyn (1995) investigated seed germination and seedling growth on fine and coarse sediments; but there have been no manipulative field studies investigating the response of *Z. texana* to specific environmental conditions.

Due to anthropogenic effects, current velocity and substrate composition in the San Marcos River are changing. Current velocity is slower due to overpumping of the Edwards Aquifer and the construction of three dams on the San Marcos River within the historic range of *Z. texana.* During the 1970's, five retention dams were constructed for flood control purposes in the upper San Marcos watershed. The retention dams have altered the frequency and magnitude of historic flooding cycles and resulted in increased sedimentation in the San Marcos River (US Fish and Wildlife Service, 1994). The effects of such historic changes in the physical characteristics of the San Marcos River on the decline of *Z. texana* population is not known.

The purpose of this study is to quantify and compare the growth response of *Z. texana* under field conditions at different flow regimes and sediment compositions.

METHODS AND MATERIALS

Three sites of similar depth (0.76-0.82 m) but different current velocity were selected near the spillway in Spring Lake, part of the historic habitat of *Z. texana*. Depth at the study site was similar to depth of wild *Z. texana* stands. Current velocity was measured with a Marsh-McBirney portable water current meter Model 201 at a depth at which submerged leaves genticulate with the current. Velocity ranged from 0.40-0.49 m/s in the fast site, 0.12-0.24 m/s in the moderate site, and 0.05-0.12 m/s in the slow site.

Coarse and fine sediments were collected from the San Marcos River near wild *Z. texana* stands. Each sediment type was thoroughly mixed and a subsample was analyzed for Kjeldhal nitrogen, phosphorus, texture and organic matter content.

Sediments were placed in 16 cm pots along with one germinated *Z. texana* seed. Seedlings were allowed to grow and become rooted for five weeks in an outdoor cement raceway on the Southwest Texas State University campus. Pots were then transported and planted in each of the three different current velocity sites in Spring Lake. There were three replicate plots in each site containing three to five pots of each sediment treatment. Plants were protected from herbivory by waterfowl with 1 m^2 floating exclosures constructed from polyvinyl chloride pipe, wire mesh, and styrofoam blocks.

After six weeks, plants were harvested. Plants badly damaged by herbivory were discarded. The number of stems (i.e. basal leaves plus reproductive culms) and leaf length were recorded. Plants were dried and aboveground parts were weighed.

Table 3-1. Texture, organic matter content, Kjeldahl N, and phosphorus concentration of sandy clay and gravel collected in the San Marcos River, Hays Co., Texas.

	Sandy clay	Gravel
Texture (%)		
Sand	56	16
Silt	23	2
Clay	22	3
Gravel	0	79
Organic Matter (%)	3.9	0.32
Nutrient (mg/kg)		
Kjeldahl N	1517	71
Phosphorus	47	15
pH	7.8	8.5

Data were analyzed by factorial ANOVA (Wilkinson, 1989) followed by a Tukey multiple comparison (Zar, 1984).

RESULTS

Textural analysis indicated sediments were sandy clay or gravel (Table 3-1). Sandy clay had greater organic matter content than gravel.

Plants in all treatments increased in mass and density during the experiment. There was a positive response by *Z. texana* to increasing flow when grown in sandy clay and a negative response to increasing flow when grown in gravel (Figure 3-1). Aboveground biomass was significantly greater on sandy clay (p<0.001), and there was significant interaction between flow and sediment on aboveground biomass (p=0.031; Table 3-2).

There was a positive relationship between current velocity and stem density for both types of sediments although the effect was greater on sandy clay than gravel (Figure 3-2).

There was significant flow effect on leaf length. Leaves were significantly longer in the moderate site when compared with leaves in the fast and slow site (p=0.002; Figure 3-3). There was no sediment effect on leaf length and there was no interaction between factors with respect to leaf length.

Table 3-2. Two-way ANOVA performed on data collected from *Z. texana* plants grown in two sediments and three flows in Spring Lake, Hays Co., Texas.

Source of variation	SS	DF	MS	F	p
Biomass					
Flow	10.660	2	5.330	2.204	0.153
Sediment	126.299	1	126.299	52.234	0.000*
Flow X Sediment	22.775	2	11.387	4.710	0.031*
Stem Density					
Flow	182.538	2	91.269	16.430	0.000*
Sediment	128.534	1	128.534	23.138	0.000*
Flow X Sediment	51.508	2	25.754	4.636	0.032*
Leaf Length					
Flow	0.493	2	0.247	10.857	0.002*
Sediment	0.088	1	0.088	3.883	0.072
Flow X Sediment	0.096	2	0.048	2.109	0.164

* Significance at 0.05 level.

DISCUSSION

There was a positive relationship between flow and stem density and aboveground biomass, but an upper threshold for growth was not observed. Macrophytes are directly affected by current through physiological enrichment (Westlake, 1967). In addition, macrophyte community structure can be shaped by current velocity. Chambers et al. (1991) found plant biomass was inversely correlated to within bed velocities between 0.01-1 m/s. Nilsson (1987) found species richness reached a peak at a surface velocity of 0.3 m/s in a fourth order stream and that species composition in slower flowing water differed from species composition in faster flowing water. *Zizania texana* with long ribbon-like leaves which provide protection from tearing in turbulent water (Reimer, 1984), prefers faster flowing water. At lower current velocities *Z. texana* leaves may be CO_2 stressed.

Plants exhibited greater aboveground biomass and stem density when grown on sandy clay. In addition, the response to sandy clay was greater in fast flowing water (0.40-0.49 m/s) than slow flowing water (0.05-0.12 m/s). A sandy clay sediment, however, is unlikely at velocities above 0.3 m/s because sandy clay is uplifted from the stream bed at 0.3 m/s (Hynes, 1970). When stem production is stimulated by fast flowing water, the resulting stem density contributes to the plant's ability to modify the substrate in its favor. Stems slow current velocity, increasing fine sediment and detritus deposition (Gregg and Rose, 1982). The result is fine sediments within stands, and coarse sediments around stands.

Physical changes in the San Marcos River include sedimentation and reduced current velocity due to construction of dams in the San Marcos River and in the watershed, and overpumping of the Edwards Aquifer. Sedimentation has a positive effect on *Z. texana* growth while low flows are not associated with good growth rates. The decline in *Z. texana* cannot be attributed to sedimentation in the San Marcos River; however, the population decline can partly be attributed to reduced flow among other possible factors.

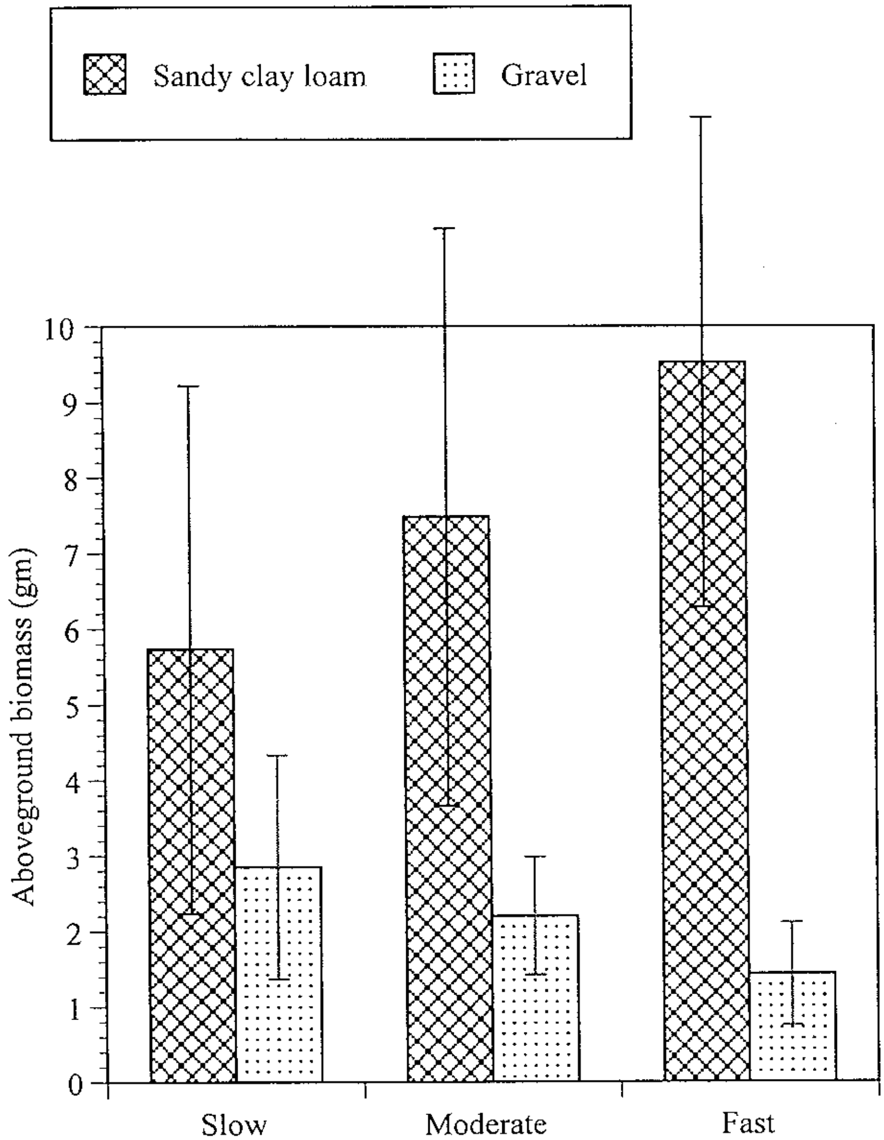

Figure 3-1. Aboveground biomass (+/-SE) of *Zizania texana* grown in two types of sediments and three current velocities in Spring Lake, Hays Co. TX.

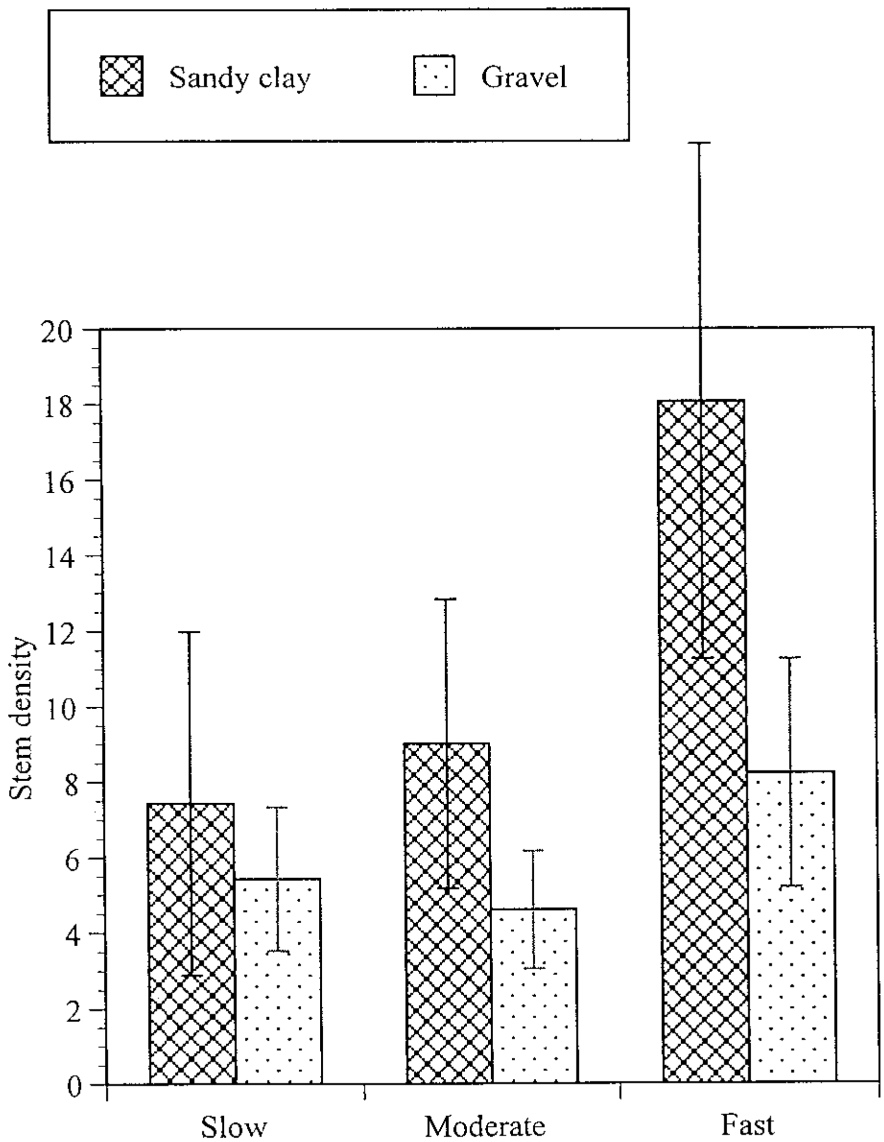

Figure 3-2. Stem density (+/-SE) of *Zizania texana* grown in two types of sediments and three current velocitites in Spring lake, hays Co., TX.

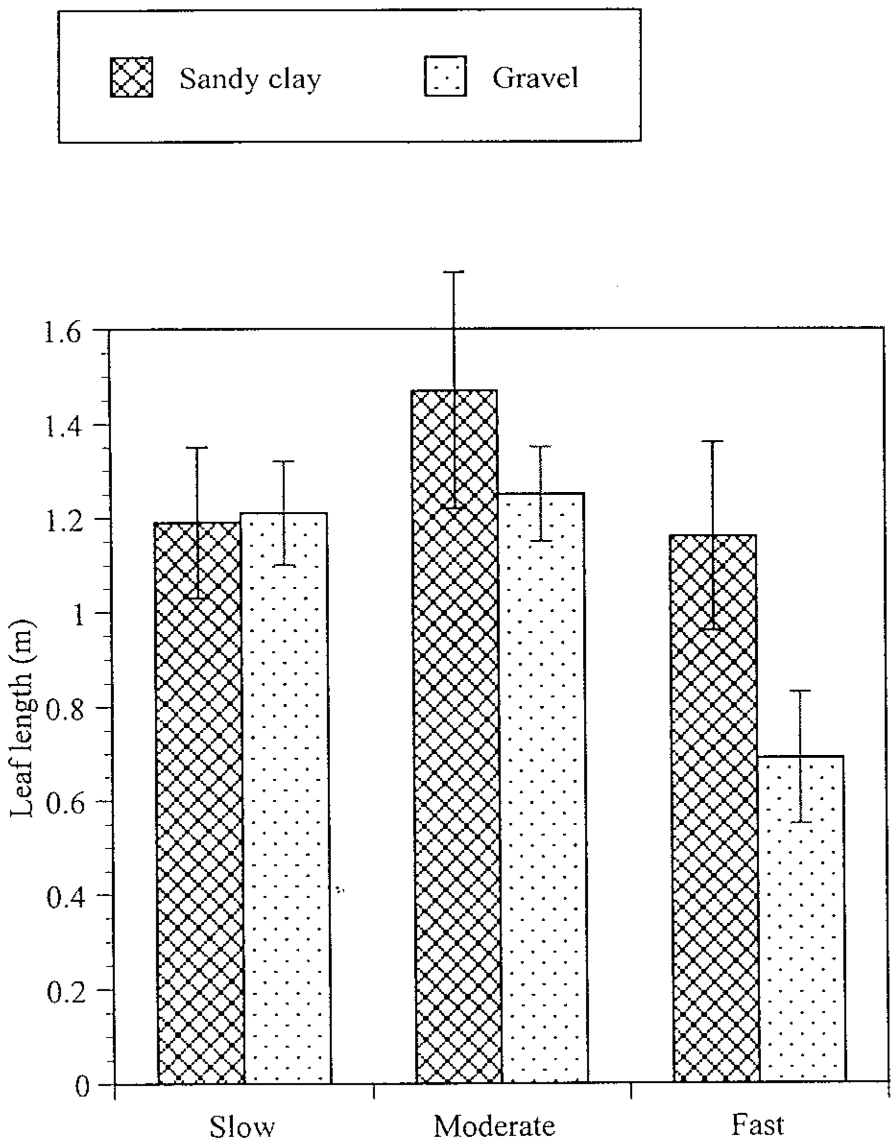

Figure 3-3. *Zizania texana* leaf length (+/- SE) from plants grown in two types of sediments and three current velocities in Spring Lake, Hays Co., TX.

CHAPTER 4.

Carbon Use By Texas Wildrice

I. CONTRACT #434-0198

The state objective for contract #434-0198 was to determine to what extent wildrice uses bicarbonate ion as a carbon source or if it is strictly a CO_2 user. There were no stated procedures for this objective.

INTRODUCTION

All photosynthetic organisms require inorganic carbon, CO_2, for photosynthesis. Terrestrial plants obtain CO_2 from the atmosphere, whereas submerged macrophytes take up inorganic carbon from the water. Inorganic carbon dissolved in the water can be in the form of CO_2 or HCO_3- or $CO_3=$. Relative concentrations of carbon species are influenced by pH. At lower pH proportionally more CO_2 is available; as pH rises, equilibrium shifts to HCO_3-, then $CO_3=$ (Figure 4-1). As pH rises to 8.5 only 0.7% of total dissolved inorganic carbon (DIC) is CO_2 (Rebsdorf, 1972). Some macrophytes are facultative HCO_3- users, others are obligate CO_2 users (Allen and Spence, 1981; Maberly and Spence, 1983; Sand-Jensen, 1983). There are no known $CO_3=$ users (Allen and Spence, 1981; Maberly and Spence, 1983).

Flowing water influences the availability of DIC in two ways. First, flowing water reduces the thickness of the stagnant boundary layer surrounding leaves. Second, as CO_2 is taken up, new CO_2 is brought by the current to the leaf surface maintaining the concentration gradient between leave surface and water. Carbon dioxide availability, therefore, is the result of the interplay between diffusion through the boundary layer and the CO_2 concentration gradient between leaf surface and surrounding water (Denny, 1993). Carbon dioxide diffuses very slowly through water, 10^4 more slowly through water than through air. As a result obligate CO_2 users receive CO_2 at a rate 300 times less than terrestrial leaves (Denny, 1993). Slow CO_2 diffusion across the boundary layer can limit photosynthesis in still or very slow moving water (Smith and Walker, 1980).

Zizania texana is an endangered macrophyte endemic to the upper reaches of the San Marcos River, Hays County, Texas (Emery, 1967; 1977; Terrell et al., 1978). In flowing water *Z. texana* produces long, ribbon-like submerged leaves. In still water only short aerial leaves are produced; submerged leaves are absent (Rose and Power, 1992; 1993). The purpose of this study was to determine to what extent the submerged leaves of the endangered species, *Z. texana*, take up HCO_3- or if the leaves are obligate CO_2 users and therefore CO_2 stressed in still water.

METHODS AND MATERIALS

To determine HCO_3- and/or CO_2 up take, we performed pH drift experiments (Allen and Spence, 1981; Maberly and Spence, 1983). During pH drift experiments plants are placed in a closed chamber and allowed to photosynthesize. As CO_2 is taken up during photosynthesis pH rises. pH is recorded at regular intervals until it stabilizes. Facultative HCO_3- users photosynthesize until pH reaches 9.5-10.5. Obligate CO_2 users stop photosynthesizing at pH

8.5-9.0 when CO_2 is depleted from the water.

Zizania texana plants used in the experiments were raised from seed on the Southwest Texas State University (SWT) campus, San Marcos, Texas and transported to Lewisville Aquatic Ecosystem Research Facility immediately prior to experimentation. Two or three plants with roots removed were placed in a glass chamber connected to flexible plastic tubing (Figure 4-2). Chamber and tubing were filled with San Marcos Spring water. Oxygen and pH probes were inserted in the plastic tubing. Water was circulated through the glass and plastic tubing by a submersible pump. The apparatus was a closed system and was placed in a water bath to maintain a constant temperature of 20° C plus or minus 1° C. Plants in the apparatus were allowed to photosynthesize, taking up CO_2 and driving up pH in the process. Oxygen and pH were recorded every minute. The experiment was terminated when pH became stabilized for 30 minutes. Plants were removed from the chamber and dried to a constant weight. Photosynthesis was measured as mg O_2 evolved/g dried plant material/hour.

RESULTS

Initial pH of the bathing solution during pH drift experiments was approximately 7.5. As plants photosynthesized, pH changed rapidly as CO_2 was taken up from solution. Change in pH slowed and eventually stopped at pH 8.5-8.7 when CO_2 was depleted (Figure 4-3).

Photosynthetic rate was high at lower pH when CO_2 was available but slowed as pH rose. Photosynthesis eventually stopped at pH 8.7 (Figure 4-4). At pH 8.7, HCO_3- concentration was 4.5 mM. In contrast, the concentration of CO_2 was 0.02 mM. This is the CO_2 compensation point for *Z. texana*, the point at which photosynthesis no longer exceeds but equals respiration. This compensation point is similar to obligate CO_2 users such as *Potomageton polygonifolius*, 0.011 mM CO_2; *Sparganium simplex*, 0.006 mM CO_2; and *Nuphar lutea*, 0.005 mM CO_2 (Maberly and Spence, 1983).

DISCUSSION

The experimental method used in this study identifies the upper pH limit for photosynthesis. Photosynthesis by *Z. texana* stopped as pH approached 8.7 when CO_2 was 0.5% of total DIC. Bicarbonate in the bathing solution at pH 8.7 was 97.5% of total DIC. At this pH there was abundant HCO_3– available for plant uptake. If *Z. texana* had the ability to take up HCO_3–, photosynthesis would continue after CO_2 was exhausted, driving pH up to 9.5-10.5. In this case, no photosynthesis occurred above 8.7 suggesting submerged leaves of *Z. texana* are obligate CO_2 users and are unable to take up HCO_3–.

Although the data show that under laboratory conditions, photosynthesis by submerged leaves is CO_2 limited at pH 8.7, in stagnant water in the field, other factors may limit photosynthesis and productivity. Sedimentation on leaves may interfere with nutrient as well as CO_2 uptake by disrupting metabolic processes. Oxygen depletion at night due to respiration by aquatic organisms may cause oxygen stress during night and early morning hours before plants begin to photosynthesize and release oxygen. Finally, toxic substances released from anaerobic breakdown of organic matter in the sediments may accumulate in stagnant water interfering with submerged leaf growth.

Flowing water influences the availability of dissolved inorganic carbon. Carbon dioxide availability is a function of the interplay between current velocity, boundary layer thickness and concentration gradient between surrounding water and leaf surface; as flow increases, boundary layer thickness decreases and CO_2 availability increases. Carbon dioxide is less likely to limit photosynthesis by obligate CO_2 users in flowing water than in stagnant water. *Zizania texana* is restricted to CO_2 uptake when other forms of inorganic carbon are plentiful and is most likely CO_2 stressed in stagnant water. Alternatively, flowing water may enhance productivity in submerged leaves by reducing the boundary layer and maintaining a high CO_2 concentration gradient between water and leaf surface.

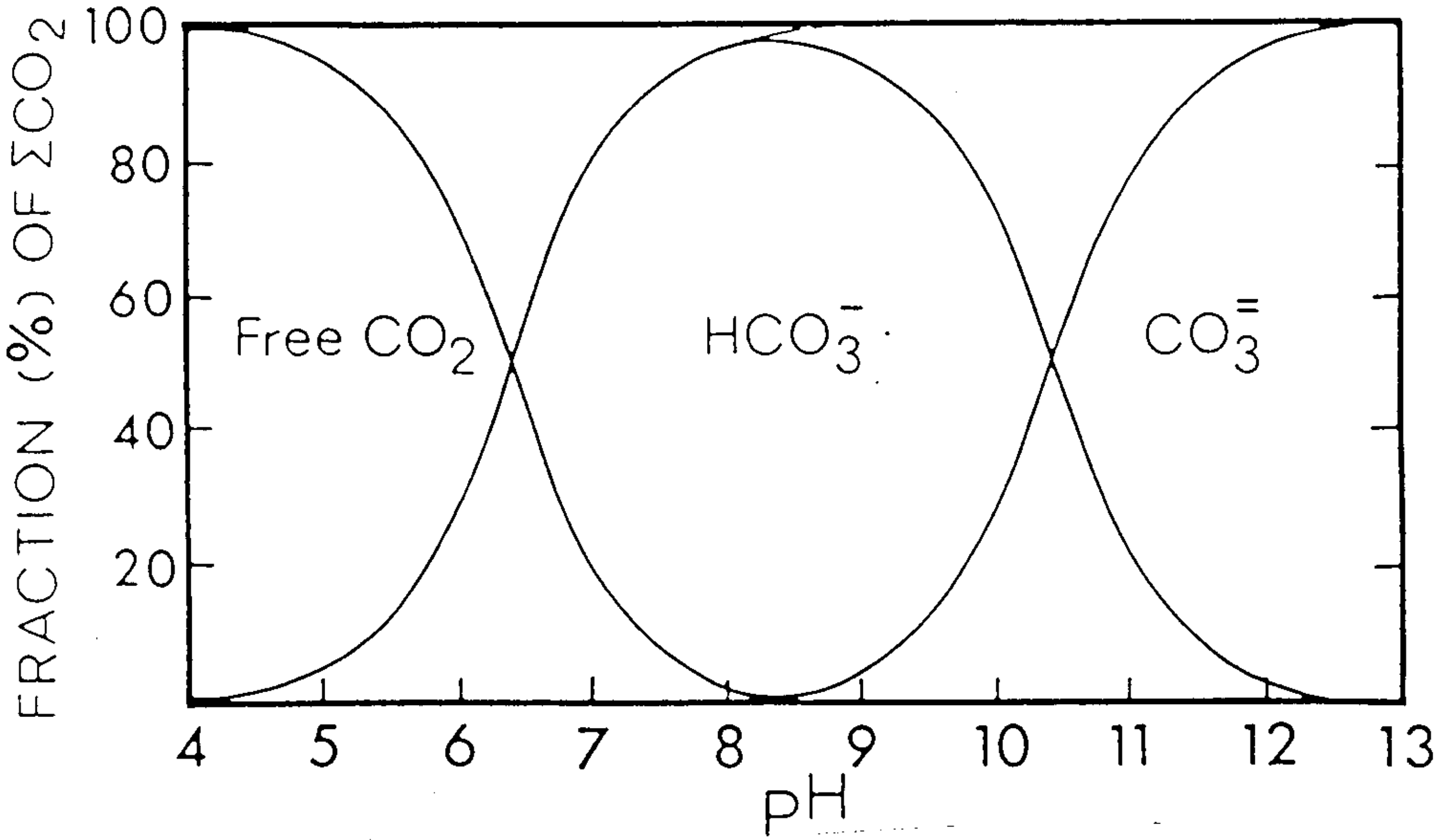

Figure 4-1 Relation between pH and the relative proportions of inorganic carbon species CO_2, HCO_3^-, and $CO_3^=$. From Wetzel, 1983.

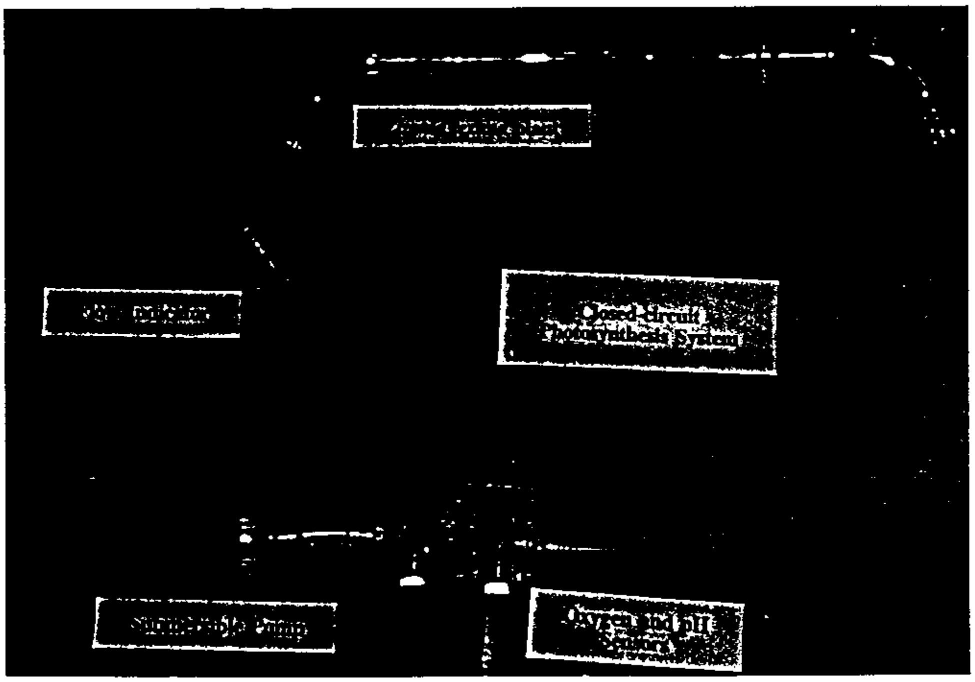

Figure 4-2 Experimental apparatus for pH drift experiment.

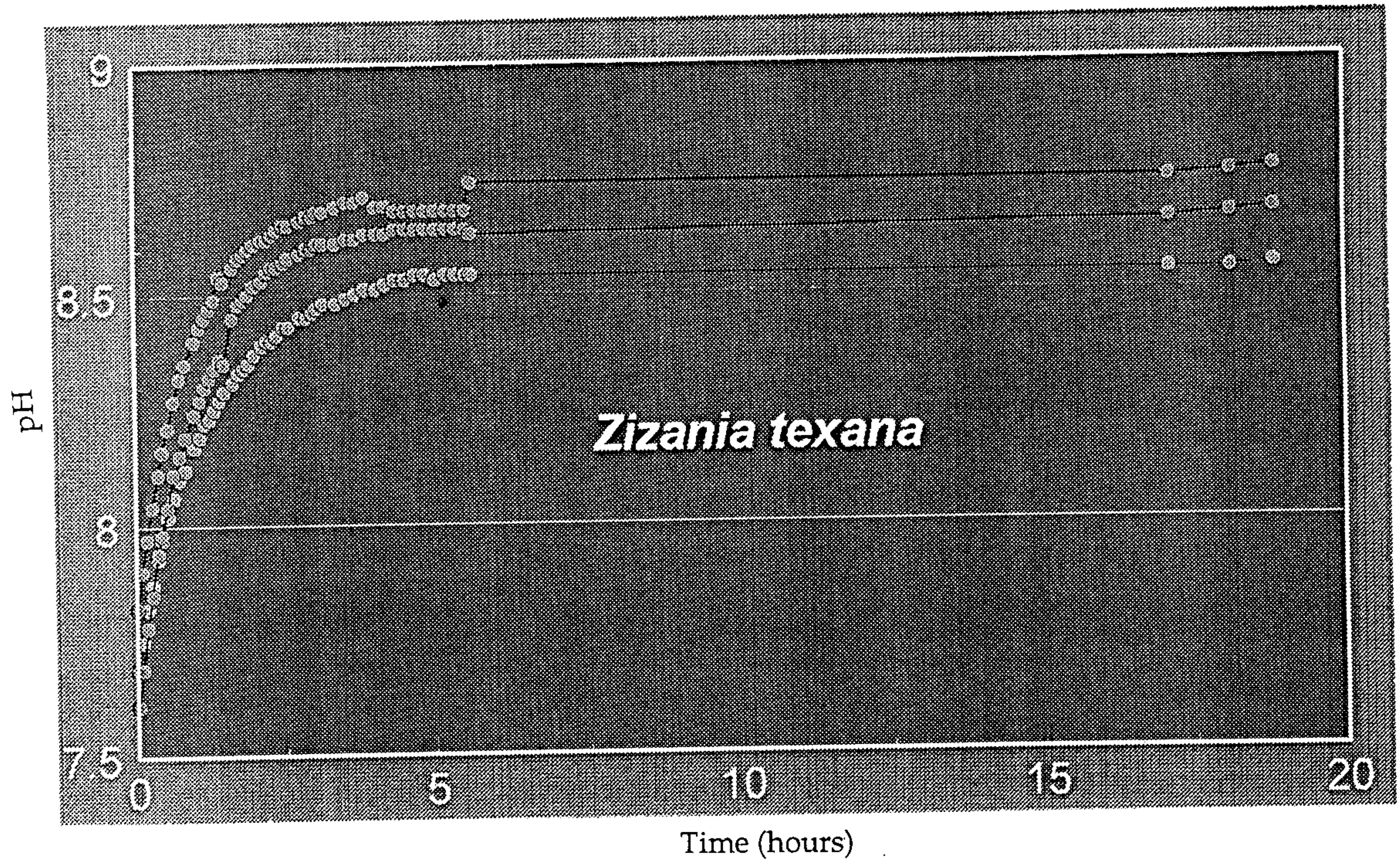

Figure 4-3 Change in pH during pH drift experiment with *Zizania texana*.

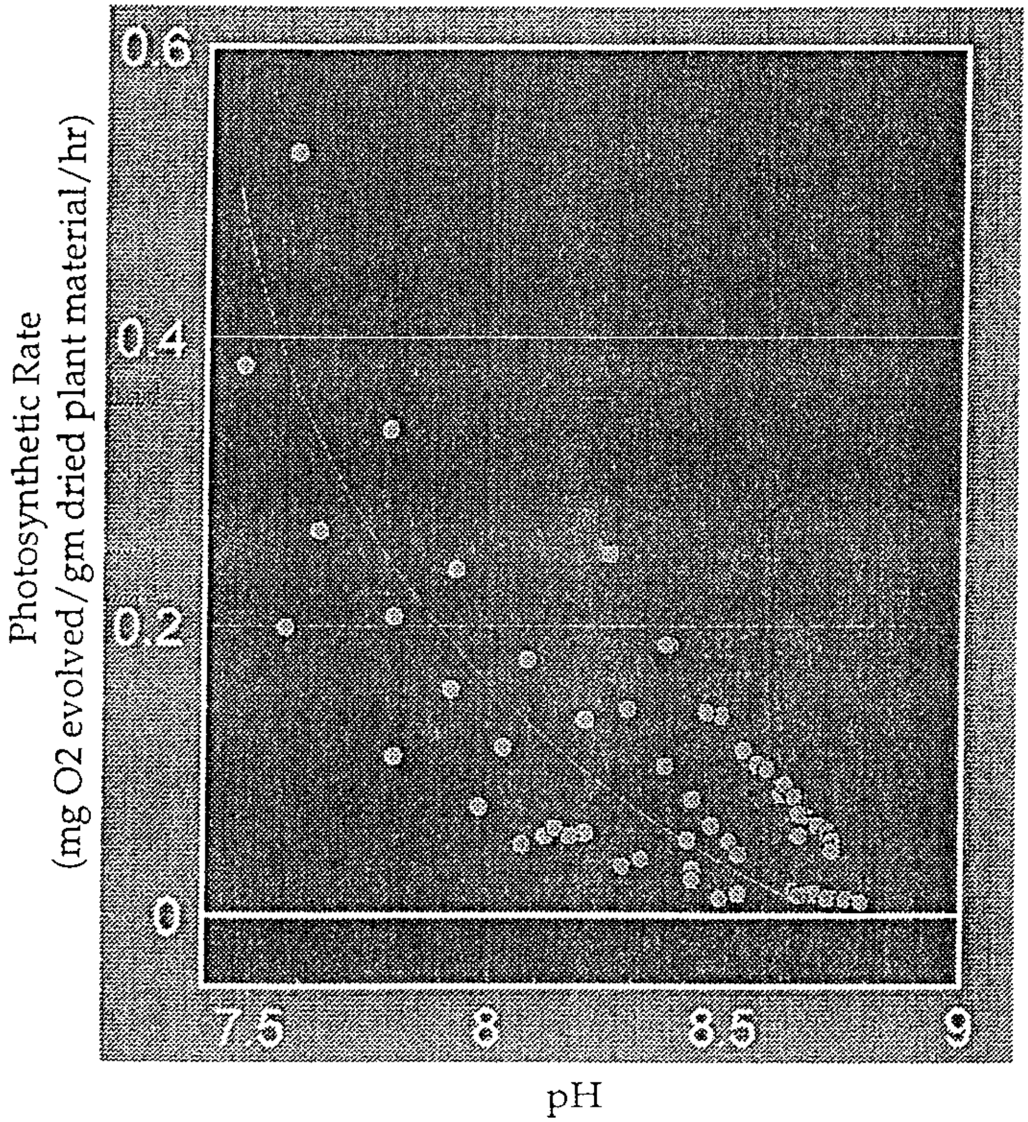

Figure 4-4. Relation between photosynthesis rate by *Zizania texana* and pH.

CHAPTER 5.

Productivity and Resource Allocation Patterns in Texas wildrice as a Function of Current Velocity

I. CONTRACT #335-0198

The stated objective for contract #335-0198 was to complete investigations on the extent velocity and substrate influence productivity and resource allocation patterns in wildrice when grown at a constant depth. There were no stated procedures for this objective.

INTRODUCTION

Harper and Ogden (1970); Hickman (1975); Van Baalen, et al. (1990); Dunn and Sharitz (1991); Madsen (1991) and Neill (1993) have reported trade-offs between reproductive allocation and vegetative allocation with changes in environmental conditions. Common environmental factors associated with allocation trade-offs for terrestrial plants include light, substrate, nutrient availability and for macrophytes, water depth and water temperature are also important. Little information is available on current velocity and its affect on allocation patterns in macrophytes.

Earlier work has suggested that current velocity may influence productivity and resource allocation patterns in *Z. texana* (Rose and Power, 1992, 1994 and Power, 1996a). The work reported here was undertaken to further identify and clarify the effects of current velocity on productivity and resource allocation patterns in *Z. texana.* over a one year time period.

METHODS

<u>Site Description</u>

This research was carried out in Spring Lake, an impoundment formed by a dam originally constructed across the San Marcos River in 1849, approximately 600 m downstream from the San Marcos Springs. On the eastern side of the dam is a spillway. Three of four experimental sites were located near the spillway. One site was located on the Southwest Texas State University (SWT) in an outdoor cement raceway. At each site pH, water depth, and current velocity were recorded on nine different dates between May, 1995 and February, 1996.

Seeds for this experiment were germinated in the lab in January, 1995, then transplanted to 15 cm plastic pots, which were lined with small plastic bags and filled with fine sediments collected from one location in Spring Lake. Pots were placed in an outdoor raceway on the SWT campus and seedlings were allowed to grow for about 6 weeks. On March 10, 1995, potted plants were transplanted into three sites in Spring Lake and one site in the raceway. There were three replicate experimental units (plots) at each site. Each replicate plot had 36 potted plants. Initially, 4 plants were harvested to obtain biomass values for newly transplanted individuals. The study design called for harvesting 4 plants from each replicate plot on nine different occasions. However, during the study period some plants were lost due to herbivory, probably by crawfish, and some plants were washed away by the current. As a result I harvested plants from each replicate plot on seven dates, May 3, June 1, June 30, July 27, September 1, October 16, and February 8.

At each harvest, plants were collected from each replicate and sediment was washed from roots. Plants were divided into vegetative parts, reproductive parts, and roots. Vegetative parts were defined as submerged leaves and reproductive parts were defined as reproductive culms with attached leaves and associated tillers. Plants were then dried at 65° C for at least 48 hours and weighed to the nearest one hundredth of a gram.

All growth measurements were analyzed with repeated measures ANOVA (Zar, 1984).

At each site current velocity was measured at 20%, 60%, and 80% depth and average velocity was calculated. Each site was named according to its current velocity: Slow Flow (raceway), No Flow, Moderate Flow and Fast Flow.

RESULTS

Plants in the No Flow site in Spring Lake were lost to herbivory prior to the second harvest and were dropped from the experiment.

Water depth was similar between sites and ranged from 0.69-0.91 m. Current velocity varied between sites. Current velocity ranged from 0-0.010 m/s in the Slow site, 0.038-0.142 m/s in the Moderate site, and 0.250-0.369 m/s in the Fast site. The pH was highest in the Slow site and very similar in the Moderate and Fast sites (Table 5-1).

Table 5-1. Ranges of environmental factors in two sites in Spring Lake and one site on the SWT campus. Data were collected on nine different days between May, 1995 and February, 1996.

	Slow	Moderate	Fast
Depth (m)	0.69-0.75	0.87-0.91	0.80-0.87
Current velocity (m/s)	0-0.010	0.038-0.142	0.250-0.369
pH	7.50-7.68	7.23-7.26	7.16-7.28

All plants increased in size over the study period. There was a significant difference in root, submerged leaf, reproductive culm and total biomass between sites and over time ($p<0.001$) and significant interaction between factors with respect to root, leaf, culm and total biomass ($p=0.001$). Plants from the Fast site showed the greatest increase in root, submerged leaf, reproductive culm and total during the study period. Plants in the Slow site showed the least increase in biomass (Figure 5-1).

The number of submerged leaves per plant was significantly different during the study ($p=0.0001$) and among sites ($p=0.0001$). Interaction between time and site with respect to number of leaves was significant ($p=0.0003$). Plants growing in the Fast site produced the greatest number of leaves/plant at each harvest while plants growing in the slow site produced the fewest number of submerged leaves in six of seven harvests. The greatest number of leaves at a single harvest was 28 leaves/plant from the Fast site on the February harvest (Figure 5-2).

The number of reproductive culms per plant was significantly different during the study ($p=0.0001$) and among sites ($p=0.0056$). Plants began producing culms between May 2, 1995 and June 1, 1995. Reproductive culms were present in every harvest from June 1, 1995 until

February 8, 1996. The greatest number of culms produced for all sites was during the February harvest with 7.8 culms/plant in the Fast site (Figure 5-2).

The proportion of biomass allocated to submerged leaves decreased significantly over the study period in all sites (p=0.0001), but did not differ between sites (p=0.2006; Figure 5-3).

Biomass allocated to roots differed significantly over time and among sites. The proportion of biomass allocated to roots increased over time (p=0.0089) and differed between sites (p=0.0001). The Fast site had the greatest proportional increase in root biomass during the study period. The slow site showed the least proportional change in root biomass.

Biomass allocated to reproductive culms differed significantly over time and among sites. The proportion of biomass allocated to culms increased over time (p=0.0089) and differed among sites (p=0.0176). Plants in the Slow site allocated more biomass to culms during the study period than either plants in the Moderate or Fast site.

DISCUSSION

Plants grown in water flowing 0.25-0.369 m/s accumulated more biomass during the study period than plants grown in water flowing 0.01-0.14 m/s. *Zizania texana* exhibited a similar response to flowing water in another study (Power, 1996a, d) and had greater survivorship when transplanted into moderate to fast flowing water in a lake (Power, 1996b). Flowing water has been shown to be an important factor influencing photosynthetic rates in macrophytes (Westlake, 1967; Smith and Walker, 1980; and Madsen and Sondergaard, 1983), macrophyte distribution (Fonseca and Kenworthy, 1987; and Nilssen, 1987); and macrophyte growth (Chambers, et al., 1991). Chambers, et al. (1991) found an inverse relationship between biomass and current velocity between 0.01-1.0 m/s. Nilssen (1987) found species richness reached a peak at about 0.3 m/s along a current gradient from 0.04-1.23 m/s, with some species growing in current velocities greater than 1 m/s. Although plant growth increased with increasing current velocity, an optimum current velocity for growth was not observed for *Z. texana* in this study.

Plants from all sites were harvested at intervals for 1 year. All plants showed initial growth in vegetative parts followed by growth in reproductive parts. Plants in the Fast site continued to increase in size throughout the study period while plants in the Moderate and Slow sites showed vey little increase in size during the same period. Plants in the Moderate site disappeared entirely at the last harvest. In this site, plants were impacted by herbivores and floating mats of vegetation. The combination of herbivory, floating vegetation mats and poor environmental conditions (current velocity) most likely contributed to their decline. In contrast plants in the Slow flow site (raceway) did not suffer from herbivory and floating vegetation mats and over wintered. At the February harvest plants in the slow flow site had a mean of 5 leaves compared with 28 leaves on plants from the Fast site.

Total biomass and reproductive biomass production peaked during the July harvest for plants slow flowing water. In contrast the July harvest corresponded to a peak in reproductive output for plants in fast flowing water while total biomass peaked during the February harvest. Continued growth throughout the study period with reproductive activity reaching a maximum during the summer months, suggests plants are tending toward a longer life span (van Lent and Verschuure, 1994).

Current velocity is a critical environmental factor influencing productivity and allocation patterns. There is an inverse relationship between current velocity and the boundary layer around leaves (Denny, 1993). The boundary layer increases the distance across which CO2 must diffuse before it is taken up by leaves. Plants in slower moving water may be CO2 stressed and as a result are less productive. In contrast, leaves in faster flowing water have a narrow boundary layer and more CO2 is available for photosynthesis and growth. Although CO_2 most likely plays a role, mechanical stimulation of the meristem may also be important in faster flowing water. At this point, the physiological mechanism for greater productivity in faster water requires further study.

Plants in slow flowing water allocated proportionally more biomass to reproductive culms (65% in October harvest) compared with plants in fast flowing water (30% in October harvest). Allocating biomass in favor of reproductive parts can be an indication of environmental stress (Harper and Ogden, 1970; Hickman, 1975) and/or a tendency toward a short life span (van Lent and Verschuure, 1994). Because all plants were harvested at the end of the study and plants could not be observed for a longer period, it is difficult to predict if the observed allocation patterns in plants in slow flowing water were an indication of a shift in life history strategy or simply a function of environmental stress.

CHAPTER 6.

Sediments, Dams and the Distribution of Texas wildrice

I. CONTRACT # 335-0198, #336-0225

The stated objective for contract #335-0198 and #336-0225 was to initiate and/or continue and complete organic matter additions and plant growth experiments. There were no stated procedures for this objective.

INTRODUCTION

Important sediment characteristics, which influence macrophyte growth, include sediment density and sediment nutrients (Barko and Smart, 1979, 1986; Chambers et al., 1989; van Wijck et al., 1992). High density sediments are often coarse in texture and are usually nutrient poor while low density sediments tend to be fine in texture with a high nutrient concentration (Barko et al., 1991). Macrophytes often exhibit robust growth on intermediate density sediments and diminished growth on high and low density sediments apparently due in part to nutrient availability (Barko et al., 1991; Barko and Smart, 1986). Among the many nutrients required by plants, nitrogen and phosphorus have been shown to be important growth limiting nutrients.

The presence of dams can effect macrophyte distribution in streams and rivers through the alteration of natural sediment characteristics and flow patterns. Periodic flooding of rivers can lead to significant transport of fine sediments downstream (Zeh and Dönne, 1994). Dams retard flood water and result in sedimentation upstream from dams while downstream sites more closely resemble natural hydrologic conditions with respect to flow patterns and sediment characteristics (Zeh and Dönne, 1994).

The San Marcos River is a thermally constant, spring fed river in Hays Co., Texas. The river has three dams along its length, originally constructed in the mid-1800's as sources of power for local industry. In addition, during the 1970's, five retention dams were constructed for flood control purposes in the upper San Marcos watershed. The local dams and retention dams have altered the frequency and magnitude of historic flooding cycles and resulted in increased sedimentation in the San Marcos River (U. S. Fish and Wildlife Service, 1994). In addition, erosion from construction on the surrounding hillsides has resulted in very coarse sediment deposition at numerous locations in the San Marcos River.

Zizania texana Hitchc. (Texas wildrice) is an endangered emergent macrophyte endemic to the San Marcos River (Silveus, 1933). *Zizania texana* is endangered, in part, because of its limited distribution and a significant population decline that occurred between 1940 and 1986 (Emery, 1967; U. S. Fish and Wildlife Service, 1994). Plants have long ribbon-like leaves and typically form dense stands in fast flowing water in the first 2.4 km of the San Marcos River (Terrell et al., 1978). Distribution maps indicate *Z. texana* is more likely to occur below dams where sediments are coarse than immediately above dams where sediments are fine (Texas Parks and Wildlife, 1989); yet results from controlled studies indicate *Z. texana* is more productive in fine sediments (Power and Fonteyn, 1995; Power, 1996a).

In this study I documented growth of *Z. texana* on a variety sediments collected above and below dams in the San Marcos River. I also attempted to assess the adequacy of Nitrogen for the growth of *Z. texana* on these sediments.

METHODS AND MATERIALS

Sediments were collected from 6 sites in the San Marcos River. Collection sites were above and below dams at Spring Lake, Rio Vista, and Thornton's Dam (Figure. 6-1). Texture of sediments was determined by the hydrometer method (Brower and Zar, 1984). Sediment density (dry weight/100 ml wet weight) and water content (g water/100 g dry sediment) were determined gravimetrically by drying known volumes in a drying oven. Dried samples were combusted at 550°C to estimate total organic matter content from loss of mass on ignition. Samples were analyzed for Kjeldhal N at Texas A&M Soil Laboratory.

Table 6-1. Gravel, sand, silt, clay, and organic matter content of six sediments collected from the San Marcos River. SD in parenthesis; n=4. Different letter indicates significant difference within column (one-way Anova, Tukey Multiple Range test).

Site	Gravel (%)	Sand (%)	Silt (%)	Clay (%)	Organic (%)
Upstream from dam					
Spring Lake	9.6	29	53	18	3.4a
Silt loam	(1.8)	(4.690)	(4.243)	(3.367)	(0.60)
Rio Vista Park	4.9	30.3	48.5	21.3	2.8b
Loam	(5.5)	(4.646)	(8.021)	(5.560)	(0.36)
Above Thornton's	16.4	27.8	52.8	19.5	2.31c
Gravelly silt loam	(14.9)	(0.500)	(6.946)	(6.758)	(0.23)
Downstream from dam					
Below Spring Lake	55.2	67.6	23.8	8.6	0.81d
Very gravelly sandy loam	(6.8)	(2.689)	(2.021)	(0.946)	(0.06)
Below Rio Vista	64.9	76	15.9	8.1	0.69e
Very gravelly sandy loam	(4.6)	(4.082)	(2.594)	(1.652)	(0.05)
Below Thornton's	75	65.8	21.6	12.6	0.83d
Very gravelly sandy loam	(4.4)	(1.848)	(1.887)	(1.493)	(0.07)

For a plant growth experiment, sediments were placed in 10 cm diameter plastic pots. One *Z. texana* seedling was placed in each pot. Pots were arranged in an outdoor cement raceway on the SWT campus in a randomized block design. There were seven replicate blocks. Untreated Edwards Aquifer water flowed through the raceway at about 0.017 m sec^{-1}, water depth was about 0.5 m. Plants were grown for eight weeks, then harvested. Plants were dried at 65 C for 48 hours, then separated into above and below ground parts and weighed.

Table 6-2. Sediment water content and sediment density in six sediments collected above and below dams in the San Marcos River, Hays Co., Texas. N=4; SD in parenthesis. Different letter indicates significant difference (one-way ANOVA, Tukey Multiple Range test).

Site	Water Content (g water/100 g soil)	Density (g dry weight/ 100 ml wet weight)	Nitrogen (%)
Upstream from dam			
Spring Lake	39.83a	0.577a	0.629a
	(4.865)	(0.042)	(0.012)
Rio Vista Park	4.004b	1.665a	0.275b
	(0.689)	(0.157)	(0.005)
Above Thornton's	2.635b	1.950a, b	0.213c
	(0.256)	(0.071)	(0.014)
Downstream from dam			
Below Spring Lake	0.527b	3.548b	0.053d,e
	(0.069)	(0.233)	(0.002)
Below Rio Vista	0.474b	3:629b	0.063d
	(0.064)	(0.209)	(0.015)
Below Thornton's	0.243b	4.578b	0.046e
	(0.040)	(0.143)	(0.008)

Sediments collected upstream and downstream of Spring Lake dam (spillway) and Thornton's dam were used for a fertilization experiment. About 1.5 kg of each sediment was placed in 15 cm plastic pots lined with clear plastic bags. The pots were then placed in the outdoor raceway on the SWT campus. One *Z. texana* seedling was placed in each pot. After 4 weeks, ammonium sulfide was injected into the sediment at one of five treatment levels. The levels were 0 mg N (control), 50 mg N, 100 mg N, 200 mg N, and 400 mg N. The top edge of the plastic bag was folded around the base of the plant and weighed down with pebbles to slow diffusion of ammonium into the surrounding water. Treatments were placed in a randomized block pattern. There were five replicate blocks. The plants were allowed to grow for five weeks after injection of N, then harvested, dried at 65 C for 48 hours, then separated into above and below ground parts and weighed. Above ground plant parts were shipped to Texas A&M Soil Laboratory for Kjeldhal N analysis.

RESULTS

Texture analysis of soils indicated that sediments collected above dams were loam, silt loam, or gravelly silt loam and sediments collected below dams were very gravelly sandy loam (Table 6-1). Sediments differed significantly with respect to density, organic matter content, water content, and %N. Sediments could not be divided into above and below dam groups based on sediment characteristics with the exception of density (Table 6-1, 6-2; all analysis one-factor ANOVA; $p<0.05$). Above dam sediments were significantly less dense than below dam sediments. Sediment density increased with increasing sand content ($p<0.05$; $r^2=0.77$; $Y = 13.937 + 13.380X$) and decreased with increasing water content ($p<0.05$; $r^2=0.997$;

$\log Y = 1.067 + -2.460\log X$). Water content was positively correlated with %N ($p<0.05$; $r^2=0.89$; $Y = 0.100 + 0.013X$). Percent N and organic matter content paralleled water content with respect to sediment density ($p<0.05$; $r^2=0.82$; $Y = 0.577 + -0.135X$ and $p<0.05$; $r^2=0.87$; $Y= 0.038 + -0.008X$ respectively).

All plants increased in size during the experimental period. There was a significant sediment effect on root, shoot, and total biomass (one-way ANOVA, $p<0.05$; Table 6-3). Plant growth, however, did not fall into significantly different groups based on above and below dam sediments. Plants had the greatest increase in root, shoot and total biomass on sediments of intermediate density and had diminished growth on either high or low density sediments (Table 6-3). Plants also showed the greatest increase in total biomass on sediments with intermediate organic matter content (Figure. 6-2).

There was no significant difference in root/shoot ratios between sites.

Table 6-3. Root, shoot, and total biomass from plants grown in sediments collected from six locations in the San Marcos River. SD in parenthesis; n=7; letter indicates significant difference among values in each column. There was no significant difference between sites with respect to root/shoot.

Site	Root	Biomass (g) Shoot	Total	R/S
Upstream from dam				
Spring Lake	0.073a	0.197a	0.266a	0.34a
	(0.036)	(0.084)	(0.121)	(0.092)
Rio Vista Park	0.328b	0.885c	1.212d	0.37a
	(0.117)	(0.258)	(0.370)	(0.059)
Above Thornton's	0.268b	0.807c	1.075c,d	0.33a
	(0.099)	(0.229)	(0.326)	(0.040)
Downstream from dam				
Below Spring Lake	0.060a	0.153a	0.213a	0.39a
	(0.014)	(0.032)	(0.044)	(0.056)
Below Rio Vista	0.093a	0.240a	0.333a	0.40a
	(0.043)	(0.099)	(0.139)	(0.056)
Below Thornton's	0.208b	0.521b	0.729b,c	0.39a
	(0.088)	(0.133)	(0.216)	(0.082)

Plants grown on fertilized and control sediments increased in size during the experiment. Two-way ANOVA indicated there was significant sediment effects on root, shoot, and total biomass ($p<0.05$); significant treatment effects on total biomass ($p<0.05$); and significant interaction between factors on total biomass ($p<0.05$; Table 6-4). Plants grown on above Thornton's dam sediments (moderate density) showed the greatest increase in total biomass for all fertilization levels (Figure. 6-3). Plants grown on sediments from above Spring Lake dam (low density) and below Spring Lake dam (high density) showed little increase in growth with N additions. Plants grown on sediments from below Thornton's dam (high density) had an intermediate growth response to N additions.

There were significant sediment and treatment effects on root/shoot ratios which decreased on all sediments with nitrogen additions (two-way ANOVA; $p<0.05$; Figure 6-4).

The relationship between shoot N concentration and shoot biomass was poor (Figure 6-5). The greatest N concentration and the most variability in N concentration occurred at the lowest shoot biomass. Thus growth response was greater with respect to sediment N than with respect to shoot N concentration (Figure. 6-3 and Figure.6-5).

DISCUSSION

The density of sediments differed significantly above and below dams. This suggests dams can be used as an indicator of relative sediment density. Dams and the resulting impoundments tend to slow current velocity. Fine sediments and organic matter settle out of the water column resulting in relatively low density sediments in impoundments. Low density sediments with high water content and high organic matter content (which contributes to %N) do not necessarily result in robust plant growth due to the distances across which nutrients must diffuse and potentially anoxic conditions from decomposition of organic matter (Barko and Smart, 1986; Bradley and Morris, 1990; van Wijk et al, 1992). In contrast, below dams, where current velocities are often greater than 0.3 ms^{-1}, fine particles are uplifted leaving sand, gravel and relatively high density sediments behind (Hynes, 1970). High density sediments tend to be nutrient poor due to low organic matter content (Barko et al, 1991).

All plants increased in size during the eight week growth experiment. However plants had the greatest increase on sediments with intermediate density (above dam sediment) and the smallest increase on low and high density sediments (above and below dam sediments). Therefore location of sediments relative to dams is not a good indicator of the sediments' potential for plant growth. A better indicator would be sediment density or organic matter content (Figure 6-2).

Low density sediments produced plants with low total biomass. Low density sediments were unconsolidated, with a high organic matter and water content. Although nitrogen was present, plants may have been nutrient limited due to the distance across which nutrients must diffuse (Barko, et al, 1991). High density sediments had relatively low %N and organic matter content. Diminished growth on these sediments may have been due to nutrient limitation.

Root/shoot ratios varied from 0.33 to 0.4. Root/shoot ratios in this range are considered high and are usually associated with plants grown on infertile sediments (Barko and Smart, 1979; Barko, et al, 1991). This suggests that plants were nutrient limited on all sediments and the variation in plant growth was governed by nutrient availability. The mechanism for plant growth and nutrient availability may have been due to low nutrient concentration in sediments (Barko et al, 1991); multiple nutrient limitation (Barko & Smart, 1986); or low oxygen levels (especially on organic sediments) which impedes nutrient uptake (Bradley and Morris, 1990; van Wijk et al, 1992).

Growth response to N additions in sediments varied significantly. Plants grown on moderately dense sediment (above Thornton's dam) showed the greatest increase in total biomass with N additions indicating N limitation on this sediment (Figure 6-3). Plants grown on high and low density sediments showed little increase in biomass with N additions. Plants grown on high and low density sediments most likely acquired additional N (as reflected in lower r:s ratio) but plant growth was still limited, possibly by P and Fe or other factors such as potentially anoxic conditions.

A number of studies have identified critical nutrient concentrations for single nutrients (Gerloff and Kromholz, 1966; Barko and Smart, 1979; Sytsma and Anderson, 1993). The critical concentration identified the point at which the nutrient was no longer limiting. It was hoped that the critical N concentration would be identified for *Z. texana* growth. However, N concentration in shoots suggested essentially no N limitation on any sediment (Figure 6-5). This clearly is not supported by growth data from above and below Thornton's Dam sediments which showed significant increases with N additions. Growth data on fertilized sediments is a better indicator of nutrient limitation than nutrient concentration in tissues. Shoot nitrogen concentration may be misleading because it assumes plants are limited by a single element and that no interaction between elements occurs (Barko and Smart, 1986).

In addition to sediment effects, plant growth by *Z. texana* has been shown to be strongly influenced by current velocity, most likely through CO_2 enrichment or mechanical stimulation of the meristem (Power, 1996b). In the San Marcos River *Z. texana* tends to be more common below dams where current is unaffected by impoundments. This suggests current velocity may play a greater role than sediments in the growth of *Z. texana* in the wild. This has important management implications. A recovery strategy for *Z. texana* is to establish healthy, self-sustaining, and reproductive populations in the San Marcos River (U. S. Fish and Wildlife Service, 1996). This will more easily be attained by taking into account the density and organic matter content of sediments in combination with current velocity.

Table 6-4. Total, root and shoot biomass from plants grown on different sediments with five levels of nitrogen additions. Two-way Anova indicated significant sediment effects on total, shoot and root biomass (p<0.05); significant treatment effects on total biomass (p<0.05); and significant interaction on total and shoot biomass (p<0.05).

		Nitrogen addition (mg)				
Sediment	Biomass	0 (control)	50	100	200	400
Spring Lake	Total	1.04 (0.457)	1.63 (0.687)	1.01 (0.553)	0.78 (0.466)	1.16 (0.304)
	Shoot	0.74 (0.31)	1.23 (0.55)	0.77 (0.40)	0.59 (0.35)	0.95 (0.28)
	Root	0.31 (0.15)	0.40 (0.18)	0.24 (0.16)	0.19 (0.12)	0.21 (0.07)
Below Spring Lake	Total	0.90 (0.581)	0.91 (0.823)	0.59 (0.291)	0.52 (0.373)	0.38 (0.244)
	Shoot	0.60 (0.31)	0.63 (0.66)	0.44 (0.21)	0.42 (0.31)	0.38 (0.18)
	Root	0.30 (0.28)	0.21 (0.17)	0.15 (0.09)	0.11 (0.07)	0.09 (0.02)
Above Thornton's	Total	2.96 (1.312)	5.80 (1.487)	6.15 (3.338)	6.75 (2.929)	7.67 (2.570)
	Shoot	2.26 (0.97)	4.73 (1.32)	5.26 (2.92)	5.69 (2.54)	6.67 (2.12)
	Root	0.71 (0.36)	1.07 (0.34)	0.90 (0.48)	1.05 (0.49)	1.0 (0.46)
Below Thornton's	Total	2.12 (1.187)	3.46 (1.827	2.37 (0.647)	2.41 (1.589)	0.95 (0.797)
	Shoot	1.31 (0.95)	2.57 (1.39)	1.87 (0.50)	1.95 (1.29)	0.43 (0.64)
	Root	0.55 (0.25)	0.89 (0.46)	0.50 (0.16)	0.46 (0.31)	0.17 (0.16)

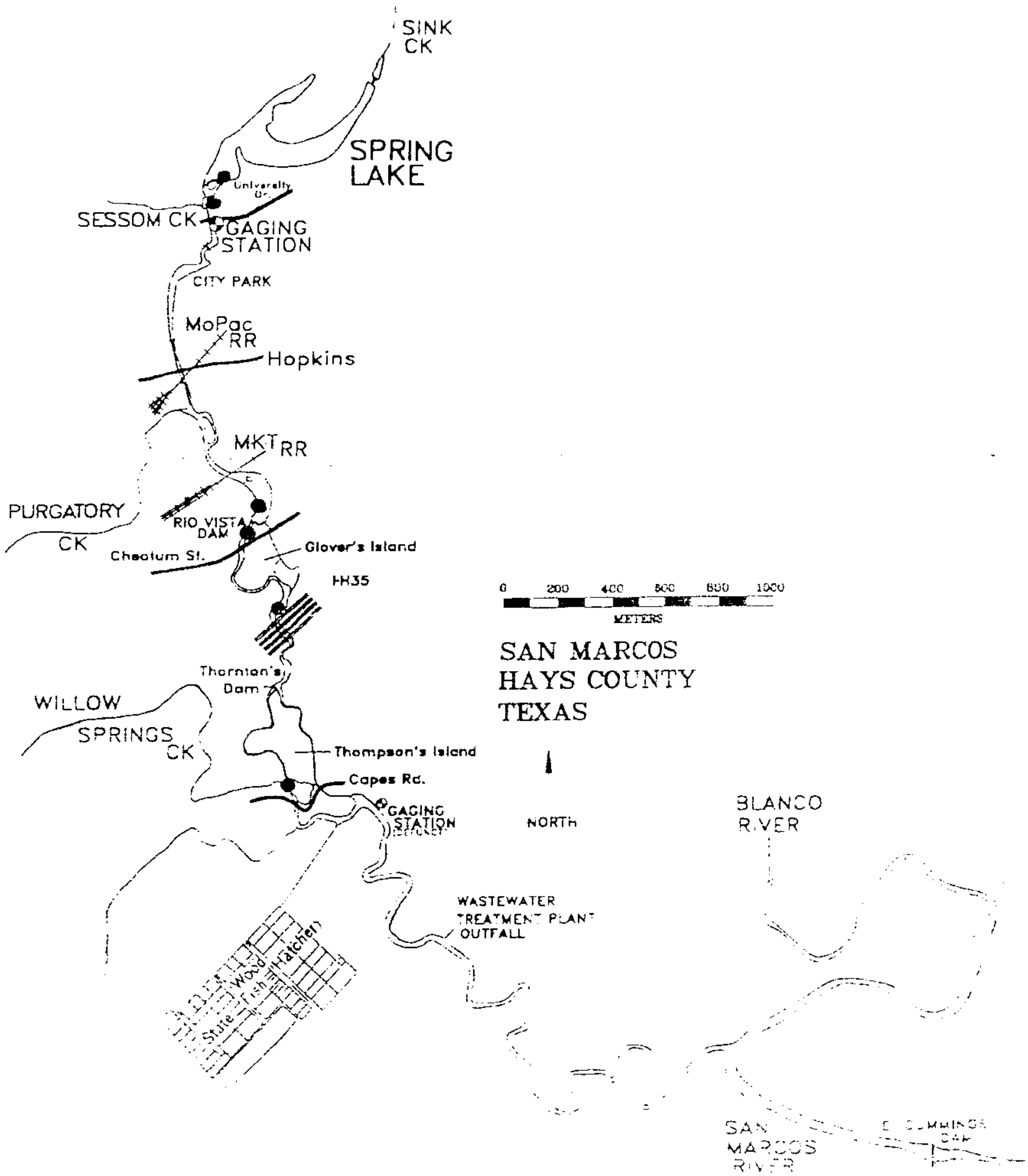

Figure 6-1 Sediment collection sites along the San Marcos River, Hays Co. Texas. Sites are indicated with dot.

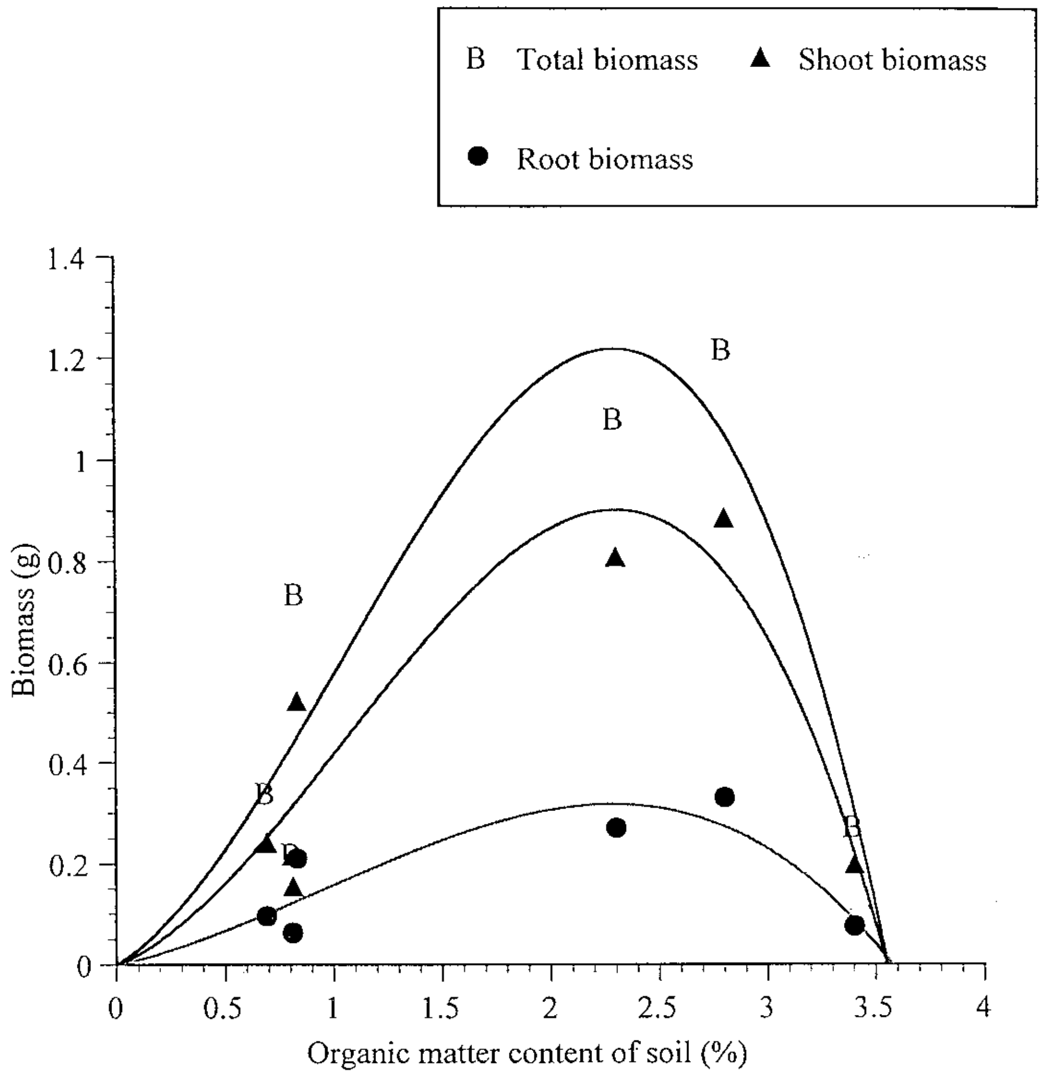

Figure 6-2. Root, shoot and total biomass of *Zizania texana* in relation to organic matter content of soils. Plants had the greatest increase in biomass when grown on soils with 0.83% - 2.8% organic matter content. Curves are computer generated.

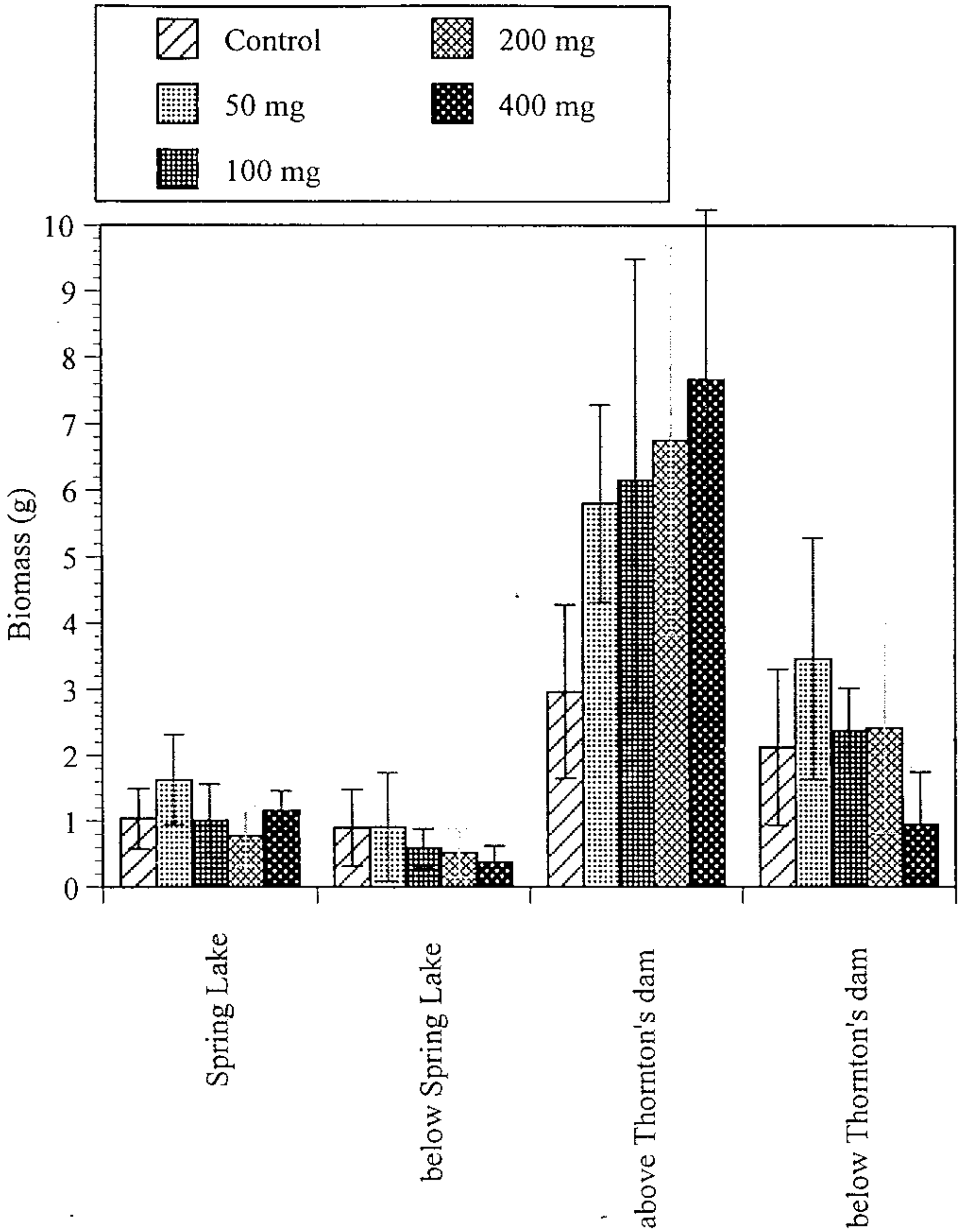

Figure 6-3. Growth of *Zizania texana* on four sediments with four levels of nitrogen additions. There was significant sediment, treatment and interaction (two-way Anova; $p<0.05$; $n=5$).

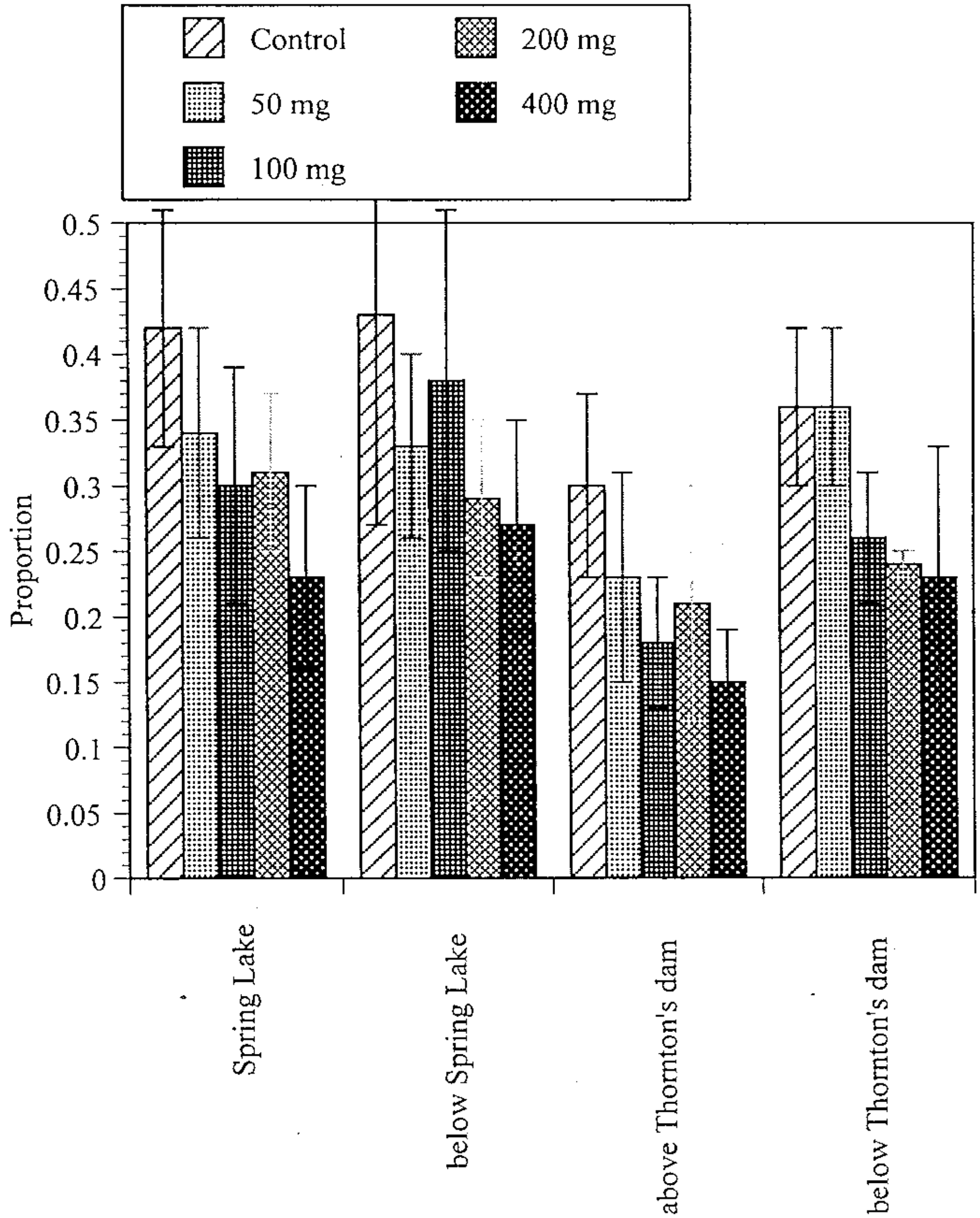

Figure 6-4. Root /shoot ratios for *Zizania texana* grown on four sediments at four levels of nitrogen additions. Sediment and treatment effects were significant (two-way Anova; $p<0.05$; $n=5$). Ratios dropped on all sediments with nitroge additions asuggesting palnt growth was limited by nitrogen availability.

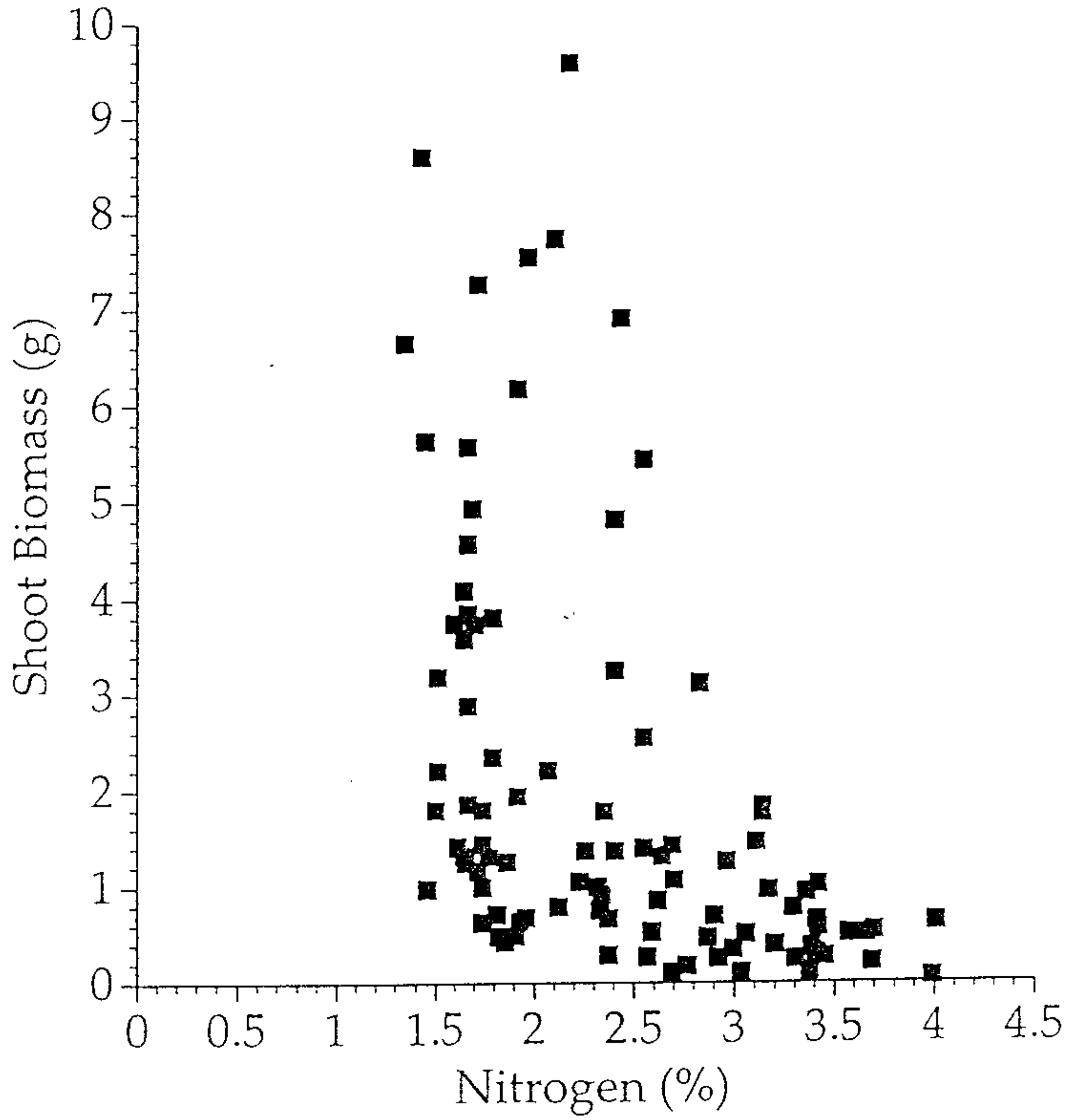

Figure 6-5 Nitrogen tissue concentration in shoots of *Z. texana*. Nitrogen shows the greatest concentration and the most variability at low shoot biomass suggesting little N limitation in plant growth. This suggestion is contrary to conclusions drawn from growth data.

CHAPTER 7

Direct and indirect effects of floating vegetation mats on Texas wildrice (*Zizania texana*)

I. Contract #336-0225

The stated objective for contract #336-0225 was initiate and/or continue and complete the quantification of the effect of floating vegetation mats on productivity and survivorship of Texas wildrice. There were no stated procedures for this objective.

INTRODUCTION

Texas wildrice (*Zizania texana* Hitchc.) is a perennial emergent aquatic macrophyte in the family Poaceae. It has long, ribbon-like, submerged leaves and reproductive culms that arise from the base of the plant. Each culm has an emergent terminal inflorescence allowing for wind pollination. *Zizania texana* is endemic to the first 2.4 km of the San Marcos River and Spring Lake in Hays Co., Texas. It commonly occurs at midchannel in relatively fast-flowing water (Silveus, 1933; Terrell et al., 1978).

Zizania texana is listed as endangered by both U. S. Fish and Wildlife Service and Texas Parks and Wildlife Department (U. S. Fish and Wildlife Service, 1985). Factors that threaten survival of *Z. texana* include reduced spring flow from the San Marcos springs, reduced water quality in the San Marcos River, competition and predation by nonnative species such as *Hydrilla verticillata* and nutria (*Myocaster coypus*), failure to reproduce sexually, and alteration of sediments in the river bottom (Emery, 1967, 1977; U. S. Fish and Wildlife Service, 1994).

More recently, floating and submerged drifting aquatic vegetation have been identified as a potential threat to *Z. texana* plants. Aquatic vegetation, including *Cerataphyllum demersum*, *H. verticillata, Egeria densa*, is mowed in Spring Lake, the headwaters of the San Marcos River, and despite attempts at harvesting the cuttings, cut vegetation drifts downstream. Cut vegetation combines with drifting macrophyte fragments and tends to accumulate behind obstructions at or above the water surface. Obstructions occur in fast or slow flowing water and can be floating *Z. texana* leaves, emergent *Z. texana* culms, other floating vegetation, fallen tree limbs, trash, or debris. Entangled vegetation can form thick mats over *Z. texana* stands in as little as one week.

Direct negative effects of floating vegetation mats may include shredding of *Z. texana* leaves by serrated leaf margins of some macrophytes (e.g., *C. demersum*) and interference with reproductive culm emergence. Indirect negative effects include blocking sunlight, which interferes with photosynthesis, and slowing current velocity, which may reduce nutrient uptake from the open water (Smith and Walker, 1980; Boeger, 1992; Rose and Power, 1994). The objective of this research was to quantify direct and indirect effects of vegetation mats on *Z. texana* stands.

METHODS AND MATERIALS

An artificial obstruction on which drifting vegetation would become entangled was created with a 1-m long by 0.075-m diameter float constructed of polyvinyl chloride pipe. Floats were anchored to the river bottom perpendicular to the direction of flow, at the leading edge of four *Z. texana* stands in the San Marcos River in July 1995. Each *Z. texana* stand was composed of closely spaced, mature individuals. A control area was marked adjacent to each float. Sites were in areas where current was great enough to wash floating vegetation downstream in the absence of an obstruction. This eliminated the need to remove vegetation mats from control areas. Vegetation accumulated upstream and downstream from the float. Six measurements were recorded in each control and treatment area. The measurements were: water depth; current velocity (measured at 20, 60, and 80% depth with a Marsh-McBirney portable water current meter model 201); photosynthetically active radiation (PAR, measured immediately below the water surface with a Li-Cor light meter model LI-185B with a LI-1935B spherical quantum sensor); leaf length (calculated as the average length of 5 or 6 leaves); stem density (no. of

Table 7-1. Environmental measurements and *Zizania texana* response (SD in parentheses) to vegetation mats. Data were recorded at the beginning of the experiment (initial) and six weeks after construction of floats that created vegetation mats (treatment); adjacent areas lack vegetation mats (control).

	Time of measurement			
	Initial		Six weeks	
Measurement	Control	Treatment	Control	Treatment
Water depth (m)	0.51a[1] (0.133)	0.57a (0.180)	0.52a (0.132)	0.47a (0.160)
Current velocity (m/s)	0.210a (0.059)	0.174a (0.095)	0.232a (0.099)	0.032b (0.009)
PAR ($\mu E/s/m^2$)	1800a (316)	1475a (709)	1530a (205)	77b (10)
Leaf length (m)	1.12a (0.278)	1.12a (0.288)	0.95a (0.131)	0.60b (0.100)
Stem density (/100 cm^2)	69.7a (20.0)	66.7a (12.4)	60.2a (12.1)	20.2b (6.40)
Percent damaged leaves	15a (0.007)	16a (0.003)	10a (0.002)	94b (0.034)

[1]Means for a measurement within a time period followed by the same letter are not significantly different ($P > 0.05$); $n = 4$.

reproductive culms + no. of submerged leaves /100 cm^2); and percent damaged leaves (calculated from 50 leaves inspected for shredding or other signs of mechanical damage). Measurements were taken at the leading edge of each stand in July and mid-August, 1995 (six weeks after construction and placement of floats).

Grab samples of vegetation mats were collected from two replicates at the end of the experimental period. Plant species were separated and identified, and percent species composition of each sample was determined.

Means from treatment and control areas at the beginning of the experiment and after six weeks were compared with a paired *t*-test (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

At time zero, there was no significant difference between control and treatment in any category measured (Table 7-1). After six weeks, current velocity, PAR, stem density, and leaf length were significantly less in the treatment areas compared with the control areas (Table 7-1). A higher percentage of leaves were damaged in treatment areas compared with control areas. In addition to being shredded, damaged leaves appeared to be paler green in color or achlorotic.

The upstream sample, composed primarily of *C. demersum,* a submerged macrophyte common in Spring Lake, was 0.35 m thick. The downstream sample was composed of vegetation common in the San Marcos River; *Potamogeton* sp. and *Sagittaria platyphylla* were the most abundant species (30% and 35%, respectively; Table 7-2). The downstream sample was 0.37 m thick.

Current velocity was significantly slower in treatment areas compared to control areas. Stationary objects, such as macrophytes, can reduce current velocities in flowing water (Gregg and Rose, 1982). Dense vegetation mats (occupying about 85% of the water column) obstructed water movement and slowed current velocity in treatment areas. In addition, stem density of plants in treatment areas declined during the study period while stem density of plants in control areas remained unchanged. A number of studies have found a positive relationship between flow and carbon uptake and photosynthetic rates (Smith and Walker, 1980; Madsen and Søndergaard, 1983). Flowing water may also provide mechanical stimulation of meristematic tissue resulting in increased stem density. The decline in stem density observed in this study may be attributed, in part, to the negative effect of decreased current velocity.

Plants in treatment areas had significantly more damaged leaves than control areas. *Ceratophyllum demersum* and *H. verticellata* have serrated leaf margins and when in contact they shred *Z. texana* leaves. Damaged leaves most likely had lower photosynthetic rates than leaves in control areas due to leaf shredding and reduced PAR below mats.

Vegetation mats may also interfere with culm emergence and pollination, contributing to sexual reproductive failure. The effect of vegetation mats on reproduction requires further study. Other direct mechanical damage to *Z. texana* by vegetation mats may include uprooting of plants. Although uprooting of plants was not observed in treatment areas, it is not uncommon to observe sediments eroding from the base of plants and eventually entire plants become uprooted. It is unknown whether uprooting of plants in the wild is due to disturbance of the sediments or caused by drag on plants from entangled vegetation fragments or some other factors.

Historic photographs of *Z. texana* and the San Marcos River (Silveus, 1933) suggest that vegetation mats are more common in the upstream site today than in the past. The increase in mats may be due to a change in species composition exhibiting a growth form more susceptible to fragmentation and drifting in Spring Lake and the San Marcos River . *Hydrilla verticellata*, a fast growing macrophyte, was introduced to Spring Lake and the San Marcos River, and *C. demersum*, a nonrooted submersed macrophyte, currently has a wider distribution in Spring Lake than historical records would indicate it had in the past (Watkins, 1930; Devall, 1940). In addition, mowing of Spring Lake, a practice initiated as early as the 1900's, releases thousands of kilograms of macrophyte fragments annually.

Table 7-2. Species composition (%) of vegetation mats sampled six weeks after construction of vegetation floats. Location 1 was downstream from confluence of Purgatory Creek and San Marcos River, adjacent to Centennial Park picnic area. Location 2 was in Sewell Park approximately 30 m downstream from Loop 82 Bridge.

	Composition (%)	
Species	Location 1	Location 2
Cabomba caroliniana	0	2
Cerataphyllum demersum	15	75
Ceratopteris thalictroides	0	1
Egeria densa	10	8
Eichhornia sp.	0	2
Hydrilla verticillata	10	7
Myriophyllum spicatum	0	2
Potamogeton sp	30	1
Sagittaria platyphylla	35	0
Utricularia sp.	0	2

Finally, reduced flooding due to the construction of flood control dams in the San Marcos River watershed and the resulting absence of scouring of the river bottom, together with reduced spring flows due to over pumping of the Edwards Aquifer, may contribute to conditions in the San Marcos River that favor increased macrophyte growth and abundance, contributing to vegetation mats.

LITERATURE CITED

Allen, E. D. and D. H. N. Spence. 1981. The differential ability of aquatic plants to utilize the inorganic carbon supply in fresh waters. New Phytologist 87:269-283.

Barko, J. W. and R. M Smart. 1978. The growth and biomass distribution of two emergent freshwater plants, *Cyperus esculentus* and *Scirpus validus*, on different sediments. Aquatic Botany, 5:109-117.

Barko, J. W. and R. M. Smart. 1979. The nutritional ecology of *Cyperus esculentus*, an emergent aquatic plant grown on different sediments. Aquatic Botany, 6:13-28.

Barko, J. W. and R. M. Smart. 1986. Sediment-related mechanisms of growth limitation in submersed macrophytes. Ecology, 67(5): 1328-1340.

Barko, J.W., D, Gunnison and S. R. Carpenter. 1991. Sediment interactions with submersed macrophyte growth and community dynamics. Aquatic Botany, 41:41-65.

Boeger, R. T. 1992. The influence of substratum and water velocity on growth of Ranunculus aquatilis L. (Ranunculaceae). Aquatic Botany, 42:351-359.

Bradley, P.M. and J. T. Morris. 1990. Influence of oxygen and sulfide concentration on nitrogen uptake kinetics in *Spartina alterniflora*. Ecology, 71(1): 282-287.

Brower, J.E. and J. H. Zar. 1984. Field & Laboratory Methods for General Ecology. Second edition, Wm. C. Brown Publishers. Dubuque, Iowa. pp 40-45.

Buckner, H. D. and W. J. Shelby. 1991. Water Resources Data Texas Water Year 1991. Vol.3 p. 232. U. S. Geological Survey.

Chambers, P. A., E. E. Prepas, M. L. Bothwell and H. R. Hamilton. 1989. Roots versus shoots in nutrient uptake by aquatic macrophytes in flowing waters. Canadian Journal of Fisheries and Aquatic Science, 46:435-439.

Chambers, P. A., E. E. Prepas, H. R. Hamilton and M. L. Bothwell. 1991. Current velocity and its effect on aquatic macrophytes in flowing waters. Ecological Applications, 1(3):249-257.

Clesceri, L. S., A. E. Greenburg, R. R. Trussel eds. 1989. Standard Methods for the examination of water and wastewater.. Pp. 2-29-2-35.

Denny, M. W. 1993. Air and Water: The biology and physics of life's media. Eberhardt, L. L. and J. M. Thomas. 1991. Designing environmental field studies. Ecological Monographs, 61(1):53-73.

Devall, L. L. 1940. A comparative study of plant dominance in a spring-fed lake. Master's Thesis, Southwest Texas State University, San Marcos, TX, 49 pp.

Dunn, C. P. and R. R. Sharitz. 1991. Population structure, biomass allocation, and phenotypic plasticity in *Murdannia keisak* (Commelinaceae). American Journal of Botany, 78(12):1712-1723.

Eberhardt, L. L. and J. M. Thomas. 1991. Designing environmental field studies. Ecological Monographs, 61(1):53-73.

Emery, W. H. P. 1967. The decline and threatened extinction of Texas wildrice (*Zizania texana* Hitchc).Southwestern Naturalist, 12(2):203-204.

Emery, W. H. P. 1977. Current status of Texas wildrice (*Zizania texana* Hitchc). Southwestern Naturalist, 22(3):393-394.

Emery, W. H. P. and M. N. Guy. 1979. Reproduction and embryo development in Texas wildrice(*Zizania texana* Hitchc.). Bulletin of the Torrey Botanical Club, 106(1):29-31.

Fonseca, M. S. and W. J. Kenworth. 1987. Effects of current on photosynthesis and distribution of seagrasses. Aquatic Botany, 27:59-78.

Fonteyn, P. J. and P. Power. 1989. Texas wildrice, Interim Report. Submitted to U. S. Fish and Wildlife Service, Region 2. pp. 1-10.

Gee, O. H., R. J. Probert and S. A. Coomber. 1994. 'Dehydrin-like' proteins and desiccation tolerance in seeds. Seed Science Research, 4:135-141.

Gerloff, G C. and Krombholz, P.H. 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. Limnology and Oceanography, 11:529-537.

Grabe, D. F., editor. 1970. Tetrazolium testing handbook for agricultural seeds. Contribution No. 29 to the Handbook on Seed Testing, prepared by the Tetrazolium Testing Committee of the Association. Published by the Association of Official Seed Analysts.

Gregg, W. W. and Rose, F. L. 1982. The effects of aquatic macrophytes on the stream microenvironment. Aquatic Botany, 14:309-324.

Harper, J. L., and J. Ogden. 1970. The reproductive strategy of higher plants. I. The concept of strategy with special reference to *Senecio vulgaris* L. Journal of Ecology, 58:681-698.

Hickman, J. C. 1975. Environmental unpredictability and plastic energy allocation strategies in the annual *Polygonum cascadense* (Polygonaceae). Journal of Ecology, 63:689-701.

Hynes, H. B. N. 1970. The Ecology of Running Waters. Liverpool University Press, Liverpool.

Kearns, C. A. and D. W. Inouye. 1993. Techniques for Pollination Biologists. University Press of Colorado.

Kardan, H. A. 1974. The rice shoot in relation to oxygen supply and root growth in seedlings germinating under water. The New Phytologist, 73:695-697.

Kennedy, R. A., S. C. H. Barrett, D. Vander Zee, and M. E. Rumpho. 1980. Germination and seedling growth under anaerobic conditions in *Echinocloa crus-galli* (Barnyard grass). Plant, Cell and Environment, 3:243-248

Losee, R. F. and R. G. Wetzel. 1993. Littoral flow rates within and around submersed macrophyte communitites. Freshwater Biology, 29:7-17.

Maberly, S. C. and D. H. N. Spence. 1983. Photosynthetic inorganic carbon use by freshwater plants. Journal of Ecology 71:705-724.

Madsen, J. D. 1991. Resource allocation at the individual plant level. Aquatic Botany, 41:678-86.

Madsen, T. V. and M. Sondergaard. 1983. The effects of current velocity on the photosynthesis of *Callitriche stagnalis* scop. Aquatic Botany, 15:187-193.

Neill, C. 1993. Growth and resource allocation of whitetop (*Scolochloa festucacea*) along a water depth gradient. Aquatic Botany, 46:235-246.

Nilsson, C., 1987. Distribution of stream-edge vegetation along a gradient of current velocity. Journal of Ecology, 75: 513-522.

Palmer, M. E. 1987. A critical look at rare plant monitoring in the United States. Biological Conservation, 39:113-127.

Pavlik, B. M. and M. G. Barbour. 1988. Demographic monitoring of endemic sand dune plants Eureka Valley, California. Biological Conservation, 46:217-242.

Power, P. 1990. Effects of oxygen concentration and substrate on seed germination and seedling growth of *Zizania texana* (Texas wildrice). Master's Thesis. Southwest Texas State University.

Power, P. 1996a. Effects of current velocity and substrate composition on growth of Texas wildrice (*Zizania texana*). Aquatic Botany, 55:199-204.

Power, P. 1996b. Reintroduction of Texas wildrice (*Zizania texana*) in Spring Lake: Some important environmental and biotic considerations. *In*: Proceedings from Second Southwestern Rare and Endangered Plant Conference: Rocky Mountain Forest & Range Experimental Station.

Power, P. 1996c. Growth of Texas wildrice (*Zizania texana*) in three sediments from the San Marcos River. Journal of Aquatic Plant Management, 34:21-23.

Power, P. 1996d. Direct and indirect effects of floating vegetation mats on Texas wildrice (*Zizania texana*). Southwestern Naturalist, 41(4):462-464.

Power, P. 1997. Moisture, seeds, and reproductive failure in Texas wildrice (*Zizania texana*). Southwestern Naturalist, 42(4):435-440.

Power, P. and Fonteyn P. J., 1995. Effects of oxygen concentration and substrate on seed germination and seedling growth of Texas wildrice (*Zizania texana*). Southwestern Naturalist, 40(1): 1-4.

Rebsdorf, A. 1972. The carbon dioxide system of freshwater. A set of tables for easy computation of the carbon dioxide system. Printed booklet. Freshwater biological Laboratory, Hillerod, Denmark.

Riemer, D. N. 1984. Introduction to Freshwater Vegetation. AVI Publishing Co., Inc., Westport, Connecticut. pp.95-106.

Rose, F. L., and P. Power. 1992. Effects of habitat and herbivory on growth and reproduction in Texas wildrice (*Zizania texana*). Report submitted to U. S. Fish and Wildlife Service, Region 2. pp. 1-20.

Rose, F. L. and P. J. Power. 1993. Continued maintenance, reintroduction, and research on Texas wildrice (*Zizania texana*). Report submitted to U. S. Fish andWildlife Service, Region 2.

Rose, F. L., and P. Power. 1994a. Continued maintenance, reintroduction and research on *Zizania texana* (Texas wildrice). III. Carbon source for submerged leaves the endangered *Zizania texana* (Texas wildrice). Report submitted to U. S. Fish and Wildlife Service, Region 2.

Rose, F. L. and P. Power. 1994b. Continued maintenance, reintroduction and research on *Zizania texana* (Texas wildrice). IV. Growth response by *Zizania texana* (Texas wildrice) under three current regimes and two sediments in Spring Lake, Hays Co., Texas. Report submitted to U. S. Fish and Wildlife Service, Region 2.

Sand-Jensen, K. 1983. Photosynthetic carbon sources of stream macrophytes. Journal of Experimental Botany. 34:198-210.

Silveus, W. A. 1932. *Zizania texana* Hitch., sp. nov. Journal of the Washington Academy of Sciences 23:454.

Silveus, W. A. 1933. Texas Grasses, Classification and Description of Grasses. The Clegg Co., San Antonio, Texas.

Smith, F. A. and N. A. Walker. 1980. Photosynthesis by aquatic plants: Effects of unstirred layers in relation to assimilation of CO_2 and HCO_3- and to carbon isotopic discrimination. New Phytologist, 86: 245-259.

Snedecor, G. W., and W. G. Cochran. 1980. Statistical Methods. Eighth ed. Iowa St. Univ. Press, Ames. Iowa, 506 pp.

Sytsma, M.D. and Anderson, L.W.J. 1993. Criteria for assessing nitrogen and phosphorus deficiency in *Myriophyllum aquaticum.* Journal of Freshwater Ecology, 8(2):155-163.

Terrell, E. E., W. H. P. Emery and H. E. Beaty. 1978. Observations on *Zizania texana* (Texas wildrice), an endangered species. Bulletin of the Torrey Botanical Club, 105:50-57.

Texas Agricultural Extension Service. 1987. Identifying and managing aquatic rodents in Texas: Beaver, Nutria and Muskrats. Texas A&M. pp. 1-46.

Texas Parks and Wildlife Department. 1990. Interim report on conservation of the upper San Marcos ecosystem: Texas wildrice (*Zizania texana*). Submitted to U. S. Fish and Wildlife Service, Region 2.

U. S. Fish and Wildlife Service. 1984. San Marcos River Recovery Plan. U. S. Fish and Wildlife Service, Albuquerque, New Mexico. pp. 41-58.

U. S. Fish and Wildlife Service. 1985. Endangered and threatened wildlife and plant taxa for listing as endangered or threatened species. Federal Register 5C(188):39526-39584.

U. S. Fish and Wildlife Service, 1994. Draft San Marcos/Comal (Revised) Recovery Plan. Albuquerque, New Mexico. pp. vii+119.

van Baalen, J., W. H. O. Ernst, J. van Andel, D. W. Janssen and H. J. M. Nelissen. 1990. Reproductive allocation in plants of *Scrophularia nodosa* grown at various levels of irradiance and soil fertility. Acta Botanica Neerl., 39(2):183-196.

van Lent, F., and J. M Verschuure. 1994. Intraspecific variability of *Zostera marina* L.

(eelgrass) in the estuaries and lagoons of the southwestern Netherlands. I. Population dynamics. Aquatic Botany, 48:31-58.

van Wijck, C., C-J. De Groot and P. Grillas. 1992. The effect of anaerobic sediment on the growth of *Potamogeton pectinatus* L.: the role of organic matter, sulphide and ferrous iron. Aquatic Botany, 44:31-49.

Vertucci, C. W., J. Crane, R. A. Porter and E. A. Oelke. 1994. Physical properties of water in *Zizania* embryos in relation to maturity status, water content and temperature. Seed Science Research 4:211-224.

Vertucci, C. W., J. Crane, R. A. Porter and E. A. Oelke. 1995. Survival of *Zizania* embryos in relation to water content, temperature and maturity status. Seed Science Research, 5:31-40.

Watkins, G. M. 1930. Vegetation of San Marcos Springs. Masters Thesis, University of Texas at Austin. pp. 1-53.

Westlake, D. F., 1967. Some effects of low-velocity currents on the metabolism of aquatic macrophytes. Journal of Experimental Botany, 18: 187-205.

Wetzel, R. G. 1983. Limnology. Saunders College Publishing, Philadelphia.

Wilkinson, L., 1989. Systat: The System for Statistics. Evanston, Ill.: SYSTAT, Inc.

Zar, J. H., 1984. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Zeh, M. and W. Donni. 1994. Restoration of spawning grounds for trout and grayling in the river High-Rhine. Aquatic Sciences, 56:59-69.

Appendix 1-1. Data from preliminary germination trials to determine conditions which would obtain consistently high percent germination. Trials included variable light and temperature regimes. Seeds were germinated either in open petri dishes or closed vials. *Zizania texana* seeds were stored 4 months at 3°C under anaerobic and aerobic conditions. Storage conditions were thus: no more than 20 seeds were placed in a vial. Vials were either capped (anaerobic) or covered with cheese cloth and placed in a beaker with an aerator (aerobic).

	Treatments		Storage Conditions	
			Aerobic No. germinated/total	Anaerobic No. germinated/total
Petri dish				
	24h L	21°C	0/5	0/5
	24h L	15/25°C	0/5	1/4
	12/12h L	21°C	0/10	0/10
			0/14	0/15
	12/12h L	15/35°C	0/8	0/7
	24h Dk	25°C	1/11	0/4
Vial				
	24h L	21°C	5/7	6/7
	12/12h L	21°C	13/13	11/13
	12/12h L	21°C	7/9	6/9
	24h Dk	21°C	6/6	3/6

Appendix 1-2. Germination of *Z. texana* seeds at different oxygen concentrations in the water. Twenty seeds were placed in a flasks containing water with different oxygen concentrations. After 7 days the number of germinated seeds was counted.

	Oxygen Concentration (ppm)					
Replicate	0.1	1.0	2.0	3.0	4.0	5.0
1	18	17	12	1	0	0
2	14	18	13	1	0	3
3	17	16	13	1	0	0
4	16	15	10	0	1	0
5	14	15	13	2	1	1
6	12	2	16	8	0	0
7	12	14	6	4	5	0
8	12	14	13	9	1	0

Appendix 1-3. *Zizania texana* seeds collected during 1989 from cultivated population at SWT. Seeds were placed in deionized water in either open vials (aerobic), closed vials (anaerobic), or closed vials containing water which had been boiled to drive off oxygen. Germinated seeds were placed in peat pots and grown for future seed production or research.

Number of Seeds						
Date of Collection	Aerobic	Anaerobic	Date of Collection	Aerobic	Anaerobic	Boiled Water
15 May	5	5	1 September	23	23	
19 May	13	12	4 September	64	64	
2 June	2	2	6 September	79	79	
5 June	9	10	8 September	22	22	
10 June	10	9	11 September	19	19	
12 June	7	6	13 September	45	45	
15 June	5	5	15 September	67	67	
19 June	5	5	19 September	62	62	
26 June	11	11	21 September	19	19	
30 June	3	4	23 September	32	32	
6 July	4	3	26 September	36	36	
10 July	1	1	28 September	105	105	
12 July	2	2	29 September	6	6	
14 July	6	6	2 October	25	25	
17 July	9	9	4 October	82	82	
19 July	10	10	8 October	50	25	
21 July	13	13	10 October	75	76	
24 July	17	17	13 October	86	85	
26 July	11	11	16 October	58	57	
28 July	18	18	19 October	34	35	
31 July	6	19	24 October	61	61	
2 August	6	6	26 October	60	60	
4 August	7	7	31 October	51	51	
7 August	7	9	3 November	39	39	
9 August	10	10	7 November	34	34	
11 August	10	10	13 November			61
14 August	13	13	14 November	61		
16 August	6	6	20 November	40		
18 August	12	12	24 November	33		
21 August	16	16	27 November	43		
23 August	20	20	30 November	21		60
25 August	27	27	4 December	5		
28 August	44	44	6 December	51		
30 August	38	37	11 December	26		
			14 December	17		

Appendix 1-4. Germination and positive tetrazolium test for *Z. texana* seeds in storage between 0 and 42 weeks. Seeds were placed in a dish filled with 300 ml water. A cover was placed over the dish. After 7 days the number of germinated seeds was counted. Ungerminated seeds were subjected to the tetrazolium test.

Time in Storage (weeks)	N	# Germinated	% Germinated	# + Tetrazolium test	% + Tetrazolium test	% Viable
0	20	1	5	19	95	100
2	20	9	45	7	35	80
4	20	19	95	0	0	95
6	20	20	100	-	-	100
8	20	16	80	3	15	95
10	20	16	80	3	15	95
12	20	19	95	0	0	95
14	20	13	65	4	20	85
16	20	18	90	2	10	100
18	20	16	80	2	10	90
19	28	24	86	1	3	89
20	41	36	88	5	12	100
21	39	32	82	1	3	85
22	15	14	93	0	0	93
24	24	19	79	1	4	83
26	20	16	80	1	5	85
28	20	19	95	0	0	95
30	20	17	85	1	5	90
32	20	13	65	2	10	75
34	20	14	70	0	0	70
36	21	12	57	0	0	57
38	20	16	80	1	5	85
40	20	10	50	4	20	70
42	77	36	47	0	0	47

Appendix 1-5. Data for seedling growth by cultivated *Z. texana*. Sand was purchased, other sediments were collected from the San Marcos River upstream of the IH35 bridge, or collected from ponds on the SWT campus.

Date	#Leaves	Length(cm)	Width (cm)	Leaf Area (cm^2)	Leaf Area/Plant
Planted in San Marcos River sediments in peat pots 14 March 89					
6 April 89	3	21	0.3	6.3	
		11	0.2	2.2	
		1	0.2	0.2	8.7
	3	19	0.3	5.7	
		9.8	0.2	1.96	
		8	0.2	1.6	9.26
	2	9.5	0.2	1.90	
		4	0.2	0.8	2.7
	3	9.5	0.2	1.9	
		4	0.2	0.8	
		1	0.2	0.2	2.9
MEAN					**5.9**
17 April 89	4	27.1	0.3	8.13	
		17.8	0.3	5.13	
		13.0	0.2	2.6	
		3.0	0.2	0.6	16.46
	4	35.3	0.3	10.59	
		14.7	0.3	4.41	
		16.0	0.2	3.2	
		8.5	0.2	1.7	19.9
	3	14.5	0.3	4.35	
		7.0	0.2	1.4	
		7.2	0.2	1.44	7.19
	4	19:2	0.3	5.76	
		3.2	0.1	0.32	
		5.2	0.2	1.04	
		2.0	0.2	0.4	7.52
MEAN					**12.8**
27 April 89	4	40	0.3	12.0	
		21	0.3	6.3	
		22	0.3	6.6	

Continued....

Appendix 1-5. Cont.

Date	#Leaves	Length(cm)	Width (cm)	Leaf Area (cm^2)	Leaf Area/Plant
		12	.3	3.6	28.5
	3	43	0.3	13.2	
27 April 89		21	0.3	6.3	
		14	0.3	4.2	23.7
	3	25	0.3	7.5	
		7	0.3	2.1	
27 April 89		2	0.3	0.6	10.2
	4	29	.3	8.7	
		18	0.3	5.4	
		5	0.3	1.5	
		3	0.3	0.9	16.5
MEAN					**19.75**
Planted in SWT pond sediments					
3 weeks after planting					
					0.3
					0.8
					5.2
					6.2
					3.2
MEAN					**3.1**
6 weeks after planting					
					17.86
					8.9
					115.8
					51.6
					43.1
MEAN					**47.5**
12 weeks after planting					
					252.1
					232.8
					55.2
					151.2
					288.1
MEAN					**195.9**

Appendix 1-5. Cont.

Date	#Leaves	Length(cm)	Width (cm)	Leaf Area (cm^2)	Leaf Area/Plant
Planted in sand					
3 weeks after planting					
					2.0
					2.0
					1.7
					2.7
					3.6
MEAN					**2.4**
6 weeks after planting					
					9.0
					6.2
					23.3
					10.6
					18.9
MEAN					**13.6**
12 weeks after planting					
					78.6
					105.6
					76.4
					41.0
					131.5
MEAN					**86.6**

Appendix 1-6. Growth and reproductive indicators of 1 year-old *Z. texana* grown in the outdoor raceway on the SWT campus. Seedlings were planted in April 1989 and measured on 13 March 1990.

Nodes with Roots	Basal Circumference (cm)	Basal Area (cm^2)	Emergent Culms	Inflorescences
3	30	72	10	1
0	19	29	0	0
7	35	97	11	0
3	31	76	9	1
3	31	76	16	1
5	35	97	11	0
3	40	127	17	1
7	75	448	11	2
3	33	87	16	1
4	56	249	20	3

Appendix 1-7. Seed production during 1990 by *Z. texana* from the cultivated population at SWT. Plants occupied about 25 m^2.

	Plant Number													
Date	1	2	3	4	5	6	7	8	9	10	Growing in mud	Growing in sand	All other	Total
2 April								2						2
5 April				9										9
16 April							2		1				44	47
17 April	2		2				4						52	60
19 April			1										26	27
23 April	5		37		26		5	14		39			46	172
24 April													80	80
26 April	10		1		3		12	13		13			7	59
30 April	1		8		13			16	1	78			116	233
1 May			2	1	5	8	2	6		8			90	122
3 May				3	9		10	10	8	32			112	184
7 May	38		40	38	11	39	15	62	41	47			237	568
8 May	14		7	14	12	5	15	26	9	29			110	241
10 May	19		63	7	28	3	42	61	34	49			341	647
14 May	13		26	4	89	13	54	94	17	33			405	748
16 May	8		19		59	8	17	53	20	52			349	630
18 May													130	130
21 May													435	435
25 May													400	400
29 May	26		85	68	21		101	111	26	151	36		511	1136
31 May	13		67	57	50		61	129	44	64	47		376	909
4 June	4		61	38	40	14	49	64	28	28	87		310	723

Continued....

Appendix 1-7. Continued.

Date	1	2	3	4	5	6	7	8	9	10	Growing in mud	Growing in sand	All other	Total
						Plant Number								
6 June	2		82	10	108	6	43	42	20	31	23		436	793
8 June	3		57	32	62	35	41	34	28	11	49		473	825
11 June	1		33	26	91	10	47	109	48	70	30		820	1285
13 June			51	13	43	5	45	50	6	16	45		516	790
15 June			52	12	16	19	37	9	3	3	71		473	695
18 June			26	23	17	11		45	12	6			390	530
20 June	3		22		10	26	24	24		6			332	447
22 June	9		58	4	8	8	17	32	4	125	81	7	368	714
25 June	12		12		4	13	17		1	37	43		244	384
27 June	4		23	5	13	14	10	13	1	92	52		343	570
29 June	4		13	9	6			73		17	4		120	246
5 July	2		9	4	17		42	26	16	76	58		190	440
10 July			24	2	11	5	17	20	3	9	63		247	301
12 July			15					3		8	42		185	253
17 July				3				15	6				303	327
19 July				1	2	3		20	2	21	2		195	246
24 July			3	3			1	55	1	26	6		382	477
26 July			1		7			15			18		171	212
31 July								6			46		187	239
2 August											36		65	101
6 August								22		3	10		205	240
9 August	7							11			5		179	202
13 August								7					106	113

Continued....

Appendix 1-7. Continued.

	Plant Number													
Date	1	2	3	4	5	6	7	8	9	10	Growing in mud	Growing in sand	All other	Total
16 August								7					120	127
20 August	4										4		50	58
23 August								17			5		120	142
27 August								6			81		194	281
30 August										12	7		118	137
TOTAL	204	0	909	386	781	246	730	1,252	380	1,177	951	7	10,699	18,737

Appendix 1-8. Destination and date of shipment of *Z. texana* from the cultivated population at SWT.

Date	Destination
15 April 1991	Dr. Ervin Oelke, U. of Minnesota (approx. 1,000 seeds)
January 1992	Dr. Robin Probert, Royal Botanic Gardens, Kew (100 seeds)
5 December 1995	Dr. Christina Walters, USDA, Ft. Collins, CO. (seeds)
6 May 1997	Wayne Kennard, U of Minnesota (seeds)
20 January 1998	Dr. Mike Antolin, Colorado State, (leaf clips)
4 June 1998	Dr. Darren Touchell, USDA, Ft. Collins, CO (leaf clips and seeds)
6 July 1998	Dr. Darren Touchell, USDA, Ft. Collins, CO (seeds)
13 July 1998	Dr. Darren Touchell, USDA, Ft. Collins, CO (seeds)
21 July 1998	Dr. Darren Touchell, USDA, Ft. Collins, CO (seeds)
9 September 1998	Dr. Darren Touchell, USDA, Ft. Collins, CO (seeds)
26 October 1998	Dr. Darren Touchell and Dr. Christina Walters, National Seed Storage Lab, Ft. Collins (9.27g seeds 2 "perenniating" structures)
8 March 1999	Dr. Darren Touchell, USDA, Ft. Collins, CO (10.0 g seeds)

Appendix 1-9. *Zizania texana* seed collection from cultivated population at SWT during 1991. Plamts occupied about 25 m^2.

Date of Collection	# Seeds	Date of Collection	# Seeds
10 April	1	27 September	154
15 April	1	30 September	218
17 April	6	2 October	172
18 April	4	4 October	107
13 May	8	7 October	139
14 May	3	9 October	134
16 May	3	11 October	116
23 May	13	14 October	184
28 May	13	16 October	142
30 May	2	18 October	148
21 June	51	23 October	285
9 July	22	25 October	170
1 August	19	28 October	218
5 August	15	30 October	128
12 August	38	1 November	11
13 August	4	4 November	10
14 August	24	8 November	48
16 August	15	11 November	32
18 August	156	15 November	123
19 August	28	18 November	93
20 August	95	22 November	106
22 August	81	27 November	112
25 August	106	2 December	78
26 August	38	4 December	35
27 August	50	6 December	20
29 August	84	12 December	78
31 August	51		
2 September	103		
3 September	85		
4 September	77		
5 September	80		
7 September	162		
9 September	151		
11 September	133		
13 September	209		
16 September	212		
18 September	120		
20 September	75		
23 September	233		
25 September	53		

GRAND TOTAL 5,685

Appendix 1-10. *Zizania texana* seed collection from cultivated population at SWT during 1992. Plants occupied about 25 m^2.

Date of Collection	# Seeds
28 April	1
1 May	61
4 May	66
6 May	37
11 May	100
13 May	81
15 May	54
18 May	52
20 May	28
26 May	172
1 June	188
3 June	183
5 June	81
8 June	141
12 June	28
15 June	76
17 June	53
24 June	191
26 June	22
30 June	54
2 July	35
7 July	33
10 July	43
14 July	31
17 July	23
20 July	17
28 July	63
20 August	32
16 September	12
24 September	40
23 October	140
12 November	54
GRAND TOTAL	2,192

Appendix 1-11. Germination and positive tetrazolium test for *Z. texana* seeds in storage between 0 and 36 weeks. Seeds were placed in a dish filled with 300 ml water. A cover was placed over the dish. After 7 days the number of germinated seeds was counted. Ungerminated seeds were subjected to the tetrazolium test.

Time in Storage (weeks)	N	# Germinated	% Germinated	# + Tetrazolium test	% + Tetrazolium test	% Viable
0	20	0	0	20	100	100
2	20	12	60	3	15	75
4	19	16	84	2	10	94
6	20	20	100	-	-	100
8	20	19	95	0	0	95
10	20	14	70	2	10	80
12	19	16	84	0	0	84
14	20	18	90	2	10	100
16	20	18	90	1	5	95
18	20	16	80	0	0	80
20	20	17	85	2	10	95
22	20	20	100	-	-	100
24	20	19	95	0	0	95
26	20	13	65	1	5	70
28	20	19	95	0	0	95
32	20	20	100	-	-	100
34	20	17	85	2	10	95
36	20	17	85	0	0	85

Appendix 1-12. Germination and seedling development of seeds collected from cultivated *Z. texana*. Seeds were placed in covered dishes for 7 days. Seven days after the cover was removed seeds were observed for chlorophyll, shoots and primary and secondary roots. 1992 data.

			Germinated Seeds			
Collection Date	# Seeds	% Germinated	% Proper Development	% Chlorophyll Roots Absent	% No Chlorophyll	Time in Storage (days)
1 May 92	61	88	74	5	20	88
4 May 92	66	95	63	22	14	107
6 May 92	37	92	88	0	13	113
11 May 92	100	86	20	8	72	122
13 May 92	81	90	70	11	19	120
15 May 92	54	83	73	7	20	132
18 May 92	52	86	18	18	64	136
20 May 92	28	71	35	30	35	141
26 May 92	172	41	70	14	15	142
1 June 92	188	58	55	14	30	136
3 June	183	9	6	0	94	145
5 June 92	81	46	5	3	86	161
8 June 92	141	64	62	3	34	210
12 June 92	28	32	0	0	100	151
24 June 92	191	71	79	8	13	194
26 June 92	22	91	100	0	0	124
30 June 92	54	67	0	22	88	128
2 July 92	35	63	9	4	86	
Mean	87.4	68.5	45.9	9.4	44.6	
SD	(60.157)	(23.932)	(33.888)	(8.925)	(34.325)	

Appendix 1-13. *Zizania texana* seed collection from cultivated population at SWT during 1993. Each seed weighed approximately 0.01g. Plants occupied about 25 m^2.

Date of Collection	# Seeds	Weight (g)	Est. # of seeds	
4 January	4			
15 April	17			
17 April	17			
19 April	26			
21 April	136			
26 April	200			
26 April	152			
28 April	452			
3 May	59			
7 May	29			
10 May	12			
12 May	17			
17 May	74			
19 May	111			
24 May	266			
26 May	103			
1 June	806			
7 June	312			
11 June	491			
14 June	1,170			
17 June		12.37	1,237	
21 June		10.37	1,037	
24 June		7.16	716	
28 June		6.15	615	
1 July		3.71	371	
6 July		3.03	303	
8 July		4.13	413	
12 July		4.00	400	
19 July		7.73	773	
22 July		4.29	429	
29 July		3.73	373	
2 August		4.11	411	
2 September	79			
20 September	144			
27 September		5.27	527	
5 October	194			
18 October	400			
25 October	370			
11 November	275			
16 November	178			
29 November	208			
TOTAL	6,302		7,078	
GRAND TOTAL				**13,380**

Appendix 1-14. Germination and seedling development of seeds collected from cultivated *Z. texana*. Seeds were placed in covered dishes for 7 days. Seven days after the cover was removed seeds were observed for chlorophyll, shoots and primary and secondary roots. 1993 data.

			Germinated Seeds			
Collection Date	N	% Germinated	% Proper Development	% Chlorophyll Roots Absent	% No Chlorophyll	Time in Storage (days)
15 April 1993	17	94	94	6	0	32
17 April 1993	17	53	44	?	33	30
19 April 1993	26	38	100	0	0	28
26 April 1993	152	89	69	7	24	38
28 April 1993	40	67	77	4	18	54
28 April 1993	100	57	74	2	24	47
28 April 1993	100	73	79	2	19	47
3 May 1993	59	88	85	0	15	49
7 May 1993	29	93	100	0	0	52
10 May 1993	12	83	70	0	30	49
12 May 1993	17	88	67	7	27	47
MEAN	51.7	74.8	78.1	2.8	17.3	
SD	(46.130)	(18.782)	(16.471)	(2.974)	(12.240)	

Appendix 1-15. *Zizania texana* seed collection from cultivated population at SWT during 1994. Each seed weighed approximately 0.01g. Plants occupied about 25 m^2.

Date of Collection	No. Seeds	Weight (g)	Est. no. of seeds	
30 March	17			
20 April	41			
25 April	71			
27 April	14			
16 May		7.67	767	
23 May		18.57	1857	
25 May		11.62	1162	
1 June		8.70	870	
7 June		19.61	1961	
10 June		15.24	1524	
13 June		12.71	1271	
21 June		21.99	2199	
27 June		15.01	1501	
5 July		19.72	1972	
10 July		30.00	3000	
15 July		15.00	1500	
21 July		18.63	1863	
26 July		7.00	700	
TOTAL	143		22,147	
GRAND TOTAL				**22,290**

Appendix 1-16. *Zizania texana* seed collection from cultivated population at SWT during 1995. Each seed weighed approximately 0.01g. Plants occupied about 25 m^2.

Date of Collection	Weight (g)	Estimated no. of seeds
7 July	1.89	189
25 July	5.25	525
27 July	4.69	469
31 July	7.44	744
3 August	6.75	675
8 August	9.45	945
10 August	4.92	492
11 August	8.89	889
15 August	12.23	1,223
21 August	19.92	1,992
24 August	20.09	2,009
31 August	25.01	2,501
18 September	40.78	4,078
27 September	55.86	5,586
4 October	21.0	210
11 October	50.08	5,008
20 October	22.86	2,286
25 October	46.21	4,621
2 November	15.12	1,512
7 November	6.71	671
GRAND TOTAL		36,625

Appendix 1-17. *Zizania texana* seed collection from cultivated population and plants collected from the wild as called for under the U.S. Fish and Wildlife Contingency Plan during 1996. Each seed weighed approximately 0.01g. Plants occupied about 25 m^2.

Cultivated Plants			Wild Plants		
Date of Collection	Weight (g)	Est. no. of seeds	Date of	Plant ID	# of Seeds
29 April	5.00	500	12 July	B2	18
3 May	15.70	1,570	18 July	B2	7
6 May	12.01	1,201	18 July	A	7
13 May	43.76	4,376	22 July	B2	11
16 May	20.20	2,020	22 July	J20	8
22 May	18.79	1,879	26 July	B2	5
3 June	19.55	1,955	26 July	B15f	3
10 June	15.90	1,590	26 July	J20	29
17 June	44.22	4,422	29 July	J20	4
20 June	18.18	1,818	29 July	B15f	8
24 June	15.93	1,593	1 August	J20	8
27 June	20.39	2,039	1 August	B15f	8
1 July	19.76	1,976	1 August	B14d	3
9 July	32.05	3,205	1 August	B2	7
12 July	10.00	1,000	5 August	B14d	13
15 July	20.14	2,014	5 August	no label	7
18 July	15.84	1,584	5 August	J20	3
22 July	16.75	1,675	8 August	no label	2
26 July	18.34	1,834	8 August	B2	1
29 July	12.78	1,278	8 August	B15f	9
1 August	10.04	1,004	8 August	B14d	27
5 August	5.14	514	14 August	B2	3
8 August	6.20	620	14 August	F11	19
14 August	9.14	914	14 August	no label	74
22 August	7.02	702	14 August	B14d	20
3 September	12.62	1,262	29 August	K37a	2
6 September	9.11	911	29 August	J20	11
17 September	7.48	748	29 August	J12	8
30 September	20.76	2,076	29 August	K10c	6
			29 August	B15f	12
			29 August	F11	63
			3 September	J20	52
			3 September	B15f	57
			3 September	A	9
			3 September	F11	17
			3 September	B14d	51
			5 September	B14d	24
			5 September	J20	18
			5 September	B15f	14
			5 September	F11	1
			5 September	B2	2
			17 September	K10c	2
TOTAL		48,280			653
GRAND TOTAL					**48,933**

Appendix 2-1. Weight of raceway grown *Z. texana*. Plants were clipped the interface between roots and leaves and returned to the lab. Plants were oven dried.

Replicate	Reproductive Material (g)	Vegetative Material (g)	Total (g)
1	0.4945	1.1742	1.6687
2		1.5280	1.5280
3	1.2007	1.2325	2.4332
4		0.7428	0.7428
5		0.8946	0.8946
6	0.9473	1.0610	2.0083
7		1.4400	1.4400
8		0.9338	0.9338
9		2.2980	2.2980
10		1.5475	1.5475
Mean	**0.8808**	**1.2852**	**1.5495**
sd	(0.2921)	(0.4488)	(0.5809)

Appendix 2-2. Survivorship of *Z. texana* in three sites in Spring Lake in 1992. Plants in all sites survived at least through July 1992 based on inflorescence data (Appendix 2-4). As plants increased in size and produced tillers, it was difficult to distinguish one plant from another.

		Number of individuals				
		Control (Exclosure)		Treatment (No exclosure)		
Site	Date	1	2	1	2	3
Wetland	20 January	10	10	10	10	
	5 February	10	10	9	1	
	21 February	10	9	0	0	
	17 March	10	9	0	0	
	13 April	9	4	0	0	
	11 May	9	2	0	0	
Intermediate	22 January	10	10	10	10	
	17 February	10	8	0	10	
	28 February	10	8	0	10	
	17 March	10	9	0	8	
	14 April	9	5	0	8	
	11 May	9	5	0	8	
Dam	10 January			6	8	10
	28 February			6	8	10
	25 March			7	8	9
	16 April			5	8	9

Appendix 2-3. Partial field notes *Z. texana* in Spring Lake.

Date	Location	Description
9 Dec 91	Wetland	plants without exclosures (planted 4 Dec 91) show 2 types of herbivory. 1) all emergent leaves and culms have been clipped off at the water line. 2) some culms have also been clipped of ~ 6" from the substrate. All age classes in exclosures appear healthy, with no signs of herbivory
16 Jan 92	Intermediate	one of the uncaged replicates shows herbivory. Stems from one plant were eaten right at the base. On other plants stems and leaves were eaten about 1 foot from base.
5 Feb 92	Wetland all	uncaged plants clipped 5-20 cm below surface
17 Feb 92	Intermediate	uncaged with 0 plants was a sandy site all others were muddy, all plants show herbivory just below water line (5-20cm) or just inside cage. Birds can put beak inside wire mesh and bite off leaves.
21 Feb 92	Intermediate	all leaves at the surface were clipped off
26 Feb 92	Wetland and Intermediate	all leaves that extend out of the cage lengthwise are eaten at the edge of the cage
4 Mar 92	Intermediate	3 female ducks were sitting on cage. There was an egg on the cage, this is the third egg since January. (Is it a turtle or a duck egg?)
17 Mar 92	Intermediate	uncaged plants are all clipped below water line but look green and healthy otherwise
11 May 92	Intermediate	Uncaged plants show few signs of herbivory. They look green and healthy. The submerged leaves are **not** at the surface.
	Dam	Shoot just breaks surface
13 May 92	Intermediate	Two shoots of uncaged plants just touching the surface
	Dam	one shoot 3 inches above surface
20 May 92	Intermediate	Teeth marks on cage, Nutria eating cage? Leaves clipped on 2 emergent culms in uncaged site
	Dam	3 emergent culms clipped
3 June 92	Dam	all emergent culms at dam site are clipped-regularly. The stems are stout and strong but they are all clipped as soon as they emerge.
8 June 92	Wetland	Dozens of emergent culms grow though wire mesh of lid of cage. They stand ~2ft above lid. Today noted that some animal walked on lid through emergent culms, chewing them off as it passed between the culms. It didn't eat the culms, just chewed them off. Probably Nutria activity.
	Intermediate	lots of clipped leaves from uncaged plants. All emergent culms are regularly clipped from uncaged plants.
30 June 92	Intermediate	Animal crawled on top of cage and chewed on stems. Same as Wetland 8 June 92. The pattern of bird herbivory appears to be more precise-less destructive. Nutria scramble around and make mincemeat out of the plant material.
26 April 93	Intermediate	Last week I rowed around Spring Lake, there were 2 new recruits outside cage at Transition (intermdiate) site

Continued ....

Appendix 2-3. Continued.

Date	Location	Description
27 May 93	Spring	meeting with Gena Janssen, Gene Sultanfus, Keith Wall. Decided to place some wildrice near springs if suitable substrate is located.
14 July 93	Dam	Planted 36 plants at dam site with Darrell Solanik and Chip Wood assisted with scuba gear. Plants planted in March were not very successful. They were overtaken by Hydrilla. Plants must be tended regularly (competition removed and cut veg cleared) Fall and winter may be best planting time Failure due to 1. intense shading from cut vegetation 2. Dapple shade from overhanging trees 3. Competition with Hydrilla
20 July 93	Dam	Cleared cut vegetation from dam site plants. Used inflatable air mattress. Cut vegetion appears to be very harmful to plants in two ways. 1. Blocks sunlight, 2. Shreds leaves, especially *Ceratophyllum demersum* and *Hydrilla*

Appendix 2-4. Inflorescences produced in 1992 by *Z. texana* grown in three sites in Spring Lake. Data are the number of inflorescences tagged with cotton string on each date. Inflorescences in control plots eventually produced seeds. Plants in treatment plots produced emergent culms but culms were clipped by herbivores before inflorescences developed (See Appendix 2-3).

		Number of inflorescences				
		Control (Exclosure)		Treatment (No exclosure)		
Site	Date	1	2	1	2	3
Wetland	21 Feb	2	0	0	0	
	6 March	2	0	0	0	
	17 March	1	0	0	0	
	30 March	7	0	0	0	
	13 April	9	0	0	0	
	27 April	17	0	0	0	
	4 May	7	0	0	0	
	11 May	9	0	0	0	
	20 May	29	0	0	0	
	26 May	24	0	0	0	
	1 June	14	0	0	0	
	8 June	18	0	0	0	
	15 June	24	0	0	0	
	24 June	28	0	0	0	
	30 June	13	0	0	0	
	7 July	18	0	0	0	
Total Inflorescences		**222**	**0**	**0**	**0**	
Intermediate	21 Feb	0	0	0	0	
	6 March	0	0	0	0	
	17 March	0	0	0	0	
	30 March	0	0	0	0	
	13 April	1	0	0	0	
	27 April	0	0	0	0	
	4 May	0	0	0	0	
	11 May	0	0	0	0	
	20 May	4	1	0	0	
	26 May	4	1	0	1	
	2 June	3	2	0	0	

Continued....

Appendix 2-4. Continued

		Number of inflorescences				
		Control (Exclosure)		Treatment (No exclosure)		
Site	Date	1	2	1	2	3
Intermediate						
	8 June	4	1	0	0	
	15 June	11	5	0	0	
	24 June	28	0	0	0	
	30 June	13	0	0	0	
	7 July	20	7	0	0	
Total Inflorescences		**87**	**17**	**0**	**1**	
Dam						
	21 Feb			0	0	0
	6 March			0	0	0
	17 March			0	0	0
	30 March			0	0	0
	13 April			0	0	0
	27 April			0	0	0
	4 May			0	0	0
	11 May			0	0	0
	20 May			0	0	0
	26 May			0	0	0
	2 June			0	0	0
Total Inflorescences				**0**	**0**	**0**

Appendix 2-5. Characteristics of *Z. texana* grown in three sites in Spring Lake. Plants were transplanted in January 1992 and harvested at three week intervals.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
Wetland												
	17 March	1	11	4	-	0	-	-	0	-	4	4
	17 March	2	20	10	-	0	-	-	0	-	16	10
	17 March	3	12	6	-	0	-	-	0	-	12	6
Mean			**14.3**	**6.7**		**0**			**0**		**10.7**	**6.7**
sd			**4.9**	**3.1**							**6.1**	**3.1**
Intermediate												
	17 March	1	22	7	-	0	-	-	0	-	5	3
	17 March	2	18	10	-	0	-	-	0	-	5	2
	17 March	3	20	7	-	0	-	-	0	-	7	1
Mean			**20**	**8**		**0**			**0**		**5.7**	**2**
sd			**2.0**	**1.7**							**1.1**	**1**
Dam												
	17 March	1	41	18	-	0	-	-	0	-	6	4
	17 March	2	85	34	-	0	-	-	0	-	5	1
	17 March	3	33	16	-	0	-	-	0	-	2	1
Mean			**53**	**22.7**		**0**			**0**		**4.3**	**2**
sd			**28**	**9.9**							**2.1**	**1.7**
Wetland												
	27 April	1	19	6	1	1	-	-	0	1.06	8	2
	27 April	2	5	9	0	0	-	-	0	0.62	9(culms)	1
	27 April	3	22	4	6/1	2/1	-	6	0	0.56	22	2
								5.2				
								7.2				
								6.4				
								5				
Mean			**15.3**	**6.3**	**3.5**	**1.5**		**6.0**	**0**	**0.75**	**13**	**1.7**
sd			**9.1**	**2.5**	**3.5**	**0.7**		**0.9**		**0.27**	**7.8**	**0.6**

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
Intermediate												
	27 April	1	54	21	2/1	0	-	20	0	1.63	2	1
								21.4				
	27 April	2	38	14	0	0	-	-	0	1.32	3	0
	27 April	3	78	29	2/1	1/1	-	14	0	-	-	
								8				
								2.2				
Mean			**56.7**	**21.3**	**1.3**	**0.3**	-	**13.1**	**0**	**1.4**	**2.5**	**0.5**
sd			**20.1**	**7.5**	**1.1**	**0.6**		**8.1**		**0.2**	**0.7**	**0.7**
Dam												
	28 April	1	135	60	0	0	-	-	-	1.85	>10	<10
	28 April	2	43	16	0	0	-	-	0	1.84	4	2
	28 April	3	109	42	1/1	0	-	23.6	0	2.05	>10	<10
					2/1			17.4				
								11.4				
Mean			**95.7**	**39.3**	**1.5/1**	**0**	-	**17.5**	**0**	**1.91**		
sd			**47.4**	**22.1**	**0.7**			**6.1**		**0.12**		
Wetland												
	8 June	1	0	14	8	1/1	28	3	0	-	13	1
								7				
								6.5				
								8				
								16.5				
								4.5				
								12.5				
								12				
					1	0		10				
					3	0		6				
								10				

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
								11.5				
	8 June	2	4	2	7	4/1	7	11	1	-	4	2
								6				
Wetland	8 June	2						3.5				
								8				
								8				
								10.5				
								20.5				
	8 June	3	5	13	4/1	0	7	12.5	2	-	0	0
								12				
								15				
								19				
					2/1			6.5			2	1
								4.5				
					2	0	6	11.5				
								10				
					5	2	9	5.5				
								17.5				
								10.5				
								8				
								11.5				
					3(tiller)	2	4	15				
								10				
								11				
					3(tiller)	1	7	10.5				1
								10				
								8				
					0	0	3	-				

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
					4	1	5	16.5				
								10				
								8				
								10				
					3	0	6	1				
								7				
								8.5				
					7	1	3	6	1		1	
Wetland	8 June	3						10.5				
								7.5				
								13				
								13				
								12				
								15.5				
Mean			**3**	**4**	**3.7**	**0.8**	**5**	**10.1**	**1.3**	**-**	**6.7**	**1.7**
sd			**2.6**	**3**	**2.3**	**1.2**	**1.7**	**4.1**	**1.5**		**5.5**	**0.6**
Intermediate												
	9 June	1	29	15	5	2	3	14		1.72	2	1
								16.5				
								19				
								15.5				
								10.5				
					3	1	4	9	1			
								22.5				
								12				
					1	0	4	3				
					3	1	5	1.5				
								9.5				
								10.5				

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
					3	1	5	10				
								14				
								6				
					6	0	4	16				
								20				
								8.5				
								13.5				
								15				
								17.5				
	9 June	2	125	31	2	0	60	7.5	3	1.55	34	24
								20.5				
Intermediate	9 June	2			5	1		9.5				
								21				
								18.5				
								12.5				
								11				
					5	1		14				
								23				
								21.5				
								7.5				
								10				
					6	2		8				
								14				
								20				
								14.5				
								10.5				
								12				
					3	0		30.5				
								15				
								11				

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
					3	0		8.5				
								23				
								12				
					4	2		16.5				
								9.5				
								11.5				
								19				
					5	2		20.5				
								14.5				
								15				
								15				
								19				
					2	0		8				
								17				
Intermediate	9 June	2			8	2		17				
								17				
								13				
								6.5				
								8.5				
								13				
								25				
								25.5				
	9 June	3	80	14	3	0	22	17	3	1.41	9	0
								21				
								13				
					2	0		10.5				
								15.5				
					6	0		21				
								14				
								19				

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
								21.5				
								18				
								19				
					6	2		3				
								17				
								22				
								18.5				
								13				
								15.5				
					2	0		16				
								18				
					4	1		14				
								21.5				
								13				
								15				
					3	0		4				
								26				
Intermediate	9 June	3						15				
					4	1		11.5				
								12.5				
								15				
								8				
					4	1		11.5				
								9.5				
								9.5				
					5	0		26				
								12.5				

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
								13.5				
								21				
								25				
Mean			**78**	**16.3**	**4.0**	**0.8**	**35.7**	**14.8**	**2.3**	**1.56**	**15.0**	**8.3**
sd			**48.0**	**13.6**	**1.7**	**0.8**	**21.1**	**5.6**	**1.1**	**0.2**	**16.8**	**13.6**
Dam												
	9 June	1	74	14	2	0	0	30.5	0	3.10	32	4
								34.5				
					4	2		12.5				
								16.5				
								36				
								25.5				
	9 June	2	44	6	2	0	0	24.5	0	3.40	17	0
								37.5				
	9 June	3	263	25	1	0	0	25.5	0	3.26	105	2
					3	1		22.5				
								2				
								10				
					1	0		31.5				
					3	0		26				
								22				
								12				
Dam	9 June	3			2	1		19				
								22				
					7			25				
								17.5				
								20				
								25				

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
								19.5				
								14				
								7.5				
					2	0		23				
								17.5				
					5	2		30				
								15.5				
								19				
								27				
								12				
					4	1		36				
								13				
								16.5				
					2	1		18.5				
								10.5				
					2	1		15.5				
								17.5				
					6	0		15.5				
								12.5				
								17.5				
								20.5				
								19.5				
								20				
					6	1		24				
								13.5				
								13				
								23.5				
Dam	9June	3						15				
								16.5				
								16				

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
					6	2		14.5				
								22				
								18.5				
								13.5				
								20.5				
					2	0		30				
								19.5				
					7	2		7				
								2				
								18				
								10				
								29.5				
								19				
								6				
Mean			**166**	**21.7**	**3.5**	**0.1**	**0**	**19.2**	**0**	**3.25**	**51.3**	**2**
sd			**186.5**	**20.6**	**2.0**			**7.7**		**0.1**	**47.1**	**2**

Appendix 2-6. Biomass of *Z. texana* harvested on 17 March 1992, 4 May 1992 and 12 June 1992 from 3 sites in Spring Lake. All plant material was oven dried to a constant weight. *Zizania texana* was first planted in January 1992. Mean weight of leaves at time of tranplanting was 1.55 g.

	Date	Plant	Roots (g)	Tiller roots (g)	Submerged leaves and reproductive culms (g)	Inflorescences (g)	Seeds (g)	Total (g)
Wetland								
	17 March	1	0.2575	0	1.2474	0	0	1.5049
	17 March	2	0.5629	0	4.7566	0	0	5.3195
	17 March	3	0.1987	0	1.4224	0	0	1.6211
Mean			**0.3397**	**0**	**2.4755**	**0**	**0**	**2.8152**
sd			**0.1955**		**1.9774**			**2.1696**
Intermediate								
	17 March	1	0.4369	0	3.0721	0	0	3.509
	17 March	2	0.4161	0	2.0995	0	0	2.5156
	17March	3	0.4600	0	1.6714	0	0	2.1314
Mean			**0.4377**	**0**	**2.2810**	**0**	**0**	**2.7187**
sd			**0.0220**		**0.7178**			**0.7109**
Dam	17 March	1	1.40	0	11.26	0	0	12.66
	17 March	2	0.79	0	11.21	0	0	12.00
	17 March	3	1.45	0	6.59	0	0	8.04
Mean			**1.213**	**0**	**9.6867**	**0**	**0**	**10.9**
sd			**0.3675**		**2.6819**			**2.50**
Wetland								
	4 May	1	0.4390	0	1.9482	0	0	2.3872
	4 May	2	0.4115	0	0.9564	0	0	1.3679
	4 May	3	0.3899	0.6360	4.2494	0	0	5.2753
Mean			**0.4135**	**0.2120**	**2.3847**	**0**	**0**	**3.0101**
sd			**0.0246**	**0.3672**	**1.6893**			**2.0268**

Continued....

Appendix 2-6 . Continued

	Date	Plant	Roots (g)	Tiller roots (g)	Submerged leaves (g)	Emergent leaves (g)	Culms (g)	Inflorescences (g)	Seeds (g)	Total (g)
Intermediate										
	4 May	1	1.12	0	13.34			0	0	14.46
	4 May	2	1.11	0	6.25			0	0	7.36
	4 May	3	2.56	0.0183	13.3821			0	0	15.9604
Mean			**1.60**	**0.0061**	**10.99**			**0**	**0**	**12.59**
sd			**0.83**	**0.0106**	**4.11**					**4.59**
Dam										
	6 May	1	4.13	0	30.96			0	0	35.09
	6 May	2	0.84	0	8.96			0	0	9.80
	6 May	3	4.23	0.0018	3.30			0	0	40.2418
Mean			**3.07**	**0.0006**	**14.41**			**0**	**0**	**28.3773**
sd			**1.93**	**0.0010**	**14.61**					**16.2933**
Wetland										
	12 June	1	1.3608	0.1701	0	1.1907	3.7705	0	0	6.4921
	12 June	2	0.2835	0.0850	0.3969	0.5386	2.6082	0.0567	0	3.9689
	12 June	3	0.6804	0.8788	1.2757	6.8607	17.8321	0.4536	0.3685	28.3498
Mean			**0.7749**	**0.3780**	**0.5575**	**2.8633**	**8.0703**	**0.1701**	**0.1228**	**12.9368**
sd			**0.5448**	**0.4358**	**0.6528**	**3.4771**	**8.4739**	**0.2471**	**0.2127**	**13.4075**
Intermediate										
	12 June	1	1.4175	0.6237	6.488	3.8270	11.25	0.50	0	24.1062
	12 June	2	9.5823	1.09	20.54	8.26	37.26	1.14	0.11	77.9823
	12 June	3	5.103	0.227	12.394	3.856	23.04	0.91	0.30	45.83
Mean			**5.3676**	**0.6469**	**13.141**	**5.314**	**23.85**	**0.85**	**0.14**	**49.3062**
sd			**4.0888**	**0.4320**	**7.056**	**2.5511**	**13.02**	**0.32**	**0.15**	**27.1057**

Continued....

Appendix 2-6. Continued

	Date	Plant	Roots (g)	Tiller roots (g)	Submerged leaves (g)	Emergent leaves (g)	Culms (g)	Inflorescences (g)	Seeds (g)	Total (g)
Dam										
	12 June	1	1.0	1.20	23.54	0	15.03	0	0	39.77
	12 June	2	1.16	0.08	28.88	0	19.64	0	0	49.76
	12 June	3	14.88	4.69	122.39	0	90.53	0	0	232.49
Mean			**5.68**	**1.99**	**58.27**	**0**	**41.73**	**0**	**0**	**107.34**
sd			7.97	**2.40**	**55.59**		**42.32**			**108.50**

Appendix 1-7. Continued.

						Plant Number								
Date	1	2	3	4	5	6	7	8	9	10	Growing in mud	Growing in sand	All other	Total
6 June	2		82	10	108	6	43	42	20	31	23		436	793
8 June	3		57	32	62	35	41	34	28	11	49		473	825
11 June	1		33	26	91	10	47	109	48	70	30		820	1285
13 June			51	13	43	5	45	50	6	16	45		516	790
15 June			52	12	16	19	37	9	3	3	71		473	695
18 June			26	23	17	11		45	12	6			390	530
20 June	3		22		10	26	24	24		6			332	447
22 June	9		58	4	8	8	17	32	4	125	81	7	368	714
25 June	12		12		4	13	17		1	37	43		244	384
27 June	4		23	5	13	14	10	13	1	92	52		343	570
29 June	4		13	9	6			73		17	4		120	246
5 July	2		9	4	17		42	26	16	76	58		190	440
10 July			24	2	11	5	17	20	3	9	63		247	301
12 July			15					3		8	42		185	253
17 July				3				15	6				303	327
19 July				1	2	3		20	2	21	2		195	246
24 July			3	3			1	55	1	26	6		382	477
26 July			1		7			15			18		171	212
31 July								6			46		187	239
2 August											36		65	101
6 August								22		3	10		205	240
9 August	7							11			5		179	202
13 August								7					106	113

Continued....

Appendix 1-7. Continued.

	Plant Number													
Date	1	2	3	4	5	6	7	8	9	10	Growing in mud	Growing in sand	All other	Total
16 August								7					120	127
20 August	4										4		50	58
23 August								17			5		120	142
27 August								6			81		194	281
30 August										12	7		118	137
TOTAL	204	0	909	386	781	246	730	1,252	380	1,177	951	7	10,699	18,737

Appendix 1-8. Destination and date of shipment of *Z. texana* from the cultivated population at SWT.

Date	Destination
15 April 1991	Dr. Ervin Oelke, U. of Minnesota (approx. 1,000 seeds)
January 1992	Dr. Robin Probert, Royal Botanic Gardens, Kew (100 seeds)
5 December 1995	Dr. Christina Walters, USDA, Ft. Collins, CO. (seeds)
6 May 1997	Wayne Kennard, U of Minnesota (seeds)
20 January 1998	Dr. Mike Antolin, Colorado State, (leaf clips)
4 June 1998	Dr. Darren Touchell, USDA, Ft. Collins, CO (leaf clips and seeds)
6 July 1998	Dr. Darren Touchell, USDA, Ft. Collins, CO (seeds)
13 July 1998	Dr. Darren Touchell, USDA, Ft. Collins, CO (seeds)
21 July 1998	Dr. Darren Touchell, USDA, Ft. Collins, CO (seeds)
9 September 1998	Dr. Darren Touchell, USDA, Ft. Collins, CO (seeds)
26 October 1998	Dr. Darren Touchell and Dr. Christina Walters, National Seed Storage Lab, Ft. Collins (9.27g seeds 2 "perenniating" structures)
8 March 1999	Dr. Darren Touchell, USDA, Ft. Collins, CO (10.0 g seeds)

Appendix 1-9. *Zizania texana* seed collection from cultivated population at SWT during 1991. Plamts occupied about 25 m^2.

Date of Collection	# Seeds	Date of Collection	# Seeds
10 April	1	27 September	154
15 April	1	30 September	218
17 April	6	2 October	172
18 April	4	4 October	107
13 May	8	7 October	139
14 May	3	9 October	134
16 May	3	11 October	116
23 May	13	14 October	184
28 May	13	16 October	142
30 May	2	18 October	148
21 June	51	23 October	285
9 July	22	25 October	170
1 August	19	28 October	218
5 August	15	30 October	128
12 August	38	1 November	11
13 August	4	4 November	10
14 August	24	8 November	48
16 August	15	11 November	32
18 August	156	15 November	123
19 August	28	18 November	93
20 August	95	22 November	106
22 August	81	27 November	112
25 August	106	2 December	78
26 August	38	4 December	35
27 August	50	6 December	20
29 August	84	12 December	78
31 August	51		
2 September	103		
3 September	85		
4 September	77		
5 September	80		
7 September	162		
9 September	151		
11 September	133		
13 September	209		
16 September	212		
18 September	120		
20 September	75		
23 September	233		
25 September	53		

GRAND TOTAL 5,685

Appendix 1-10. *Zizania texana* seed collection from cultivated population at SWT during 1992. Plants occupied about 25 m^2.

Date of Collection	# Seeds
28 April	1
1 May	61
4 May	66
6 May	37
11 May	100
13 May	81
15 May	54
18 May	52
20 May	28
26 May	172
1 June	188
3 June	183
5 June	81
8 June	141
12 June	28
15 June	76
17 June	53
24 June	191
26 June	22
30 June	54
2 July	35
7 July	33
10 July	43
14 July	31
17 July	23
20 July	17
28 July	63
20 August	32
16 September	12
24 September	40
23 October	140
12 November	54
GRAND TOTAL	2,192

Appendix 1-11. Germination and positive tetrazolium test for *Z. texana* seeds in storage between 0 and 36 weeks. Seeds were placed in a dish filled with 300 ml water. A cover was placed over the dish. After 7 days the number of germinated seeds was counted. Ungerminated seeds were subjected to the tetrazolium test.

Time in Storage (weeks)	N	# Germinated	% Germinated	# + Tetrazolium test	% + Tetrazolium test	% Viable
0	20	0	0	20	100	100
2	20	12	60	3	15	75
4	19	16	84	2	10	94
6	20	20	100	-	-	100
8	20	19	95	0	0	95
10	20	14	70	2	10	80
12	19	16	84	0	0	84
14	20	18	90	2	10	100
16	20	18	90	1	5	95
18	20	16	80	0	0	80
20	20	17	85	2	10	95
22	20	20	100	-	-	100
24	20	19	95	0	0	95
26	20	13	65	1	5	70
28	20	19	95	0	0	95
32	20	20	100	-	-	100
34	20	17	85	2	10	95
36	20	17	85	0	0	85

Appendix I-12. Germination and seedling development of seeds collected from cultivated *Z. texana*. Seeds were placed in covered dishes for 7 days. Seven days after the cover was removed seeds were observed for chlorophyll, shoots and primary and secondary roots. 1992 data.

			Germinated Seeds			
Collection Date	# Seeds	% Germinated	% Proper Development	% Chlorophyll Roots Absent	% No Chlorophyll	Time in Storage (days)
1 May 92	61	88	74	5	20	88
4 May 92	66	95	63	22	14	107
6 May 92	37	92	88	0	13	113
11 May 92	100	86	20	8	72	122
13 May 92	81	90	70	11	19	120
15 May 92	54	83	73	7	20	132
18 May 92	52	86	18	18	64	136
20 May 92	28	71	35	30	35	141
26 May 92	172	41	70	14	15	142
1 June 92	188	58	55	14	30	136
3 June	183	9	6	0	94	145
5 June 92	81	46	5	3	86	161
8 June 92	141	64	62	3	34	210
12 June 92	28	32	0	0	100	151
24 June 92	191	71	79	8	13	194
26 June 92	22	91	100	0	0	124
30 June 92	54	67	0	22	88	128
2 July 92	35	63	9	4	86	
Mean	87.4	68.5	45.9	9.4	44.6	
SD	(60.157)	(23.932)	(33.888)	(8.925)	(34.325)	

Appendix 1-13. *Zizania texana* seed collection from cultivated population at SWT during 1993. Each seed weighed approximately 0.01g. Plants occupied about 25 m^2.

Date of Collection	# Seeds	Weight (g)	Est. # of seeds	
4 January	4			
15 April	17			
17 April	17			
19 April	26			
21 April	136			
26 April	200			
26 April	152			
28 April	452			
3 May	59			
7 May	29			
10 May	12			
12 May	17			
17 May	74			
19 May	111			
24 May	266			
26 May	103			
1 June	806			
7 June	312			
11 June	491			
14 June	1,170			
17 June		12.37	1,237	
21 June		10.37	1,037	
24 June		7.16	716	
28 June		6.15	615	
1 July		3.71	371	
6 July		3.03	303	
8 July		4.13	413	
12 July		4.00	400	
19 July		7.73	773	
22 July		4.29	429	
29 July		3.73	373	
2 August		4.11	411	
2 September	79			
20 September	144			
27 September		5.27	527	
5 October	194			
18 October	400			
25 October	370			
11 November	275			
16 November	178			
29 November	208			
TOTAL	6,302		7,078	
GRAND TOTAL				**13,380**

Appendix 1-14. Germination and seedling development of seeds collected from cultivated *Z. texana*. Seeds were placed in covered dishes for 7 days. Seven days after the cover was removed seeds were observed for chlorophyll, shoots and primary and secondary roots. 1993 data.

			Germinated Seeds			
Collection Date	N	% Germinated	% Proper Development	% Chlorophyll Roots Absent	% No Chlorophyll	Time in Storage (days)
15 April 1993	17	94	94	6	0	32
17 April 1993	17	53	44	?	33	30
19 April 1993	26	38	100	0	0	28
26 April 1993	152	89	69	7	24	38
28 April 1993	40	67	77	4	18	54
28 April 1993	100	57	74	2	24	47
28 April 1993	100	73	79	2	19	47
3 May 1993	59	88	85	0	15	49
7 May 1993	29	93	100	0	0	52
10 May 1993	12	83	70	0	30	49
12 May 1993	17	88	67	7	27	47
MEAN	51.7	74.8	78.1	2.8	17.3	
SD	(46.130)	(18.782)	(16.471)	(2.974)	(12.240)	

Appendix 1-15. *Zizania texana* seed collection from cultivated population at SWT during 1994. Each seed weighed approximately 0.01g. Plants occupied about 25 m^2.

Date of Collection	No. Seeds	Weight (g)	Est. no. of seeds	
30 March	17			
20 April	41			
25 April	71			
27 April	14			
16 May		7.67	767	
23 May		18.57	1857	
25 May		11.62	1162	
1 June		8.70	870	
7 June		19.61	1961	
10 June		15.24	1524	
13 June		12.71	1271	
21 June		21.99	2199	
27 June		15.01	1501	
5 July		19.72	1972	
10 July		30.00	3000	
15 July		15.00	1500	
21 July		18.63	1863	
26 July		7.00	700	
TOTAL	143		22,147	
GRAND TOTAL				**22,290**

Appendix 1-16. *Zizania texana* seed collection from cultivated population at SWT during 1995. Each seed weighed approximately 0.01g. Plants occupied about 25 m^2.

Date of Collection	Weight (g)	Estimated no. of seeds
7 July	1.89	189
25 July	5.25	525
27 July	4.69	469
31 July	7.44	744
3 August	6.75	675
8 August	9.45	945
10 August	4.92	492
11 August	8.89	889
15 August	12.23	1,223
21 August	19.92	1,992
24 August	20.09	2,009
31 August	25.01	2,501
18 September	40.78	4,078
27 September	55.86	5,586
4 October	21.0	210
11 October	50.08	5,008
20 October	22.86	2,286
25 October	46.21	4,621
2 November	15.12	1,512
7 November	6.71	671
GRAND TOTAL		**36,625**

Appendix 1-17. *Zizania texana* seed collection from cultivated population and plants collected from the wild as called for under the U.S. Fish and Wildlife Contingency Plan during 1996. Each seed weighed approximately 0.01g. Plants occupied about 25 m^2.

Cultivated Plants			Wild Plants		
Date of Collection	Weight (g)	Est. no. of seeds	Date of	Plant ID	# of Seeds
29 April	5.00	500	12 July	B2	18
3 May	15.70	1,570	18 July	B2	7
6 May	12.01	1,201	18 July	A	7
13 May	43.76	4,376	22 July	B2	11
16 May	20.20	2,020	22 July	J20	8
22 May	18.79	1,879	26 July	B2	5
3 June	19.55	1,955	26 July	B15f	3
10 June	15.90	1,590	26 July	J20	29
17 June	44.22	4,422	29 July	J20	4
20 June	18.18	1,818	29 July	B15f	8
24 June	15.93	1,593	1 August	J20	8
27 June	20.39	2,039	1 August	B15f	8
1 July	19.76	1,976	1 August	B14d	3
9 July	32.05	3,205	1 August	B2	7
12 July	10.00	1,000	5 August	B14d	13
15 July	20.14	2,014	5 August	no label	7
18 July	15.84	1,584	5 August	J20	3
22 July	16.75	1,675	8 August	no label	2
26 July	18.34	1,834	8 August	B2	1
29 July	12.78	1,278	8 August	B15f	9
1 August	10.04	1,004	8 August	B14d	27
5 August	5.14	514	14 August	B2	3
8 August	6.20	620	14 August	F11	19
14 August	9.14	914	14 August	no label	74
22 August	7.02	702	14 August	B14d	20
3 September	12.62	1,262	29 August	K37a	2
6 September	9.11	911	29 August	J20	11
17 September	7.48	748	29 August	J12	8
30 September	20.76	2,076	29 August	K10c	6
			29 August	B15f	12
			29 August	F11	63
			3 September	J20	52
			3 September	B15f	57
			3 September	A	9
			3 September	F11	17
			3 September	B14d	51
			5 September	B14d	24
			5 September	J20	18
			5 September	B15f	14
			5 September	F11	1
			5 September	B2	2
			17 September	K10c	2
TOTAL		48,280			653
GRAND TOTAL					48,933

Appendix 2-1. Weight of raceway grown *Z. texana*. Plants were clipped the interface between roots and leaves and returned to the lab. Plants were oven dried.

Replicate	Reproductive Material (g)	Vegetative Material (g)	Total (g)
1	0.4945	1.1742	1.6687
2		1.5280	1.5280
3	1.2007	1.2325	2.4332
4		0.7428	0.7428
5		0.8946	0.8946
6	0.9473	1.0610	2.0083
7		1.4400	1.4400
8		0.9338	0.9338
9		2.2980	2.2980
10		1.5475	1.5475
Mean	**0.8808**	**1.2852**	**1.5495**
sd	(0.2921)	(0.4488)	(0.5809)

Appendix 2-2. Survivorship of *Z. texana* in three sites in Spring Lake in 1992. Plants in all sites survived at least through July 1992 based on inflorescence data (Appendix 2-4). As plants increased in size and produced tillers, it was difficult to distinguish one plant from another.

		Number of individuals				
		Control (Exclosure)		Treatment (No exclosure)		
Site	Date	1	2	1	2	3
Wetland	20 January	10	10	10	10	
	5 February	10	10	9	1	
	21 February	10	9	0	0	
	17 March	10	9	0	0	
	13 April	9	4	0	0	
	11 May	9	2	0	0	
Intermediate	22 January	10	10	10	10	
	17 February	10	8	0	10	
	28 February	10	8	0	10	
	17 March	10	9	0	8	
	14 April	9	5	0	8	
	11 May	9	5	0	8	
Dam	10 January			6	8	10
	28 February			6	8	10
	25 March			7	8	9
	16 April			5	8	9

Appendix 2-3. Partial field notes *Z. texana* in Spring Lake.

Date	Location	Description
9 Dec 91	Wetland	plants without exclosures (planted 4 Dec 91) show 2 types of herbivory. 1) all emergent leaves and culms have been clipped off at the water line. 2) some culms have also been clipped of ~ 6" from the substrate. All age classes in exclosures appear healthy, with no signs of herbivory
16 Jan 92	Intermediate	one of the uncaged replicates shows herbivory. Stems from one plant were eaten right at the base. On other plants stems and leaves were eaten about 1 foot from base.
5 Feb 92	Wetland all	uncaged plants clipped 5-20 cm below surface
17 Feb 92	Intermediate	uncaged with 0 plants was a sandy site all others were muddy, all plants show herbivory just below water line (5-20cm) or just inside cage. Birds can put beak inside wire mesh and bite off leaves.
21 Feb 92	Intermediate	all leaves at the surface were clipped off
26 Feb 92	Wetland and Intermediate	all leaves that extend out of the cage lengthwise are eaten at the edge of the cage
4 Mar 92	Intermediate	3 female ducks were sitting on cage. There was an egg on the cage, this is the third egg since January. (Is it a turtle or a duck egg?)
17 Mar 92	Intermediate	uncaged plants are all clipped below water line but look green and healthy otherwise
11 May 92	Intermediate	Uncaged plants show few signs of herbivory. They look green and healthy. The submerged leaves are **not** at the surface.
	Dam	Shoot just breaks surface
13 May 92	Intermediate	Two shoots of uncaged plants just touching the surface
	Dam	one shoot 3 inches above surface
20 May 92	Intermediate	Teeth marks on cage, Nutria eating cage? Leaves clipped on 2 emergent culms in uncaged site
	Dam	3 emergent culms clipped
3 June 92	Dam	all emergent culms at dam site are clipped-regularly. The stems are stout and strong but they are all clipped as soon as they emerge.
8 June 92	Wetland	Dozens of emergent culms grow though wire mesh of lid of cage. They stand ~2ft above lid. Today noted that some animal walked on lid through emergent culms, chewing them off as it passed between the culms. It didn't eat the culms, just chewed them off. Probably Nutria activity.
	Intermediate	lots of clipped leaves from uncaged plants. All emergent culms are regularly clipped from uncaged plants.
30 June 92	Intermediate	Animal crawled on top of cage and chewed on stems. Same as Wetland 8 June 92. The pattern of bird herbivory appears to be more precise-less destructive. Nutria scramble around and make mincemeat out of the plant material.
26 April 93	Intermediate	Last week I rowed around Spring Lake, there were 2 new recruits outside cage at Transition (intermdiate) site

Continued ....

Appendix 2-3. Continued.

Date	Location	Description
27 May 93	Spring	meeting with Gena Janssen, Gene Sultanfus, Keith Wall. Decided to place some wildrice near springs if suitable substrate is located.
14 July 93	Dam	Planted 36 plants at dam site with Darrell Solanik and Chip Wood assisted with scuba gear. Plants planted in March were not very successful. They were overtaken by Hydrilla. Plants must be tended regularly (competition removed and cut veg cleared) Fall and winter may be best planting time Failure due to 1. intense shading from cut vegetation 2. Dapple shade from overhanging trees 3. Competition with Hydrilla
20 July 93	Dam	Cleared cut vegetation from dam site plants. Used inflatable air mattress. Cut vegetion appears to be very harmful to plants in two ways. 1. Blocks sunlight, 2. Shreds leaves, especially *Ceratophyllum demersum* and *Hydrilla*

Appendix 2-4. Inflorescences produced in 1992 by *Z. texana* grown in three sites in Spring Lake. Data are the number of inflorescences tagged with cotton string on each date. Inflorescences in control plots eventually produced seeds. Plants in treatment plots produced emergent culms but culms were clipped by herbivores before inflorescences developed (See Appendix 2-3).

		Number of inflorescences				
		Control (Exclosure)		Treatment (No exclosure)		
Site	Date	1	2	1	2	3
Wetland	21 Feb	2	0	0	0	
	6 March	2	0	0	0	
	17 March	1	0	0	0	
	30 March	7	0	0	0	
	13 April	9	0	0	0	
	27 April	17	0	0	0	
	4 May	7	0	0	0	
	11 May	9	0	0	0	
	20 May	29	0	0	0	
	26 May	24	0	0	0	
	1 June	14	0	0	0	
	8 June	18	0	0	0	
	15 June	24	0	0	0	
	24 June	28	0	0	0	
	30 June	13	0	0	0	
	7 July	18	0	0	0	
Total Inflorescences		**222**	**0**	**0**	**0**	
Intermediate	21 Feb	0	0	0	0	
	6 March	0	0	0	0	
	17 March	0	0	0	0	
	30 March	0	0	0	0	
	13 April	1	0	0	0	
	27 April	0	0	0	0	
	4 May	0	0	0	0	
	11 May	0	0	0	0	
	20 May	4	1	0	0	
	26 May	4	1	0	1	
	2 June	3	2	0	0	

Continued....

Appendix 2-4. Continued

		Number of inflorescences				
		Control (Exclosure)		Treatment (No exclosure)		
Site	Date	1	2	1	2	3
Intermediate						
	8 June	4	1	0	0	
	15 June	11	5	0	0	
	24 June	28	0	0	0	
	30 June	13	0	0	0	
	7 July	20	7	0	0	
Total Inflorescences		**87**	**17**	**0**	**1**	
Dam						
	21 Feb			0	0	0
	6 March			0	0	0
	17 March			0	0	0
	30 March			0	0	0
	13 April			0	0	0
	27 April			0	0	0
	4 May			0	0	0
	11 May			0	0	0
	20 May			0	0	0
	26 May			0	0	0
	2 June			0	0	0
Total Inflorescences				**0**	**0**	**0**

Appendix 2-5. Characteristics of *Z. texana* grown in three sites in Spring Lake. Plants were transplanted in January 1992 and harvested at three week intervals.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
Wetland												
	17 March	1	11	4	-	0	-	-	0	-	4	4
	17 March	2	20	10	-	0	-	-	0	-	16	10
	17 March	3	12	6	-	0	-	-	0	-	12	6
Mean			**14.3**	**6.7**		**0**			**0**		**10.7**	**6.7**
sd			**4.9**	**3.1**							**6.1**	**3.1**
Intermediate												
	17 March	1	22	7	-	0	-	-	0	-	5	3
	17 March	2	18	10	-	0	-	-	0	-	5	2
	17 March	3	20	7	-	0	-	-	0	-	7	1
Mean			**20**	**8**		**0**			**0**		**5.7**	**2**
sd			**2.0**	**1.7**							**1.1**	**1**
Dam												
	17 March	1	41	18	-	0	-	-	0	-	6	4
	17 March	2	85	34	-	0	-	-	0	-	5	1
	17 March	3	33	16	-	0	-	-	0	-	2	1
Mean			**53**	**22.7**		**0**			**0**		**4.3**	**2**
sd			**28**	**9.9**							**2.1**	**1.7**
Wetland												
	27 April	1	19	6	1	1	-	-	0	1.06	8	2
	27 April	2	5	9	0	0	-	-	0	0.62	9(culms)	1
	27 April	3	22	4	6/1	2/1	-	6	0	0.56	22	2
								5.2				
								7.2				
								6.4				
								5				
Mean			**15.3**	**6.3**	**3.5**	**1.5**		**6.0**	0	**0.75**	**13**	**1.7**
sd			**9.1**	**2.5**	**3.5**	**0.7**		**0.9**		**0.27**	**7.8**	**0.6**

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
Intermediate												
	27 April	1	54	21	2/1	0	-	20	0	1.63	2	1
								21.4				
	27 April	2	38	14	0	0	-	-	0	1.32	3	0
	27 April	3	78	29	2/1	1/1	-	14	0	-	-	
								8				
								2.2				
Mean			**56.7**	**21.3**	**1.3**	**0.3**	-	**13.1**	**0**	**1.4**	**2.5**	**0.5**
sd			**20.1**	**7.5**	**1.1**	**0.6**		**8.1**		**0.2**	**0.7**	**0.7**
Dam												
	28 April	1	135	60	0	0	-	-	-	1.85	>10	<10
	28 April	2	43	16	0	0	-	-	0	1.84	4	2
	28 April	3	109	42	1/1	0	-	23.6	0	2.05	>10	<10
					2/1			17.4				
								11.4				
Mean			**95.7**	**39.3**	**1.5/1**	0	-	**17.5**	**0**	**1.91**		
sd			**47.4**	**22.1**	**0.7**			**6.1**		**0.12**		
Wetland												
	8 June	1	0	14	8	1/1	28	3	0	-	13	1
								7				
								6.5				
								8				
								16.5				
								4.5				
								12.5				
								12				
					1	0		10				
					3	0		6				
								10				

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
								11.5				
	8 June	2	4	2	7	4/1	7	11	1	-	4	2
								6				
Wetland	8 June	2						3.5				
								8				
								8				
								10.5				
								20.5				
	8 June	3	5	13	4/1	0	7	12.5	2	-	0	0
								12				
								15				
								19				
					2/1			6.5			2	1
								4.5				
					2	0	6	11.5				
								10				
					5	2	9	5.5				
								17.5				
								10.5				
								8				
								11.5				
					3(tiller)	2	4	15				
								10				
								11				
					3(tiller)	1	7	10.5				1
								10				
								8				
					0	0	3	-				

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
					4	1	5	16.5				
								10				
								8				
								10				
					3	0	6	1				
								7				
								8.5				
					7	1	3	6	1		1	
Wetland	8 June	3						10.5				
								7.5				
								13				
								13				
								12				
								15.5				
Mean			**3**	**4**	**3.7**	**0.8**	**5**	**10.1**	**1.3**	**-**	**6.7**	**1.7**
sd			**2.6**	**3**	**2.3**	**1.2**	**1.7**	**4.1**	**1.5**		**5.5**	**0.6**
Intermediate												
	9 June	1	29	15	5	2	3	14		1.72	2	1
								16.5				
								19				
								15.5				
								10.5				
					3	1	4	9	1			
								22.5				
								12				
					1	0	4	3				
					3	1	5	1.5				
								9.5				
								10.5				

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
					3	1	5	10				
								14				
								6				
					6	0	4	16				
								20				
								8.5				
								13.5				
								15				
								17.5				
	9 June	2	125	31	2	0	60	7.5	3	1.55	34	24
								20.5				
Intermediate	9 June	2			5	1		9.5				
								21				
								18.5				
								12.5				
								11				
					5	1		14				
								23				
								21.5				
								7.5				
								10				
					6	2		8				
								14				
								20				
								14.5				
								10.5				
								12				
					3	0		30.5				
								15				
								11				

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
					3	0		8.5				
								23				
								12				
					4	2		16.5				
								9.5				
								11.5				
								19				
					5	2		20.5				
								14.5				
								15				
								15				
								19				
					2	0		8				
								17				
Intermediate	9 June	2			8	2		17				
								17				
								13				
								6.5				
								8.5				
								13				
								25				
								25.5				
	9 June	3	80	14	3	0	22	17	3	1.41	9	0
								21				
								13				
					2	0		10.5				
								15.5				
					6	0		21				
								14				
								19				

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
								21.5				
								18				
								19				
					6	2		3				
								17				
								22				
								18.5				
								13				
								15.5				
					2	0		16				
								18				
					4	1		14				
								21.5				
								13				
								15				
					3	0		4				
								26				
Intermediate	9 June	3						15				
					4	1		11.5				
								12.5				
								15				
								8				
					4	1		11.5				
								9.5				
								9.5				
					5	0		26				
								12.5				

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
								13.5				
								21				
								25				
Mean			**78**	**16.3**	**4.0**	**0.8**	**35.7**	**14.8**	**2.3**	**1.56**	**15.0**	**8.3**
sd			**48.0**	**13.6**	**1.7**	**0.8**	**21.1**	**5.6**	**1.1**	**0.2**	**16.8**	**13.6**
Dam												
	9 June	1	74	14	2	0	0	30.5	0	3.10	32	4
								34.5				
					4	2		12.5				
								16.5				
								36				
								25.5				
	9 June	2	44	6	2	0	0	24.5	0	3.40	17	0
								37.5				
	9 June	3	263	25	1	0	0	25.5	0	3.26	105	2
					3	1		22.5				
								2				
								10				
					1	0		31.5				
					3	0		26				
								22				
								12				
Dam	9 June	3			2	1		19				
								22				
					7			25				
								17.5				
								20				
								25				

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
								19.5				
								14				
								7.5				
					2	0		23				
								17.5				
					5	2		30				
								15.5				
								19				
								27				
								12				
					4	1		36				
								13				
								16.5				
					2	1		18.5				
								10.5				
					2	1		15.5				
								17.5				
					6	0		15.5				
								12.5				
								17.5				
								20.5				
								19.5				
								20				
					6	1		24				
								13.5				
								13				
								23.5				
Dam	9June	3						15				
								16.5				
								16				

Continued....

Appendix 2-5. Continued.

Site	Date	Plant	Submerged leaves	Culms	Nodes/ culm	Rooting nodes (tillers)	Emergent leaves	Internode length (cm)	Inflorescences	Longest leaf length (m)	Clipped leaves	Chewed leaves
					6	2		14.5				
								22				
								18.5				
								13.5				
								20.5				
					2	0		30				
								19.5				
					7	2		7				
								2				
								18				
								10				
								29.5				
								19				
								6				
Mean			**166**	**21.7**	**3.5**	**0.1**	**0**	**19.2**	**0**	**3.25**	**51.3**	**2**
sd			**186.5**	**20.6**	**2.0**			**7.7**		**0.1**	**47.1**	**2**

Appendix 2-6. Biomass of *Z. texana* harvested on 17 March 1992, 4 May 1992 and 12 June 1992 from 3 sites in Spring Lake. All plant material was oven dried to a constant weight. *Zizania texana* was first planted in January 1992. Mean weight of leaves at time of tranplanting was 1.55 g.

	Date	Plant	Roots (g)	Tiller roots (g)	Submerged leaves and reproductive culms (g)	Inflorescences (g)	Seeds (g)	Total (g)
Wetland								
	17 March	1	0.2575	0	1.2474	0	0	1.5049
	17 March	2	0.5629	0	4.7566	0	0	5.3195
	17 March	3	0.1987	0	1.4224	0	0	1.6211
Mean			**0.3397**	**0**	**2.4755**	**0**	**0**	**2.8152**
sd			**0.1955**		**1.9774**			**2.1696**
Intermediate								
	17 March	1	0.4369	0	3.0721	0	0	3.509
	17 March	2	0.4161	0	2.0995	0	0	2.5156
	17March	3	0.4600	0	1.6714	0	0	2.1314
Mean			**0.4377**	**0**	**2.2810**	**0**	**0**	**2.7187**
sd			**0.0220**		**0.7178**			**0.7109**
Dam	17 March	1	1.40	0	11.26	0	0	12.66
	17 March	2	0.79	0	11.21	0	0	12.00
	17 March	3	1.45	0	6.59	0	0	8.04
Mean			**1.213**	**0**	**9.6867**	**0**	**0**	**10.9**
sd			**0.3675**		**2.6819**			**2.50**
Wetland								
	4 May	1	0.4390	0	1.9482	0	0	2.3872
	4 May	2	0.4115	0	0.9564	0	0	1.3679
	4 May	3	0.3899	0.6360	4.2494	0	0	5.2753
Mean			**0.4135**	**0.2120**	**2.3847**	**0**	**0**	**3.0101**
sd			**0.0246**	**0.3672**	**1.6893**			**2.0268**

Continued....

Appendix 2-6 . Continued

Date	Plant	Roots (g)	Tiller roots (g)	Submerged leaves (g)	Emergent leaves (g)	Culms (g)	Inflorescences (g)	Seeds (g)	Total (g)
Intermediate									
4 May	1	1.12	0	13.34			0	0	14.46
4 May	2	1.11	0	6.25			0	0	7.36
4 May	3	2.56	0.0183	13.3821			0	0	15.9604
Mean		**1.60**	**0.0061**	**10.99**			**0**	**0**	**12.59**
sd		**0.83**	**0.0106**	**4.11**					**4.59**
Dam									
6 May	1	4.13	0	30.96			0	0	35.09
6 May	2	0.84	0	8.96			0	0	9.80
6 May	3	4.23	0.0018	3.30			0	0	40.2418
Mean		**3.07**	**0.0006**	**14.41**			**0**	**0**	**28.3773**
sd		**1.93**	**0.0010**	**14.61**					**16.2933**
Wetland									
12 June	1	1.3608	0.1701	0	1.1907	3.7705	0	0	6.4921
12 June	2	0.2835	0.0850	0.3969	0.5386	2.6082	0.0567	0	3.9689
12 June	3	0.6804	0.8788	1.2757	6.8607	17.8321	0.4536	0.3685	28.3498
Mean		**0.7749**	**0.3780**	**0.5575**	**2.8633**	**8.0703**	**0.1701**	**0.1228**	**12.9368**
sd		**0.5448**	**0.4358**	**0.6528**	**3.4771**	**8.4739**	**0.2471**	**0.2127**	**13.4075**
Intermediate									
12 June	1	1.4175	0.6237	6.488	3.8270	11.25	0.50	0	24.1062
12 June	2	9.5823	1.09	20.54	8.26	37.26	1.14	0.11	77.9823
12 June	3	5.103	0.227	12.394	3.856	23.04	0.91	0.30	45.83
Mean		**5.3676**	**0.6469**	**13.141**	**5.314**	**23.85**	**0.85**	**0.14**	**49.3062**
sd		**4.0888**	**0.4320**	**7.056**	**2.5511**	**13.02**	**0.32**	**0.15**	**27.1057**

Continued....

Appendix 2-6. Continued

	Date	Plant	Roots (g)	Tiller roots (g)	Submerged leaves (g)	Emergent leaves (g)	Culms (g)	Inflorescences (g)	Seeds (g)	Total (g)
Dam										
	12 June	1	1.0	1.20	23.54	0	15.03	0	0	39.77
	12 June	2	1.16	0.08	28.88	0	19.64	0	0	49.76
	12 June	3	14.88	4.69	122.39	0	90.53	0	0	232.49
Mean			**5.68**	**1.99**	**58.27**	**0**	**41.73**	**0**	**0**	**107.34**
sd			7.97	**2.40**	**55.59**		**42.32**			**108.50**

Appendix 2-7. Alkalinity and pH of water collected from 8 locations in Spring Lake and the San Marcos River. On 3 January 1994 250 ml water was collected from each site, placed in bottles, packed in ice, and transported to Lewisville Aquatic Ecosystem Research Facility, Lewisville, Texas for analysis. All samples were collected at about 30 cm below the water surface.

Site	Alkalinity	pH
From spring at base of steps in front of Aquarena Springs Inn	264	7.36
	245	7.35
30 m downst4ream form ferry dock on NW shore directly over reintroduction site	252	7.29
	250	7.17
30 m above confluence of Purgatory Ck. and San Marcos River	253	7.76
	257	7.8
Immediately upstream from large *Z. texana* stand located downstream from the confluence of Purgatory Ck and San Marcos River	250	8.11
	251	8.16
At intake for A. E. Wood State Fish Hatchery	249	7.89
	241	7.91
Downstream from A. E. Wood State Fish Hatchery outflow	241	7.91
	246	7.93
Downstream from sewage treatment facility	245	7.84
	243	7.81
Upstream from sewage treatment facility	246	7.93
	242	7.93

Appendix 2-8. Seasonal monitoring of *Z. texana* at the dam site in Spring Lake. Transects were 10 m x 0.25 m and were placed randomly, parallel to the current. Plants intersecting the transect were measured. Occasionally fewer than four plants intersected the transect.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
8/3/94	1	20	10	157	12	1.5 2.5 2.1 1.1 1.5 2.2	1.82	+	+
8/3/94	1	15	15	177	6	2.2 1.8 2.1 1.6 1.7 1.3	1.76	+	+
8/3/94	2	20	10	157	22	2.4 2.6 1.5 2.8 2.5 2.3	2.35	+	0
8/3/94	2	30	20	471	20	2.1 1.75 3.0 2.2 2.3 2.3	2.27	+	+
8/3/94	2	20	5	314	10	1.5 2.5 1.8 2.4 1.8 2.3	2.05	+	+
8/3/94	2	25	5	98	10	2.1	2.37	+	+
MEAN				**229**	**13.3**		**2.10**		
SD				**138**	**6.3**		**0.27**		

Continued....

Appendix 2-8. Seasonal monitoring of *Z. texana* at the dam site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
22/7/94	1	20	15	236	17	1.43	2.01	+	±
						1.75			
						1.89			
						2.29			
						2.31			
						2.4			
22/7/94	1	10	22	188	24	1.8	2.03	+	+
						1.78			
						1.97			
						2.03			
						2.3			
						2.3			
22/7/94	1	5	5	20	10	1.17	1.64	+	0
						1.22			
						1.64			
						1.75			
						2.02			
						2.02			
22/7/94	1	10	10	78.5	13	1.78	2.04	+	+
						1.8			
						2.09			
						2.04			
						2.2			
						2.35			
22/7/94	2	15	10	117.8	14	2.16	2.3	+	+
						1.83			
						2.62			
						2.44			
						2.65			
						2.12			
MEAN				**128**	**15.6**		**2.0**		
SD				**85.8**	**5.3**		**0.2**		

Continued....

Appendix 2-8. Seasonal monitoring of *Z. texana* at the dam site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
21/9/94		15	10	118	23	1.12	1.34	+	+
						1.25			
						1.3			
						1.35			
						1.59			
						1.46			
21/9/94		2	2	3.1	8	1.0	1.31	+	+
						1.07			
						1.08			
						1.52			
						1.57			
						1.62			
21/9/94		30	12	283	32	1.21	1.72	+	+
						1.74			
						1.82			
						1.79			
						1.97			
						1.80			
21/9/94		22	12	207	44	1.33	2.32	+	+
						1.87			
						2.28			
						2.70			
						2.41			
						2.65			
						2.97			
21/9/94		7	12	66	22	1.13	1.6	+	+
						1.26			
						1.57			
						1.8			
						1.33			
						1.64			
						2.44			
MEAN				**141**	**25.8**		**1.7**		
SD				**103.4**	**13.3**		**0.4**		

Continued.....

Appendix 2-8. Seasonal monitoring of *Z. texana* at the dam site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
12/12/94	1	10	9	71	19	1.03	1.69	+	+
						1.21			
						1.5			
						2.0			
						2.08			
						2.3			
12/12/94	1	12	15	141	29	1.28	1.93	-	-
						2.15			
						2.3			
						2.09			
						1.9			
						1.85			
12/12/94	1	4	4	13	4	1.35	1.67	-	-
						1.5			
						1.8			
						1.75			
						1.9			
						1.7			
12/12/94	1	7	5	27	21	1.35	1.76	-	-
						1.8			
						1.95			
						1.75			
						1.90			
						1.8			
12/12/94	1	3	2	5	4	1.3	1.65	-	-
						1.4			
						1.8			
						1.9			
						1.7			
						1.8			

Continued....

Appendix 2-8. Seasonal monitoring of *Z. texana* at the dam site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
12/12/94	2	15	12	141	24	1.25	1.61	+	+
						1.3			
						1.75			
						1.80			
						1.85, 1.70			
12/12/94	2	10	8	63	13	1.30	1.67	+	+
						1.30			
						1.65			
						1.95			
						2.0			
						1.85			
12/12/94	2	6	5	24	12	1.0	1.26		
						1.4			
						1.3			
						1.25			
						1.1			
						1.5			
12/12/94	2	7	7	38	16	2.0	1.82	+	+
						1.7			
						2.05			
						1.95			
						1.8			
						1.4			
12/12/94	2	5	5	20	9	1.4	1.42		
						1.3			
						1.65			
						1.25			
						1.4			
						1.5			
MEAN				**54**	**15**		**1.65**		
SD				**50**	**8**		**0.19**		

Continued....

Appendix 2-8. Seasonal monitoring of *Z. texana* at the dam site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
1/3/95	1	13	11	112	32	1.6	2.09	+	+
						2.01			
						2.05			
						2.6			
						2.5			
						1.80			
1/3/95	1	5	6	24	13	1.5	2.56	+	+
						2.95			
						3.25			
						3.15			
						2.0			
						2.54			
1/3/95	1	11	10	86	24	1.5	2.04	+	+
						1.5			
						2.2			
						2.56			
						2.8			
						1.7			
1/3/95	1	8	9	57	14	1.78	1.87	+	0
						1.93			
						1.88			
						2.0			
						1.62			
						2.04			
1/3/95	1	11	12	104	27	2.14	2.1	+	0
						2.1			
						1.6			
						2.32			
						2.03			
						2.44			
1/3/95	2	8	7	44	18	1.82	2.17		
						2.6			

Continued....

Appendix 2-8. Seasonal monitoring of *Z. texana* at the dam site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
						2.15			
						2.31			
						2.2			
						1.94			
1/3/95	2	8	9	57	24	1.5	1.73		
						1.4			
						1.83			
						1.92			
					1.83, 1.91				
1/3/95	2	8	8	50	17	1.93	2.06		
						2.12			
						2.08			
						1.79			
						2.31			
						2.13			
1/3/95	2	9	9	64	20	2.1	2.06		
						1.81			
						2.25			
						1.96			
						2.0			
						2.25			
1/3/95	2	7	7	38	14	2.12	2.07		
						2.05			
						1.96			
						2.21			
						2.14			
						1.92			
MEAN				**63**	**20**		**2.07**		
SD				**28**	**6**		**0.21**		
12/5/95		missing data***	missing data		22	2.0	2.22	+	+

Continued....

Appendix 2-8. Seasonal monitoring of *Z. texana* at the dam site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
						2.0			
						2.05			
						2.1			
						2.68			
						2.48			
12/5/95					4	0.6	1.92	+	+
						1.95			
						2.7			
						2.2			
						2.15			
12/5/95					18	0.6	1.05	+	+
						0.72			
						0.75			
						1.0			
						2.18			
12/5/95					8	0.7	1.62	+	+
						1.69			
						2.25			
						2.01			
						1.45			
12/5/95					32	1.4	1.87	+	+
						1.96			
						1.81			
						1.98			
						2.2			
MEAN					**17**		**1.74**		
SD					**11**		**0.44**		
1/8/95					32	1.52	1.80		
						1.76			
						1.89			
						1.80			

Continued....

Appendix 2-8. Seasonal monitoring of *Z. texana* at the dam site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
						2.03			
1/8/95					25	1.93	1.98		
						1.71			
						2.05			
						2.26			
						1.96			
1/8/95					29	1.38	2.15		
						1.82			
						3.60			
						2.0			
						1.95			
1/8/95					13	1.14	1.78		
						2.06			
						1.91			
						2.19			
						1.59			
1/8/95					38	1.54	1.82		
						1.85			
						1.72			
						1.69			
						3.31			
MEAN					**27**		**1.91**		
SD					**9**		**0.16**		

Continued.....

Appendix 2-8. Seasonal monitoring of *Z. texana* at the dam site in Spring Lake, continued.

Date d/m/y	Submerged leaves (#)	Culms (#)	Stem density /100 cm^2	Emergent culms (#)	Clipped culms (#)	Nodes with roots	Nodes with roots +/0
15/8/95	13	5	0	5	7		
15/8/95	39	7	0	7	7		
15/8/95	8	2	0	2	3		
15/8/95	22	1	0	0	0		
15/8/95	42	5	0	1	2		
15/8/95	9	1	0	0	1		
15/8/95	28	2	0	1	2		
15/8/95	24	1	0	0	1		
17/8/95	6	7	0	5	6		
17/8/95	39	5	0	5	4		
17/8/95	2	1	0	1	5		
MEAN	**21**	**3**	**0**	**2**	**3**		
SD	**14**	**2**		**2**	**2**		
13/11/95	42	5	0	0	7		
13/11/96	6	2	0	1	3		
13/11/96	6	1	0	1	2		
13/11/96	26	2	0	1	3		
MEAN	**20**	**2.5**	**0**	**1**	**4**		
SD	**17**	**2**		**0.5**	**2**		
19/2/96	23	6	0	0	5		

***new field assistant. Values collected by new assistant appeared to be an order of magnitude greater than values collected by previous assistants

Appendix 2-9. Seasonal monitoring of *Z. texana* at the intermediate site in Spring Lake. Transects were 10 m x 0.25 m and were placed randomly, parallel to the current. Plants intersecting the transect were measured.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
11/3/94	*	2	2	3.1	2	0.7**	0.75	+	0
						0.8			
30/5/94	*	20	10	157	10	0.5	0.83		
						0.74			
						0.85			
						0.92			
						1.21			
						0.75			
14/9/94	no plants								
12/12/94	no plants								
1/3/95	no plants								
12/5/95	no plants								
1/8/95	no plants								
15/8/95	no plants								
13/11/95	no plants								
19/2/96	no plants								

*1 plant located, no transect
**plant had 2 leaves, total

Appendix 2-10. Seasonal monitoring of *Z. texana* at the spring site in Spring Lake. Transects were 10 m x 0.25 m and were placed randomly, parallel to the current. Plants intersecting the transect were measured.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
8/3/94	1	25	20	393	15	1.6	1.83	+	0
						2.0			
						2.1			
						2.1			
						1.7			
						1.5			
8/3/94	1	30	35	825	9	1.3	1.46	+	0
						1.5			
						1.65			
						1.35			
						1.55			
						1.40			
8/3/94	2	10	10	78	5	1.4	1.27	+	+
						1.4			
						1.6			
						1.3			
						1.1			
						0.8			
8/3/94	2	20	20	314	6	1.3	1.39	+	+
						1.45			
						1.2			
						1.6			
						1.5			
						1.3			
8/3/94	2	30	15	353	9	0.9	1.17	+	+
						1.4			
						1.7			
						1.2			
						0.8			

Continued....

Appendix 2-10. Seasonal monitoring of *Z. texana* at the spring site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
						1.0			
8/3/94	2	5	10	39	4	1.4	1.19	+	0
						1.1			
						1.15			
						1.25			
						1.25			
						1.0			
8/3/94	2	15	30	353	15	1.2	1.2	+	+
						1.3			
						1.1			
						1.6			
						0.95			
						1.05			
MEAN				**336**	**9**		**1.36**		
SD				**258**	**4**		**0.23**		
23/5/94	1	10	10	78	9	1.8	1.77	+	+
						2.8			
						1.5			
						1.6			
						1.7			
						1.2			
23/5/94	1	20	20	314	13	0.9	1.6	+	+
						2.0			
						1.7			
						1.6			
						1.5			
						1.9			

Continued....

Appendix 2-10. Seasonal monitoring of *Z. texana* at the spring site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
23/5/94	1	20	20	314	23	1.8	1.82	+	+
						2.5			
						0.9			
						2.0			
						2.3			
						1.4			
23/5/94	1	10	20	157	14	1.5	1.53	+	+
						1.4			
						1.7			
						1.0			
						1.7			
						1.9			
23/5/94	1	15	30	353	14	1.8	2.17	+	+
						2.1			
						2.3			
						2.3			
						2.4			
						2.1			
23/5/94	2	20	30	471	21	1.2	1.38	+	+
						1.1			
						1.5			
						1.7			
						2.0			
						0.8			
23/5/94	2	10	15	118	10	2.6	2.07	+	+
						2.4			
						2.1			
						1.5			
						1.8			
						2.0			

Continued....

Appendix 2-10. Seasonal monitoring of *Z. texana* at the spring site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
23/5/94	2	10	5	39	6	1.5 2.0 2.1 1.9 1.7 2.0	1.87	+	0
23/5/94	2	10	10	78	8	1.7 1.8 2.2 2.1 2.0 1.9	1.95	+	+
23/5/94	2	10	15	118	14	2.2 2.2 1.7 1.6 1.8 1.4	1.82	+	+
23/5/94	2	30	45	1,060	28	2.3 1.9 1.8 2.5 2.2 1.6	2.05	+	+
MEAN				**282**	**14**		**1.82**		
SD				**293**	**7**		**0.24**		
14/9/94		10	8	63	19	1.81 1.70 2.0 1.75	1.72	+	+

Continued....

Appendix 2-10. Seasonal monitoring of *Z. texana* at the spring site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
						1.32			
14/9/94		5	8	31	15	1.23	1.45	+	+
						2.05			
						1.05			
						1.28			
						1.62			
						1.47			
14/9/94		8	9	56	14	1.28	1.42	+	+
						1.4			
						2.05			
						1.68			
						0.75			
						1.39			
14/9/94		4	5	16	7	0.88	1.27	+	0
						1.32			
						1.47			
						1.05			
						1.32			
						1.59			
MEAN				**41**	**14**		**1.46**		
SD				**22**	**5**		**0.19**		

Note: 14/9/94 all leaves clipped at/below water. Nutria?waterfowl?cutterboat?

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
16/11/94		5	5	20	6	1.1	1.02	+	0
						0.95			
						1.2			
						1.18			
						1.0			
						0.7			
16/11/94		1	1	0.8	2	0.6	0.67		
						0.75			

Continued....

Appendix 2-10. Seasonal monitoring of *Z. texana* at the spring site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
16/11/94		1	1	0.8	1	0.5	0.5	+	0
16/11/94	1	2	2	3	6	0.6	0.72	+	0
						0.55			
						0.7			
						0.4			
						1.0			
						1.1			
16/11/94	1	2	2	3	7	0.75	0.85	+	+
						0.8			
						1.1			
						0.9			
						0.68			
16/11/94	2	4	4	13	6	1.3	0.85	+	+
						1.25			
						1.10			
						0.8			
						0.1			
						0.55			
16/11/94	2	3	3	7	4	0.3	0.42	+	+
						0.5			
						0.2			
						0.7			
16/11/94	2	2	2	3	6	1.2	0.78	+	0
						1.15			
						0.35			
						0.7			
						0.6			
						0.7			

Continued....

Appendix 2-10. Seasonal monitoring of *Z. texana* at the spring site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
16/11/94		1	1	0.8	2	0.75	1.02		
						1.3			
MEAN				**6**	**4**		**0.76**		
SD				**6**	**2**		**0.21**		
Note: 16/11/94 Plants covered with silt and vegetation. Work at archeological site(?) may contribute to siltation.									
22/2/95	1	5	4	16	11	1.27	1.56	0	0
						1.38			
						1.45			
						1.78			
						1.80			
						1.71			
22/2/95	1	2	2	3	3	1.55	1.76	0	0
						1.60			
						1.62			
						1.71			
						2.0			
						2.07			
22/2/95	1	1	1	0.8	2	0.9	1.1	0	0
						1.1			
						1.2			
						1.19			
22/2/95	1	1	1	0.8	4	1.4	1.89	0	0
						1.8			
						1.93			
						2.15			
						2.10			
						1.95			
22/2/95	1	1	1	0.8	2	0.6	1.05	0	0
						1.5			
22/2/95	2	1	1	0.8	2	0.8	1.54	0	0

Continued....

Appendix 2-10. Seasonal monitoring of *Z. texana* at the spring site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
						1.45			
						2.0			
						1.9			
22/2/95	2	1	1	0.8	4	0.41	0.48	+	+
						0.52			
						0.3			
						0.68			
22/2/95	2	3	4	9	16	0.93	1.1	+	+
						1.02			
						1.17			
						1.3			
						1.03			
						1.0			
22/2/95	2	4	5	16	7	0.96	1.29	+	=
						1.2			
						1.43			
						1.35			
						1.32			
						1.47			
22/2/95	2	4	4	13	10	0.92	1.1	+	+
						0.83			
						1.17			
						0.75			
						1.32			
						1.61			
MEAN				**6**	**6**		**1.29**		
SD				**7**	**5**		**0.41**		
10/5/95	1	missing data***	missing data		10	0.5	1.11	+	+
						0.65			
						1.20			

Continued....

Appendix 2-10. Seasonal monitoring of *Z. texana* at the spring site in Spring Lake, continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm²)	Stem density /100 cm²	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
						2.20			
						1.0			
10/5/95	1				19	0.6	1.44	+	+
						1.4			
						1.1			
						2.5			
						1.6			
MEAN					14		1.3		
SD					6		0.2		
1/8/95					15	0.50	0.5		
						0.38			
						0.52			
						0.54			
						0.55			
1/8/95					38	1.52	1.10		
						1.49			
						0.82			
						0.70			
						0.96			
1/8/95					9	0.28	0.40		
						0.29			
						0.29			
						0.57			
						0.59			
1/8/95					15	1.50	1.60		
						1.80			
						1.0			
						2.0			
						1.7			
MEAN					**19**		**0.90**		
SD					**13**		**0.56**		

Continued....

Appendix 2-10. Seasonal monitoring of *Z. texana* at the spring site in Spring Lake, continued.

15/8/95 no plants
13/11/95 no plants
19/2/96 no plants

***new field assistant. Values collected by new assistant appeared to be an order of magnitude greater than values collected by previous assistants

Appendix 2-11. Seasonal monitoring of *Z. texana* at the spillway site in Spring Lake. Transects were 10 m x 0.25 m and were placed randomly, parallel to the current. Plants intersecting the transect were measured.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
18/5/95	1	10	10	78	34	0.5	0.85	+	0
						0.9			
						1.0			
						1.0			
						0.85			
18/5/95	1	10	10	78	14	0.62	0.91	0	0
						0.85			
						1.0			
						1.0			
						1.1			
18/5/95	1	10	10	78	34	1.01	1.23	+	+
						1.25			
						1.08			
						1.45			
						1.38			
18/5/95	1	10	10	78	19	0.7	1.05	+	0
						0.83			
						1.23			
						1.24			
						1.25			
18/5/95	1	10	10	78	45	0.8	0.99	+	+
						0.8			
						1.02			
						1.13			
						1.2			
18/5/95	2	10	10	78	45	0.51	0.69	+	+
						0.64			
						0.64			
						0.82			
						0.85			

Continued....

Appendix 2-11. Seasonal monitoring of *Z. texana* at the spillway site in Spring Lake continued.

Date d/m/y	Transect	Plant intercept length (cm)	Plant intercept width (cm)	Basal area (cm^2)	Stem density /100 cm^2	Submerged leaf length (m)	Leaf length ave.	Culms +/0	Nodes with roots +/0
18/5/95	2	10	15	118	46	0.59	0.71	+	+
						0.67			
						0.67			
						0.78			
						0.85			
18/5/95	2	10	12	94	30	0.89	0.99	0	0
						0.96			
						0.98			
						1.06			
						1.08			
18/5/95	2	1	1	0.8	2	0.35	0.51	0	0
						0.67			
18/5/95	2	10	5	39	23	0.6	0.77	0	0
						0.62			
						0.68			
						0.98			
MEAN				**72**	**29**		**0.87**		
SD				**32**	**15**		**0.21**		
1/8/95					46	0.62	0.9		
						0.87			
						1.18			
						0.85			
						0.99			
1/8/95					40	0.86	1.08		
						1.0			
						1.24			
						1.2			
						1.1			
1/8/95					31	1.15	0.76		
						0.61			

Continued....

Appendix 2-11. Seasonal monitoring of *Z. texana* at the spillway site in Spring Lake, continued.

Date d/m/y	Submerged leaves (#)	Culms (#)	Emergent culms (#)	Clipped culms (#)	Nodes with roots	
					0.59	
1/8/95				73	0.22	0.68
					0.38	
					1.00	
					1.00	
					0.79	
MEAN				**54**		**0.9**
SD				**21**		**0.18**
17/8/95	6	1	0	0	0	
17/8/95	21	0	0	0	0	
17/8/95	51	2	0	1	1	
17/8/95	59	4	0	0	4	
17/8/95	24	4	0	1	0	
17/8/95	12	0	0	0	0	
17/8/95	9	0	0	0	0	
17/8/95	9	0	0	0	0	
17/8/95	18	1	0	0	0	
MEAN	**23**	**1**	**0**	**0.2**	**0.5**	
SD	**19**	**2**		**0.4**	**1**	
13/11/95	0	1	0	0	2	
13/11/95	12	0	0	0	0	
13/11/95	22	2	0	0	0	
13/1195	37	5	0	0	3	
13/11/95	2	0	0	0	0	
13/11/95	10	1	0	0	0	

Continued

Appendix 2-11. Seasonal monitoring of *Z. texana* at the spillway site in Spring Lake, continued.

Date d/m/y	Submerged leaves (#)	Culms (#)	Emergent culms (#)	Clipped culms (#)	Nodes with roots
MEAN	**14**	**1.5**	**0**	**0**	**1**
SD	**14**	**1.9**			**1.3**
19/2/96	45	2	0	0	0
19/2/96	22	2	0	0	1
19/2/96	15	3	0	1	2
19/2/96	18	3	0	3	2
19/2/96	24	6	0	0	6
19/2/96	27	0	0	0	0
19/2/96	47	6	0	0	5
19/2/96	3	2	0	0	1
19/2/96	3	0	0	0	0
19/2/96	12	0	0	0	0
MEAN	**22**	**2**	**0**	**0.4**	**2**
SD	**15**	**2**		**1.0**	**2**

TEXAS
PARKS AND WILDLIFE DEPARTMENT

4200 Smith School Road Austin, Texas 78744

February 18, 1988

Ms. Paula Powers
Department of Biology
Southwest Texas State University
San Marcos, Texas 78666

Dear Ms. Powers:

Let me thank you once again for your efforts in transplanting the Texas Wild Rice plant from the San Marcos River to the raceways at Southwest Texas State University. This activity, although simple in action and time involved, was an essential part of the mitigation required for the construction of a water intake structure at the San Marcos State Fish Hatchery.

Your efforts are to be commended.

Sincerely,

Raymond W. Neck

RAYMOND W. NECK, Biologist
Parks Division
Master Planning Branch

RWN:smg

MANAGEMENT AND CONTINUED RESEARCH ON *ZIZANIA TEXANA*

DRAFT INTERIM REPORT

CONTRACT NO: 331-0253

March 15,1992

[illegible]
[illegible] Texas State [illegible]
[illegible]

INTRODUCTION

During the month of December, there was significant flooding of Spring Lake and the San Marcos River. This flooding occurred immediately after we had finished planting Texas wildrice in the experimental sites. The flooding caused significant damage to some of the experiment, many plants were carried away by the current, as were four of the six cages. Fortunately we retrieved three of the lost cages. Because of this damage and limited plant material in the raceway on campus, we modified the experiment in the following manner:

1. Two replicates for each treatment at each site
2. One age class (4-6 month)
3. Included a growth experiment

The growth experiment was added because Texas wildrice clearly shows two developmental pathways in the San Marcos River and in the raceway on the SWT campus. River populations show mainly vegetative growth while raceway populations show mainly reproductive growth. The developmental pathway taken appears to be triggered by environmental conditions to which the plant is exposed.

For the experiment, at each site, thirty, six month-old plants were placed in cages (except the dam site). At six week intervals, five plants will be harvested. The plant material well be returned to the lab, divided into vegetative and reproductive parts, dried and weighed. This study will determine how reproductive effort and energy allocated for vegetative parts is effected by envorinmental conditions, most importantly water depth.

Finally, between December 15, 1991 and March 15, 1992, we accomplished the following tasks.

1. Tested seeds in storage for viability to 24 weeks
2. Additional plants added to existing plants in Spring Lake to bring total to [illegible]
3. [illegible]
4. [illegible]
5. [illegible]

APPENDIX 2-7

SEED PRODUCTION - 1990

Date	Plant 1	2	3	4	5	6	7	8	9	10	Growing in mud	Growing in sand	All other	Total
April 2								2						2
April 5				9										9
April 16							2		1				44	47
April 17	2		2				4						52	60
April 19			1										26	27
April 23	5		37		26		5	14		39			46	172
April 24														80
April 26	10		1		3		12	13		13			7	59
April 30	1		8		13			16	1	78			116	233
Total	18	0	49	9	42	0	23	45	2	130	0	0	291	689
May 1			2	1	5	8	2	6		8			90	122
May 3				3	9		10	10	8	32			112	184
May 7	38		40	38	11	39	15	62	41	47			237	568
May 8	14		7	14	12	5	15	26	9	29			110	241
May 10	19		63	7	28	3	42	61	34	49			341	647
May 14	13		26	4	89	13	54	94	17	33			405	748
May 16	8		19		59	8	17	53	20	52			349	630
May 18														130
May 21														435
May 25														400
May 29	26		85	68	21		101	111	26	151	36		511	1136
May 31	13		67	57	50		61	129	44	64	47		376	909

Appendix continued

August 9	7							11			5		179	202
August 13								7					106	113
August 16								7					120	127
August 20	4										4		50	58
August 23								17			5		120	142
August 27								6			81		194	281
August 30										12	7		118	137
Total	11	0	0	0	0	0	0	70	0	15	148	0	1157	1401

PROPOSAL

Federal contract No: ~~54646~~ 14-16-0002-84-222

Submitted to

United States Department of the Interior
Fish and Wildlife Service
Endangered Species Office
Post Office Box 1306
Albuquerque, New Mexico 87103

Submitted by

Southwest Texas State University
Department of Biology
San Marcos, Texas 78666

Title of ~~Proposed~~ Research: "Investigation of Recovery Techniques for *Zizania texana*"

Principal Investigator: Paul J. Fonteyn
Associate Professor
Department of Biology

Amount Requested: $10,000

Proposed Duration of Project: 9/1/86 - 8/31/88

Endorsement:

Paul J. Fonteyn
Associate Professor
Department of Biology
(512) 245-2178

Paula Power
Research Associate
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(512) 245-2178

INVESTIGATION OF RECOVERY TECHNIQUES FOR *ZIZANIA TEXANA*

INTRODUCTION

The population monitoring of *Zizania texana* for the past 20 years has revealed the rapid decline of this species (Emery 1967, 1977; Fonteyn and Vaughan, 1986). We propose, therefore, to conduct a series of field experiments to develop the techniques needed to ensure the recovery of this species in the San Marcos River. Based on our previous work with *Zizania*, we developed the hypothesis that *Zizania texana* was outcompeted by exotic species in its historic native habitat along the banks of the San Marcos River and was subsequently forced to exist in deeper channel regions. In these regions, populations can exist but only marginally; little if any sexual reproduction occurs. To test the hypothesis, exotic competitors (primarily *Colocasia esculenta* - Elephant Ear) currently occupying the banks of the San Marcos River will be removed by various means, followed by the reintroduction of *Zizania*. Additionally, we will establish a population at the Aquatic Station at Southwest Texas State University (SWTSU) to create a seed bank and nursery for future experiments and reintroduction to historic native habitat.

EXPERIMENTAL DESIGN

Fifteen protected areas will be selected along the upper San Marcos River. Five contiguous 2x2 m treatment plots will be delinated in each area (Fig. 1). The treatments will be: (1) Control, (2) Interplanting *Colocasia* with *Zizania*, (3) *Colocasia* manually removed, (4) *Colocasia* removed by single application of herbicide, (5) *Colocasia* removed by multiple applications of herbicide.

To determine the effect of small mammal herbivory (primarily *Myocastor coypus* -Nutria) on the establishment of *Zizania*, one half of each 2x2 m treatment plot will be fenced producing 2x1 m subplots.

In treatment areas 2-5, 10 individuals of *Zizania* will be planted in each subplot. Prior to planting, each individual will be clipped to 20 cm. Increases in culm length will be monitored monthly for 18 months to determine growth rate. Plants will not be clipped to determine dry-weight increase because such treatment could prevent successful establishment. Sexual and asexual reproduction and mortality will also be monitored.

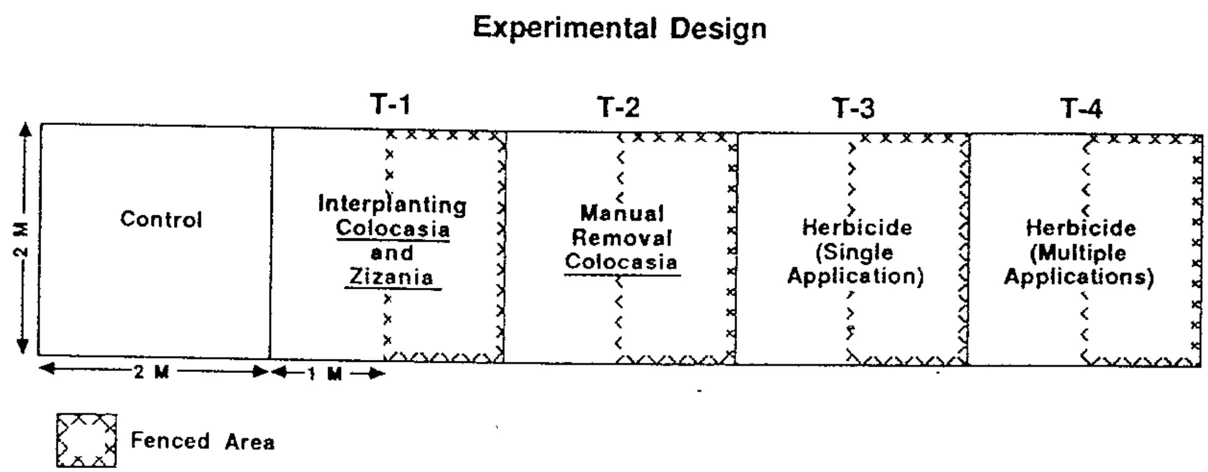

Fig. 1

Schedule of Main Activities

September 1986 - August 1987

September -

1. Selection of experimental sites.
2. Growth of Z. texana in raceways of SWTSU.
3. Establishment of Z. texana population in raceways of SWTSU.

October -

1. Manual removal of Colocasia
2. Application of herbicide to treatment plots 3 and 4. Number and time of repeat applications of herbicide to treatment 4 plots will be determined by an expert from Texas Parks and Wildlife.

November -

Texture analysis of soil at experimental sites and sites of current occupation by Zizania.

December -

Construction of fences.

January 1987 -

Construction of fences.

February -

Observation of effects of Colocasia treatments.

March -

Placement of raceway grown Zizania into treatment plots.

April - August

1. Observations of Zizania growth.

2. Observation of growth of Zizania in raceways at SWTSU.

3. Collection of seeds produced by population in raceways.

September 1987 - August 1988

September - December

1. Continued monitoring of treatment plots.

2. If sufficient seed production occurs investigate germination and seedling establishment requirements.

3. Assess Colocasia reestablishment. If reinvading, explore alternative removal methods.

January - August

1. Continued monitoring of treatment plots.

2. Analysis of data and submittal of report.

Importance of Proposed Project to the Endangered Species Program

The experiments proposed would help provide the data needed to formulate a practical and workable plan to ensure the survival and reestablishment of Zizania. The project directly addresses several items oulined in the Action Plan Narrative of the San Marcos Recovery Plan. This study may also assist in the recovery of another endangered species in the San Marcos River -- Gambusia georgei -- for which Zizania texana vegetation is the prime habitat (Robert Edwards, personal communication).

Literature Cited

Emery, W.H.P. 1967. The decline and threatened extinction of Texas Wildrice. Southwest Nat. 12(2): 203-204.

__________. 1977. Current status of Texas Wildrice. Southwest Nat. 22(3): 393-394.

Fonteyn, P. and J. Vaughan. 1986. Interim report. SWTSU.

BUDGET JUSTIFICATION

INSTITUTION: Southwest Texas State University

PRINCIPAL INVESTIGATOR: Paul J. Fonteyn

A. SALARIES AND WAGES		
Principal Investigator - Paul J. Fonteyn	$4,483	
Graduate Student	2,956	
Total Salaries and Wages		$ 7,439
B. EMPLOYEE BENEFITS (18.8% Salaries & Wages)		$ 1,401
C. ~~SUPPLIES~~		
Chemicals	$ 100	
Laboratory Materials	50	
Total Supplies		$ 150
D. OTHER DIRECT COST		
To include but not limited to:		
Communications and Duplicating		$ 100
TOTAL DIRECT COST		$ 9,090
F. INDIRECT COST (10% Direct Cost)		$ 910
TOTAL PROJECT COST		$10,000

Reprinted from

Aquatic botany

Aquatic Botany 55 (1996) 199–204

Effects of current velocity and substrate composition on growth of Texas wildrice (*Zizania texana*)

Paula Power *

Biology Department, Southwest Texas State University, San Marcos, TX 78666, USA

Accepted 28 June 1996

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Aquatic Botany 55 (1996) 199–204

Aquatic botany

Effects of current velocity and substrate composition on growth of Texas wildrice (*Zizania texana*)

Paula Power *

Biology Department, Southwest Texas State University, San Marcos, TX 78666, USA

Accepted 28 June 1996

Abstract

Zizania texana Hitchc., an endangered macrophyte has declined in number during the past 50 years. To determine the effects of substrate composition and current velocity on plant growth, plants were grown in pots containing either fine or coarse sediment at three sites in Spring Lake, Hays Co., TX. Each site was similar in depth but differed in current velocity. Plants were allowed to grow for 6 weeks and then harvested. Growth was greater on fine sediments. There was significant interaction between flow and sediment with respect to aboveground biomass and stem density and, there was significant flow effect on leaf length and stem density. Stem density was greater in fast flowing water (0.40–0.49 $m\,s^{-1}$) than either moderate (0.12–0.24 $m\,s^{-1}$) or slow flowing (0.05–0.12 $m\,s^{-1}$) water. The decline in *Zizania texana* population can be attributed, in part, to physical changes to *Zizania texana* habitat.

Keywords: Current velocity; Endangered; Macrophyte; Photosynthesis; Texas wildrice; Sediment; *Zizania texana*

1. Introduction

Macrophyte growth in streams is influenced, in part, by current velocity and sediment composition. Low to moderate current velocities have a positive effect on metabolism by delivering nutrients, including CO_2, to the boundary layer surrounding submerged leaves (Westlake, 1967; Smith and Walker, 1980). Sediments are also a source of nutrients for rooted macrophytes (Chambers et al., 1989). Fine sediments tend to be more fertile than coarse sediments, allowing for more biomass production in plants grown on fine

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sediments (Barko and Smart, 1978, 1979). Conversely, rooted macrophytes can alter stream microhabitat characteristics. Macrophyte beds alter flow characteristics, provide more substrate surface area and have more fine particle sediments when compared with unvegetated areas (Gregg and Rose, 1982; Losee and Wetzel, 1993).

Zizania texana Hitchc. (Texas wildrice), an endangered emergent macrophyte in the family Poaceae, has long ribbon-like submerged leaves and emergent reproductive culms (Silveus, 1933). Plants typically form dense stands in fast flowing water and only occur in the first 2.4 km of the San Marcos River, Hays Co., TX (Terrell et al., 1978). The upper reaches of the San Marcos River are characterized by numerous riffles and pools; its headwaters are springs arising from the Edwards Aquifer. *Zizania texana* is endangered, in part, because of its limited distribution and a significant population decline that occurred between 1940 and 1967 (Devall, 1940; Emery, 1967 and US Fish and Wildlife Service, 1994). At present, there have been few ecological studies on *Zizania texana*. Terrell et al. (1978) provide descriptive observations on the ecology of *Zizania texana* and Power and Fonteyn (1995) investigated seed germination and seedling growth on fine and coarse sediments; but there have been no manipulative field studies investigating the response of *Zizania texana* to specific environmental conditions.

Due to anthropogenic effects, current velocity and substrate composition in the San Marcos River are changing. Current velocity is slower due to overpumping of the Edwards Aquifer and the construction of three dams on the San Marcos River within the historic range of *Zizania texana*. During the 1970s, five retention dams were constructed for flood control purposes in the upper San Marcos watershed. The retention dams have altered the frequency and magnitude of historic flooding cycles and resulted in increased sedimentation in the San Marcos River (US Fish and Wildlife Service, 1994). The effects of such historic changes in the physical characteristics of the San Marcos River on the decline of the *Zizania texana* population is not known. The purpose of this study is to quantify and compare the growth response of *Zizania texana* under field conditions at different flow regimes and sediment compositions.

2. Methods and materials

Three sites of similar depth (0.76–0.82 m) but different current velocity were selected near the spillway in Spring Lake, part of the historic habitat of *Zizania texana*. Depth at the study site was similar to depth of wild *Zizania texana* stands. Current velocity was measured with a Marsh–McBirney portable water current meter Model 201 at a depth at which submerged leaves geniculate with the current. Velocity ranged from 0.40–0.49 $m\,s^{-1}$ in the fast site, 0.12–0.24 $m\,s^{-1}$ in the moderate site, and 0.05–0.12 $m\,s^{-1}$ in the slow site.

Coarse and fine sediments were collected from the San Marcos River near wild *Zizania texana* stands. Each sediment type was thoroughly mixed and a subsample was analyzed for Kjeldhal nitrogen, phosphorus, texture and organic matter content.

Sediments were placed in 16 cm pots along with one germinated *Zizania texana* seed. Seedlings were allowed to grow and become rooted for 5 weeks in an outdoor cement raceway on the Southwest Texas State University campus. Pots were then transported

Table 1
Texture, organic matter content, Kjeldahl N, and phosphorus concentration of sandy clay and gravel collected in the San Marcos River, Hays Co., TX

	Sandy clay	Gravel
Texture (%)		
Sand	56	16
Silt	23	2
Clay	22	3
Gravel	0	79
Organic matter (%)	3.9	0.32
Nutrient (mg kg^{-1})		
Kjeldahl N	1517	71
Phosphorus	47?	15
pH	7.8	8.5

and planted in each of the three different current velocity sites in Spring Lake. There were three replicate plots in each site containing three to five pots of each sediment treatment. Plants were protected from herbivory by waterfowl with 1 m^2 floating exclosures constructed from polyvinyl chloride pipe, wire mesh, and styrofoam blocks.

After 6 weeks, plants were harvested. Plants badly damaged by herbivory were discarded. The number of stems (i.e. basal leaves plus reproductive culms) and leaf length were recorded. Plants were dried and aboveground parts were weighed.

Data were analyzed by factorial ANOVA (Wilkinson, 1989) followed by a Tukey multiple comparison (Zar, 1984).

3. Results

Textural analysis indicated sediments were sandy clay or gravel (Table 1). Sandy clay had greater organic matter content than gravel.

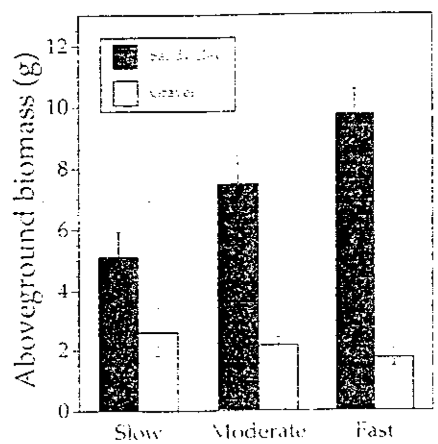

Fig. 1. Aboveground biomass (± SE) of *Zizania texana* grown in two types of sediments and three current velocities in Spring Lake, Hays Co., TX.

Table 2
Two-way ANOVA performed on data collected from *Zizania texana* plants grown in two sediments and three flows in Spring Lake, Hays Co., TX

Source of variation	Sum of squares	d.f.	Mean square	*F*	*P*
Biomass					
Flow	10.660	2	5.330	2.204	0.153
Sediment	126.299	1	126.299	52.234	0.000 *
Flow X sediment	22.775	2	11.387	4.710	0.031 *
Stem density					
Flow	182.538	2	91.269	16.430	0.000 *
Sediment	128.534	1	128.534	23.138	0.000 *
Flow X sediment	51.508	2	25.754	4.636	0.032 *
Leaf Length					
Flow	0.493	2	0.247	10.857	0.002 *
Sediment	0.088	1	0.088	3.883	0.072
Flow X sediment	0.096	2	0.048	2.109	0.164

* Significance at 0.05 level.

Plants in all treatments increased in mass and density over the course of the experiment. There was a positive response by *Zizania texana* to increasing flow when grown in sandy clay and a negative response to increasing flow when grown in gravel (Fig. 1). Aboveground biomass was significantly greater on sandy clay ($P < 0.001$), and there was significant interaction between flow and sediment on aboveground biomass ($P = 0.031$; Table 2).

There was a positive relationship between current velocity and stem density for both types of sediments although the effect was greater on sandy clay than gravel (Fig. 2).

There was significant flow effect on leaf length. Leaves were significantly longer in the moderate site when compared with leaves in the fast and slow site (Fig. 3;

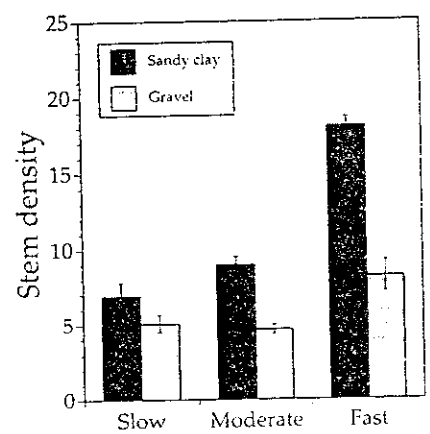

Fig. 2. Stem density (± SE) of *Zizania texana* grown in two types of sediments and three current velocities in Spring Lake, Hays Co., TX.

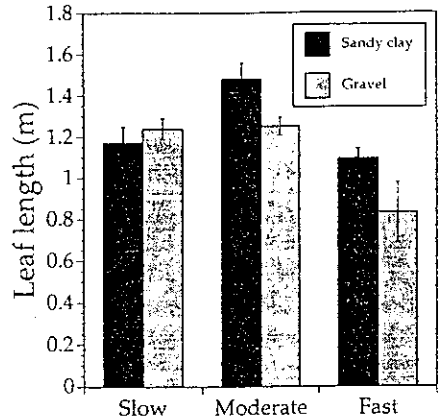

Fig. 3. *Zizania texana* leaf length (±SE) from plants grown in two types of sediments and three current velocities in Spring Lake, Hays Co., TX.

$P = 0.002$). There was no sediment effect on leaf length and there was no interaction between factors with respect to leaf length.

4. Discussion

There was a positive relationship between flow and stem density and aboveground biomass, but an upper threshold for growth was not observed. Macrophytes are directly affected by current through physiological enrichment (Westlake, 1967). In addition, macrophyte community structure can be shaped by current velocity. Chambers et al. (1991) found plant biomass was inversely correlated to within bed velocities between 0.01–1 $m\,s^{-1}$. Nilsson (1987) found species richness reached a peak at a surface velocity of 0.3 $m\,s^{-1}$ in a fourth order stream and that species composition in slower flowing water differed from species composition in faster flowing water. *Zizania texana*, with long ribbon-like leaves which provide protection from tearing in turbulent water (Reimer, 1984), prefers faster flowing water. At lower current velocities *Zizania texana* leaves may be CO_2 stressed.

Plants exhibited greater aboveground biomass and stem density when grown on sandy clay. In addition, the response to sandy clay was greater in fast flowing water (0.40–0.49 $m\,s^{-1}$) than slow flowing water (0.05–0.12 $m\,s^{-1}$). A sandy clay sediment, however, is unlikely at velocities above 0.3 $m\,s^{-1}$ because sandy clay is uplifted from the stream bed at 0.3 $m\,s^{-1}$ (Hynes, 1970). When stem production is stimulated by fast flowing water, the resulting stem density contributes to the plant's ability to modify the substrate in its favor. Stems slow current velocity, increasing fine sediment and detritus deposition (Gregg and Rose, 1982). The result is fine sediments within stands, and coarse sediments around stands.

Physical changes in the San Marcos River include sedimentation and reduced current velocity due to construction of dams in the San Marcos River and in the watershed, and overpumping of the Edwards Aquifer. Sedimentation has a positive effect on *Zizania texana* growth while low flows are not associated with good growth rates. The decline in *Zizania texana* cannot be attributed to sedimentation in the San Marcos River; however, the population decline can partly be attributed to reduced flow among other possible factors.

Acknowledgements

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References

Barko, J.W. and Smart, R.M., 1978. The growth and biomass distribution of two emergent freshwater plants, *Cyperus esculentus* and *Scirpus validus*, on different sediments. Aquat. Bot., 5: 109–117.

Barko, J.W. and Smart, R.M., 1979. The nutritional ecology of *Cyperus esculentus*, an emergent aquatic plant, grown on different sediments. Aquat. Bot., 6: 13–28.

Chambers, P.A., Prepas, E.E., Bothwell, M.L. and Hamilton, H.R., 1989. Roots versus shoots in nutrient uptake by aquatic macrophytes in flowing waters. Can. J. Fish. Aquat. Sci., 46: 435–439.

Chambers, P.A., Prepas, E.E., Hamilton, H.R. and Bothwell, M.L., 1991. Current velocity and its effect on aquatic macrophytes in flowing waters. Ecol. Appl., 1(3): 249–257.

Devall, L.L., 1940. A comparative study of plant dominance in a spring-fed lake. Master's Thesis, Southwest Texas State University, San Marcos, TX, 49 pp.

Emery, W.H.P., 1967. The decline and threatened extinction of Texas wildrice (*Zizania texana* Hitchc.). Southwest. Nat., 12(2): 203–204.

Gregg, W.W. and Rose, F.L., 1982. The effects of aquatic macrophytes on the stream microenvironment. Aquat. Bot., 14: 309–324.

Hynes, H.B.N., 1970. The Ecology of Running Waters. Liverpool University Press, Liverpool.

Losee, R.F. and Wetzel, R.G., 1993. Littoral flow rates within and around submersed macrophyte communities. Freshwater Biol., 29: 7–17.

Nilsson, C., 1987. Distribution of stream-edge vegetation along a gradient of current velocity. J. Ecol., 75: 513–522.

Power, P. and Fonteyn, P.J., 1995. Effects of oxygen concentration and substrate on seed germination and seedling growth of Texas wildrice (*Zizania texana*). Southwest. Nat., 40(1): 1–4.

Reimer, D.N., 1984. Introduction to Freshwater Vegetation. AVI, Westport, CT, pp. 95–106.

Silveus, W.A., 1933. Texas Grasses, Classification and Description of Grasses. Clegg., San Antonio, TX.

Smith, F.A. and Walker, N.A., 1980. Photosynthesis by aquatic plants: effects of unstirred layers in relation to assimilation of CO_2 and HCO_3^- and to carbon isotopic discrimination. New Phytol., 86: 245–259.

Terrell, E.E., Emery, W.H.P. and Beaty, H.E., 1978. Observations on *Zizania texana* (Texas wildrice), an endangered species. Bull. Torrey Bot. Club, 105: 50–57.

US Fish and Wildlife Service, 1994. Draft San Marcos/Comal (Revised) Recovery Plan. Albuquerque, NM, pp. vii + 119.

Westlake, D.F., 1967. Some effects of low-velocity currents on the metabolism of aquatic macrophytes. J. Exp. Bot., 18: 187–205.

Wilkinson, L., 1989. Systat: The System for Statistics. SYSTAT, Evanston, IL.

Zar, J.H., 1984. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, NJ.

USDA Forest Service
General Technical Report RM-GTR-283

August 1996

Southwestern Rare and Endangered Plants: Proceedings of the Second Conference

September 11-14, 1995
Flagstaff, Arizona

Technical Coordinators:

Joyce Maschinski
The Arboretum at Flagstaff
Flagstaff, Arizona

H. David Hammond
Northern Arizona University
Flagstaff, Arizona

Louella Holter
Bilby Research Center, NAU
Flagstaff, Arizona

Rocky Mountain Forest and Range
Experiment Station
U.S. Department of Agriculture
Fort Collins, Colorado

Maschinski, Joyce; Hammond, H. David, and Holter, Louella, tech. eds. 1996. Southwestern rare and endangered plants: Proceedings of the Second Conference; 1995 September 11-14; Flagstaff, AZ. Gen. Tech. Rep. RM-GTR-283. Fort Collins, Co: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. 328 pp.

Abstract

These contributed papers review the current status of plant conservation in the southwestern U.S. in the current legislative arena, citing specific cases from surveys, and genetic, demographic, and ecological studies. In addition, broad issues affecting the paradigms of conservation of rare plant species in an ecosystem management context are reviewed.

Key Words: plant conservation, genetics, demography, reproductive biology, monitoring, endangered species

Cover illustration of *Clematis hirsutissima* var. *arizonica* by Anne E. Grodor.

Reintroduction of Texas Wildrice (*Zizania texana*) in Spring Lake: Some Important Environmental and Biotic Considerations

PAULA J. POWER

Biology Department, Southwest Texas State University, San Marcos 78666

Abstract: Texas wildrice (*Zizania texana* Hitchc.), an endangered macrophyte, is endemic to the San Marcos River and Spring Lake in Hays County, Texas. Its population declined dramatically between 1940 and 1967. In a reintroduction program that was initiated in 1992, plants were transplanted into five microhabitats at a density of 10 plants/m^2. Seasonal monitoring identified an initial increase in plant size followed by high mortality in three of five sites. Data suggest that stem density is a good indicator of future transplant success and that current velocity is an important environmental factor associated with transplant success. However, herbivores have continually clipped reproductive culms just below the water surface. This limits transplants to clonal reproduction.

Introduction

Texas wildrice (*Zizania texana* Hitchc.) is an emergent macrophyte in the family Poaceae. It has long ribbon-like submerged leaves and reproductive culms that arise from the base of the plant. Each culm has an emergent terminal inflorescence, allowing for wind pollination. Culms root at the nodes, producing tillers, which is a form of clonal reproduction.

Zizania texana is endemic to the first 2.4 km of the San Marcos River and Spring Lake, Hays County, Texas, the headwaters of which are springs arising from the Edwards Aquifer. The springs are located in Spring Lake. Historically, *Z. texana* was abundant in Spring Lake, the San Marcos River, and adjoining irrigation ditches (Watkins 1930, Silveus 1933, Devall 1940). During the first half of this century, *Z. texana*, like many macrophytes, was considered a pest species and efforts were made to control the growth of the plant (Watkins 1930, Emery 1967). Between 1933 and 1967 the population declined significantly, and after 1977 *Z. texana* occurred only in the first 2.4 km of the San Marcos River (Emery 1977).

Zizania texana is now listed as an endangered species by both the U.S. Fish and Wildlife Service and the Texas Parks and Wildlife Department (U.S. Fish and Wildlife Service 1985). Factors threatening the survival of *Z. texana* include reduced spring flow from the San Marcos springs due to overpumping of the Edwards Aquifer, reduced water quality in the San Marcos River from nonpoint source pollution, competition and predation by nonnative species such as nutria (*Myocaster coypus*), absence of sexual reproduction, and alteration of sediments in the river bottom (U.S. Fish and Wildlife Service 1984). Alteration of the river sediments includes deposition of fine organic and inorganic particles due to reduction in frequency and magnitude of historic flooding cycles, and coarse gravel deposition from soil erosion in the immediate water shed.

Twice during the past 20 years *Z. texana* has been transplanted from captive populations on the Southwest Texas State (SWT) campus into the San Marcos River in attempts to increase the naturally occurring population. There were no long-term survivors in either case (W.H.P. Emery, personal communication, Fonteyn and Power 1990).

Monitoring a plant population concerns the quantitative assessment of the population over time using data derived from individual plants, which allows managers to detect important changes in health, vigor, and reproductive potential, as well as identify demographic trends such as adult survivorship and seedling recruitment. These measurements are vital in evaluating the long-term success of a reintroduction program (Palmer 1987, Pavlik and Barbour 1988). The objective of this project, which will be completed in 1996, is the reintroduction of *Z. texana* into different microhabitats in Spring Lake, and the monitoring of the status of the transplants in each microhabitat. The outcome of the study will provide managers with important information necessary for decisions with respect to San Marcos River and watershed management, as well as insights into appropriate environmental conditions for more successful transplantation into the San Marcos River.

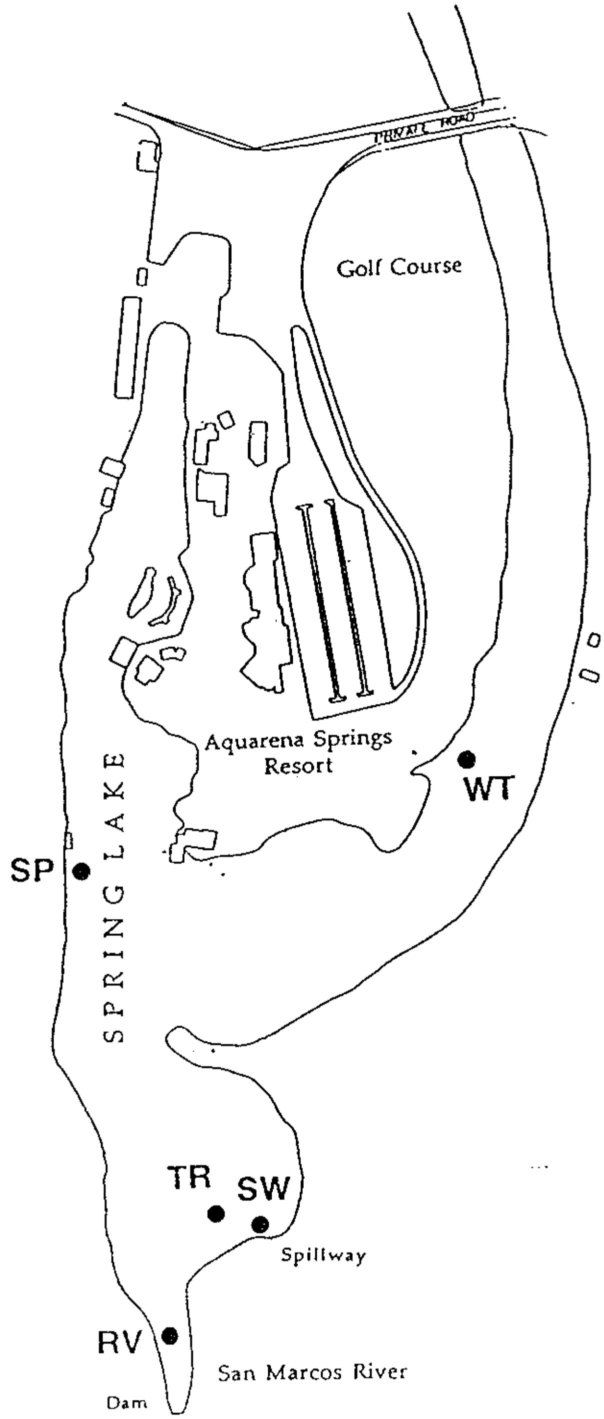

Figure 1. Spring Lake, San Marcos, Hays County, Texas. *Zizania texana* was reintroduced into Spring Lake in five sites between 1992 and 1995. Sites were Wetland (WT), Transition (TR), Riverine (RV), Springs (SP) and Spillway (SW).

Table 1. Ranges for current velocity, water depth, light, and soil characteristics found in *Zizania texana* reintroduction sites in Spring Lake. Observations and measurements were collected between 1992 and 1995; + = presence; 0 = absence.

	WT	TR	RV	SP	SW
Light	Full sun	Full sun	Partial sun	Full sun	Partial sun
Sediment	Soft mud	Soft mud	Soft mud	Soft mud	Mud/sand/gravel
Water depth (m)	0.31	0.76	0.91–2.04	2.3–4.3	0.70–1.13
Current velocity (m/s)	0	0.027	0.038–0.079	0.030–0.038	0.088–0.345
Survivorship	0	0	+	0	+

Table 2. *Zizania texana* in five reintroduction sites in Spring Lake, Hays County, Texas; + = presence, 0 = absence.

Year	WT	TR	RV	SP	SW
1992	+	+	+		
1993	0	+	+		
1994	0	0	+	+	
1995	0	0	+	0	+

Table 3. The data below represent reproductive activity by *Zizania texana* grown in two sites in Spring Lake during summer 1995. Culms represent sexual reproductive activity; tillers represent asexual reproductive activity. Data were collected from three transects in each site; SD in parentheses.

	No. Culms/ Plant	Percent Clipped Culms	No. Emergent Inflorescences	No. Tillers/ Plant
RV	3.44 (1.073)	53 (31.4)	0	3.58 (1.843)
SW	1.25 (1.146)	8.3 (14.4)	0	0.42 (0.723)

herbivores. Culms that were not clipped were all well below the water surface. There were no emergent inflorescences at either site.

Discussion

Monitoring plants has identified some important relationships between environmental factors and health and vigor of *Z. texana* transplants. For example, plants in sites with no detectable to slow current had high mortality, while plants in sites with moderate to fast current velocity had survivors. Rose and Power (1994) examined the relationship between current velocity and culm production, and suggested that culm production may increase in flowing water either by mechanical stimulation of the meristem or physiological enrichment. In contrast, there was no consistent relationship between survivorship and water depth, suggesting that water depth does not play as critical a role in transplant success (Table 1).

During 1994 and 1995, losses in leaf length and stem density were followed by periods of recovery in some sites (Figures 4 and 5). Plants in TR failed to recover from losses of plant material in 1994 and plants in SP failed to recover in 1995. Damage to leaves and stems was probably due, in part, to floating and drifting mats of aquatic vegetation, which is mechanically cut upstream by Aquarena Springs, a nature theme park. Despite attempts to harvest cuttings, some cuttings drift downstream, form mats that tend to accumulate in spring and summer during peak growing periods, and then

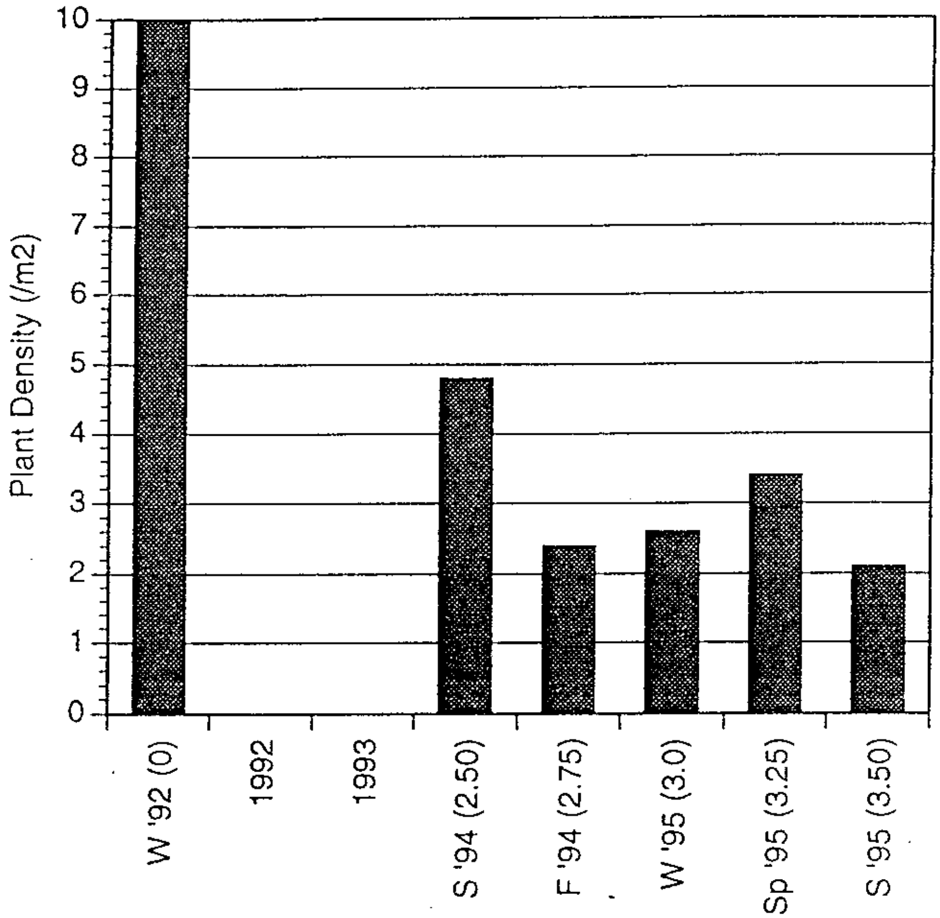

Figure 2. Plant density of *Zizania texana* transplants in Spring Lake at RV site between winter 1992 and summer 1995. No data are available for 1992 and 1993. Time since transplanting in parentheses.

break up during fall and winter. The mats, composed primarily of *Cerataphyllum demersum*, become entangled in *Z. texana* leaves, shredding them. The mats block sunlight and slow current velocity (Rose and Power 1995). *Zizania texana* plants appear to recover from the damage attributed to the mats during fall and winter.

In summer 1995, plants in SW and RV produced reproductive culms and asexual tillers (Table 3). It is unknown at this time whether the lower number of culms and tillers in SW compared with RV is attributable to the age of transplants or environmental conditions.

Reproductive failure has been noted many times as a serious threat to the survival of *Z. texana*, but there has been no documentation of the cause of reproductive failure (Emery 1967, 1977, Terrell et al. 1978, U.S. Fish and Wildlife Service 1984). In this study we identified herbivory as an important contributor to reproductive failure. Over half the culms at the RV site were clipped and 8.3 percent of culms were clipped at the SW site. All culms close to the surface were clipped, precluding seed production. Field observations suggest that some important herbivores include crawfish, near the base of the plant; nutria, which was first introduced to Hays County after 1960 (Texas Agricultural Extension Service 1987); and resident waterfowl, including swans, mallards, and muscovy ducks, which are encouraged by Aquarena Springs employees with supplemental feed to remain throughout the year for the amusement of Aquarena Springs guests. With the loss of all inflorescences by herbivory, transplants are limited to clonal reproduction.

Will *Z. texana* persist in Spring Lake? Historically, emergent reproductive culms were a common occurrence in the San Marcos River and

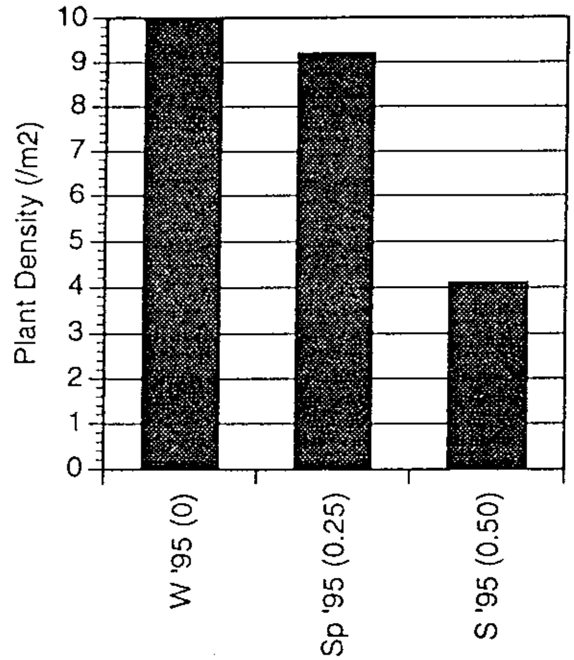

Figure 3. Plant density of *Zizania texana* transplants in Spring Lake at SW site between winter 1995 and summer 1995. Time since transplanting in parentheses.

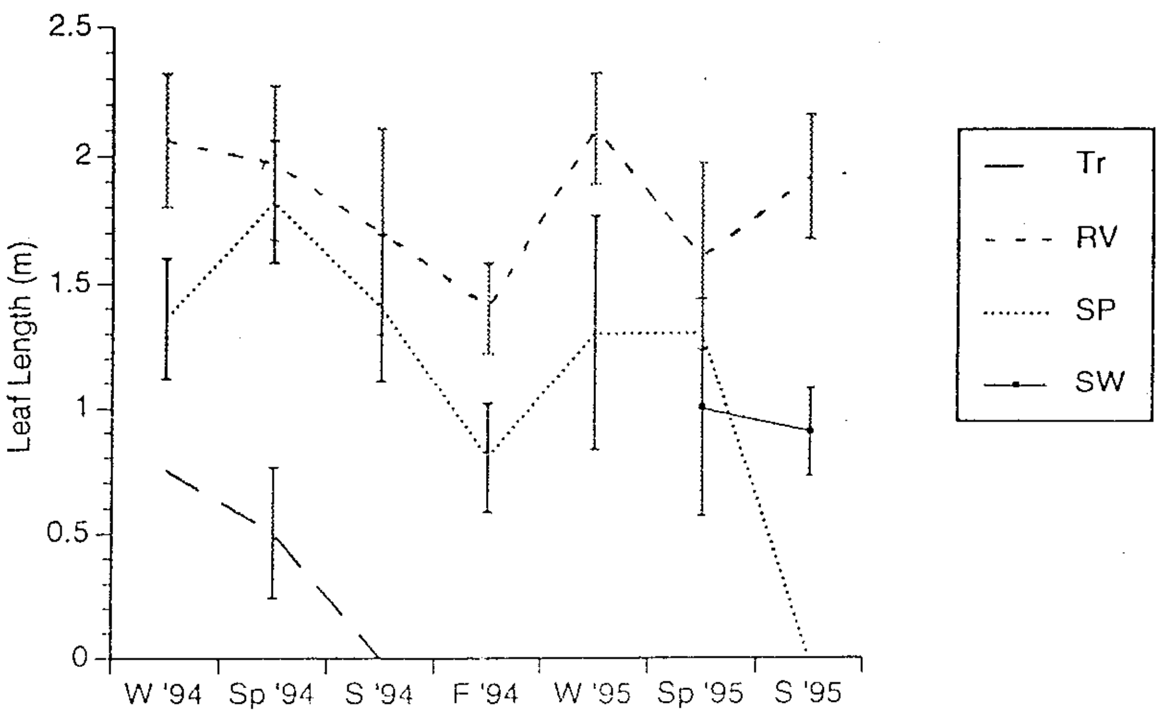

Figure 4. Leaf length of *Zizania texana* transplants grown in TR, RV, SP, and SW sites in Spring Lake.

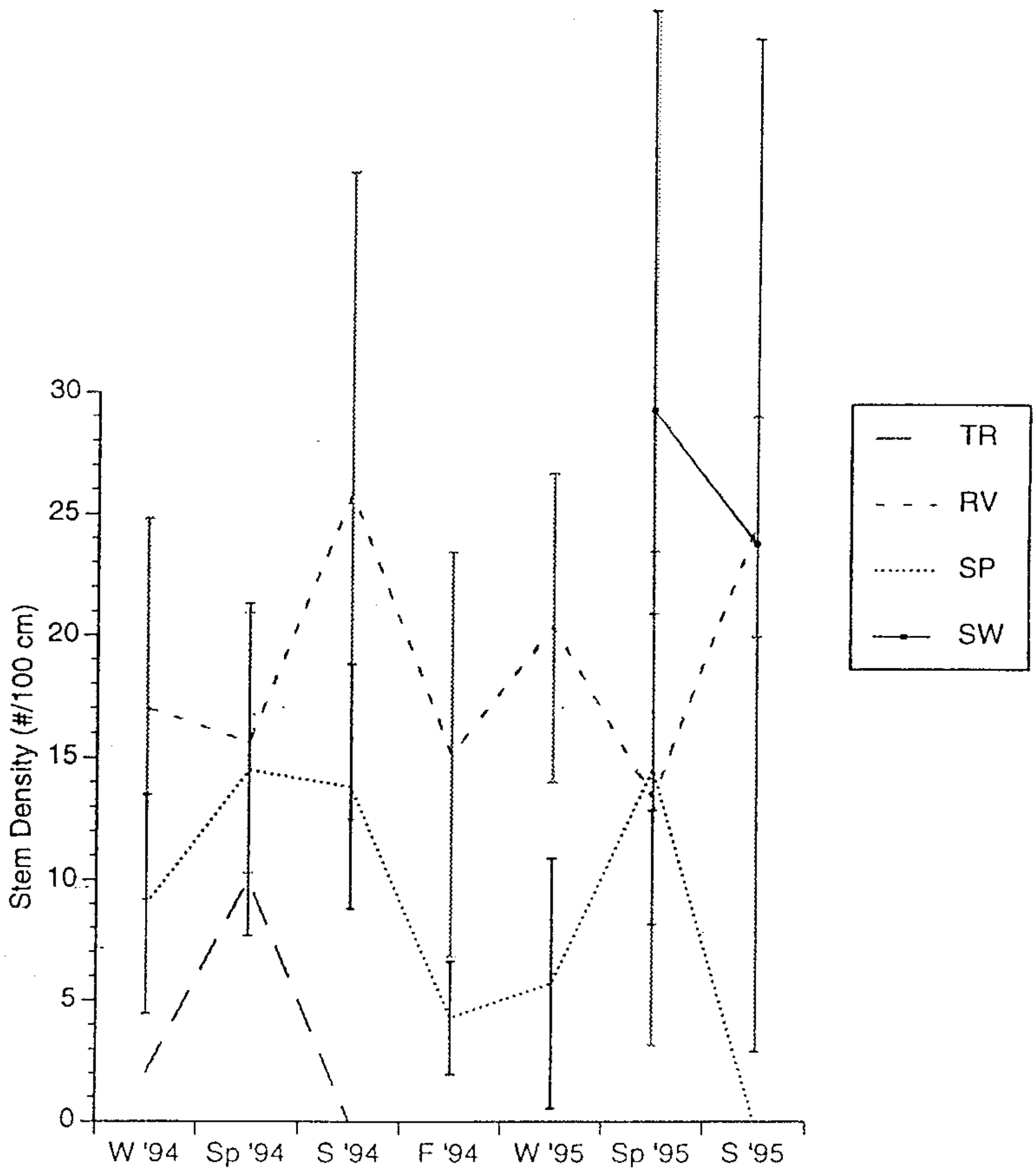

Figure 5. Stem density of *Zizania texana* transplants grown in TR, RV, SP, and SW sites in Spring Lake.

Spring Lake (Devall 1940, Silveus 1933, Watkins 1930). Today, however, reproductive culms are under an onslaught of herbivore activity, and there is no new recruitment by seed. In addition, literally tons of drifting aquatic vegetation become entangled in *Z. texana* plants, blocking sunlight, slowing current velocity, and damaging leaves. *Zizania texana*'s existence in Spring Lake is further jeopardized by reduced spring flow from the Edwards Aquifer and possibly competition for space and light from introduced macrophytes. *Zizania texana* has persisted in RV and SW despite these insults. Monitoring these sites through 1996 will provide the additional data necessary to draw conclusions about the long-term status of *Z. texana* in Spring Lake.

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Literature Cited

Devall, L.L. 1940. A comparative study of plant dominance in a spring fed lake. Master's thesis, Southwest Texas State University.

Eberhardt, L.L. and J.M. Thomas. 1991. Designing environmental field studies. Ecological Monographs 61(1):53–73.

Emery, W.H.P. 1967. The decline and threatened extinction of Texas wildrice (*Zizania texana* Hitchc.). Southwestern Naturalist 12(2):203–204.

Emery, W.H.P. 1977. Current status of Texas wildrice (*Zizania texana* Hitchc.). Southwestern Naturalist 22(3):393–394.

Fonteyn, P.J. and P. Power. 1989. Texas wildrice, interim report. Submitted to U.S. Fish and Wildlife Service, Region 2. pp. 1–10.

Palmer, M.E. 1987. A critical look at rare plant monitoring in the United States. Biological Conservation 39:113–127.

Pavlik, B.M. and M.G. Barbour. 1988. Demographic monitoring of endemic sand dune plants Eureka Valley, California. Biological Conservation 46:217–242.

Rose, F.L. and P. Power. 1992. Effects of habitat and herbivory on growth and reproduction in Texas wildrice (*Zizania texana*). Report submitted to U.S. Fish and Wildlife Service, Region 2. pp. 1–20.

Rose, F.L. and P. Power. 1994. Continued maintenance, reintroduction and research on *Zizania texana* (Texas wildrice). IV. Growth response by *Zizania texana* (Texas wildrice) under three current regimes and two sediments in Spring Lake, Hays County, Texas. Report submitted to U.S. Fish and Wildlife Service, Region 2.

Rose, F.L. and P. Power. 1995. Continued maintenance, reintroduction and research on *Zizania texana* (Texas wildrice). VIII. Direct and indirect effects of aquatic vegetation mats on Texas wildrice (*Zizania texana*). Submitted to U.S. Fish & Wildlife Service, Region 2.

Silveus, W.A. 1933. Texas Grasses, Classification and Description of Grasses. The Clegg Co., San Antonio, Texas.

Terrell, E.E., W.H.P. Emery and H.E. Beaty. 1978. Observations on *Zizania texana* (Texas wildrice), an endangered species. Bulletin of the Torrey Botanical Club 105:50–57.

Texas Agricultural Extension Service. 1987. Identifying and Managing Aquatic Rodents in Texas: Beaver, Nutria and Muskrats. Texas A&M. pp. 1–46.

U.S. Fish and Wildlife Service. 1984. San Marcos River Recovery Plan. U.S. Fish and Wildlife Service, Albuquerque, New Mexico. pp. 41–58.

U.S. Fish and Wildlife Service. 1985. Endangered and threatened wildlife and plant taxa for listing as endangered or threatened species. Federal Register 5C(188):39526–39584.

Watkins, G.M. 1930. Vegetation of San Marcos Springs. Master's thesis, University of Texas at Austin. pp. 1–53.

J. Aquat. Plant Manage. 34: 21-23

Growth of Texas Wildrice (*Zizania texana*) in Three Sediments from the San Marcos River

PAULA POWER[1]

ABSTRACT

A conservation population of the endangered Texas wildrice has been maintained in an outdoor raceway on the Southwest Texas State University campus since 1986. To determine a suitable planting medium for raceway grown plants, seeds were germinated, then planted in three soils: clay, sandy clay loam, and gravel. After 6 weeks of growth, plants were dried, separated into above and below ground parts and weighed. Plants produced the greatest biomass in sandy clay loam and the least in clay. Plants grown in clay (with intermediate nutrient concentration) had the highest r:s, 0.74, which suggests sediment texture as well as nutrient concentration may play an important role in growth of this endangered species.

Key words: macrophyte, nutrient concentration, Texas wildrice, *Zizania texana*, root to shoot ratio, endangered species.

INTRODUCTION

Texas wildrice (*Zizania texana* Hitchc.) is an emergent aquatic macrophyte in the family Poaceae. Its distribution is limited to the first 2.4 kilometers of the spring fed, thermally constant San Marcos River, Hays County, Texas. Texas wildrice typically occurs adjacent to the deepest part of the river channel in gravel or soft, muddy sediments forming dense stands which vary in aerial coverage from approximately 0.45 m^2 to 194 m^2 (Texas Parks and Wildlife Department 1990).

Texas wildrice is listed as an endangered species by both U.S. Fish and Wildlife Service and Texas Parks and Wildlife Department. Factors which threaten its survival include reduced spring flow from the San Marcos springs, reduced water quality in the San Marcos River, competition and predation by nonnative species such as Nutria (*Myocaster coypus*) and *Hydrilla verticillata*, absence of sexual reproduction in the wild, and alteration of sediments in the river bottom (U.S. Fish and Wildlife Service 1984). Alteration of the river sediments includes deposition of fine organic and inorganic particles due to reduction in frequency and magnitude of historic flooding cycles, and coarse gravel deposition from soil erosion in the immediate water shed. To protect plants from extinction from threats in the wild and also for research purposes, attempts have been made to propagate Texas wildrice outside the San Marcos River. Terrell et al. (1978) had limited success growing mature Texas wildrice plants in Beltsville, Maryland. Emery (1977) briefly grew Texas wildrice in an outdoor raceway on the Southwest Texas State University (SWT) campus, San Marcos, Texas; but, they were not maintained after Emery's retirement in the late 1970's. In 1986 Power collected small clumps of Texas wildrice from the San Marcos River which were planted in peat pots containing native sediments and placed in flowing water on the SWT campus. A conservation population of Texas wildrice has since been maintained on the SWT campus.

Growth by Texas wildrice is significantly influenced by the sediment in which it is grown (Power and Fonteyn 1995). It is unknown, however, to what extent nutrients and sediment, separately or in combination, influence plant growth. This study was initiated to elaborate on the nutritional requirements of Texas wildrice and the potential role sediment texture may play in plant growth. The objective was to determine growth response by Texas wildrice when grown in sediments collected from the species' natural habitat. The research will improve our understanding of the ways in which sediments effect plant growth; and, it may benefit decisions on management with respect to the conservation population, habitat alteration of the San Marcos River and watershed, and future reintroduction programs of this endangered species.

METHODS AND MATERIALS

Three sediment types were selected for the growth experiment, 1) a moderately fine sediment from Sewell Park in the San Marcos River; 2) a coarse sediment from the confluence of Sessom Creek and the San Marcos River; and, 3) a very fine soil adjacent to the San Marcos River. Thirty 10 cm peat pots were filled with one of three soil types and placed randomly in a raceway. Water flowed through the raceway at 0.015 m/s from the Edwards Aquifer via an artesian well on the SWT campus. Water depth was 0.4 m. One germinated Texas wildrice seed was placed in each pot. After six weeks growth, plants were harvested. Soil was gently washed from the roots. Plants were returned to the lab and divided into above and below ground parts. Plant material was dried and weighed.

Subsamples of each soil type were shipped to Texas A&M Soil Testing Laboratory for texture, organic matter, and nutrient analysis.

Data were analyzed by single factor ANOVA followed by Tukey Multiple Comparison. Proportional data were transformed by arcsine transformation before analysis (Zar 1984).

RESULTS AND DISCUSSION

On the basis of textural analysis, sediments were designated clay, sandy clay loam, and gravel (Texas A&M Soil Testing Laboratory). Clay was a fine soil, consisting of 62% clay,

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TABLE 1. TEXTURE AND ORGANIC MATTER CONTENT OF THREE SOILS: CLAY, SANDY CLAY LOAM AND GRAVEL, COLLECTED IN AND AROUND THE SAN MARCOS RIVER, SAN MARCOS, TEXAS.

Texture (%)	Clay	Sandy clay loam	Gravel
[illegible]	3	56	16
[illegible]	35	23	2
[illegible]	62	22	3
Gravel	0	0	79
Organic matter (%)	1.7	3.9	0.32

35% silt and only 3% sand. Sandy clay loam, a moderately fine soil, consisted of nearly equal parts silt and clay and 56% sand. Gravel consisted of 79% gravel (particles larger than 2mm), 16% sand and negligible amounts of clay and silt (Table 1). Sandy clay loam had the greatest organic matter content, 3.9%, followed by clay, 1.7% and gravel, 0.32%. Organic matter most likely contributed to the physiological enrichment of the sediments, especially sandy clay loam (Sand-Jensen and Søndergaard 1979).

Soils were analyzed for pH, NO_3, Kjeldahl N, available P, K, Ca, Mg, Ž, Fe, Mn, Cu, Na, and S. Soil nutrient concentrations are presented in Table 2. In water, N, P, Z, Mn and Cu coprecipitate and are taken up by the roots (Barko et al. 1991). Of the nutrients which coprecipitate, P and especially N are commonly considered important limiting nutrients for macrophytes (Barko and Smart 1979; Barko et al. 1991). Sandy clay loam had double the Kjeldahl nitrogen concentration of clay, and Kjeldahl nitrogen concentration of gravel was negligible. Available phosphorus concentration was ighest in clay followed by sandy clay loam.

Total biomass of plants grown in sandy clay loam (0.790 \ was significantly greater when compared with plants ʹn in gravel (0.138 gm) and clay (0.025 gm). Root and ot biomass were also significantly greater in plants grown in sandy clay loam when compared with plants grown in gravel and clay (Table 3). Nitrogen most likely limited plant growth in gravel and clay sediments. In contrast, phosphorus

TABLE 2. NUTRIENT CONTENT (MG/KG DRY SOIL) OF THREE SOILS: CLAY, SANDY CLAY LOAM AND GRAVEL, COLLECTED IN AND AROUND THE SAN MARCOS RIVER, SAN MARCOS, TEXAS. TEXAS WILDRICE SEEDLINGS WERE PLANTED IN EACH SOIL TYPE.

Nutrient	Clay	Sandy clay loam	Gravel
*NO_3	1	1.3	0.3
*Kjeldahl N	776	1517	71
*Phosphorus	55	47	15
Potassium	335	55	15
Calcium	18893	18413	4830
Magnesium	322	840	225
*Zinc	0.1	2.2	0.6
*Iron	19	83	5
*Manganese	5.6	5.2	1.2
*Copper	1.6	0.8	0.4
Sodium	25	54	17
Sulfur	304	1750	428
pH	8.2	7.8	8.5

Nutrients which coprecipitate; their source is sediment. All other nutrients i.e. salts which dissolve in water and are most likely taken up by shoots (Barko et al. 1991).

TABLE 3. SHOOT BIOMASS, ROOT BIOMASS, TOTAL BIOMASS (GM/DRY WEIGHT) AND ROOT TO SHOOT RATIO OF TEXAS WILDRICE GROWN IN THREE SEDIMENTS. LETTER FOLLOWING MEAN INDICATES SIGNIFICANT DIFFERENCE USING TUKEY'S MULTIPLE COMPARISON PROCEDURE AT THE 0.05 LEVEL OF SIGNIFICANCE. SD IN PARENTHESIS.

Biomass	Clay	Sandy clay loam	Gravel
Shoot	0.014a[1]	0.616b	0.094a
	(0.006)	(0.205)	(0.043)
Root	0.011a	0.174b	0.044a
	(0.007)	(0.051)	(0.015)
Total	0.025a	0.790b	0.138a
	(0.013)	(0.254)	(0.057)
R:S	0.74a	0.29b	0.50c

[1]Means followed by standard deviation.

was probably not a limiting factor because clay, with the highest phosphorus concentration, produced plants with lowest total biomass.

On infertile sediments, plants allocate more biomass to belowground structures to maximize volume of soil occupied by roots, resulting in relatively high root to shoot ratios (Barko et al. 1991). In this study, however, a different biomass allocation pattern developed than was expected. Plants grown in clay soils (with intermediate nutrient concentration) had the highest root to shoot ratio, 0.74 (Table 3). This suggests plants grown in clay allocate more biomass to below ground parts in an effort to maximize nutrient uptake, and, nutrients, although present in relatively moderate amounts, are inaccessible to plant roots. It is possible small, closely packed clay particles may impede root penetration making nutrients essentially inaccessible for uptake..

Plants grown in the most nutrient depauperate soil, gravel, had an intermediate root to shoot ratio, 0.50. Sandy clay loam, not surprisingly, had a low root to shoot ratio, 0.29 (Table 3). High root to shoot ratio of plants grown in gravel relative to sandy clay loam suggests nutrient limitation on gravel sediments. This may be due to both low nutrient concentration and low nutrient availability because of the greater diffusion distance between gravel particles (Barko and Smart 1986).

In conclusion, high root to shoot ratio of plants grown in clay which had intermediate nutrient concentrations; and, low productivity by plants grown in gravel and clay suggest that soil texture as well as nutrient concentration play an important role in Texas wildrice growth. Plant growth limitation on very fine sediments may be due to textural considerations and on coarse sediments, limitation may be due to nutrient considerations. When selecting sediments for a planting medium for Texas wildrice, either for a conservation population or other purposes, such as reintroduction programs or research projects when high productivity is desirable, coarse, as well as very fine sediments, should be avoided. Sediment selection should focus on moderately fine sediments with high nutrient concentrations.

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LITERATURE CITED

Barko, J. W., D. Gunnison, and S. R. Carpenter. 1991. Sediment interactions with submersed macrophyte growth and community dynamics. Aquatic Botany 41: 41-65.

Barko, J. W. and R. M. Smart. 1979. The nutritional ecology of *Cyperus esculentus*, an emergent aquatic plant, grown on different sediments. Aquatic Botany 6: 13-28.

[Barko], J. W. and R. M. Smart. 1986. Sediment-related mechanisms of growth [li]mitation in submersed macrophytes. Ecology 67(5): 1328-1340.

Emery, W. H. P. 1977. Current status of Texas wildrice (*Zizania texana* Hitchc.). Southwestern Naturalist 22: 393-394.

Power, P. and P. J. Fonteyn. 1995. Effects of oxygen concentration and substrate on seed germination and seedling growth in *Zizania texana* (Texas wildrice). Southwestern Naturalist 40(1): 1-4.

Sand-Jensen, K. and M. Søndergaard. 1979. Distribution and quantitative development of aquatic macrophytes in relation to sediment characteristics in oligotrophic Lake Kalgaard, Denmark. Freshwater Biology 9: 1-11.

Terrell, E. E., W. H. P. Emery, and H. E. Beaty. 1978. Observations on *Zizania texana* (Texas wildrice), an endangered species. Bulletin of the Torrey Botanical Club 105(1): 50-57.

Texas Parks and Wildlife Department. 1990. Interim report on conservation of the upper San Marcos ecosystem: Texas wildrice (*Zizania texana*). Submitted to U.S. Fish and Wildlife Service, Region 2.

U.S. Fish and Wildlife Service. 1984. *San Marcos River Recovery Plan.* U.S. Fish and Wildlife Service, Albuquerqe, New Mexico.

Zar, J. H. 1984. Biostatistical Analysis, 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

DIRECT AND INDIRECT EFFECTS OF FLOATING VEGETATION MATS ON TEXAS WILDRICE (*ZIZANIA TEXANA*)

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Texas wildrice (*Zizania texana*) is a perennial emergent aquatic macrophyte in the family Poaceae. It has long, ribbonlike, submerged leaves and reproductive culms that arise from the base of the plant. Each culm has an emergent terminal inflorescence allowing for wind pollination. *Zizania texana* is endemic to the first 2.4 km of the San Marcos River and Spring Lake in Hays Co., Texas. It commonly occurs at mid-channel in relatively fast-flowing water (Silveus, 1933; Terrell et al., 1978).

Zizania texana is listed as endangered by both U.S. Fish and Wildlife Service and Texas Parks and Wildlife Department (U.S. Fish and Wildlife Service, 1985). Factors that threaten survival of *Z. texana* include reduced spring flow from the San Marcos springs, reduced water quality in the San Marcos River, competition and predation by nonnative species such as *Hydrilla verticillata* and nutria (*Myocaster coypus*), failure to reproduce sexually, and alteration of sediments in the river bottom (Emery, 1967, 1977; U.S. Fish and Wildlife Service, 1994).

More recently, floating and submerged drifting aquatic vegetation have been identified as a potential threat to *Z. texana* plants. Aquatic vegetation, including *Ceratophyllum demersum*, *H. verticillata*, *Egeria densa*, is mowed in Spring Lake, the headwaters of the San Marcos River, and despite attempts at harvesting the cuttings, cut vegetation drifts downstream. Cut vegetation combines with drifting macrophyte fragments and tends to accumulate behind obstructions at or above the water surface. Obstructions occur in fast or slow flowing water and can be floating *Z. texana* leaves, emergent *Z. texana* culms, other floating vegetation, fallen tree limbs, trash, or debris. Entangled vegetation can form thick mats over *Z. texana* stands in as little as one week.

Direct negative effects of floating vegetation mats may include shredding of *Z. texana* leaves by serrated leaf margins of some macrophytes (e.g., *C. demersum*) and interference with reproductive culm emergence. Indirect negative effects include blocking sunlight, which interferes with photosynthesis, and slowing current velocity, which may reduce nutrient uptake from the open water (Smith and Walker, 1980; Boeger, 1992). The objective of this research was to quantify direct and indirect effects of vegetation mats on *Z. texana* stands.

An artificial obstruction on which drifting vegetation would become entangled was created with a 1-m long by 0.075-m diameter float constructed of polyvinyl chloride pipe. Floats were anchored to the river bottom perpendicular to the direction of flow, at the leading edge of four *Z. texana* stands in the San Marcos River in July 1995. Each *Z. texana* stand was composed of closely spaced, mature individuals. A control area was marked adjacent to each float. Sites were in areas where current was great enough to wash floating vegetation downstream in the absence of an obstruction. This eliminated the need to remove vegetation mats from control areas. Vegetation accumulated upstream and downstream from the float. Six measurements were recorded in each control and treatment area. The measurements were: water depth; current velocity (measured at 20, 60, and 80% depth with a Marsh-McBirney portable water current meter model 201); photosynthetically active radiation (PAR, measured immediately below the water surface with a Li-Cor light meter model LI-185B with a LI-1935B spherical quantum sensor); leaf length (calculated as the average length of 5 or 6 leaves); stem density (no. of reproductive culms + no. of submerged leaves/100 cm^2); and percent damaged leaves (calculated from 50 leaves inspected for shredding or other signs of mechanical damage). Measurements were taken at the leading edge of each stand in July and mid-August, 1995 (six weeks after construction and placement of floats).

Grab samples of vegetation mats were collected from two replicates at the end of the experimental period. Plant species were separated and identified, and percent species composition of each sample was determined

TABLE 1—Environmental measurements and *Zizania texana* response (*SD* in parentheses) to vegetation mats. Data were recorded at the beginning of the experiment (initial) and six weeks after construction of floats that created vegetation mats (treatment); adjacent areas lack vegetation mats (control).

	Time of measurement			
	Initial		Six weeks	
Measurement	Control	Treatment	Control	Treatment
Water depth (m)	0.51a[1]	0.57a	0.52a	0.47a
	(0.133)	(0.180)	(0.132)	(0.160)
Current velocity (m/s)	0.210a	0.174a	0.232a	0.032b
	(0.059)	(0.095)	(0.099)	(0.009)
PAR ($\mu E/s/m^2$)	1800a	1475a	1530a	77b
	(316)	(709)	(205)	(10)
Leaf length (m)	1.12a	1.12a	0.95a	0.60b
	(0.278)	(0.288)	(0.131)	(0.100)
Stem density (/100 cm^2)	69.7a	66.7a	60.2a	20.2b
	(20.0)	(12.4)	(12.1)	(6.40)
Percent damaged leaves	15a	16a	10a	94b
	(0.007)	(0.003)	(0.002)	(0.034)

[1] Means for a measurement within a time period followed by the same letter are not significantly different ($P > 0.05$); $n = 4$.

Means from treatment and control areas at the beginning of the experiment and after six weeks were compared with a paired *t*-test (Snedecor and Cochran, 1980).

At time zero, there was no significant difference between control and treatment in any category measured (Table 1). After six weeks, current velocity, PAR, stem density, and leaf length were significantly less in the treatment areas compared with the control areas (Table 1). A higher percentage of leaves were damaged in treatment areas compared with control areas. In addition to being shredded, damaged leaves appeared to be paler green in color or achlorotic.

The upstream sample, composed primarily of *C. demersum*, a submerged macrophyte common in Spring Lake, was 0.35 m thick. The downstream sample was composed of vegetation common in the San Marcos River; *Potamogeton* sp. and *Sagittaria platyphylla* were the most abundant species (30% and 35%, respectively; Table 2). The downstream sample was 0.37 m thick.

Current velocity was significantly slower in treatment areas compared to control areas. Stationary objects, such as macrophytes, can reduce current velocities in flowing water (Gregg and Rose, 1982). Dense vegetation mats (occupying about 85% of the water column) obstructed water movement and slowed current velocity in treatment areas. In addition, stem density of plants in treatment areas declined during the study period while stem density of plants in control areas remained unchanged. A number of studies have found a positive relationship between flow and carbon uptake and photosynthetic rates (Smith and Walker, 1980; Madsen and Søndergaard, 1983). Flowing water may also provide mechanical stimulation of meristematic tissue resulting in increased stem density. The decline in stem density observed in this study

TABLE 2—Species composition (%) of vegetation mats sampled six weeks after construction of vegetation floats. Location 1 was downstream from confluence of Purgatory Creek and San Marcos River, adjacent to Centennial Park picnic area. Location 2 was in Sewell Park approximately 30 m downstream from Loop 82 Bridge.

	Composition (%)	
Species	Location 1	Location 2
Cabomba caroliniana	0	2
Ceratophyllum demersum	15	75
Ceratopteris thalictroides	0	1
Egeria densa	10	8
Eichhornia sp.	0	2
Hydrilla verticillata	10	7
Myriophyllum spicatum	0	2
Potamogeton sp.	30	1
Sagittaria platyphylla	35	0
Utricularia sp.	0	2

may be attributed, in part, to the negative effect of decreased current velocity.

Plants in treatment areas had significantly more damaged leaves than control areas. *Ceratophyllum demersum* and *H. verticellata* have serrated leaf margins and when in contact they shred *Z. texana* leaves. Damaged leaves most likely had lower photosynthetic rates than leaves in control areas due to leaf shredding and reduced PAR below mats.

Vegetation mats may also interfere with culm emergence and pollination, contributing to sexual reproductive failure. The effect of vegetation mats on reproduction requires further study.

Other direct mechanical damage to *Z. texana* by vegetation mats may include uprooting of plants. Although uprooting of plants was not observed in treatment areas, it is not uncommon to observe sediments eroding from the base of plants and eventually entire plants become uprooted. It is unknown whether uprooting of plants in the wild is due to disturbance of the sediments or caused by drag on plants from entangled vegetation fragments or some other factors.

Historic photographs of *Z. texana* and the San Marcos River (Silveus, 1933) suggest that vegetation mats are more common in the upstream site today than in the past. The increase in mats may be due to a change in species composition exhibiting a growth form more susceptible to fragmentation and drifting in Spring Lake and the San Marcos River. *Hydrilla verticellata,* a fast growing macrophyte, was introduced to Spring Lake and the San Marcos River, and *C. demersum*, a nonrooted submersed macrophyte, currently has a wider distribution in Spring Lake than historical records would indicate it had in the past (Watkins, 1930; Devall, 1940). In addition, mowing of Spring Lake, a practice initiated as early as the 1900's, releases thousands of kilograms of macrophyte fragments annually.

Finally, reduced flooding due to the construction of flood control dams in the San Marcos River watershed and the resulting absence of scouring of the river bottom, together with reduced spring flows due to over pumping of the Edwards Aquifer, may contribute to conditions in the San Marcos River that favor increased macrophyte growth and abundance, contributing to vegetation mats.

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Literature Cited

Boeger, R. T. 1992. The influence of substratum and water velocity on growth of *Ranunculus aquatilis* L. (Ranunculaceae). Aquatic Botany 42:351–359.

Devall, L. L. 1940. A comparative study of plant dominance in a spring-fed lake. Unpublished M.S. thesis, Southwest Texas State University, San Marcos, Texas.

Emery, W. H. P. 1967. The decline and threatened extinction of Texas wildrice (*Zizania texana* Hitchc.). Southwestern Naturalist 12(2):203–204.

Emery, W. H. P. 1977. Current status of Texas wildrice (*Zizania texana* Hitchc.). Southwestern Naturalist 22(3):393–394.

Gregg, W. W., and F. L. Rose. 1982. The effects of aquatic macrophytes on the stream microenvironment. Aquatic Botany 14:309–324.

Madsen, T. V., and M. Søndergaard. 1983. The effects of current velocity on the photosynthesis of *Callitriche stagnalis* Scop. Aquatic Botany 15:187–193.

Silveus, W. A. 1933. Texas grasses, classification and description of grasses. The Clegg Co., San Antonio, Texas, 473–475 pp.

Smith, F. A., and N. A. Walker. 1980. Photosynthesis by aquatic plants: Effects of unstirred layers in relation to assimilation of CO_2 and HCO_3^- and to carbon isotopic discrimination. New Phytologist 86:245–259.

Snedecor, G. W., and W. G. Cochran. 1980. Statistical Methods. Eighth ed. Iowa St. Univ. Press, Ames, Iowa.

Terrell, E. E., W. H. P. Emery, and H. E. Beaty. 1978. Observations on *Zizania texana* (Texas wildrice), an endangered species. Bulletin of the Torrey Botanical Club 105:50–57.

U. S. Fish and Wildlife Service. 1985. Endangered and threatened wildlife and plant taxa for listing as endangered or threatened species. Federal Register 50(188):39526–39584.

U. S. Fish and Wildlife Service. 1994. Draft San Marcos/Comal (Revised) Recovery Plan. Albuquerque, New Mexico.

Watkins, G. M. 1930. Vegetation of San Marcos Springs. Unpublished M.S. thesis, University of Texas at Austin.

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MOISTURE, SEEDS, AND REPRODUCTIVE FAILURE IN TEXAS WILDRICE (*ZIZANIA TEXANA*)

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ABSTRACT—The population of Texas wildrice (*Zizania texana*), an endangered macrophyte endemic to the San Marcos River, Texas, declined between 1940 and 1967. The decline has been attributed to reproductive failure among other factors. Panicles in the wild can be pushed under water for periods of time by floating bits of vegetation, swimming waterfowl and recreational users of the river. In this study, the potential role of wetting developing panicles in reproductive failure was investigated. Wetting was achieved by misting panicles for 30 seconds 3 times/day, or by submerging panicles for 3 or 6 hours each day during floral and seed development. Control panicles were left undisturbed. In addition, seeds collected from treated panicles were categorized as having firm, soft, or liquid endosperm. Control panicles developed significantly faster (9.4 days) than panicles submerged for 6 hours (14.8 days). Seed set was significantly lower when panicles were submerged for 3 and 6 hours. There was a significant interaction between treatments and seed endosperm category. The greater the wetting time the greater the proportion of seeds with liquid endosperm and thus inviable seeds. In addition, seeds with soft and liquid endosperm were smaller and contained more moisture than seeds with firm endosperm indicating seeds had not gone through a critical period of dry matter accumulation and that there was a difference in maturity level among seed categories.

Successful sexual reproduction in angiosperms has three sequential stages: pollination, fertilization, and seed maturation (Lyons et al., 1989). During each stage of reproduction there are hazards which may result in reproductive failure. During pollination of anemophyllous plants (wind pollinated) weather can pose a hazard. Anthers generally do not open unless the weather is warm and dry, because pollen is rapidly washed out of the air during rain (Faegri and van der Pijl, 1979). In addition, anemophyllous plants are dependent on high pollen density to successfully pollinate, because they lack specific animal pollinators to efficiently distribute pollen. High pollen density is most likely achieved by large closely spaced populations (Faeri and van der Pijl, 1979).

For most seeds, desiccation is the final stage during seed development, it leads to a state of metabolic quiescence before germination. Seeds which do not achieve low moisture levels during development, may not germinate upon imbibition (Kermode and Bewley, 1985). Apparently, conditions which impede desiccation pose a hazard during seed development and reduce successful germination and seedling growth (Kermode and Bewley, 1985).

Zizania texana, an anemophyllous, perennial macrophyte, is a member of the family Poaceae. It is listed as endangered by both the state and federal government (U.S. Fish and Wildlife Service, 1985). Emergent culms produce terminal panicles during spring, summer, and fall months, and long ribbon-like submerged leaves are produced throughout the year. Panicles are monoecious with separate male and female spikelets. Lower branches bear one-flowered staminate spikelets and upper branches bear one-flowered pistillate spikelets (Gould, 1975). Floral development is similar to *Z. palustris* as described by Archibold and Weichel (1986) and *Z. aquatica var. aquatica* as described by Weir and Dale (1960). Pistillate spikelets emerge from the leaf sheath and stigmas are exserted over a number of days followed by emergence of staminate spikelets and anther dehiscence.

Although historically an abundant species in the San Marcos River, Hays Co., Texas, *Z. texana* declined dramatically between 1940 and 1986 (Emery, 1967, 1977; Vaughn, 1986). Presently, *Z. texana* occurs mostly as small-to-moderate-sized fragmented stands scattered along the first 2.4 km of the San Marcos River. Its population decline has been attributed to a number of factors including sexual reproductive failure (Emery, 1977; Vaughn, 1986). Reproductive failure can be attributed, in part, to herbivory by nutria (*Myocaster coypus*), an introduced rodent and herbivory by waterfowl (U.S. Fish and Wildlife Service, 1994; Power, 1995). Historical records suggest in the past, emergent panicles and seed set were common (Silveus, 1933). In contrast, at the present time, few panicles emerge from the river water in the wild. In high current areas, panicles can be drawn underwater periodically. Panicles can also be pushed under water for periods of time by bits of free floating vegetation, swimming waterfowl, or recreational users of the river (Power, 1996). The effect of submerging developing panicles on pollination and seed maturation is not well understood.

The objective of this study was to determine if wetting or submerging reproductive culms during floral maturation, pollination and seed development poses a significant threat to reproductive success.

METHODS AND MATERIALS—Initially, a bagging experiment was performed to test the ability of panicles to self pollinate. Secondly, misting panicles with a hand held mister and gently submerging panicles was used as a method of examining if wetting panicles affected the 1) timing of panicle development; 2) stigma receptivity; 3) pollen viability; 4) seed set; and 5) seed development. Each aspect of reproduction was examined separately.

Plants were grown in 10 cm diameter peat pots in sediments collected from the San Marcos River. Pots were placed in an outdoor cement raceway on the Southwest Texas State University campus. Water flowed into the raceway through a valve at one end and out a drain at the other end. The raceway is about 50 m long and about 1 m wide. Water depth was approximately 50 cm. Plants were closely spaced along about 40 m of the raceway. Panicles were erect and emergent during the study which took place between May and August, 1996.

A bagging experiment examined the ability of *Z. texana* to self pollinate. Panicles were: 1) bagged and allowed to self pollinate; 2) bagged and emasculated as anthers emerged from the sheath; and 3) left unbagged. There were eight replicate panicles for each treatment. Bags were made from 7 cm wide dialysis tubing cut to lengths long enough to completely cover panicles. Bags were secured with staples. When seeds were evident on control panicles, all control and treated panicles were harvested and percent seed set for each panicle was calculated as number of seeds present/number of pistillate spikelets/panicle.

To determine if wetting affected the duration of panicle development, pistillate spikelets were tagged when they first emerged from the leaf sheath and then either 1) misted for 30 seconds at 10:00 AM, 2:00 PM and 6:00 PM; 2) submerged for 3 hours; 3) submerged for 6 hours; or 4) left undisturbed (control). Each day the presence of pistillate spikelets with exerted stigmas, staminate spikelets, and dehisced anthers was recorded. Treatments continued each day until all anthers dehisced. There were seven replicate panicles for each treatment.

To determine if wetting affected stigma receptivity, panicles were tagged and either 1) misted 3 times/day; 2) submerged for 3 hours each day; or 3) left undisturbed (control). Three to seven pistillate spikelets with newly exserted stigmas were collected from panicles on five consecutive days. Spikelets were returned to the lab and immediately placed in capillary tubing containing hydrogen peroxide. Stigmas which bubbled were considered receptive (Kearnes and Inouye, 1993).

To determine if wetting affected pollen viability, panicles were tagged and either 1) misted 3 times/day; 2) submerged for 3 hours/day; 3) submerged for 6 hours/day; or 4) left undisturbed (control). There were four replicate panicles for each treatment. For three days during anther dehiscence, three open anthers were collected from each replicate panicle before 10:00 AM. Pollen from each anther was shaken onto a slide containing a drop of lactophenol-aniline blue. A cover slip was placed on the slide and pollen grains under the cover slip were counted. Viable pollen grains stained dark blue, and non-viable grains stained faintly or not at all (Kearnes and Inouye, 1993). Percent viable pollen was calculated as number of viable pollen grains/total number of pollen grains.

To determine the effects of wetting on seed set and seed development, panicles were tagged and either 1) misted 3 times/day; 2) submerged for 3 hours/day; 3) submerged for 6 hours/day; or 4) left undisturbed (control). There were thirteen replicate panicles for each treatment. Treatments began when pistillate stigmas were first exserted and continued for 16 consecutive days with the exception of day number 6, 13 and 14. After the 16th day, all anthers had dehisced and harvesting of seeds commenced. Seeds were collected by gently dragging the fingers along the central axis of the panicle. Seeds were harvested for the next six days. Percent seed set was calculated as number of seeds harvested/number of pistillate rachilla.

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Collected seeds were stained using tetrazolium chloride (Kearnes and Inouye, 1993), but viable and nonviable seeds could not be distinguished. As an alternative to the tetrazolium chloride treatment, the endosperm from five to seven seeds from each treatment was categorized as firm, soft, or liquid. Endosperm was categorized firm, soft, or liquid in part because seeds with a firm endosperm are likely to germinate given appropriate environmental conditions and seeds with a liquid endosperm will not germinate (pers. observ.; Power and Fonteyn, 1995).

Collected seeds were shipped to the National Seed Storage Lab in Ft. Collins, CO for additional measurements. Dry weight and moisture content (water in tissue (g)/dry weight (g)) were determined for embryos and endosperm. Data were pooled during laboratory analysis. In addition, embryos were assayed for dehydrin, a protein expressed late in seed development. Seeds were assayed using Western Blot analysis and an antibody for dehydrin protein.

RESULTS—Inflorescences were terminal and determinate. Flowers developed from the apex downward and were protogynous (stigmas were receptive before anthers dehisced). In all treatments, the central axis of the inflorescence elongated and mature pistillate spikelets emerged from the leaf sheath followed by staminate spikelets. The pistillate stage, staminate stage, and full panicle development took fewer days in the control treatment than in the 6-hour submerge treatment (Table 1, 1-way ANOVA, $P < 0.05$ for each). Control panicles developed in significantly fewer days (9.4) than panicles submerged for 6 hours each day (14.8). Mist treatment and 3-hour treatment were intermediate between the control and 6-hour treatment.

Neither bagged nor bagged and emasculated flowers produced any seed, suggesting inflorescences do not self pollinate or exhibit agamospermy. In contrast, 66% (± 27% SD) of unbagged pistillate spikelets were pollinated and developed seed (1-way ANOVA, $P < 0.05$).

Misting and submerging panicles had no significant effect on stigma receptivity or pollen viability when compared with control panicles (1-way ANOVA, $P > 0.05$). Sixty-six to 100% of the stigmas from all treatments bubbled upon exposure to hydrogen peroxide until the fourth day of treatment. Pollen viability was high from all treatments. Pollen from control treatment was 95 ± 2% viable, 94 ± 3% viable from mist treatment, 93 ± 2% viable from 3-

TABLE 1—Timing and sequence of floral development in *Zizania texana* from plants grown on the Southwest Texas State University campus. Pistillate stage is the number of days between emergence of first pistillate spikelet and exsertion of first anther, staminate stage is the number of days between exsertion of first anther and first anther dehiscence, full panicle development is the number of days until all anthers have dehisced, total is the number of days between the beginning of the pistillate stage and the end of full panicle development. A 1-way ANOVA was performed on each measured stage followed by a Tukey multiple range test. All ANOVAs were significant ($P < 0.05$). The SD is in parentheses and $n = 7$ for each stage. Means within a row for a given stage with the same superscript are not significantly different.

	Number of days			
	Control	Mist	3-hour dip	6-hour dip
Pistillate stage	2.1^{b} (0.378)	$1.5^{a,b}$ (0.837)	3.6^{c} (1.902)	3.2^{c} (0.408)
Staminate stage	$3.1^{a,b}$ (1.345)	$4.3^{b,c}$ (0.816)	5.1^{c} (1.676)	5.3^{c} (1.211)
Full panicle development	$4.1^{a,b}$ (1.345)	$5.3^{b,c}$ (0.816)	$5.7^{b,c}$ (1.211)	6.3^{c} (1.211)
Total	$9.4^{a,b}$ (2.936)	$11.0^{b,c}$ (2.366)	$13.3^{b,c}$ (3.615)	14.8^{c} (2.562)

hour treatment, and 87 ± 9% viable from 6-hour treatment (with no significant difference, 1-way ANOVA, $P > 0.05$).

Mean seed set was 32–54%, with the highest value in the control (Fig. 1). There was a significant difference in seed set between control and treatment panicles (1-way ANOVA; $P = 0.018$). Tukey multiple range test determined seed set was significantly greater in control panicles than in either the 3 or 6 hour submerged treatments, but seed set in the mist treatment was intermediate between the control and submerged treatments (Fig. 1).

Control and treatment panicles produced firm, soft, and liquid seeds. Two-way ANOVA indicated that there was a significant difference in the proportion of seeds in the three seed categories and that there was significant interaction between treatment and seed category; the proportion of liquid and soft seeds increased with increasing wetting time (Table 2).

Embryos and endosperm from seeds with

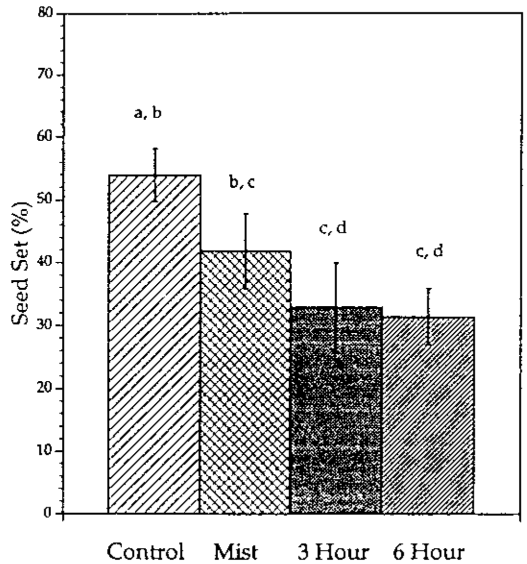

FIG. 1—Seed set in *Zizania texana* after sixteen consecutive treatment days. Seeds were collected daily until all seeds had fallen from treatment and control panicles. A 1-way ANOVA indicated significant differences between treatments ($P < 0.05$). Two bars with the same letter are not significantly different (Tukey multiple range test).

TABLE 2—Texture of seeds collected from *Zizania texana* after three experimental treatments and control. Seeds were pricked with a scalpel and categorized as firm, soft, or liquid. The endosperm of firm seeds appeared slightly chalky, the endosperm of soft seeds was very soft or slightly runny, the endosperm of liquid seeds was liquid. A 2-way ANOVA was performed followed by a Tukey multiple range test. There was a significant difference in seed texture and significant interaction between treatment and texture. The SE is in parentheses and means followed by the same superscript are not significantly different.

	Seeds (%)			
	Control $n = 7$	Mist $n = 6$	3-hour dip $n = 5$	6-hour dip $n = 6$
Firm	76.0^a	71.3^a	44.1^b	44.8^b
	(5.59)	(6.37)	(12.21)	(11.39)
Soft	12.0^b	14.0^b	29.3^b	26.2^b
	(3.40)	(2.93)	(5.95)	(5.59)
Liquid	12.1^b	14.7^b	26.5^b	29.0^b
	(3.46)	(4.16)	(15.96)	(8.74)

firm endosperm were larger than seeds with soft or liquid endosperm. Seeds with soft and liquid endosperm contained more water than seeds with firm endosperm, suggesting seeds with a soft or liquid endosperm had not gone through a period of desiccation (Table 3). Western blot analysis showed strong banding for two proteins at 23.4 and 25 Kd in seeds with firm endosperm. Seeds with soft endosperm showed faint banding for one protein at 23.4 Kd. Seeds with liquid endosperm were not assayed due to insufficient sample size.

DISCUSSION—In this study *Z. texana* panicles did not self pollinate when bagged. Self incompatibility occurs within the genus (Weir and Dale, 1960; Rogosin, 1954) and seems to be the case in *Z. texana*. Bagging, however, may have caused higher temperatures within bags which could have interfered with fertilization and embryo development giving a false indication of self-incompatibility although this possibility seems remote.

TABLE 3—Dry weight and water content of embryos and endosperm of *Zizania texana* seed. Smaller embryo weight and higher water content of soft and liquid seeds suggests a difference in maturity stage among categories. Data were pooled during laboratory analysis and were not analyzed statistically.

		Embryo		Endosperm	
	% Embryo present	Dry weight (mg)	Water (g)/ Dry weight (g)	Dry weight (mg)	Water (g)/ Dry weight (g)
Firm	93	0.241	1.715	3.524	0.464
	$n = 45$	$n = 3$	$n = 3$	$n = 3$	$n = 3$
Soft	82	0.092	4.654	1.286	1.551
	$n = 22$	$n = 5$	$n = 5$	$n = 3$	$n = 3$
Liquid	45	0.026	1.506	0.655	1.730
	$n = 20$	$n = 9$	$n = 9$	$n = 3$	$n = 3$

Panicle development can be divided into three stages, pistillate stage, staminate stage, and full panicle development. The pistillate stage begins with the emergence of the first pistillate spikelet and ends with the exsertion of the last stigma. The staminate stage begins with the emergence of the first anther and ends with the emergence of the last anther. Full panicle development is the time between the dehiscence of the first and last anther. Between the beginning of the pistillate stage and the end of the staminate stage stigmas from one panicle are receptive but anthers from the same panicle have not yet dehisced. Thus, the timing of floral development and stigma receptivity seems to promote outcrossing. This is in agreement with Emery and Guy (1979) who noted that pistillate spikelets showed enlarged ovaries 1 to 2 days after they were exserted from the leaf sheath indicating pistillate flowers were pollinated before anthers from the same panicle dehisced. Outcrossing is favored in large, dense stands with many inflorescences at various developmental stags. This condition is lacking in the fragmented Texas wildrice population. Reproductive failure in the wild in this species may occur, at least in part, at the pollination stage because there simply are not enough emergent panicles with receptive stigmas and dehiscing anthers at the same time and place to have effective pollination.

Wetting panicles apparently impedes reproductive success during pollination. Seed set was greatest in control panicles when compared with misting, and the 3- and 6-hour dipping treatments. Treatments began each day at about 10:00 AM. At this time of day, anthers had dehisced and occasionally clouds of pollen were visible in the air when anthers were disturbed. Misting may have washed pollen from the air preventing pollination from taking place and submerging panicles may simply have prevented pollen from reaching stigmas.

Wetting panicles also apparently impedes reproductive success during seed development. Three-hour and 6-hour dipping treatments had significantly fewer firm seeds than control or mist treatments and therefore fewer seeds likely to germinate. Results also showed that firm seeds had larger embryos and more dry matter accumulation than soft or liquid seeds. These data coupled with different staining patterns for firm and soft seeds indicate a difference in maturity stage among seed categories, but it is unclear whether this is due to arrested development or incipient abortion (or both). Germinability and seedling growth is not fully expressed unless seeds go through a period of desiccation during development (Kermode and Bewley, 1985) and a slight reduction in water potential is necessary for desiccation and dry matter accumulation during development (Vertucci and Farrent, 1995). Misting and submerging panicles may have altered water potentials inside and outside seeds, interupting the desiccation process.

Conditions in the wild which cause panicles to remain wet for long periods of time, such as floating vegetation mats, resident waterfowl, recreational users of the river, or other factors may contribute to pollination failure and reduced seed set, or developmental failure and fewer mature seeds which are capable of germinating, thus contributing to overall reproductive failure in *Z. texana*.

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Literature Cited

Archibold, O. W., and B. J. Weichel. 1986. Variation in wild rice (*Zizania palustris*) stands across northern Saskatchewan. Canadian Journal of Botany 64:1204–1211.

Emery, W. H. P. 1967. The decline and threatened extinction of Texas wildrice (*Zizania texana* Hitchc.). Southwestern Naturalist 12:203–204.

Emery, W. H. P. 1977. Current status of Texas wildrice (*Zizania texana* Hitchc.). Southwestern Naturalist 22:393–394.

Emery, W. H. P., and M. N. Guy. 1979. Reproduction and embryo development in Texas Wildrice (*Zizania texana* Hitchc.). Bulletin of the Torrey Botanical Club 106:29–31.

Faegri, K., and L. van der Pijl. 1979. The Principles of Pollination Ecology. Pergamon Press, Oxford, England.

Gould, F. W. 1975. The Grasses of Texas. Texas A&M University Press, College Station, Texas.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for Pollination Biologists. University Press of Colorado, Boulder.

Kermode, A. R., and J. D. Bewley. 1985. The role of maturation drying in the transition from seed development to germination. Journal of Experimental Botany 36:1906–1915.

LYONS, E. E., N. M. WASER, M. V. PRICE, J. ANTONOVICS, AND A. F. MOTTEN. 1989. Sources of variation in plant reproductive success and implications for concepts of sexual selection. American Naturalist 134:409–433.

POWER, P. 1995. Reintroduction of Texas wildrice (*Zizania texana*) in Spring Lake: Some important environmental and biotic considerations. In: J. Maschinske, H. D. Hammond, and L. Holter, editors. Southwestern Rare and Endangered Plants: Proceedings of the Second Conference. General Technical Report RM-GTR, U. S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experimental Station, Ft. Collins, Colorado. Pp. 179–186.

POWER, P. 1996. Direct and indirect effects of floating vegetation mats on Texas wildrice (*Zizania texana*). Southwestern Naturalist 41:462–464.

POWER, P., AND P. J. FONTEYN. 1995. Effects of oxygen concentration and substrate on seed germination and seedling growth of *Zizania texana* (Texas wildrice). Southwestern Naturalist 40:1–4.

ROGOSIN, A. 1954. An ecological history of Wild Rice. Department of Conservation, Minneapolis, Minnesota.

SILVEUS, W. A. 1933. Texas grasses, classification and description of grasses. The Clegg Co., San Antonio, Texas.

U. S. FISH AND WILDLIFE SERVICE. 1985. Endangered and threatened wildlife and plant taxa for listing as endangered or threatened species. Federal Register 50:39526–39584.

U. S. FISH AND WILDLIFE SERVICE. 1994. Draft San Marcos/Comal (Revised) Recovery Plan. Albuquerque, New Mexico.

VAUGHAN, J. E. JR. 1986. Population and autecological assessment of *Zizania texana* Hitchc. (Poaceae) in the San Marcos River. M.S. Thesis, Southwest Texas State University, San Marcos, Texas.

VERTUCCI, C. W., AND J. M. FARRANT. 1995. Acquisition and loss of desiccation tolerance. In: J. Kigel, and G. Galili, editors. Seed development and germination. Marcel Dekker, Inc., New York. Pp. 237–271.

WEIR, C. E., AND H. M. DALE. 1960. A developmental study of Wild Rice, *Zizania aquatica* L. Canadian Journal of Botany 38:719–739.

EFFECTS OF OXYGEN CONCENTRATION AND SUBSTRATE ON SEED GERMINATION AND SEEDLING GROWTH OF TEXAS WILDRICE (*ZIZANIA TEXANA*)

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ABSTRACT—We examined effects of O_2 concentration on germination of Texas wildrice (*Zizania texana*), an endangered plant endemic to the spring fed, thermally constant San Marcos River. Seeds had significantly higher germination at 0.1, 1.0, and 2.0 ppm O_2 and lower germination at 3.0, 4.0, and 5.0 ppm O_2. Depth and substrate significantly influenced percent germination; seeds buried in clay had the highest percent germination and seeds placed on the surface of sand had the lowest percent germination. At each of three different time intervals (3, 6, and 12 weeks after planting), seedlings grown in clay had significantly greater leaf area than seedlings grown in sand. These data show O_2 level to be an important environmental cue for Texas wildrice. Low O_2 levels trigger germination in anaerobic sediments; such sediments ultimately provide the most suitable substrate for growth.

Texas wildrice (*Zizania texana*), an endangered perennial macrophyte, is endemic to the spring fed, thermally constant San Marcos River in Hays County, Texas. Texas wildrice has narrow, long blades that float on or below the surface of the water and erect reproductive culms that rise above the water surface allowing for wind pollination. It forms large vegetative stands that are often associated with but not restricted to the main channel of the river (Terrell et al., 1978).

During the past 55 years, Texas wildrice has declined dramatically due to habitat destruction, removal of the species from the river, and the absence of seed production (Emery, 1967, 1977). Research on germination requirements could provide insight into appropriate habitat conditions for seedling survival (Fenner, 1985), a critical stage in the life history of the species.

Some aquatic macrophytes require low oxygen levels for seed germination (Kennedy et al., 1980; Bonnewell et al., 1983; Pons and Schroder, 1986). A. Rogosin (in litt.) and Simpson (1966) suggested that low oxygen levels may influence germination in *Z. aquatica* seeds but others achieved good germination without maintaining artificially low oxygen levels (Simpson, 1966; Atkins et al., 1987). We hypothesized that dissolved oxygen concentration in water affects germination of Texas wildrice. We tested the effects of oxygen concentration on seed germination and the effects of sediments with different oxygen availability on seedling growth and seedling survivorship.

MATERIALS AND METHODS—Seeds were collected during summer and fall 1989 from plants growing outdoors in a cement raceway on the Southwest Texas State University (SWT) campus, Hays Co., Texas. The raceway, originally constructed for raising sport fish, is continuously supplied with water from an artesian well. Water depth and flow are controlled with an adjustable valve. Seeds were hulled and placed in vials immediately after collection. Vials were filled to within 1 mm of the top with deionized water and stored at 3°C.

Germination was measured in deionized water at six oxygen concentrations: 0.1, 1.0, 2.0, 3.0, 4.0, and 5.0 ppm. A concentration of 5.0 ppm oxygen was achieved by bubbling air into a five gallon carboy filled with deionized water. Nitrogen was bubbled into the carboy to obtain 0.1, 1.0, 2.0, 3.0, and 4.0 ppm oxygen concentrations. Oxygen levels in the water were determined using a YSI model 57 oxygen meter with a YSI model 5739 oxygen probe. When the desired concentration was reached, water was carefully siphoned

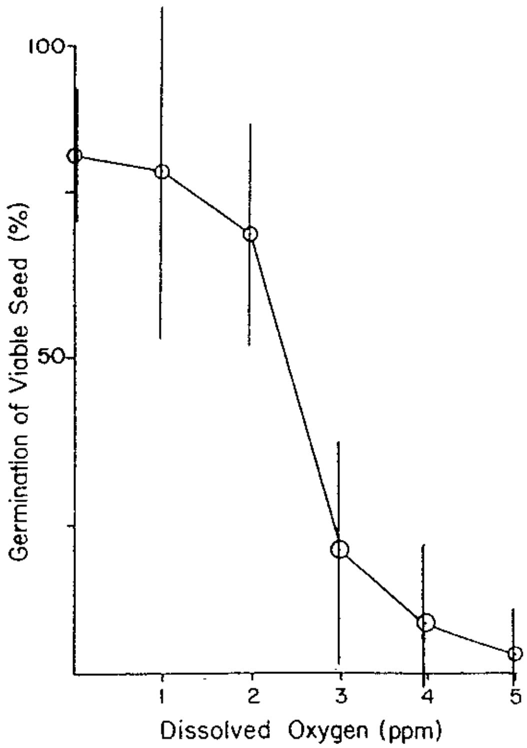

FIG. 1—Percent germination of Texas wildrice seed in water with six different dissolved oxygen concentrations. Vertical bars indicate *SD*.

into 250 ml Erlenmyer flasks containing 20 seeds. Flasks were immediately stoppered and placed in an environmental chamber set at 21°C with a 12-h light:12-h dark photoperiod. There were eight replicates for each treatment. Germinated seeds were counted daily for 4 days. A seed was considered germinated when the shoot extended through the seed coat.

Because seeds placed in higher oxygen levels failed to germinate, seeds initially placed in water at higher oxygen levels were placed in water at low oxygen levels to determine germination response. The water was decanted from treatments with 3.0, 4.0, and 5.0 ppm oxygen and replaced with water containing 0.1 ppm oxygen. Flasks were stoppered and returned to the environmental chamber. Germinated seeds were counted after 7 days.

Ungerminated seeds from all treatments were tested for viability by splitting the seed lengthwise and placing it in a solution of 0.12 gm tetrazolium chloride and 50 ml deionized water. Viable embryos stained dark red after 24 h. Nonviable embryos were white to pale pink (Simpson, 1966).

Percent germination of viable seed was calculated for each treatment. The data were transformed with an arcsine transformation and analyzed using a one-way ANOVA followed by Scheffe pairwise comparison of means.

To determine if the results of the laboratory oxygen experiment would be repeated under field conditions, seeds were planted in two sediments: clay rich in organic matter collected from ponds on the SWT campus and sand with little or no organic matter purchased at a local hardware store and rinsed prior to the experiment. These sediments were selected because each has a different oxygen demand and therefore different oxygen availability. Clay rich in organic matter has a high biological oxygen demand and therefore low dissolved oxygen levels. In contrast, sand has little or no organic matter, and hence a low biological oxygen demand and high dissolved oxygen availability. Plastic pots were filled with clay or sand sediments and placed in water 50 cm in depth in a raceway on the SWT campus. Seeds were placed on the surface and 1.5 cm deep in both sediments. There were four replicates with 20 seeds for each treatment. Current velocity was 0.015 m/s, not rapid enough to wash out seeds placed on the sediment surface. After 10 days all seeds were retrieved from each pot and germinated seeds were counted. Percent germination was calculated using viable seeds only.

Germination data for seeds placed in two substrates and two depths were transformed with an arcsine transformation then analyzed with a two-factor analysis of variance with depth and substrate as the main factors followed by Tukey Multiple Comparison test (Zar, 1984).

To determine survivorship and growth in two substrates, seeds were planted 1.5 cm deep in sand and clay in plastic pots and placed in a raceway on the SWT campus. Survivorship was determined by counting the number of seedlings in each substrate at 2, 3, 6, and 12 weeks after planting. Because Texas wildrice is an endangered species, we felt it appropriate to determine growth by measuring leaf area as an alternative to harvesting plants and weighing biomass. To this end, length and width of all leaves of each plant were measured 3, 6, and 12 weeks after planting. A *t*-test was used to analyze productivity data.

RESULTS—Dissolved oxygen concentration in the water significantly (ANOVA, $P < 0.001$) influenced germination in Texas wildrice (Fig. 1). Highest percent germination of viable seed in the laboratory occurred at the lowest oxygen levels: 84.5% at 0.1 ppm and 79.1% at 1.0 ppm (Fig. 1). Lowest percent germination occurred at the highest oxygen levels: 7.0% at 4.0 ppm and 3.2% at 5.0 ppm. Combined treatment 0.1, 1.0, 2.0 had significantly higher germination than combined treatment 3.0, 4.0, 5.0 ($P < 0.001$, Scheffe pairwise comparison). Seeds that did not germinate at 3.0, 4.0, and 5.0 ppm oxygen readily germiffe pairwise comparison

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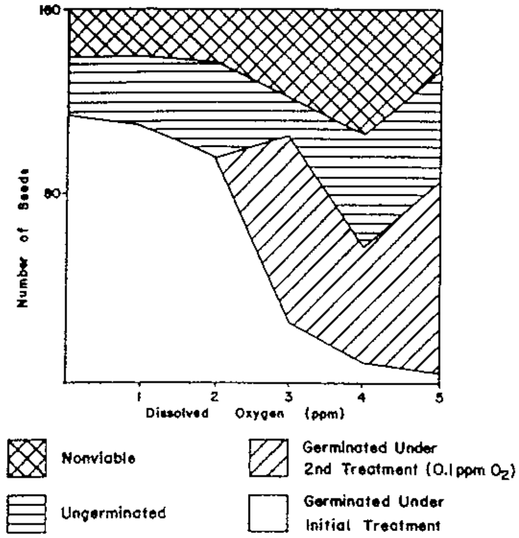

FIG. 2—Proportion of Texas wildrice seeds which were nonviable, remained ungerminated after treatment, germinated under initial oxygen concentration treatment, and germinated at low oxygen concentration after initial treatment at higher oxygen concentration.

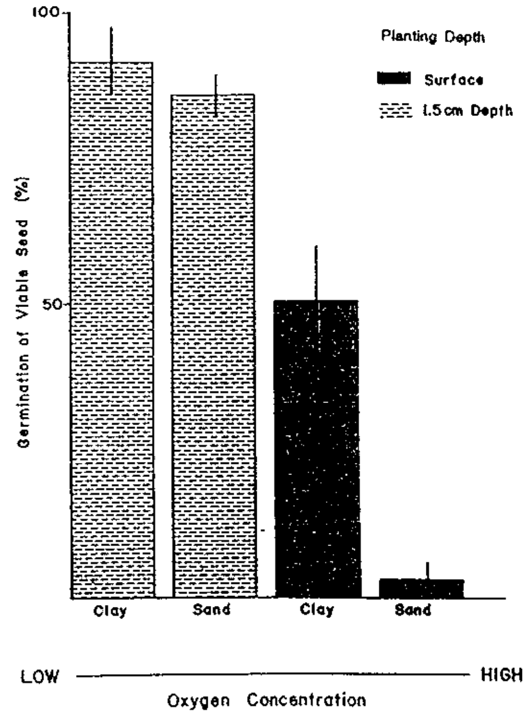

FIG. 3—Germination of Texas wildrice seeds in two different sediments and two different planting depths. Assumed oxygen concentration decreases with increasing depth. Vertical bars represent *SD*.

nated when placed under low oxygen conditions (Fig. 2).

The two-factor ANOVA indicated that buried seeds had significantly higher percent germination when compared with seeds placed on the sediment surface regardless of sediment type (Fig. 3). In addition, germination was greater in seeds placed on the surface of clay when compared with seeds placed on the surface of sand (Fig. 3). However, the Tukey multiple comparison test indicated no difference in percent germination between buried treatments.

Seedlings grown in clay had nearly twice the leaf area of those grown in sand at each time interval. Leaf area (in cm^2) after 3, 6, and 12 weeks in clay was 2.9 (0.4 *SE*), 46.5 (4.9), and 195.8 (41.7) respectively; corresponding values for sand were 1.9 (0.2), 22.4 (3.8), and 86.6 (15.2). The effects of substrate were significant for each age group ($P < 0.05$). After seeds germinated, there was some mortality; however once established, the chance of survival was the same regardless of substrate.

DISCUSSION.—Low oxygen concentration is a characteristic of waterlogged soils (Ponnamperuma, 1972), and a germination requirement for many wetland and aquatic species (Kennedy et al., 1980; Bonnewell et al., 1983; Pons and Schroder, 1986). This requirement allows seed to respond to suitable sites that provide favorable conditions for initial seedling growth (Fenner, 1985). Percent germination increased with low dissolved oxygen concentration and anaerobic sediment conditions. Buried seeds in both sediment types showed the greatest percent germination, presumably where the sediments were low in dissolved oxygen (Ponnamperuma, 1972). The clay-water interface should have a higher oxygen concentration than the area 1.5 cm below the surface due to the oxygen concentration gradient between sediment and water, and, as expected, seeds at the clay-water interface had lower percent germination than buried seeds. Sandy soil with very little organic matter and low biological oxygen demand probably had high oxygen concentration at the sand-water interface, resulting in low percent germination (Fig. 3).

Texas wildrice seeds that germinated and grew in clay rich in organic matter produced more leaf material than did seeds that germinated and grew in sand low in organic matter. The growth of Texas wildrice seedlings in clay may have been due to nutrients released from decomposing organic matter rather than some quality of the sediments. Nevertheless, clay, the sediment that produced significantly greater germination, also produced larger seedlings.

Sandy soil can provide a suitable substrate for seed germination of Texas wildrice only if the seeds are buried. Burial is possible in a natural habitat since the moving water on the river bed continuously rolls particles on the bottom. Eventually seeds could lodge against larger stationary objects and become buried by smaller rolling particles. Seedlings growing in sand, where they have smaller leaves and probably a smaller root system, would not withstand disturbance encountered during the seedling stage as successfully as seedlings growing in clay.

The substrate of the San Marcos River is patchy. Soft, muddy areas can be found adjacent to sandy-gravely areas. In a riverine habitat with a patchy substrate, low oxygen can be an important environmental cue for seed germination. A low-oxygen cue triggers seed germination under conditions that enhance seedling survival and growth. Low oxygen is encountered when seeds are buried in coarse sandy sediments. The low oxygen requirement would ensure that seeds germinate only when buried in coarse substrates and would reduce the likelihood of newly germinated seeds being carried away by the current. Low oxygen is also encountered both at and below the surface of fine sediments with abundant organic matter. Fine clay sediments with abundant organic matter provide positive conditions for germination and subsequently abundant plant growth.

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LITERATURE CITED

ATKINS, T. A., A. G. THOMAS, AND J. M. STEWART. 1987. The germination of wild rice seed in response to diurnally fluctuating temperatures and after-ripening period. Aquatic Botany, 29:245–259.

BONNEWELL, V., W. L. KOUKKARE, AND D. C. PRATT. 1983. Light, oxygen, and temperature requirements for *Typha latifolia* seed germination. Canadian J. Bot., 61:1330-1336.

EMERY, W. H. P. 1967. The decline and threatened extinction of Texas wildrice. Southwestern Nat., 12:203–204.

———. 1977. Current status of Texas wildrice. Southwestern Nat., 22:393–394.

FENNER, M. 1985. *Seed Ecology*. Chapman Hall. New York.

KENNEDY, R. A., S. C. H. BARRETT, D. VANDER ZEE, AND M. E. RUMPHO. 1980. Germination and seedling growth under anaerobic conditions in *Echinochloa crus-galli* (barnyard grass). Plant, Cell and Environ., 3:245–248.

PONNAMPERUMA, J. N. 1972. Chemistry of submerged soils. Advanced Agron., 24:26–96.

PONS, L. L., AND H. F. J. M. SCHRODER. 1986. Significance of temperature fluctuation and oxygen concentration for germination of the rice field weeds *Fimbristylis littoralis* and *Scirpus juncoides*. Oecologia, 68:315–319.

SIMPSON, G. M. 1966. A study of germination in the seed of Wildrice (*Zizania aquatica*). Canadian J. Bot., 44:1–9.

TERRELL, E. E., W. H. P. EMERY, AND H. E. BEATY. 1978. Observations on *Zizania texana* (Texas wildrice), an endangered species. Bull. Torrey Bot. Club, 105:50–57.

ZAR, J. H. 1984. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, New Jersey.