

**ECOLOGICAL LIMITATIONS FOR SOUTHERN WILD RICE ASSOCIATED
WITH BACKWATER LAKES OF THE ILLINOIS AND
UPPER MISSISSIPPI RIVER VALLEYS**

A Thesis

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The Graduate College of
Missouri State University

In Partial Fulfillment

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Master of Science, Biology

By

Bethany R. Dalrymple

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ABSTRACT

Records from the Illinois Natural History Survey of the late 1930s and early 1940s indicate that natural wild rice (*Zizania aquatica* L.var. *aquatica*) populations were common in backwater lakes throughout the Illinois River Valley; however, only a single population was documented in this region between 2001 and 2007. This single population, located in Spring Lake near Pekin, Illinois, provided seed for this study. After several seasons of trials to assess germination requirements, plugs were successfully produced in April, 2007; and were used for the present out-planting study. The purpose of this study was to determine the range of physiological tolerances of *Z. aquatica* var. *aquatica* to 19 water and soil variables, as it has not been previously studied in *Z. aquatica* L. var. *aquatica* populations from this particular region. One month old plugs of wild rice were planted at 15 different sites within the southern half of the Upper Mississippi River Valley during the 2007 growing season. Plants at all sites survived to reproductive stages with an average seed viability of 89.5%, although final plant height ranged from (180.4 cm to 378.7 cm). Interestingly, water and soil pH ranged widely across study sites (water: 5.6-11.1; soil: 5.0-7.8) but were not found to correlate with plant growth during any phenology stage. Due to the success of these plants across a wide and variable range of edaphic conditions, it is suspected that *Z. aquatica* L. var. *aquatic* maintains an extensive physiological tolerance range and therefore a narrow tolerance range does not explain the loss of naturally occurring populations in this region.

KEYWORDS: *Zizania aquatica*, backwater lake, hydrology, physiological, tolerance

This abstract is approved as to form and content

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CHAPTER 1: AN INTRODUCTION TO BACKWATER LAKE ECOSYSTEMS OF THE ILLINOIS AND UPPER MISSISSIPPI RIVER VALLEYS AND THEIR ASSOCIATED VEGETATION

Backwater Lakes of the Illinois and Upper Mississippi River Valleys

Definition. Backwater lakes are not actually lakes in the true sense; they are a type of wetland associated with riparian systems. A lake, by definition, has a developed profundal zone, which is a deep-water section where light does not penetrate. Backwater lakes are shallow throughout and therefore lack this defining characteristic. Wetlands are generally defined as areas where terrestrial and aquatic systems converge, but are difficult to define because of the great diversity that exists in water/land convergence zones (Mitsch and Gosselink 2000). Keddy (2000) attempts to provide an all-inclusive definition for wetlands by describing a wetland as “an ecosystem that arises when inundation by water produces soils dominated by anaerobic processes and forces the biota, particularly rooted plants, to exhibit adaptations to tolerate flooding.” Similarly, the legal definition of wetlands is, “those areas that are inundated or saturated by surface water or groundwater at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions” (Wetland Training Institute, Inc. 1987). This legal definition is jointly shared by the U.S. Army Corps of Engineers and the Environmental Protection Agency, and is listed in the Federal Registry (33 CFR 328.3(b); 40 CFR 230.3(t)). A wetland is legally defined by three characteristics: (1) inundation of water, (2) reduced

oxygen levels in the soil, and (3) presence of biota that can tolerate flooding and anaerobic conditions (Keddy 2000). Accordingly, wetlands are delineated using three characteristics: (1) presence of permanently shallow or frequent, temporarily flooded areas, (2) hydric soils, and (3) hydrophytic plants (Wetland Training Institute Inc. 1987, Mitsch and Gosselink 2000).

Backwater lakes found within the Upper Mississippi and Illinois River valleys are ecosystems characterized as shallow water wetlands that maintain permanent water levels of less than a meter in depth. The Canadian Wetland Classification System describes shallow water wetlands as “distinct wetlands transitional between those wetlands that are saturated or seasonally wet (bog, fen, marsh or swamp) and aquatic ecosystems (lakes), which usually have a developed profundal zone” (National Wetlands Working Group 1997). Shallow waters of backwater lakes experience gaseous and nutrient exchange, oxidation, decomposition, and highly variable ionic concentration, while the stable hydrology provides a substrate for rooted, submerged, emergent and floating hydrophytic vegetation (National Wetlands Working Group 1997).

Geological Formation. Approximately 21,000 years ago, the Wisconsinian glaciation diverted the Mississippi River channel from the Illinois Valley to its present channel. Upon the retreat of the massive ice sheet, glacial melt flood waters formed the Des Plaines and Kankakee rivers which joined to create the present Illinois River. The Illinois River carved a new channel that diverted its channel into the deep, broad, ancient valley of the Mississippi River. The large channel capacity, low volume of flow and slow rate of fall in the Illinois River combined to form the unique bottomland lakes associated with the Illinois Valley of the Illinois and Upper Mississippi Rivers. This

phenomenon occurred over eons of time as the faster-moving waters of the main channel met the slower-moving backwaters to result in sediment deposition along a shear that eventually created natural levees. These barrier island-like structures that separated the main channel waters from the adjacent backwaters formed the present backwater lakes of the Upper Mississippi River Valley (Turner 1936, Bellrose et al 1979, Heitmeyer and Westphall 2007).

Functions. Backwater lakes are important in controlling flood waters, maintaining water quality, serving as sediment sinks and water storage reservoirs (Morris 1991). Shallow water wetlands are known to perform several important biogeochemical functions as a result of permanent inundation that results in anoxic substrates. These anoxic substrates contain large amounts of denitrifying bacteria that obtain their energy by metabolizing nitrogen compounds with the unique enzyme, *nitrase reductase*. As a result, nitrate ions are reduced to nitrogen gas (Galloway et al 1995). Shallow water wetlands are also responsible for transferring globally significant amounts of methane and reduced sulfur to the atmosphere (Morris 1991, Keddy 2000, Mitsch and Gosselink 2000). Sparks (1995) states that nearly 35% of the total nitrogen entering large river watersheds is retained or lost through denitrification within shallow water impoundments; therefore, that nitrogen is not transferred to the ocean.

Backwater lakes function as refugia for migratory waterfowl (Low and Bellrose 1944, Bellrose et al 1979, Sparks 1995, Sparks et al 1998), shorebirds, marsh birds, aquatic invertebrates, amphibians, and several native fish species (Sparks 1995, Sparks et al 1998). For example, nine plant and animal species that are listed as federally threatened or endangered along with 50 that are listed as rare, threatened or endangered

by states bordering the Upper Mississippi River System depend on large river floodplains and backwater lakes (Bellrose 1941, Morris 1991, Sparks et al 1998).

Aquatic Vegetation

Food Web Functions. As the major primary producer of wetland ecosystems, aquatic plants form the basis of most food webs, with the exception of photosynthetic cyanobacteria and algae. In fact, wetlands have been called “biological supermarkets” due to the extensive foodchains that they support (Mitsch and Gosselink 2000). The most recognized consumers of aquatic vegetation are migratory waterfowl (Bellrose 1954); however, ducks and geese are not the only wildlife species that depend on aquatic vegetation as a major food source. Fish (Hansson et al 1987), muskrats (Bellrose 1950), and several macroinvertebrate species (Smock and Harlowe 1983, Sheldon 1987) are also highly dependent on wetland vegetation. Historically, humans have also taken advantage of, and in some cases subsisted on, wetland vegetation. The ancient Babylonian, Egyptian and Aztec civilizations incorporated wetlands and their plant resources into their culture (Mitsch and Gosselink 2000). Today, while nearly 50% of wetlands have been lost worldwide, humans are still dependent on wetland plants for sources of food and fuel (Mitsch and Gosselink 2000). Rice, wild rice, cranberries, peat and sphagnum moss are all resources of wetlands that are commercially harvested.

Water Quality Functions. Aquatic plants improve water clarity by trapping suspended solids and particulates from the water column while holding bottom substrates in place (Mickle 1993). Water quality is also improved through interactions with microorganisms as well as physical/chemical processes including sedimentation, adsorption and precipitation (Gersberg et al 1986). Aquatic plants are also known to decrease

pollutants through uptake of inorganic nutrients, heavy metals, and dissolved organic compounds (Mickle 1993). Submerged aquatic plants provide attachment surfaces for nitrifying bacteria, making them suitable for use in alternative wastewater treatment systems (Gersburg et al 1986, Eriksson and Weisner 1999).

Vegetation of the Backwater Lakes. Shallow water emergent plants grow near the waters edge in natural backwater lake environments and usually only reproduce vigorously in permanent shallow water habitat. Living in permanent shallow water (< 0.5 m) is a tradeoff for emergent plants. On the one hand, they are protected from drought and extreme temperature variations and enjoy an abundant nutrient source. On the other hand, light may be inadequate due to high plankton density or suspended solids and reproduction could be impacted by water fluctuations during the germination and seedling stage. Shallow water plants must produce seed with the ability to germinate under water and produce leaf structure that can photosynthesize and assimilate enough energy for growth through the water column to the waters surface to reach the floating leaf stage (Leck and Simpson 1993).

Shallow water associations of southern wild rice (*Zizania aquatica* L. var. *aquatica*), green arrow arum (*Peltandra virginica* L.) , pickerelweed (*Pontederia cordata* L.), pondweed (*Polygonum amphibium*), giant bur-reed (*Sparganium eurycarpum*), spatterdock or yellow water lily (*Nuphar variegatum*), softstem bulrush (*Scirpus validus*), woolgrass (*Scirpus cyperinus*), duck potatoes (*Sagittaria latifolia*), rice cutgrass (*Leersia oryzoides*) , Walters millet (*Echinochloa walteri*) and narrow leaf cattail (*Typha angustifolia*), can/may occur in permanent fresh shallow water with muck substrates (Bellrose et al. 1979). For germination, seeds from all of the above species, with the

exception of *Typha* (cattail), cannot dry out and must be stratified in water for 5-7 months at 1-3 degrees centigrade (Muenscher, 1936).

Status of Backwater Lake Vegetation. Much of the aquatic vegetation of the Mississippi and Illinois River backwater lakes have been profoundly affected by human manipulations to the main river channels and surrounding land usage since the early 1900s (Jackson and Starrett 1959, Bellrose et al 1979) . Compounding effects of urban pollution from the Chicago Sanitary and Ship Canal, creation of levees and drainage districts, dredging, and increased water levels for navigation through the locks and dams have greatly altered backwater lake habitats (Turner 1936, Bellrose et al 1979, Sparks 1995, Sparks et al 1998). The 1979 Illinois Natural History Survey bulletin gives a description of water level changes and the associated increases in sedimentation and alterations in vegetation structure in the backwater lakes along the Illinois River for several years following operational dredging to the main channel below the Starved Rock lock and dam in 1938. During this period, many of the backwater lakes experienced increased water levels upon construction of levees. The higher water levels then resulted in greater wave action and increased turbidity that all but eliminated much of the aquatic vegetation. Turbidity levels of these backwater lakes have also increased greatly over the years as sheet erosion from intensively farmed agricultural lands in the watershed has added large amounts of clay and fine silt particles to the main river channel. The backwater lakes that are directly connected to the river were the first to experience deposition of flocculent false bottoms over the original firm bottoms. This flocculent material is constantly resuspended in the water column by wave action from high wind fetch and the activity of rough fish (*i.e.* Asian carp) (Jackson and Starrett 1959), thus

preventing light penetration and successful growth in most native shallow water species. Today, managers of backwater lake systems continually struggle to reestablish native shallow water plant communities in the presence of these factors with little success.

Ecological Restoration

The book, *Foundations of Restoration Ecology* (Falk et al 2006) points out the need for a connection between the practical applications of restoration and the underlying theories of ecology. Falk et al (2006) believe that ecological restoration of a strict sense involves attempting to return a system to some historical state. Unfortunately, this is not a reasonable goal in most scenarios. More realistically speaking, it should be the aim of restoration projects to “move a damaged ecological system to a state that is within some acceptable limits relative to a less disturbed system” (Falk et al 2006).

Preserving genetic variability during restoration is key to maintaining evolutionary fitness for changing environmental conditions (Falk et al. 2006, Groom et al 2006). Distributing genetic variability within and among populations is important in promoting the establishment of self-sustaining populations (Ingvarsson 2002, Falk et al 2006). As population genetics studies have shown, populations that are completely isolated are unlikely to persist within a variable environment (Booth and Grime 2003). Gene exchange among individuals and sub-populations (meta-populations) tends to increase heterozygosity and overall evolutionary fitness (Groom et al 2006).

Wetlands create patchy habitat distributions that resemble fragmented landscapes. The establishment of meta-populations within neighboring habitat units provides a connectivity that allows greater genetic variation to exist (Falk et al 2006). When small, established populations are isolated, a phenomenon known as in-breeding depression

usually occurs. Populations that experience in-breeding depression are comparatively short-lived and lack the ability to adapt to changing environments due to a reduction in heterozygosity (Ivey et al 2004, Groom et al 2006).

Restoration projects face both the challenge to utilize sufficient diversity that allows adaptation to new circumstances, and the challenge to avoid the adverse effects of introducing genotypes that are poorly adapted to the local environment (Falk et al 2006). Results from experiments testing the effects of out-breeding depression have shown that it is important to establish meta-populations of native plants using seed sources that are found within the same general ecotype (Daehler et al 1999, Groom et al 2006). For example, when re-establishing a bottomland hardwood forest with oak trees, it is important to use a genetic stock from a bottomland that has adapted to local flooding regimes versus one from an upland system. By introducing genetics from an upland stock, the fitness of bottomland populations may be reduced through the input of individuals with genes that are not adapted for local conditions. This phenomenon is referenced as out-breeding depression.

Ecological Restoration Applied to Backwater Lakes

Ecosystem Approach. Managing at the ecosystem level rather than the species level has recently become the basis of most restoration practices that occur on federal and state lands (Heitmeyer and Westphall 2007). The species-orientated approach has shown several short-comings that ultimately result from the lack of available information on the many species that reside within the ecosystem and underlying abiotic forces driving their presence and growth. For example, by focusing management decisions only on a few charismatic species, other species are either left with sub-optimal habitat conditions or

completely eliminated (Sparks 1995). In contrast to the species-oriented approach, ecosystem management has the goal of maintaining or recovering the biotic integrity of an ecosystem (Sparks 1995). Biotic integrity includes elements such as genes, species and populations along with the processes that generate them such as disturbance, selection and nutrient cycling. In wetland ecosystems, the sediment and water quality and flow regimes are prime factors affecting biotic integrity; therefore, it is important that management practices maintain or restore the processes of erosion, sediment deposition, colonization and succession so that the system can repair and rejuvenate itself (Sparks 1995).

Surrounding Land Use. Increases in the sediment load and changes in the water regime of backwater lakes (e.g. more rapid, frequent fluctuations and deep permanent inundation) have led to the loss of native aquatic plants that were adapted to pre-dam water and sediment regimes (Sparks 1995). Historically, backwater lakes were protected from heavy sediment loads and drastic water fluctuations by low, broad natural levees and wetlands that served as buffers from the main channel. The presence of native trees also protected them from high fetch winds that re-suspend bottom sediments. Today, the watersheds surrounding most backwater lakes are intensively farmed. Tree removal and poor land use practices have greatly increased sediment loads that enter these systems. Therefore, erosion control throughout the watershed and surrounding tributaries should be of the highest concern for an ecosystem management approach. Controlling erosion in these areas would effectively reduce the occurrence of flocculent false bottoms that constantly re-suspend and increase turbidity to levels that are unsuitable for germination.

Erosion control measures would also reduce the excessive nutrients and pesticides that enter the main channels and cause algal bloom formation with eventual hypoxia.

Water Regime. Water regime within backwater lake systems may be the most critical factor affecting plant biodiversity. Historically, backwater lakes were filled with clear water from precipitation, snow melt, tributaries and rising ground water; these sources made it difficult for silty river waters to infiltrate (Sparks et al 1998). Backwater lakes were also much shallower during the low flow season than where they are kept today. Lower water levels provided less surface area for winds to blow over and churn bottom sediments. Today, dam operations often invert the natural water regimes of these areas by draining them in moderate floods during the spring and fall and flooding them during the fall growing season (Sparks et al 1998). For this reason, it is often necessary for federal and state waterfowl refuges to actively manage wetland units using pumps and gates to expose mudflats for moist soil vegetation during the summer growing months (Sparks et al 1998). Unfortunately, moist soil plants only define one type of community that exists in these dynamic systems. Often, shallow water communities are neglected at the expense of management focusing solely on moist soil communities for use by dabbling ducks during the major waterfowl migrations. Moist soil communities consist of plant species that require mud flats to germinate and are then subsequently inundated. This is very different from the shallow water communities that must germinate through the water column. Perhaps the general disregard of this community type is due to lack of information, social interest or feasibility of restoring and maintaining. However, if an ecosystem approach was used, the restoration of all known community types for the preservation of the system's genetic and evolutionary fitness would occur.

Plant Recruitment. Efforts to restore shallow water communities are best focused on establishment of small, protected plant colonies at strategic water depths based on species ecology (Dick et al 2005). Once these colonies are established, they can serve as founder populations that provide recruitment opportunities in adjacent un-vegetated areas when conditions are favorable (Dick et al 2005). Exclosures benefit the establishing plant colonies by effectively reducing herbivory damage (Dick et al 2004).

Summary

Backwater lakes of the Illinois and Upper Mississippi River Valleys are unique wetland systems that provide a host of ecological and biogeochemical services. Providing wildlife habitat may be the most obvious service; however, backwater lakes are also responsible for improving water quality and play a critical roll in nitrogen reduction, uptake, and immobilization. Unfortunately, the magnitude of these services has been greatly diminished since the installation of lock and dam systems to the main river channels in the late 1930s. Changes in hydrology coupled with poor land use practices in the surrounding watershed have nearly eliminated aquatic vegetation within backwater lakes, which forms the basis of critical ecological and biogeochemical functions. Aquatic plants of backwater lake ecosystems have evolved to grow in stable, permanent, shallow water. Today, most backwater lakes in this region either experience a great deal of hydrological fluctuation or are maintained at high water levels for boating and fishing recreation during critical growth periods for these species. Without proper management in place, aquatic vegetation communities and associated functions may be lost. An ecosystem approach that addresses sediment and water quality and flow regimes to

preserve biotic integrity of the system as a whole should be a goal as opposed to managing for a few charismatic and/or game species.

CHAPTER 2: ECOLOGICAL LIMITATIONS FOR SOUTHERN WILD RICE POPULATIONS IN THE ILLINOIS AND UPPER MISSISSIPPI RIVER VALLEYS

Introduction

Description. Southern Wild Rice (*Zizania aquatica* L.var. *aquatica*), a native to North America, is an annual aquatic member of the tribe Oryzeae within the family Poaceae (Warwick and Aiken 1986, Oelke 1993, Zaitchik et al 2000). Unlike the common name suggests, it is not a wild species of white rice (*Oryza sativa* L.); however it grows in similar flooded conditions and produces a highly nutritious seed (Anderson 1976, Oelke 1993). This particular variety of *Z. aquatica* grows predominantly along the Atlantic Seaboard from southern Ontario and Quebec and from Florida and Louisiana with patches ranging inland from northern New York to Wisconsin and southern Illinois.

Southern Wild Rice can be distinguished from its commercially grown sister species, Northern Wild Rice (*Zizania palustris* L. var. *palustris*) using several physical characteristics. For example, *Z. aquatica* is a much taller plant, reaching 240 to 320 cm in height (Dore 1969); whereas, *Z. palustris* has a range from 90 to 240 cm (Dore 1969). *Z. aquatica* also exhibits wider leaves (2.5-5.0 cm) and smaller seeds than *Z. palustris* (Dore 1969). The pistillate lemmas and paleas of *Z. aquatica* are described as chartaceous (papery) and covered uniformly with prickly hairs while *Z. palustris* has coriaceous (leathery) lemmas and paleas that have prickly hairs restricted to rows above the vascular bundles (Dore 1969, Dore and McNeill 1980, Terrell and Wergin 1981, Gould and Shaw 1983, Aiken 1986, Duvall and Biesboer 1988). *Z. aquatica* is a monoecious plant with a panicle inflorescence that displays pistillate spikelets on the upper branches and staminate spikelets on the lower branches (Weir and Dale 1960, Liu et al 1998, Zaitchik

et al 2000). The transition zone between male and female branches is not always discrete, with several containing hermaphrodite flowers that are able to self-pollinate (Liu et al 1998, Zaitchik et al 2000). Most mature plants will produce several tillers (stems), each of which form a seed head (Oelke 1993).

Uses. *Z. aquatica* was once a staple for early North American inhabitants including the Ojibway, Menomini, Cree, Algonquin and Sioux tribes (Moyle 1944, Vennum 1988, Oelke 1993). However, today *Z. palustris* makes up the majority of wild rice consumed by humans; it was first commercialized in the 1970s. Moreover, the discovery of a more shatter-resistant population of *Z. palustris* has led to the development of varieties that can be easily harvested. On the other hand, *Z. aquatica* has not been grown commercially due to its greater height and smaller, shattering seeds, which make it difficult to harvest.

The main consumers of *Z. aquatica* seed are waterfowl that make yearly migrations over the course of the Atlantic and Mississippi flyways. The high protein and carbohydrate content along with less than 1 per cent fat (Anderson 1976, Oelke et al 1997) make it an important source of energy for long flights (Moyle 1944). Wild rice has been reported to make up nearly 50 percent of the gut content in wild ducks from the Minnesota lakes in autumn (Moyle 1944). The stems of *Z. aquatica* provide roosting and loafing areas for adult waterfowl as well as brood cover for the young (Dore 1969). Wild rice plants have a relatively high requirement for nutrients to produce a single pound of dry matter (Oelke et al 1997). This quality allows the wild rice to serve as a nutrient sink, which in turn translates to potential use in alternative water treatment systems.

Status. Little is known of the current status of *Z.a. var. aquatica* throughout the Mississippi and Illinois River valleys. The general consensus based on natural history surveys from the late 1930s suggests a great reduction in the occurrence of populations that existed before the installation of the lock and dam system. However, it is difficult to validate this assumption based on general taxonomical terms due to the fact that both of the annual species of *Zizania* grow sympatrically throughout the Great Lakes regions. It is assumed that references made of ‘wild rice’ throughout southern and central Illinois are most likely that of *Z.a. var. aquatica* based on height descriptions of 12-15 ft. As previously mentioned, *Z. palustris* is a much smaller plant.

Objectives. In general, the goal of this research was to determine what environmental factors might be limiting native populations of Southern Wild Rice (*Z.a. var. aquatica*) within backwater lakes throughout the Illinois and Upper Mississippi River Valley regions. Water quality, soil quality and plant growth measurements were compared between 15 outplanting sites within the southern half of the Upper Mississippi River Valley to determine tolerance ranges for *Z.a. var. aquatic*. Water and soil parameters were also correlated to plant growth parameters to determine if relationships existed between them. Correlations, though not indicative of causation, may show patterns that will lead to a greater understanding of optimal growth conditions for future inquiries.

Methods

Seed Source and Plant Propagation. Seeds of *Z.a. var. aquatica* were collected from a natural population found in Spring Lake (Tazewell County, Illinois) during August, 2001. They were then transferred to a shallow frog pond located in Annada,

Missouri where they have successfully grown and re-seeded themselves every year since. This pond population has provided a seed supply for preliminary germination trials. Observational data from these trials eventually led to successful germination and plug propagation in a controlled greenhouse environment at Forest Keeling Nursery located near Elsberry, Missouri.

In order to produce plugs for this study, seeds were harvested in late August and immediately placed in plastic baggies with water. The baggies of wild rice were then placed in a refrigerator set at 36 degrees Fahrenheit for a six month stratification period, then emptied into plastic dish tubs with fresh water and placed in a greenhouse for three weeks. Once seeds had germinated, they were transferred to larger tubs containing a growth mixture, patented by Forest Keeling Nursery (Elsberry, Missouri), at a density of 1 seedling per square inch. Water and urea fertilizer were added as needed to maintain healthy growth for one month.

Site Selection. Fifteen study sites located on public and private lands throughout the southern half of the Upper Mississippi River valley (including the Illinois River Valley) (Table 1; Figure 1) were planted with one month old *Z.a. var aquatica* plugs during the first week of June, 2007. All sites are found within the native historical range of *Z. aquatica*. Outplanting areas were chosen based on a single requirement. Land managers that were willing to participate were asked to locate accessible areas that held water between 1 and 30 centimeters in depth from April to September. This criterion was determined through observations of the pond source population, which tended to regenerate in a 1-30 cm range of depth.

Outplanting Strategy. At each site, herbivore damage prevention measures were taken by placing round 1-m diameter galvanized wire mesh enclosures around groupings of 3 wild rice plugs (1 stem/plug). The enclosures were held in place with bamboo stakes for the duration of the study to prevent herbivory attempts by muskrats, coots, and geese. Inter-specific competition was reduced by removing all other plants from inside the enclosures to 60 cm beyond the circumference. Depending on the size of the study sites, 5-9 enclosures were constructed pseudo- replication at each site.

Site Visits. Sites were visited monthly throughout the growing season (3 visits per site). Due to monetary constraints and the distance between sites, this period length was most feasible. The first round of samplings took place 25 days after outplanting (60 days after germination). The second took place 55 days after outplanting (90 days after germination). The final samplings of the growing season occurred 85 days after outplanting (120 days after germination). During each period, water quality readings, water depth, water samples, soil samples, maximum plant height, total stems, and maximum leaf width were measured/collected. Digital photographs and field notes were also taken at each site each visit. In addition, seeds were collected from each site during the final sampling period to measure seed viability.

Data Collection. Water and soil variables along with duplicates (2 per site per visit) were collected haphazardly at each site to ensure unbiased samplings.

Water Quality and Depth. Water quality measurements were taken during field visits using a Horiba U-22XD Multi-Parameter Water Quality Monitoring System. This instrument measures the following: pH (0-14), conductivity (S/cm), turbidity (NTU), dissolved oxygen (mg/L), temperature (Celsius), total dissolved solids (g/L), and

Oxidation/Reduction potential (mV). Several readings were taken during each visit to get an overall site average. Before each field visit, this instrument was calibrated and standardized to the manufacturer's specifications. Water depths were recorded in centimeters for each site by placing the end of a meter stick on the surface of the substrate within each of the exclosures.

Water Sample Collection and Analyses. Water samples were collected using 500mL, sterilized water bottles. Each bottle was placed 5cm below the water surface with the lid covering the mouth. Once submerged to this depth, the lid was removed to allow water to infiltrate. Samples that contained large amounts of debris were dumped and re-collected until they were relatively clear. All samples were then placed in iced coolers during transport until they could be stored in a freezer. This method of preservation was chosen because of the lag in time between collections and analyses. Water samples were analyzed by the Ozarks Environmental and Water Resources Institute (OEWRI, Missouri State University in Springfield, MO) for the following variables: non-purgable organic carbon, total nitrogen and total phosphorus.

Soil Sample Collection and Analyses. Soil samples were collected inside and outside of the plants' root zones during each visit. A scoop with a mesh bottom was used to collect the top 5 cm of sediment. Each sample was then placed in a gallon size Ziploc bag for transport. All samples were then air dried and finely ground to < 2mm for analyses. Soil samples were analyzed by the Ozarks Environmental and Water Resources Institute (OEWRI) for particle size, percent nitrogen, percent sulfur, pH and percent organic matter.

Plant Growth Variables. At each site, mean maximum plant height was determined by measuring the tallest stem within each exclosure and dividing by the total number of exclosures. Wild rice plants also grow vegetatively by tillering, or sending out new stems, each producing a seed head. As a measure of reproductive fitness, I counted all flower producing stems in each exclosure. Mean total number of stems per exclosure was calculated by counting all stems at the site and dividing by the total number of exclosures. Average maximum leaf width for each site was determined by measuring the widest section of the main leaf for the tallest stem in each exclosure, then dividing by the total number of exclosures. During the final round of site visits, seeds were collected at each site by carefully shaking seed heads inside a 5-gallon bucket. Seeds were then stored in individual Ziploc bags with water, transported in an iced cooler, and placed in a refrigerator for a six month stratification period. After the six month period, seeds from each site were placed in separate, clear plastic containers with 2 to 4 cm of water and set in the MSU biology department greenhouse. Over the course of a three week period, seeds broke dormancy and germinated. All germinated and non-germinated seeds were counted to calculate the percent germination for each site.

Statistical Analyses. Mean maximum plant height, total number of stems, and maximum leaf width were analyzed at each site each month. Exclosures within a site were subject to the same water and soil conditions and therefore treated as measures of pseudo-replication. In order to identify potential relationships between growth variation and environmental conditions at different stages in the plant's life history, nineteen water and soil variables were correlated to the three plant growth variables for each visit using Spearman Correlation Coefficient ($n = 15$ when comparing site means).

Results

Plant Growth Variation. Plants used for this study originated from a single seed source to reduce genetic variation among the outplanting site populations. Mean maximum plant height, total number of stems and maximum leaf width were all significantly different between study sites (Figures 2-4; Table 2). Although vegetative growth differed across sites, all populations survived to reproductive growth stages and produced highly viable seed (Figure 5). Therefore, no site consisted of a suite of conditions that disallowed growth and reproduction.

Site Variation. Study sites consisted of closed ponds, spring-fed wetlands and backwater areas open to the Mississippi River. Variation of water source inputs resulted in a wide range of extant conditions among study sites. Ranges of tolerance for water pH, temperature, depth, turbidity, total phosphorus, and non-purgable organic carbon are provided in Table 3.

Study sites presented substrates that ranged from silty-muck to sand. Ranges of tolerance for mean soil pH, particle size, percent clay content, percent sand content, percent silt content, and percent organic matter are provided in Table 4.

Correlations with Plant Growth (Spearman Correlation Coefficient). June samplings indicate that plant height has a positive correlation to both the percent nitrogen and percent organic matter in the soil (Figures 6 and 7). These relationships are also present in July samplings (Figures 8 and 9). No significant correlations were found for plant height during the August samplings.

There were no significant correlations detected between the mean total number of stems and any of the soil or water variables tested during the June and July samplings.

However, data from the August samplings indicate that mean total number of stems has positive correlations with percent clay and percent nitrogen in the soil as well as water temperature and organic carbon in the water (Figures 10-13).

Water depth was negatively correlated to mean maximum leaf width for June samplings (Figure 14). No correlations were detected during the July visit; however, percent organic matter and particle diameter of the soil were positively correlated for August samplings (Figures 15 and 16).

Discussion

Notes on Germination. Germination requirements for seeds that were collected from the source population in Spring Lake were not known initially, as I was not able to discover primary literature that addressed this subject for *Z.a. var. aquatica*. Throughout the many preliminary germination trials that preceded the present outplanting study, several observations were noted, which include the following:

It is not completely clear as to whether light, temperature, or a combination of the two is responsible for germination. I did however observe early germination in wild rice seeds that were held in the dark, cold refrigerator. The ability to germinate under these conditions seems to be associated with fungi, as it was present in all early germination instances. I suspect that the fungi weakened the hard outer seed coat. This is plausible, as other studies have shown, that wild rice seeds will germinate once the pericarp has been damaged (Aiken 1986). Early germination would cause plants to emerge before the season's last frost in this Midwest region, which would surely result in death during the floating leaf stage. This would likely not be the case in southerly extremes of *Z.a. var. aquatica*'s geographical range where temperatures remain warm year-round. Perhaps a

relationship with fungi is necessary for germination in these warmer regions where a cold stratification period is not possible. This relationship has yet to be addressed in primary research. Additionally, I noticed that the darker, more mature seeds seemed to be the last to germinate when placed in the greenhouse. This may indicate an adaptive mechanism by which this species increases survivorship by having different growth stages present during a chance flood event.

Hydrology Management and Germination. Currently, moist soil management is the exclusive technique employed for providing waterfowl forage in the majority of public areas of the region studied. Moist soil management involves water level drawdown of wetland units in order to expose mud-flats for germination of millets, smart weeds, and other water-tolerant emergents. After plants have colonized the mud flat, wetland units are then re-flooded to provide easy access for waterfowl. However, wild rice seed and other shallow water adapted plants must germinate through the water column, and therefore current management practices may be limiting germination. Additionally, the early stages of growth are by far the most vulnerable (Meeker 1993). Once wild rice germinates and reaches the delicate floating leaf stage, water levels must remain stable. If flooding occurs during this stage, roots may be pulled up from the substrate (Lee and Stewart 1980) or the plant may not have enough energy to continue growing through the deeper water column. Finally, turbidity may greatly limit photosynthesis through the water column and heavy sedimentation loads can potentially bury young plants (Lee and Stewart 1980).

Outplanting Study. Few studies have focused on the basic edaphic requirements of *Z.a. var. aquatica*. Earlier studies refer to wild rice in the general sense, as it was not

previously subdivided into species until more recent genetic and morphological studies (Warwick and Aiken 1986, Duvall and Biesboer 1988). For this reason, many of the variables for this study were chosen based on studies which were presumably carried out on the closely related sister species, *Z. palustris* (Moyle 1944, Thomas and Stewart 1969, Lee and Stewart 1980). Since *Z. palustris* is grown commercially, some of these studies are aimed more heavily at grain production; whereas this study addressed overall survivorship and is the first of its kind to address growth and growth conditions of *Z.a. var. aquatica* within the southern half of the Upper Mississippi River valley.

Plant growth variables for this study included: mean maximum height, total number of stems, and maximum leaf width. Though vegetative growth was highly variable between sites, all plants survived to final growth stages and produced viable seed. Successful growth and development within diverse soil and water conditions imply that *Z.a. var. aquatica* has a wide range of physiological tolerances. The optimal range of conditions may be much narrower, but nevertheless this species is quite hardy. A limited physiological tolerance range does not seem to justify the decline in *Z.a. var. aquatica* populations within the southern half of the Upper Mississippi River valley.

Data and observations from this study offer new insight for factors associated with wild rice growth requirements for three phenology stages (primary vegetative growth, flowering, and seed producing stages).

Plant Height. During the June sampling period, young plants were allocating energy exclusively towards vegetative growth. Results show that mean plant height was positively correlated to the percent organic matter and the percent nitrogen in the soil. The same relationship held true during July when plants were in the flowering stage.

This finding seems plausible, as the high rate of denitrification makes nitrogen a limiting factor in the anaerobic sediments of wetlands. The positive correlation to percent organic matter is most likely attributed to water holding capacity of the soil. Data for August (during the seed production stage) did not indicate a significant relationship between plant height and any of the soil or water chemistry variables tested.

Stems. There were no significant correlations between mean number of stems and any of the environmental factors tested in this study for June and July. Soil chemistry parameters not included in this study may have played a larger role in tillering. Peden (1982) was able to correlate soil phosphorus, sodium, calcium, zinc, soluble potassium, copper, manganese, chloride and iron to wild rice growth. Though Peden did not directly relate these soil nutrients to mean number of stems, grain weight was used as the primary measurement for growth. Since each stem produces a seed head, assumptions can be inferred; however, further studies are needed to make direct relationships.

Conversely, data from August did reveal correlations between stem count and environmental factors, which can most likely be attributed to hydrology. The month of August presented unseasonal rain events in the north that resulted in regional flooding at several of the Iowa and northern Illinois sites while late summer drought plagued the south, causing sites in southern Illinois and Missouri to dry up. It is suspected that the extremes in hydrology between sites drove differences between the northern and southern sites that may have otherwise gone un-noticed. Plants growing at sites with drought conditions experienced early senescence that was visible by yellowing leaves and drying stems. Stems of this condition were not counted in the total number of stems; therefore,

this value decreased at some sites. A positive correlation between mean total number of stems and percent clay in the soil suggests that sites with greater water holding capabilities (those with high clay content) were able to better support wild rice during drought conditions (Table 5). A positive relationship between mean number of stems and increased water temperatures may indicate that wild rice preferred shallow water levels that tended to increase in temperature as the growing season progressed, as opposed to flooding waters that tended to be much cooler (Table 6). Water temperature is most likely not the factor influencing growth; however, higher water levels certainly change anaerobic conditions which most likely explains differences in growth. Coincidentally, the sites that experienced flooding had cooler water on average than the non-flooding sites, thus an indirect relationship was revealed through correlation analysis.

Leaf Width. A negative correlation between maximum leaf width and water depth during the June visit may be further indication of the importance of hydrology in the earlier growth stages. One month old wild rice plugs were outplanted within a range of water depth between 10 and 30 cm. Differences in water depth within this specified range were simply a matter of what was present at the different sites during the time of outplanting. Perhaps plants that had a majority of their leaves inundated experienced carbon assimilation stress as opposed to plants that had a majority of their leaves above water. Even though carbon dioxide is usually just as concentrated in water as it is the air, the diffusion coefficient is greatly reduced in aqueous solutions; thus preventing optimal rates of photosynthesis (Keddy 2000).

Percent organic matter of the soil was positively correlated to maximum leaf width during the August samplings. The high water holding capacity of organic matter

may explain this relationship. Plants within moist soils did not experience early senescence during August. Additionally, a positive correlation was present between mean particle size of the soil and maximum leaf width during August. This is most likely attributed to the coincidence that none of the sites with high sand content went completely dry, thus plants did not experience early senescence.

Conclusions. Results of this study indicate that water chemistry does not affect survival and reproductive success in *Z.a. var. aquatica*. Soil chemistry may explain variation in vegetative growth between sites; however, none of the various soil conditions at the different sites eliminated growth or reduced reproductive success in this species. On the contrary, *Z.a. aquatica* seems to be able to tolerate most soil and water conditions that might be present in a wetland system. Based on the hardiness of this plant, I conclude that declines in populations within this region are not attributed to a narrow physiological tolerance range in the species. Further studies are needed to more fully explain this phenomenon. Hydrology effects seem to be the most logical area of future inquiry. Results of Thomas and Stewart (1969) indicate that dry weight yield of wild rice (in general) decreased by water depths less than 8 cm and greater than 110 cm. Though specific water depth preferences for *Z.a. var. aquatic* were not addressed, much data from this study indicate that hydrology is most likely a key limiting factor to the success in growth and maintenance for *Z.a. var. aquatica*.

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Table 1. Outplanting site name, GPS location, and ownership information

Outplanting Site		
(Site Number²) Name [Abbreviation³]	GPS Location¹	Ownership
(1) Delair [D]	N39 26.478 W90 58.642	USFWS-Clarence Cannon
(2) Sny Levee Pond [S]	N39 19.609 W90 47.325	IDNR-Sny Levee District
(3) Norm Stone [NS]	N41 05.927 W91 08.562	Private
(4) Milton Hayes [MH]	N41 09.273 W91 07.803	Private
(5) Ivan Keller [IK]	N41 13.955 W91 07.777	Private
(6) UpperMiss 1 [UM1]	N42 03.240 W90 07.265	USFWS-Savannah District
(7) UpperMiss. 2 [UM2]	N42 01.784 W90 07.126	USFWS-Savannah District
(8) Chautauqua [CH]	N40 22.477 W89 58.782	USFWS-Middle Miss.
(9) Wetlands Forever [WF]	N38 34.852 W88 57.422	Private
(10) Cypress Creek [CC]	N37 18.671 W89 02.640	IDNR-Region 2 South
(11) Cache River [CR]	N37 18.743 W89 01.204	USFWS-Cypress Creek
(12) Davis Minton 1 [DM1]	N36 42.778 W90 08.099	Private
(13) Davis Minton 2 [DM2]	N36 42.387 W90 08.088	Private
(14) Davis Minton 3 [DM3]	N36 42.217 W90 08.158	Private
(15) Cato Sleugh [CS]	N37 03.765 W90 02.758	Private

¹ GPS locations are mapped in Figure 1

² Site numbers are used in Figures 2-5

³ Site abbreviations are used in Figures 6-16

Table 2. Final growth measurements for *Z. aquatica* var. *aquatica*

	Mean (+/- 1 SD)	Min.	Max.
Max. Height (cm)	286.3 (57.6)	180.4	378.7
Total Stems	16.9 (6.2)	6.5	27.8
Max. Leaf Width (cm)	4.4 (1.0)	2.8	6.4

Table 3. Water chemistry (range of tolerance, mean and standard deviation) for *Zizania aquatica* L. var. *aquatica* plug outplantings.

Variable	Range of Tolerance	Mean	SD
pH (+/-1)	5.6 -11.1	8.4	1.2
Conductivity (mS/m)	0.99 – 99.00	31.89	22.35
Turbidity (NTU)	1.9 – 953.0	202.8	231.4
Dissolved Oxygen (mg/L)	0.07 – 18.90	9.27	4.89
Temperature (Celsius)	14.8 – 34.8	27.1	4.3
Total Dissolved Solutes (g/L)	0.02 – 0.77	0.24	0.16
Oxidation Reduction Potential (mV)	-168.0 – 412.0	118.5	134.7
Non-purgable Organic Carbon (mg/L)	3.27 – 26.99	13.77	6.44
Total Phosphorus (mg/L)	0.02 – 0.76	0.31	0.18
Total Nitrogen (mg/L)	0.21 – 5.91	1.32	1.14

Table 4. Soil chemistry and texture (range of tolerance, mean, standard deviation) for *Zizania aquatica* L. var. *aquatica*

Variable	Range of Tolerance	Mean	SD
pH (+/-1)	5.0 – 7.8	6.3	0.8
% Organic Matter	0.20 – 33.76	6.44	7.40
Particle Diameter (µm)	16.9 – 469.1	93.2	113.9
% Clay	0.0 – 27.0	15.0	7.6
% Sand	1.9 – 100.0	29.6	30.1
% Silt	0.0 – 83.5	55.4	24.0
% Nitrogen	0.02 – 1.12	0.26	0.24
% Sulfur	0.01 – 0.44	0.06	0.09

Table 5. Mean total number of stems at sites that experienced drought during August samplings (Soil Texture < 20% Clay vs. Soil Texture > 20% Clay).

	Mean	SD	Minimum	Maximum
Soil Texture <20% Clay	13.1	3.6	10.0	16.7
Soil Texture >20%Clay	24.1	3.8	20.2	27.8

Table 6. Mean total number of stems per enclosure for *Z.a.* var. *aquatic* in relation to water temperature of flooded and non-flooded sites during August samplings.

	Temperature (Celsius)		Total Stems	
	Mean	SD	Mean	SD
Flooded	24.9	3.5	11.8	3.8
Non- Flooded	29.7	1.3	22.8	2.8

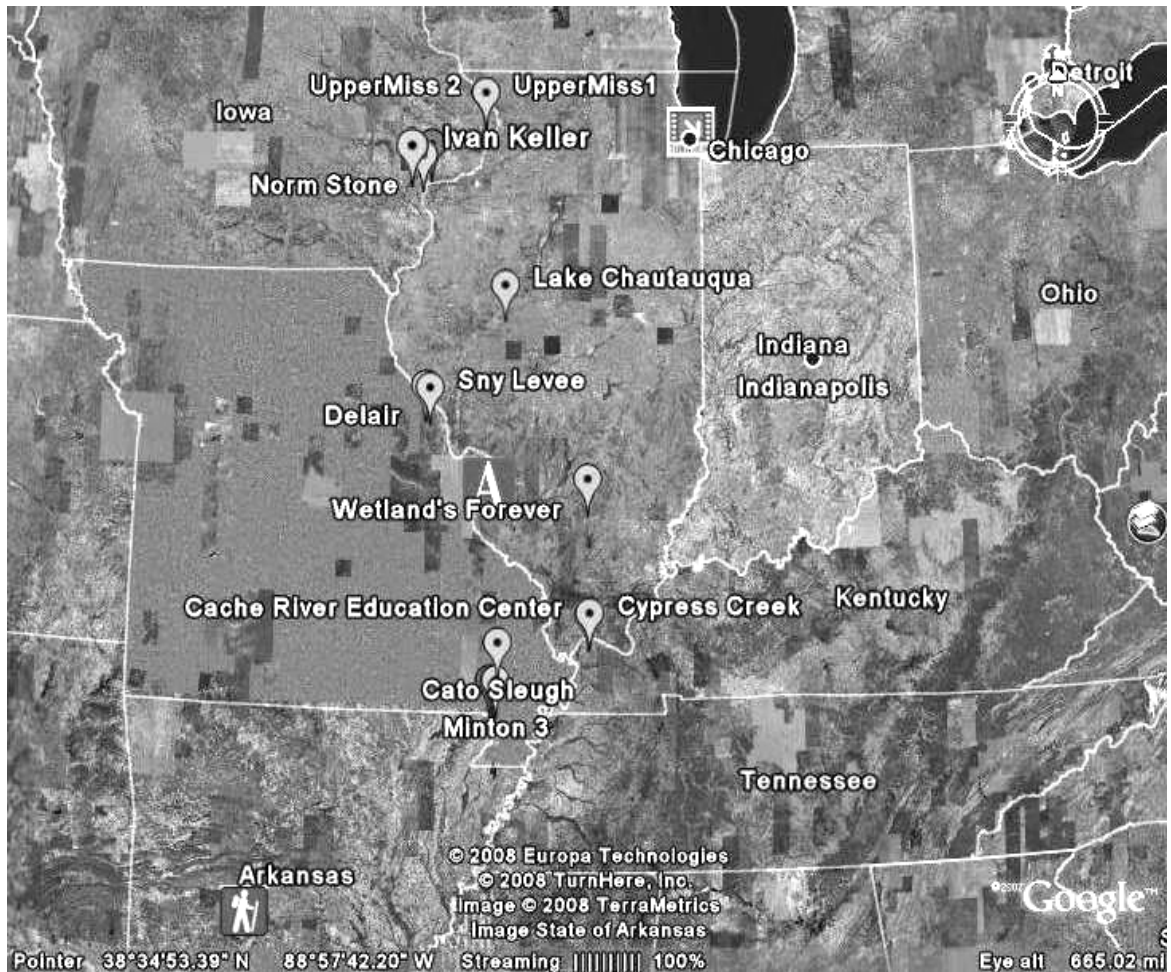


Figure 1. Locations of Southern Wild Rice (*Zizania aquatic* L. var. *aquatic*) outplanting sites in Missouri, Illinois and Iowa. These sites fall within the southern half of the Upper Mississippi River Valley. Created in GoogleEarth

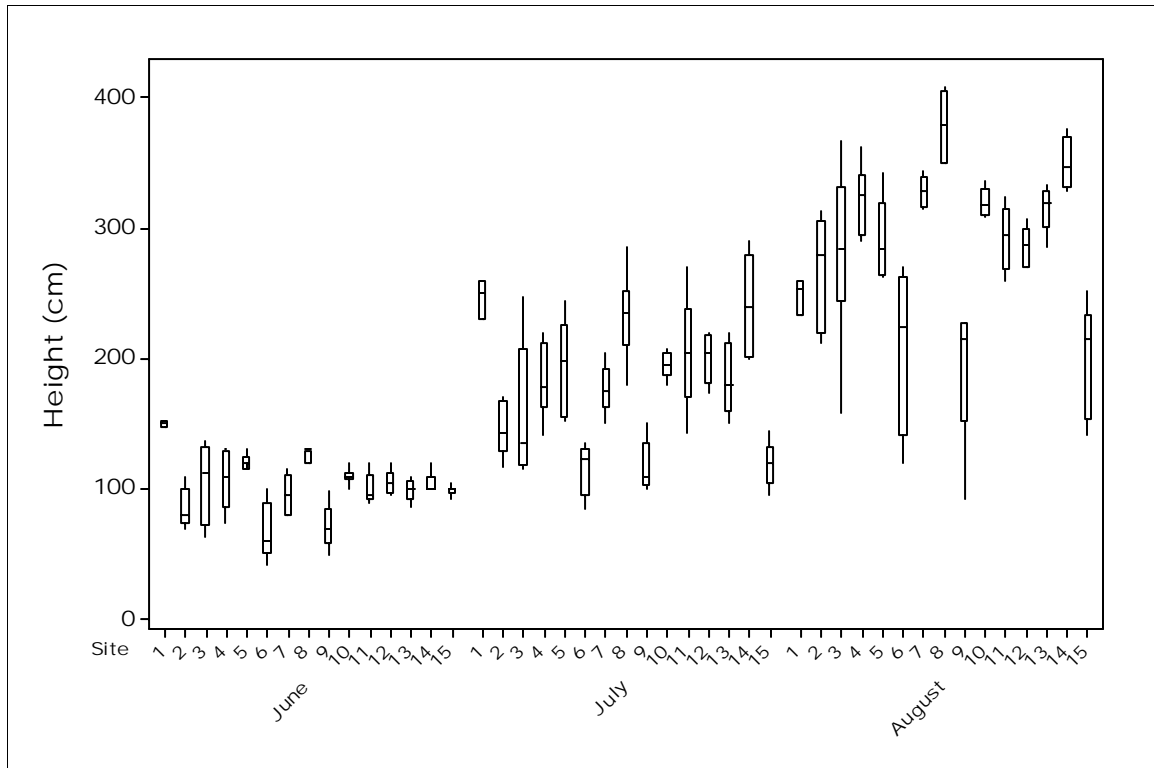


Figure 2. Mean maximum plant height (cm) of *Zizania aquatica* var. *aquatica* at the outplanting sites for the 2007 growing season. Among- site variation was significant (Kruskal-Wallis; $p < 0.001$) for all three months. Boxes encompass standard deviation; midpoints within boxes represent site median and lines extending above and below boxes represent minimum and maximum values. June mean 103.08 ± 20.33 ; July mean 180.0 ± 42.5 ; August mean 286.3 ± 57.6

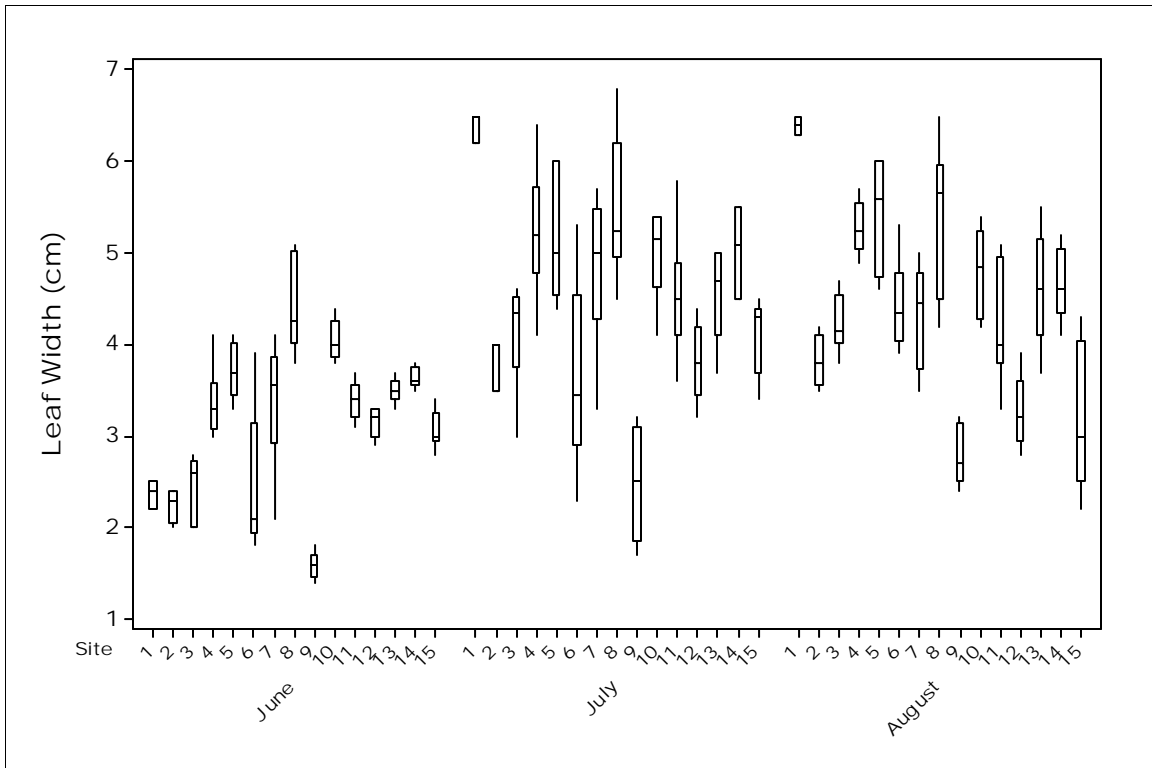


Figure 3. Mean maximum leaf width (cm) of *Zizania aquatica* var. *aquatica* at the outplanting sites during the 2007 growing season. Leaf width was taken at the widest section of the leaves. Among site variation was significant (Kruskal-Wallis; $p < 0.001$) for all three months. Boxes encompass standard deviation; midpoints within boxes represent site median and lines extending above and below boxes represent minimum and maximum values. June mean 3.12 ± 0.76 ; July mean 4.71 ± 1.03 ; August mean 4.44 ± 0.97 .

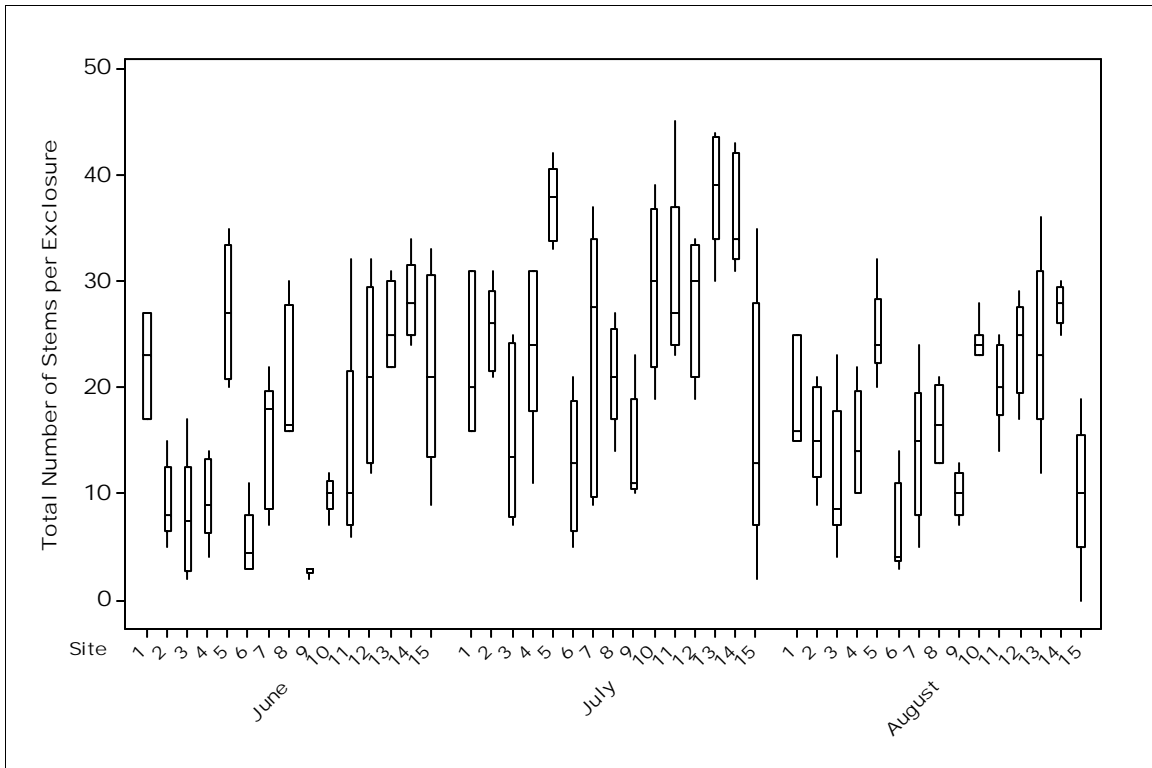


Figure 4. Mean total number of stems per exclosure at the outplanting sites during the 2007 growing season. *Zizania aquatica* var. *aquatica* can/may tiller (produce new stems) throughout its growing period. Among-site variation was significant (Kruskal-Wallis; $p < 0.001$) for all three months. Boxes encompass standard deviation; midpoints within boxes represent site median and lines extending above and below boxes represent minimum and maximum values. June mean 16.07 ± 8.29 ; July mean 25.01 ± 8.45 ; August mean 16.85 ± 6.18 .

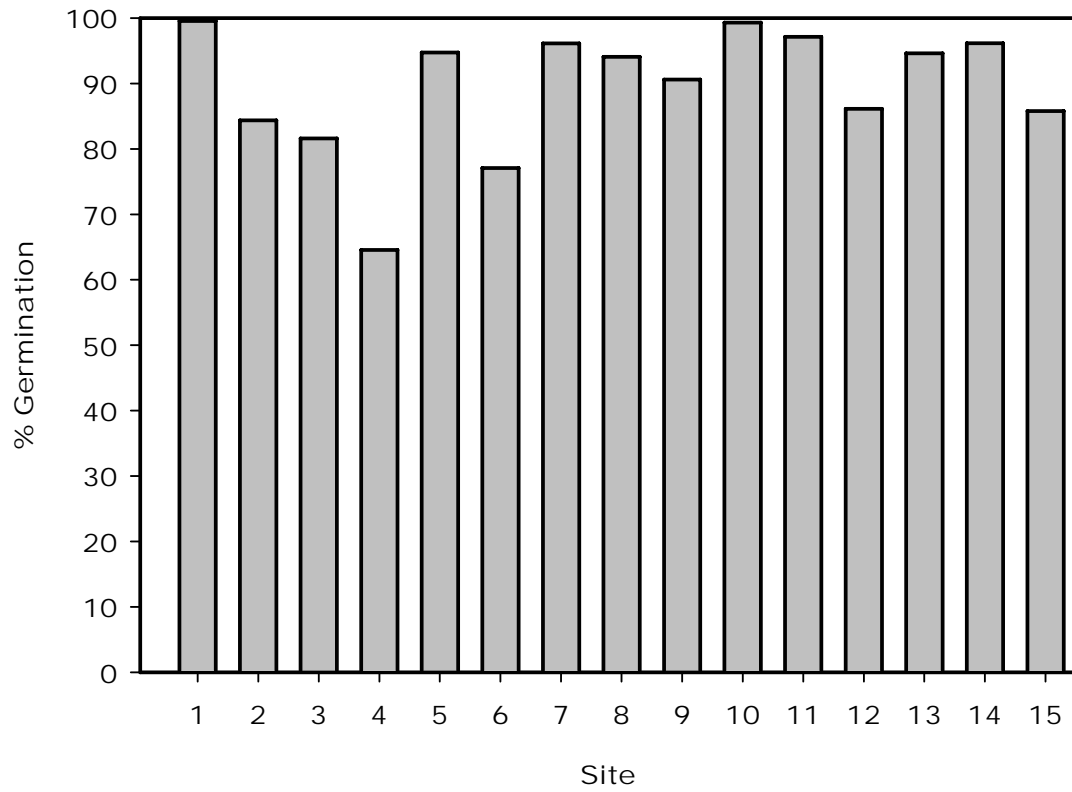


Figure 5. Percent germination of Southern Wild Rice seeds collected from the outplanting sites during August 2007. Average seed viability was 89.5% across sites.

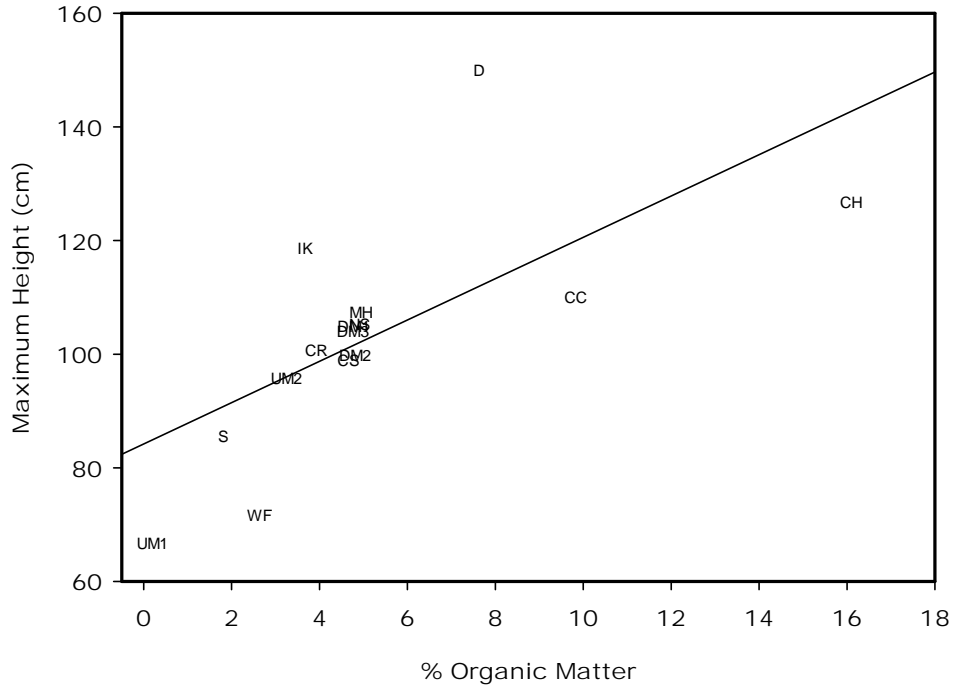


Figure 6. Mean maximum plant height (cm) vs. % Organic matter in the soil for June samplings of *Zizania aquatica* var. *aquatica* (Spearman Correlation Coefficient = 0.825; $p < 0.001$; $n = 15$). Abbreviations are defined in Table 1.

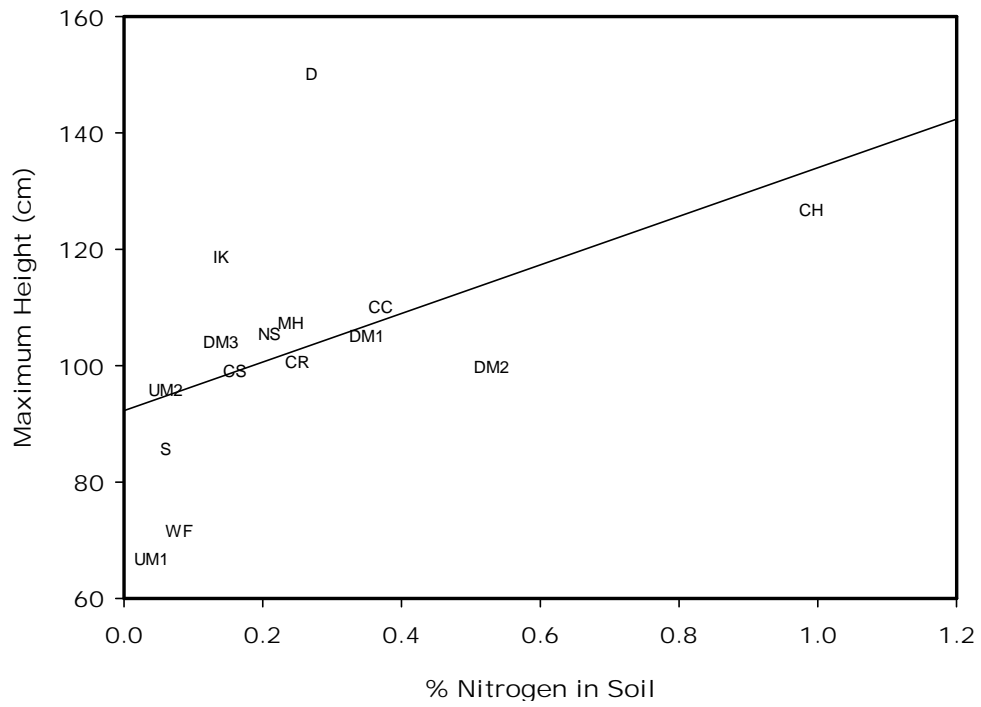


Figure 7. Mean maximum plant height (cm) vs. % nitrogen in the soil during June samplings of *Zizania aquatica* var. *aquatica* (Spearman Correlation Coefficient = 0.676; $p = 0.005$; $n = 15$). Abbreviations are defined in Table 1.

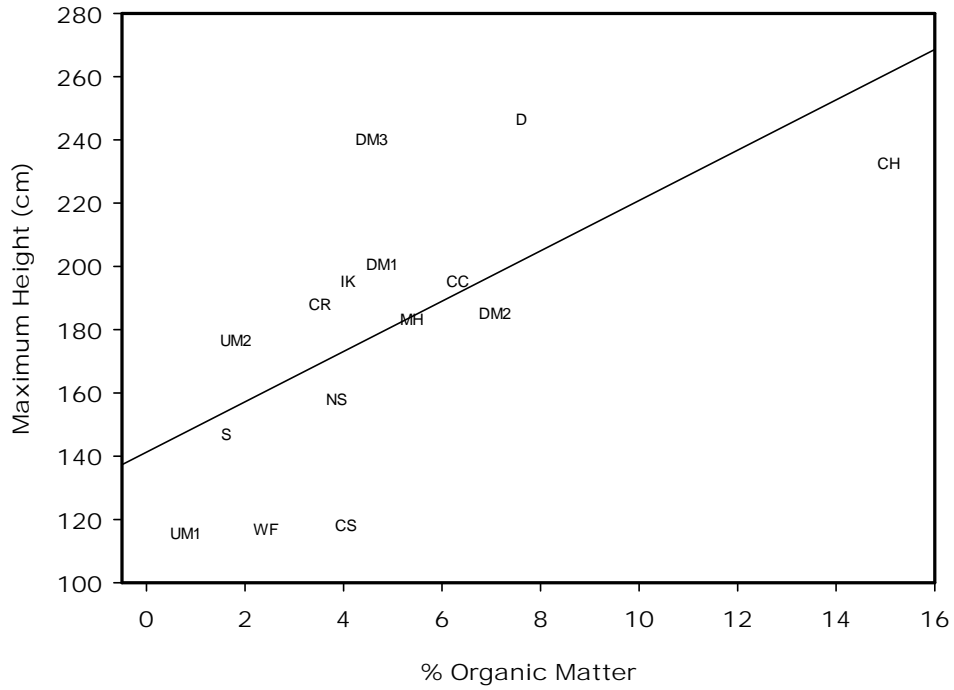


Figure 8. Mean maximum plant height (cm) vs. % organic matter in the soil during July samplings of *Zizania aquatica* var. *aquatic* (Spearman Correlation Coefficient = 0.754; $p < 0.001$; $n = 15$). Abbreviations are defined in Table 1.

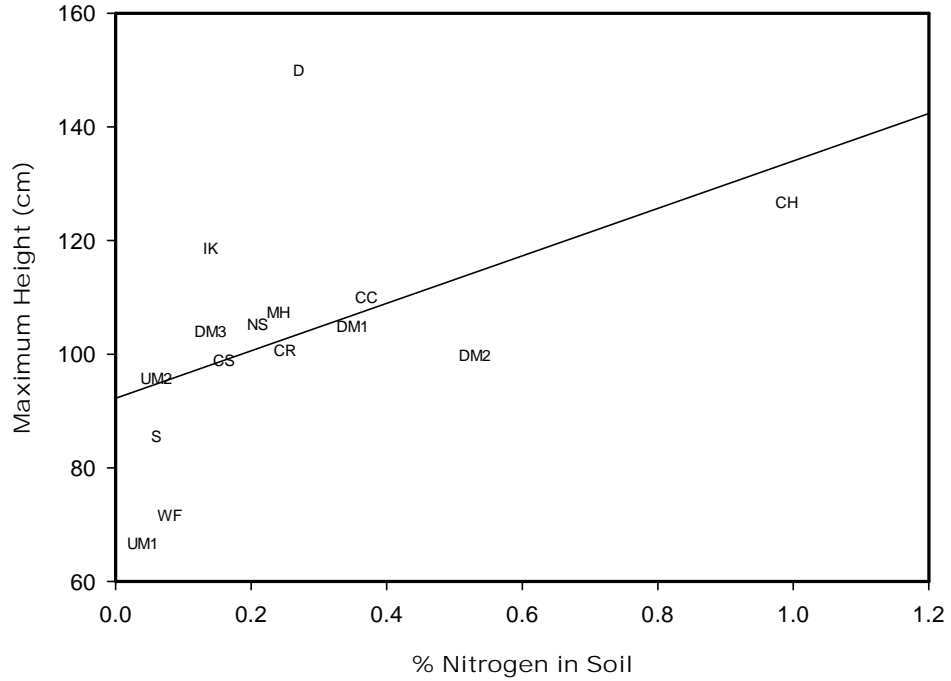


Figure 9. Mean maximum plant height (cm) vs. % nitrogen in the soil during July samplings of *Zizania aquatic* var. *aquatic* (Spearman Correlation Coefficient = 0.676; $p = 0.005$; $n = 15$). Abbreviations are defined in Table 1.

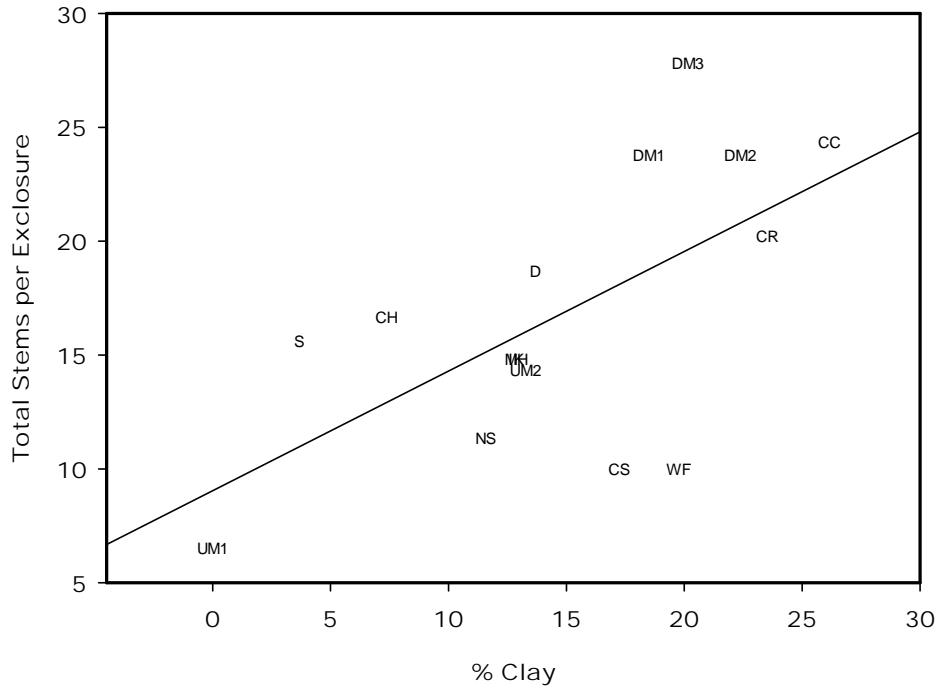


Figure 10. Mean total number of stems per exclosure vs. % clay the soil during August samplings of *Zizania aquatica* var. *aquatica* (Spearman Correlation Coefficient = 0.602; $p = 0.017$; $n = 15$). Abbreviations are defined in Table 1.

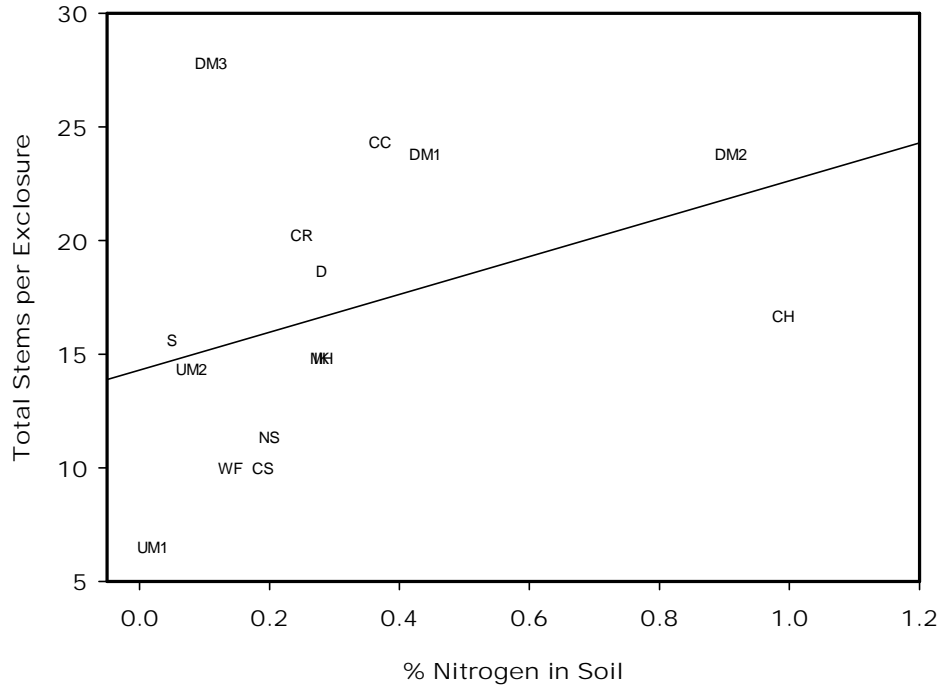


Figure 11. Mean total number of stems per exclosure vs. % nitrogen in the soil during August samplings of *Zizania aquatica* var. *aquatica* (Spearman Correlation Coefficient = 0.525; $p = 0.043$; $n = 15$). Abbreviations are defined in Table 1.

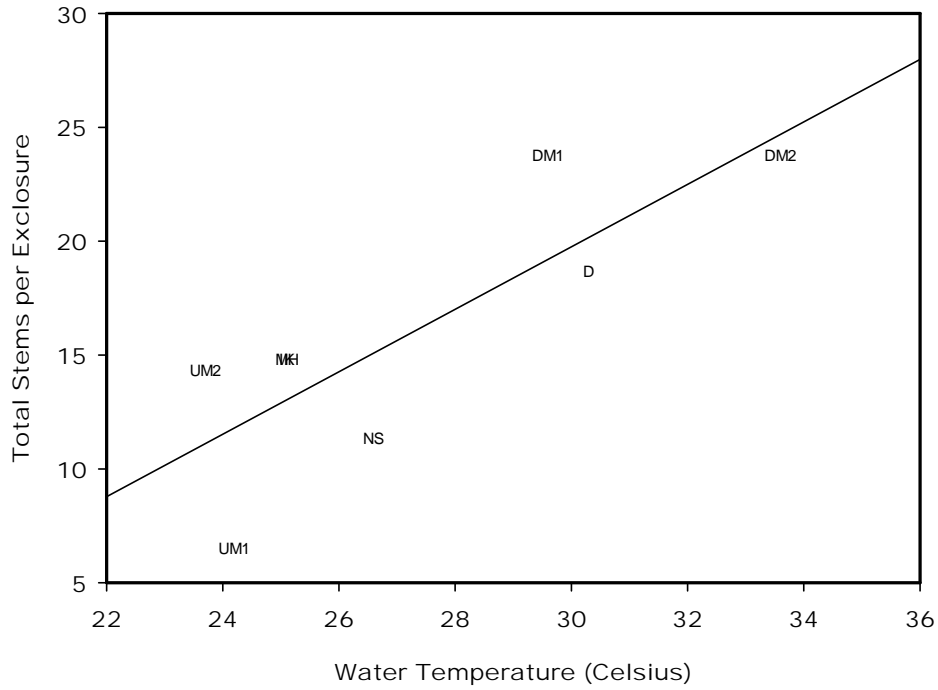


Figure 12. Mean total number of stems per exclosure vs. water temperature (Celsius) during August samplings of *Zizania aquatica* var. *aquatica* (Spearman Correlation Coefficient = 0.764; $p = 0.021$; $n = 7$). Abbreviations are defined in Table 1. Note: Sites 2, 8, 9, 10, 11, 14, and 15 experienced insufficient water levels for water variable data during the month of August.

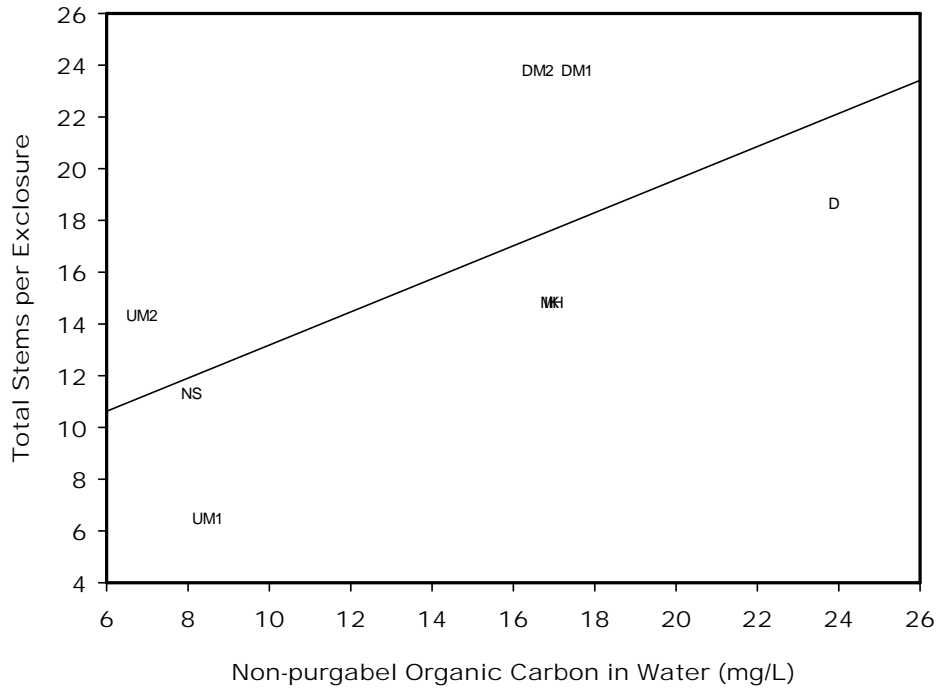


Figure 13. Mean total number of stems per exclosure vs. non-purgable organic carbon in water (mg/L) during August samplings of *Zizania aquatica* var. *aquatica* (Spearman Correlation Coefficient = 0.679; $p = 0.047$; $n = 8$). Abbreviations are defined in Table 1. Note: Sites 2, 8, 9, 10, 11,14, and 15 experienced insufficient water levels for water variable data during the month of August.

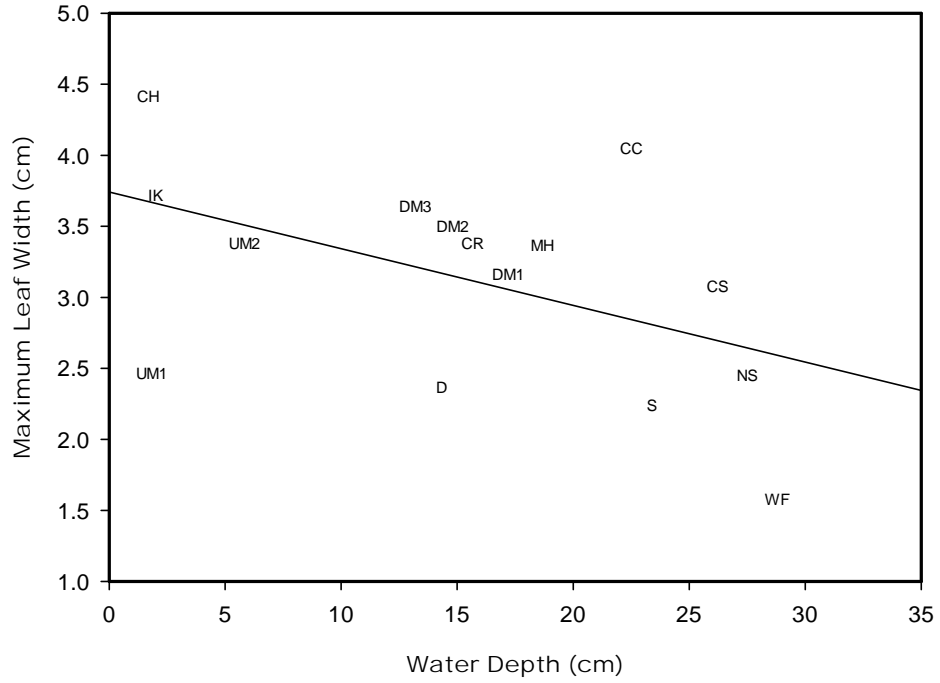


Figure 14. Mean maximum leaf width vs. water depth (cm) during June samplings of *Zizania aquatica* var. *aquatica* (Spearman Correlation Coefficient = -0.568; $p = 0.026$; $n = 15$). Abbreviations are defined in Table 1.

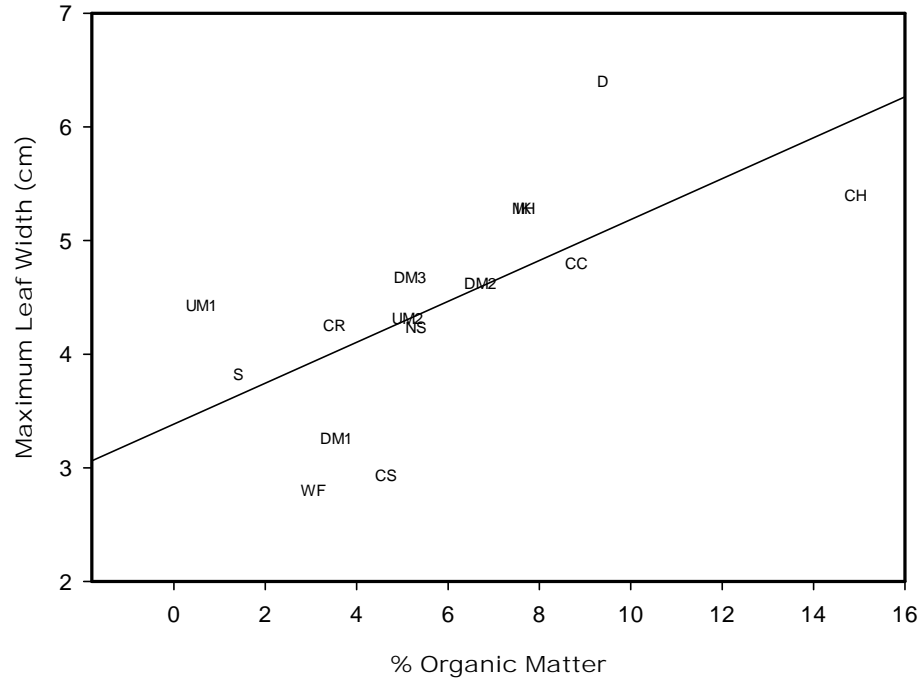


Figure 15. Mean maximum leaf width (cm) vs. % organic matter in the soil during August samplings of *Zizania aquatica* var. *aquatica* (Spearman Correlation Coefficient = 0.803; $p < 0.001$; $n = 15$). Abbreviations are defined in Table 1.

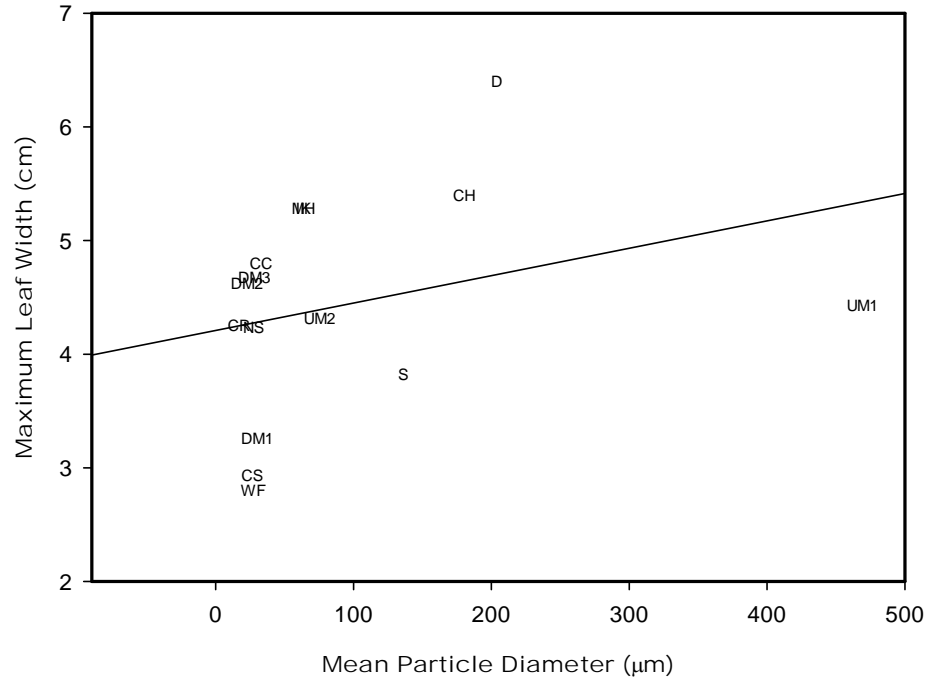


Figure 16. Mean maximum leaf width vs. soil particle diameter for August samplings of *Zizania aquatica* var. *aquatica* (Spearman Correlation Coefficient = 0.586; $p = 0.026$; $n = 15$). Abbreviations are defined in Table 1.