ESTABLISHMENT OF NATIVE AQUATIC VEGETATION IN CONJUNCTION WITH AN INTEGRATED INVASIVE AQUATIC VEGETATION MANAGEMENT PROGRAM

A Thesis

by

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ABSTRACT

Invasive aquatic vegetation is problematic in many Texas lakes and reservoirs and requires management for control. Most often an integrated pest management program incorporating biological, mechanical or chemical means is implemented. Establishment of non-native plants can also be deterred by utilizing an ecological method of planting native vegetation into the empty niches of a disturbed ecosystems that do not have a propagule bank. However, native vegetation establishment can be delayed by herbivory resulting in the need for protection of plants. Lake Raven was chosen as the study site because an integrated pest management approach using chemical, mechanical and biological means has been implemented to control invasive aquatic plants. The most recent herbicide treatments were fluridone on June 6, 2014 and glyphosate in May and August 2014. Native plant restoration was conducted in July 2014 in niches opened from the management of invasive aquatic vegetation. Six deep water and six shallow water species of native aquatic plants were planted in protective exclosures along the shoreline. Plants were given one month to establish before half the treatment exclosures were opened to potential herbivory. Analysis of covariance was used to determine if herbicide and herbivory limited native plant survival. The herbicide application had a significant effect on deep water plants, but did not have a significant effect on shallow water plants. There was not a significant effect associated with herbivory for any of the plant species, which is likely due to remaining invasive aquatic vegetation. Future research is needed to develop an integrated pest management program that incorporates ecological method without limitations from herbicide application.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

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CHAPTER I

INTRODUCTION

Vegetation is an important component of aquatic environments. However, invasive aquatic plant species can be detrimental to an aquatic ecosystem. Aquatic vegetation provides habitat, refuge and food for many organisms in freshwater systems (Dibble et al., 1996). Submersed aquatic vegetation can oxygenate the water, create a temperature gradient and recycle sediment nutrients (Barko and Smart, 1981; Brönmark and Weisner, 1992). Native plant communities enhance the aquatic environment by providing diverse fish and waterfowl habitat, improving water quality, increasing resilience to disturbances and providing resistance to non-native plant invasion (Brönmark and Weisner, 1992; Smart et al., 1996). Disturbances can include water level changes, high turbidity, high nutrient concentrations or construction operations (Smart and Doyle, 1995; Smart et al., 1996). When native plants are established, they have the ability resist invasion because they utilize ecosystem resources making those resources unavailable to non-native plants (Symstad, 2000; Fargione et al., 2003; Maron and Marler, 2007).

Studies have shown that some fish species are influenced by the amount of aquatic vegetation present in the water body. Non-nesting adult largemouth bass *Micropterus salmoides* have been observed most often in association with aquatic vegetation (Annett et al., 1996). Juvenile largemouth bass are found near structure, but most individuals are found nearby aquatic vegetation (Annett et al., 1996). Fishes including largemouth bass, bluegill *Lepomis macrochirus* and orange-spotted sunfish *Lepomis humilis* will select complex habitat for refuge and foraging with vegetated areas used most frequently (Crowder and Cooper, 1982; Durocher et al., 1984; Wiley et al., 1984; Dibble, 1993). However, dense aquatic vegetation beds reduce predator movement, limiting foraging to the periphery (Colle and Shireman, 1980; Trebitz et al., 1997). A reduction in dense stands of invasive aquatic vegetation improves largemouth bass condition by increasing the availability of prey items (Colle and Shireman, 1980). Fish growth rates and feeding efficiency increase by managing excessive amounts of aquatic vegetation most often created by non-native aquatic plants (Colle and Shireman, 1980; Bettoli et al., 1992; Hoyer and Canfield Jr, 1996; Trebitz et al., 1997).

Many non-native aquatic plant species are adapted to colonize disturbed sites and will often establish before native plant communities develop (Smart et al., 1996). Hydrilla *Hydrilla verticillata*, for example, can colonize a wide range of depths, has multiple means of reproduction and several dormant forms that will resume growing when suitable conditions return (Swarbrick et al., 1981). The competitive nature of some invasive aquatic plants can lead to a monospecific plant community (Smart and Doyle, 1995). Some invasive aquatic plant species, such as hydrilla, form dense canopies at the surface of the water which limit light penetration and inhibit competition from submersed native plant species (Haller and Sutton, 1975). When left unimpeded by herbivory, hydrilla will take over shallow water bodies and outcompete native plants (Dick et al., 1995). In some instances, overabundant vegetation resulting from non-native species introduction can have detrimental effects on fishery resources (Smart et al., 2009). Dense canopies often formed by invasive plants can lead to fish stress created by extreme daily fluctuations in temperature, dissolved oxygen and pH (Bowes et al., 1979; Smart et al., 2009). Invasive Eurasian watermilfoil *Myriophyllum spicatum* and hydrilla canopies have higher rates of solar irradiation absorption compared to the open structure of native plant canopies (Smart et al., 2009). Dense aquatic vegetation increases the amount of carbon dioxide removed from the water through photosynthesis resulting in higher water pH (Smart et al., 1994; Smart et al., 2009). Nutrients from the soil are taken up by plants and then may be released when plants decay due to herbicide treatment or senescence (Nichols and Shaw, 1986; Jewell, 1971; Peverly and Johnson, 1979). As a result, increased productivity and noxious algal blooms put pressure on the oxygen resources (Jewell, 1971). This becomes a problem when non-native aquatic plants overtake a water body because they do not have a natural herbivore (Harris, 1988).

Native aquatic plant establishment can act to prevent invasive plant establishment. Disturbed ecosystems including large Texas reservoirs, often remain un-vegetated because they do not have a propagule bank of native plants and are often inhospitable for initial establishment (Smart and Doyle, 1995; Doyle et al., 1997). Man-made reservoirs have variable hydrology when impounded within a flowing water system, can have high nutrient loading rates and are likely to recover slowly after a disturbance (Smart and Doyle, 1995). These characteristics of young water bodies promote the spread of weedy species and growth of species that form surface mats (Smart and Doyle, 1995). Native plant establishment can be the most effective and financially feasible method for controlling invasive aquatic vegetation (Lodge et al., 2006). Risk of future establishment and spread of non-native plants can be minimized by combining native plant establishment and non-native species control in an Integrated Pest Management program (IPM) (Shea and Chesson, 2002; Chadwell and Engelhardt, 2008).

Integrated Pest Management combines elements of biological, mechanical, chemical or ecological approaches for managing invasive species (Jordan et al., 2003; Clout and Williams, 2009). A biological approach combats invasive species by using host specific natural parasites or herbivores (DeBach and Rosen, 1991). Hydrilla flies *Hydrellia pakistanae*, alligator weed flea beetles *Agasicles hygrophila*, grass carp *Ctenopharyngodon idella* and giant salvinia weevils *Cyrtobagous salviniae* are organisms used in this approach to manage invasive aquatic vegetation in Texas (Chilton II, 1998). A mechanical approach subjects the plants to unfavorable conditions or destruction. Mechanical methods can include benthic barriers, cutting or physical removal by hand or machines (Clout and Williams, 2009). Mechanical approaches are labor intensive and usually only a short-term solution to a non-native aquatic plant invasion (Chilton II, 1998; Clout and Williams, 2009). Chemical approaches utilize herbicides that are toxic to aquatic plants or inhibit production of essential proteins or hormones (Chilton II, 1998). Many herbicides can be expensive and are therefore generally most cost effective on small infestations (Clout and Williams, 2009). Based on the mechanism of their action on plant tissues, herbicides are grouped as either contact or systemic. Contact herbicides are fast acting by causing cellular damage to the tissues contacted, but are ineffective on plant tissues beneath the sediment (Madsen, 1997). Systemic herbicides are translocated throughout the entire plant and inhibit production of essential proteins or hormones rather than causing cellular damage which generally makes them slower acting, but they result in total morality of the plant (Madsen, 1997). Combining mechanical, biological or chemical management methods with an ecological approach provides the best opportunity for successful restoration (Smart and Doyle, 1995).

An ecological approach should focus on restoration by mitigating invasive species to reduce their impact on the environment (Smart and Doyle, 1995; Madsen, 1997; Jordan et al., 2003). The ecological approach is an experimental method for managing invasive aquatic plants (Chilton II, 1998; Dick et al., 2004). Establishing native aquatic vegetation acts as an ecological approach by filling niches previously opened by the other IPM methods and then deterring invasive plant establishment or spread (Chilton II, 1998).

Previous research indicates that native aquatic plants have competed well with some invasive aquatic plants. In a small scale experiment, hydrilla and wild celery *Vallisneria americana* produced similar biomasses when grown alone (Smart, 1993). Wild celery has shown to be an effective competitor against hydrilla, especially when wild celery has access to sediment nitrogen (Smart, 1993). Hydrilla outcompeted wild celery when planted at the same time, but wild celery's competiveness increased when given a four week preemption period (Smart, 1993; Doyle and Smart, 1995). American lotus *Nelumbo lutea* also shows ability to reduce Eurasian water-milfoil growth as it is not able to grow beneath an established American lotus colony canopy (Doyle and Smart, 1995). Submersed arrowhead *Sagittaria subulata* has been found to resist the initial invasion of hydrilla with fewer shoots and roots of hydrilla compared to hydrilla grown alone (Sutton, 1990). However submersed arrowhead is generally found in shallow pools growing to a height of about one and a half feet while hydrilla is known to grow at depths greater than thirty-nine feet (Clausen, 1941; Swarbrick et al., 1981; Langeland, 1996). Other experiments have shown that when hydrilla is planted in slender arrowhead *Sagittaria graminea* shoot and root weight is about 90 percent lower than when hydrilla was planted alone (Sutton, 1986). American pondweed *Potamogeton nodosus* has also show to effectively compete with hydrilla especially when the American pondweed has emerged from the sediment prior to hydrilla introduction (Spencer and Ksander, 2000). However, established American pondweed may not effectively reduce hydrilla infestations if American pondweed does not produce more tubers and persist throughout the hydrilla growing season (Spencer and Ksander, 2000). Herbivory also weakens the ability of invasive aquatic plants to compete with native aquatic plants. Wild celery, for example, is able to compete better with hydrilla when the competitive advantage of biomass is reduced using hydrilla flies (Van et al., 1998). However, herbivores including grass carp, crayfish *Orconectes spp.* and red-eared sliders *Trachemys scripta elegans* are known to eat or cause damage to both native and non-native aquatic plant species.

Grass carp are a non-native, herbivorous species used as a biological method for managing invasive aquatic species such as hydrilla (Cross, 1969). Grass carp feeding rates are greatest during morning hours and are dependent on the age and size of fish as well as species of plants present and ambient temperature (Dyke et al., 1984; Hockin et al., 1989). Grass carp diets and food preferences tend to vary between water bodies which may be the result of water chemistry affecting plant palatability, nutritional value and chemical composition (Chilton II and Muoneke, 1992). Despite differences in plant growing conditions, grass carp prefer tender, young growth of submersed vegetation to emergent and floating plants which are often only eaten in the absence of more palatable species (Fischer, 1968; van Dyke et al., 1984; Chilton II and Muoneke, 1992; Masser, 2002). Grass carp will graze on native plants including wild celery, muskgrass *Chara spp.*, pondweeds, common duckweed *Lemna minor* and Southern naiad *Najas guadalupensis* (Fischer, 1968; Hestand and Carter, 1978; Pine and Anderson, 1991; Bonar et al., 1993; Masser, 2002). Invasive plants including Eurasian watermilfoil, giant salvinia *Salvinia molesta*, water hyacinth *Eichhoria crassipes* and highly preferred hydrilla will also be consumed (Pine and Anderson, 1991; Chilton II and Muoneke, 1992; Masser, 2002). Grass carp will not readily consume water pennywort *Hydrocotyle ranunculoides* or wild celery in the presence of hydrilla, *Nitella spp.*, *Potamogeton spp.* or coontail *Ceratophyllum demersum* (Hestand and Carter, 1978; Johnston et al., 1983; Miller and King, 1984).

Although introduced grass carp pose a large threat to native aquatic vegetation, native herbivores must not be overlooked. Little research has been conducted on the aquatic plant preferences of red-eared sliders, but it is evident they will eat American pondweed, wild celery and Southern naiad *Najas guadalupensis* (Dick et al., 1995). Overall red-eared sliders prefer free floating fragments of wild celery, but feeding preferences change between male, female, adult and subadults (Dick et al., 1995). When exclosure wires are found compromised while establishing native plants, the damage is generally attributed to red-eared sliders (Doyle et al., 1997). Crayfish will also cause damage to native plants by clipping the shoots with wild celery and pondweeds being the most susceptible (Lodge and Lorman, 1987; Chambers et al., 1990).

Protection using exclosures prevents plant damage by herbivory and dramatically benefits native aquatic vegetation during establishment (Lodge, 1991; Smart et al., 1996; Doyle et al., 1997). Plants have a greater chance to remain permanently established if they were protected from grazing for the first year after planting (Carter and Rybicki, 1985). Wild celery, American pondweed, and Illinois pondweed *Potamogeton illinoensis* are three of the native plant species most vulnerable to herbivory (Webb et al., 2012). When protected by exclosures, water stargrass *Heteranthera dubia*, wild celery and American pondweed have shown high survival rates and the ability to expand within exclosures (Doyle et al., 1997; Smart et al., 1998). In lakes with high herbivore pressure, expansion outside of small exclosures is limited (Doyle et al., 1997; Smart et al., 1998).

Given adequate protection from herbivores, native aquatic plant establishment is possible; however, establishment may be challenged by herbicide application. Systemic herbicides, such as fluridone and glyphosate, are commonly used for management of aquatic plant species (McCowen et al., 1979; Lopez, 1993). Glyphosate is effective when applied to the surface of floating leaved and emerged aquatic plants, but does not threaten submersed aquatic plants (Forney and Davis, 1981). Glyphosate binds to soil and organic matter in the water column making it unavailable for plant absorption (Giesy et al., 2000; Solomon, 2003). Glyphosate requires a short contact time, is fast acting and very little glyphosate enters the water column when applied on floating plants (Chilton II, 1998).

Fluridone is effective when treating whole ponds and can be used selectively in waterbodies two hectares or larger (Langeland, 1996). The reliance on fluridone in large scale hydrilla management has resulted in fluridone resistant hydrilla (Netherland and Jones, 2015). The continued use of fluridone in a system overtime can lead to the hydrilla stand being primarily fluridone resistant (Michel et al., 2004; Netherland and Jones, 2015). Mesocosm studies have revealed fluridone selectivity is due the differences in tolerance to concentration and exposure period for each plant species (Langeland, 1996; Netherland et al., 1997). Chlorosis, a reduction in plant pigment levels, leaves new growth white to pale in color and is the initial symptom of plants exposed to fluridone (McCowen et al., 1979; Netherland et al., 1997). Fluridone will impede hydrilla growth when applied at a concentration of at least five parts per billion (MacDonald et al., 1993). American pondweed, Illinois pondweed, white water lily *Nymphaea odorata* and wild celery have the potential to recover from treatment at the same concentration (Netherland et al., 1997; Madsen et al., 2002; Pedlow et al., 2006).

Once biological and chemical management methods are in place, plant establishment using founder populations is the most cost effective method to create habitat and ecosystem stability (Smart et al., 1996). Propagules for native plants can be found in some commercial nurseries, collected from the field, or propagated where resources are available (Smart et al., 1996). When possible, it is best to use mature peat potted transplants or dormant tubers for establishment because bare root transplants suffer higher mortality and are more susceptible to uprooting (Smart et al., 1996; Smart et al., 1998). Site selection should include areas of low turbidity, protected from wind action and water no deeper than six feet (Smart and Dick, 1999). Historic water level fluctuations should be considered in choosing a proper planting depth (Smart and Dick, 1999). Generally, submersed plants should be planted at a depth of two to three feet and emergent up to one foot (Smart and Dick, 1999; Webb et al., 2012). A fine textured substrate is best for planting to allow the roots to penetrate and anchor the plant (Smart and Dick, 1999). Protection for newly planted plants can be provided by building exclosures. Materials for exclosures include various fencing materials and stakes which make exclosures suitable to deter herbivores (Smart and Dick, 1999; Webb et al., 2012). Fencing may be polyvinyl chloride-coated galvanized wire or orange plastic fencing (Smart et al., 1996; Webb et al., 2012). Wire mesh size of two by two inch to two by four inch is small enough to provide protection from most herbivores, but also large enough for plant fragments to spread outside of the exclosure (Doyle et al., 1997; Webb et al., 2012). Circular exclosures, three to six feet in diameter, are adequate for protecting small groups of plants (Webb et al., 2012). Once established, native aquatic plant communities within an exclosure improve chances of success for additional plant establishment throughout a reservoir (Doyle and Smart, 1995; Smart et al., 1996).

Native aquatic plant establishment is challenged both by herbivory and herbicide application. Field studies reveal that removing herbivory pressure can allow native plants to establish. Mesocosm studies show that aquatic herbicides such as fluridone are species selective at low concentrations and exposure times which may allow for plant recovery following treatment (Netherland et al., 1997). Protected native plants have been found to fill and expand outside of an exclosure while unprotected plants are damaged or killed by unidentified herbivores (Webb et al., 1994; Doyle et al., 1997). Results from native aquatic vegetation establishment experiments have been inconsistent, motivating the need of further research (Chilton II, 1998).

This was a TPWD contracted study designed to examine the establishment success of newly planted native aquatic plant species to herbivory after fluridone treatment to remove non-native hydrilla. Lake Raven has a history of invasive aquatic plants with an integrated pest management program using biological and chemical control in place. A previous grass carp stocking and herbicide application opened niches for native aquatic plants and thus made it an ideal location for this study. Native aquatic vegetation susceptibility to fluridone was determined by planting after the herbicide application and measured by percent of chlorotic vegetation. Establishment success, measured as a percent of plant survival, was determined by planting within exclosures and exposing plants to herbivores once established. A secondary objective was to record the frequency of grazing on select native aquatic plant species by herbivores through the use of underwater recording devices. The water had low turbidity and relatively constant levels allowing underwater cameras to record herbivore movements within native aquatic vegetation exclosures. This study allowed the opportunity to test the null hypothesis that herbivory does not affect plant abundance when accounting for plant loss from herbicide.

CHAPTER II

MATERIALS AND METHODS

Study Site

Texas Parks and Wildlife Department (TPWD) re-impounded Lake Raven in 1956 for recreational use following repairs. Lake Raven is a lentic, man-made, 203 acre reservoir located in Huntsville State Park, Huntsville, Texas. The relatively constant water level makes it an ideal study site for aquatic vegetation establishment. In addition, Lake Raven contains the non-native, invasive aquatic plants hydrilla, water hyacinth, alligator weed, and giant salvinia. Lake Raven has a history of integrated pest management using herbicide application, biological control and mechanical removal which further makes it an appropriate choice for a study site. Herbicides have been used for control efforts of all invasive species in 2009, 2010, 2012 and 2014. Most recently, Lake Raven was treated using glyphosate in May and August of 2014 and fluridone in June 2014. Biological control efforts include grass carp, hydrilla flies and alligator weed flea beetles. In both 2001 and 2009 for hydrilla management, four hundred adult grass carp were stocked in Lake Raven. In 2014, 6,000 alligator weed flea beetles were released. While invasive species persist in the lake, a reduction in invasive vegetation cover gave the opportunity to conduct native plant restoration.

The Prairie Branch arm of Lake Raven (Figure 1) was chosen to plant native aquatic vegetation. A bathymetric survey revealed ideal slopes and depths for planting. Prairie Branch has fine textured sediment bed consisting primarily of organic debris. Avoiding the main body of the lake also minimized any damage from wind fetch. Archeological protected areas in Lake Raven are not found within Prairie Branch. High use areas, especially the boat ramp, are outside of Prairie Branch which reduced potential human disturbance.

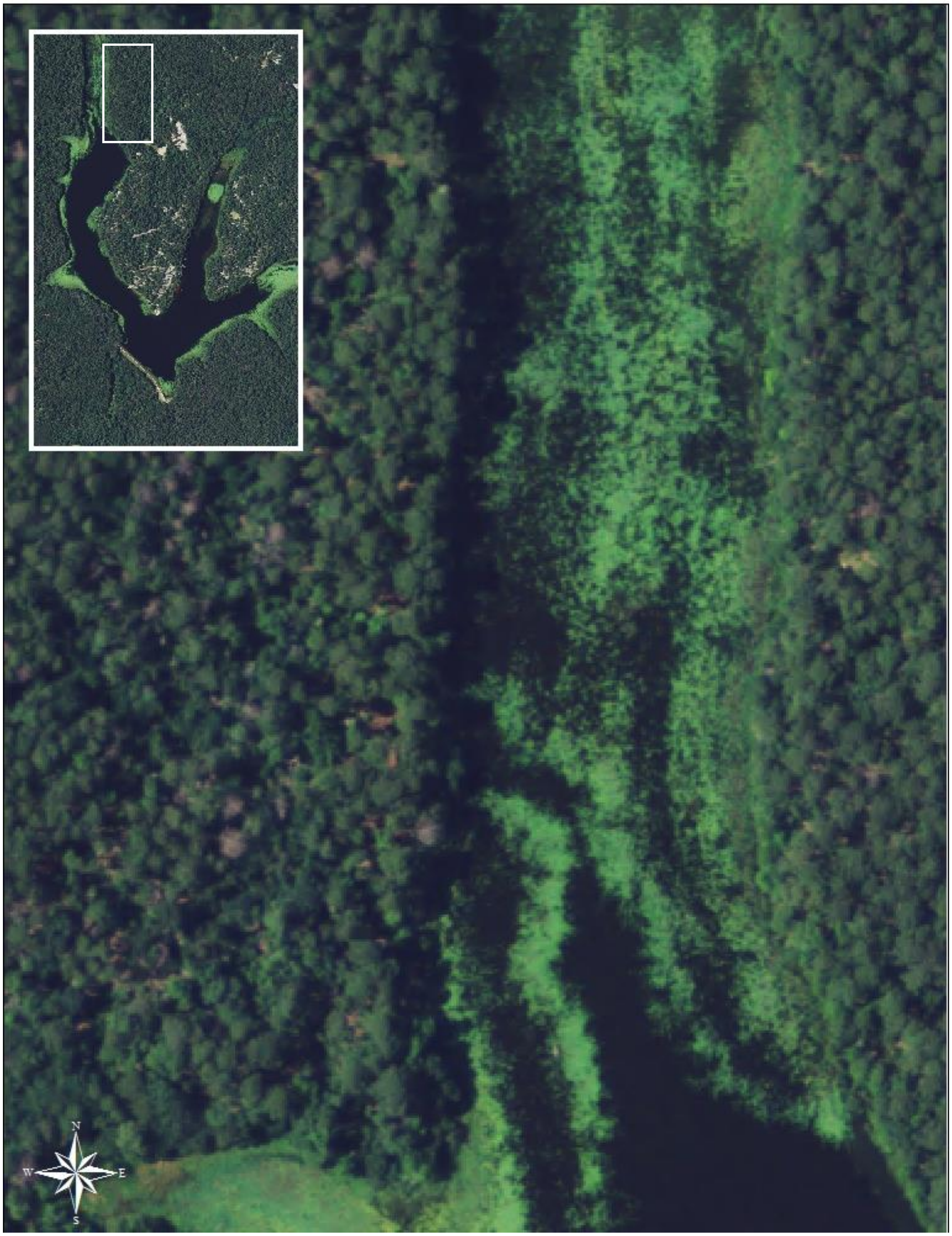

Figure 1: Prairie Branch, Lake Raven in Huntsville State Park

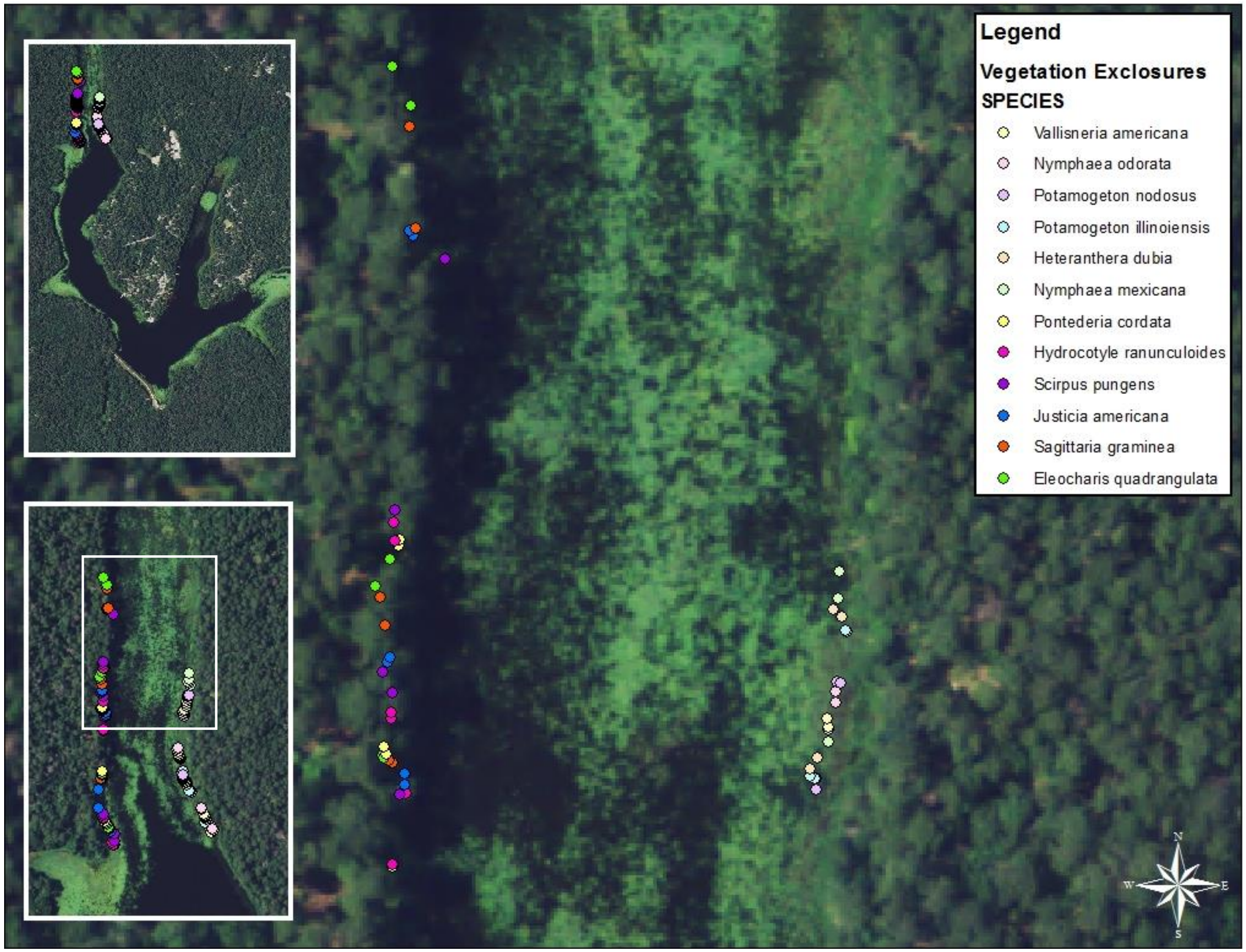

Figure 2: Native aquatic plant exclosures in the North half of Prairie Branch. Exclosures on East side at 3-4 feet. Exclosures on West side at 1-2 feet.

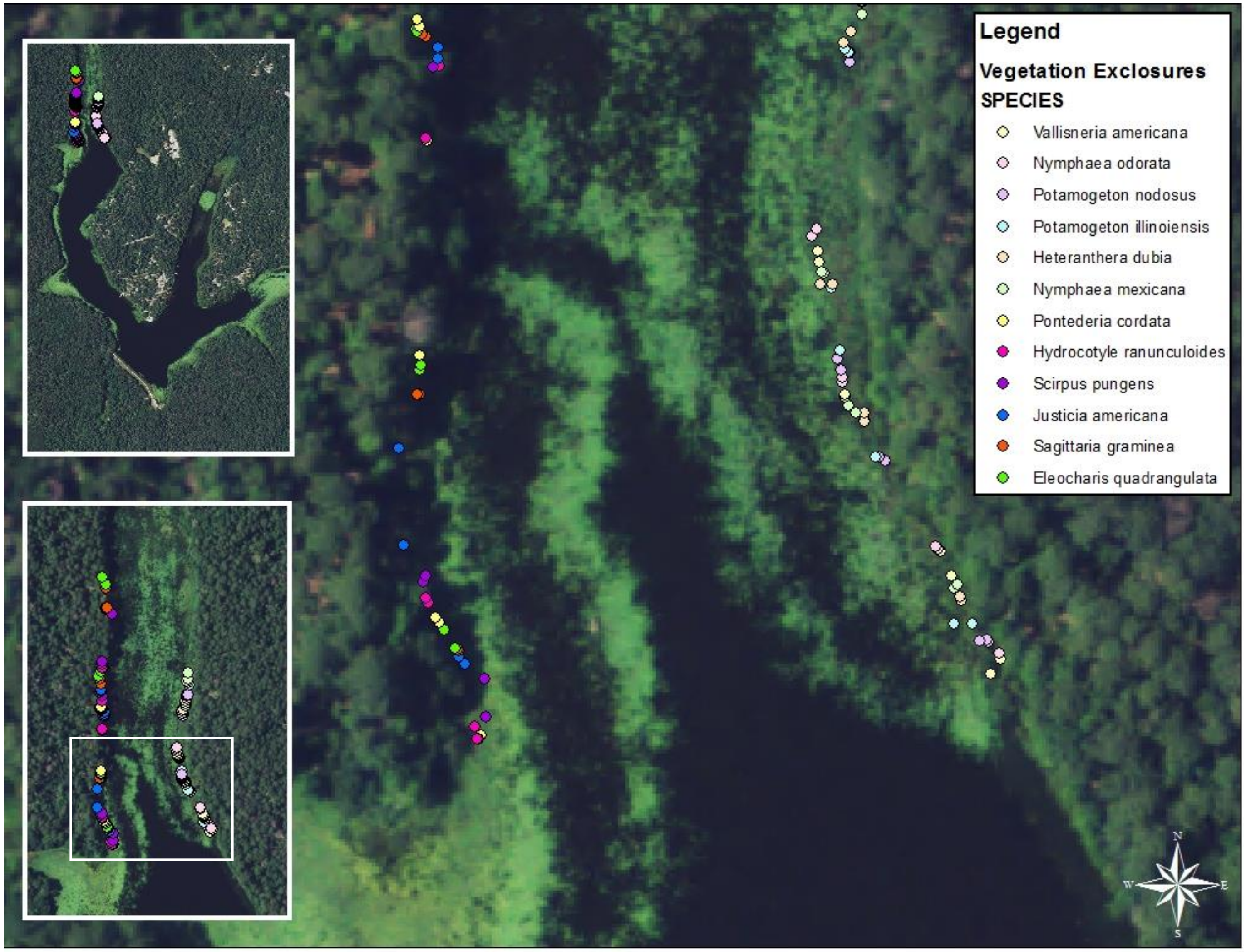

Figure 3: Native aquatic plant exclosures in the South half of Prairie Branch. Exclosures on East side at 3-4 feet. Exclosures on West side at 1-2 feet.

Field Methods

Treatment of hydrilla using 2500 pounds (3.11 pounds/acre foot) of fluridone for a 5 to 7 ppb concentration of fluridone (SonarOne, SePro Corporation, Carmel, Indiana) was conducted on June 6, 2014. Granular pellets were distributed throughout Lake Raven excluding Prairie Branch to avoid direct application in the selected planting area. Two water samples were taken in Prairie Branch on August 7, 2014. One sample was taken at in the Northwest end of Prairie Branch and the other sample was taken in the Southeast end.

Polyvinyl chloride coated, 14 gauge, welded wire, six foot high, with two inch by four inch mesh and rebar posts was used to build 120 enclosed exclosures for native plant protection. Wire mesh was cut into 25 feet lengths then rolled for transportation to Lake Raven. Rebar was cut into 8 feet lengths before transport. Twelve native aquatic plant species were selected for their availability, diverse growth phenotypes and knowledge of their life history. The deep water plants species selected for planting were wild celery, white water lily, American pondweed, Illinois pondweed, water stargrass and yellow water lily *Nymphaea Mexicana.* The shallow water plants selected for planting were pickerelweed *Pontederia cordata*, water pennywort, American bulrush *Schoenoplectus americanus*, American waterwillow *Justicia americana*, bull tongue *Sagittaria lancifolia L.* and squarestem spikerush *Eleocharis quadrangulata*. Mature plants were purchased from Joe Snow Aquatic Plants in Argyle, Texas. Plants were delivered and placed in the native aquatic plant nursery at TPWD Inland Fisheries Division 3E office near Snook, Texas on June 11, 2014. Plants remained in the TPWD nursery until planting.

Exclosures were built and positioned between June 18-25, 2014. The space for each exclosure was opportunistically selected within Prairie Branch where exclosures could be built at least one foot apart along the shoreline avoiding any woody debris and maintaining adequate planting depth (Figure 2, 3). In preparation of building each exclosure, the spaces were manually cleared of any existing vegetation by hand removal and rakes. Each length of wire mesh formed a seven foot diameter circular exclosure by joining the ends and wrapping the free wire tabs around the intact mesh end which created a seam. Exclosures were held in place using four lengths of rebar. Sixty exclosures for deep water plants, American pondweed, Illinois pondweed, water stargrass, wild celery, white water lily and yellow water lily, were built in three to four feet of water. Sixty exclosures for shallow water plants, American bulrush, American waterwillow, bull tongue, squarestem spikerush, water pennywort and pickerelweed were built in one to two feet of water. Exclosures were georeferenced using a handheld GPS and mapped in ArcView (Figure 1).

The native aquatic plants were planted in the exclosures on June 26, 27 and 30, 2014. A water sample was not taken prior to planting to determine the fluridone concentration because it was assumed that the herbicide had degraded below harmful conditions. The native aquatic plants were transported in covered plastic tubs with water from the TPWD facility to Lake Raven to prevent desiccation. Tubs remained closed and shaded until plants were planted to reduce plant stress. All planting materials and plants were transported by boat to the planting sites. Exclosures were opened halfway down the seam to access the inside for planting. Any animals larger than the mesh found trapped within the exclosures were removed. Any vegetation missed while building exclosures was removed. Five plants of a single specie were planted in each exclosure in a central rosette pattern. A 13.5 inch long trowel was used to measure an equidistance between plants. Side by side exclosures were planted in pairs per plant species. There were five paired replicates for each species. Sequence for planting the species was determined using a random number generator. Deep water plants were sequenced wild celery, white water lily, American pondweed, Illinois pondweed, water stargrass, and yellow water lily. Shallow plants were sequenced pickerelweed, water pennywort, American bulrush, American waterwillow, bull tongue, and squarestem spikerush. Plants were monitored throughout the initial growth period for survival and chlorosis and exclosures checked for breeches. Plants in each exclosure were counted and recorded as an indication of survival. Percent chlorosis was determined by a visual inspection of each exclosure to the nearest ten percent. Pictures were taken of each exclosure on July 7, 10, 19, 24 and 31 as a visual reference.

An exclosure of each pair was selected to be opened using a coin toss. Shallow water exclosures were open on August 4 and deep water exclosures were opened on August 6. Plants in each exclosure were counted prior to each exclosure being opened and on September 2 to determine susceptibility to herbivory. Surface pictures taken for a visual reference of herbivory, growth, and chlorosis continued on August 8, 14, 17, 25 and September 2. A trail camera was placed at the opening of one open exclosure of pickerelweed, water pennywort, bull tongue, squarestem spikerush, wild celery, white water lily, American pondweed, Illinois pondweed, water stargrass, and yellow water lily. Trail cameras were enclosed in a waterproof plastic container with a desiccant packet and weight. A bungee cord was used to secure the container to wire mesh and rebar at the opening of the exclosure. Trail cameras were set to take pictures every 2 minutes from dawn to dusk until being removed on September 2. Pictures from the trail cameras were used to document presence of herbivores or other aquatic animals. Pictures from the submerged trail cameras were collected on August 11, 17, 21, 25, 28 and September 2. Due to technical problems with cameras, pictures were not taken for the water stargrss between August 8 and August 11, Illinois pondweed between August 17 and 21, and yellow water lily between August 17 and 21. In each respective situation, no remaining plants were present when the cameras were checked. As a result of the technical problems and a lack of remaining plants for observation, no pictures were taken for the water stargrass, and the only pictures taken for the Illinois pondweed and yellow water lily occurred between August 8 and 17.

Analysis

Analysis of Covariance (ANCOVA) was conducted to determine if the effects of herbivory on each species and plant type were significant while accounting for plant loss due to herbicide. The homogeneity of regression was tested to determine that there was not an interaction between the change in plant abundance associated with herbicide and herbivory exposure for any plant species or plant type. The change in plant abundance from the time of planting to the exclosures being opened was used to quantify the effect of herbicide. One exclosure of each pair was opened to determine change in plant abundance when exposed to herbivory. Significance was set at $\alpha = 0.05$. The null hypothesis that neither herbivory nor herbicide are associated with the change in plant abundance was determined using the ANOVA statistic. If this null hypothesis was rejected, the effect test was used to determine if herbivory, herbicide or both were associated with the change in plant abundance. Descriptive statistics were determined for the change in plant abundance associated with herbicide and herbivory. The pictures indicating that the pickerelweed, water pennywort, bull tongue, squarestem spikerush, wild celery, white water lily, American pondweed, Illinois pondweed, and yellow water lily exclosures were utilized were counted and compared to the total number of pictures taken for each plant species. The number of pictures with a presence of herbivores out of total pictures with an animal was used to compare which species was most preferred. Individual herbivores were also counted for frequency of presence for each plant species. Descriptive statistics for percent chlorosis was determined after one and two months of plant establishment.

CHAPTER III

RESULTS

Herbicide

Fluridone concentration 2 months after treatment was unexpectedly 9.1 ppb in the Southeast end and 9.0 in the Northwest end of Prairie Branch in Lake Raven. This was 2 ppb above the anticipated concentration. Chlorosis was observed on the hydrilla from the fluridone treatment; however, an increase in open water was not observed in Prairie Branch before or during the study. In April 2015, ten months after the fluridone treatment, TPWD surveys detected a one percent increase in open water in Lake Raven compared to June 2014. Chlorosis was observed on ten of the native plant species (Table 1) from fluridone exposure one month after planting. White water lily was the most affected plant species with 100 percent chlorosis after the first month of fluridone exposure. Water pennywort and wild celery were the only plant species that did not have any chlorotic vegetation after one month of fluridone exposure. The null hypothesis that herbivory, herbicide or both was related to the change in plant abundance was rejected for some of the plant species. Further details of ANCOVA revealed that herbicide was the only factor associated with the change in plant abundance for white water lily, American pondweed, Illinois pondweed, water stargrass and yellow water lily (Table 2). Relative to the initial planting, the number of deep water plants decreased significantly (mean, 3.05; SD, 1.57; $P = 0.000$), but there was not a significant difference in the number of shallow water plants (mean, 0.08; SD, 0.46; $P = 0.195$) associated with the herbicide application. The fluridone application did not result in a significant decrease in the number of plants for white water lily (mean, 2.1; SD, 1.2; $P = 0.031$), American pondweed (mean, 3.0; SD, 1.3; $P = 0.003$), Illinois pondweed (mean, 4.3; SD, 0.9; $P = 0.000$), water stargrass (mean, 4.1; SD, 1.0; $P = 0.000$) and yellow water lily (mean, 3.5; SD, 1.2; $P = 0.000$).

Observation during the second month of establishment revealed an increase in chlorosis (Table 1) for water pennywort (7.0 percent), American bulrush (18.0 percent) and squarestem spike rush (18 percent). There was an average decrease in chlorosis for American pondweed (12.5 percent) pickerel weed (10.0 percent), American waterwillow (6.0 percent) and bull tongue (10.2 percent) during the second month of herbicide exposure. There were no Illinois pondweed, water stargrass or yellow water lily plants at the end of the study for comparison.

Table 1- Average percent chlorosis observed for each plant species

Plant Species	**August Chlorosis**	**September Chlorosis**
Wild Celery	0.0	0.0
White Water Lily	100.0	100.0
American Pondweed	80.0	67.5
Illinois Pondweed*	41.7	-
Water Stargrass*	50.0	-
Yellow Water Lily*	97.5	-
Pickerel Weed	29.0	19.0
Water Pennywort	0.0	7.0
American Bulrush	26.0	44.0
American Waterwillow	87.0	81.0
Bull tongue	48.0	37.8
Squarestem Spikerush	41.0	59.0

**No plants for observation in September*

Herbivory

Opening the exclosures to herbivory did not create a significant change in plant abundance when accounting for change in plant abundance due to herbicide (Table 2). Even though plant loss was observed after opening the exclosures, the loss occurred slowly over the one month exposure to herbivory. Plant growth was not quantified; however, growth was observed for all remaining plants from June to September. By plant type, deep water plants had a greater change in abundance than the shallow water plants; however, the change was not significant for neither deep water plants (P=0.461) nor shallow water plants (P=0.770). By plant species, wild celery had the greatest decrease in plant abundance associated with herbivory, but the change was not significant (P=0.884). American bulrush and squarestem spikerush did not exhibit any change in plant abundance after exposure to herbivory.

Table 2- ANCOVA results for the percent change in plant abundance associated with herbicide application and herbivory for each species

Plant Species	**ANOVA Statistic Prob > F**	**Percent Δ in plant abundance associated with Herbicide**	**Percent Δ in plant abundance associated with Herbivory**
Wild Celery	P = 0.395	-26.0 ± 28.0 (P = 0.231)	-48.0 ± 34 (P = 0.884)
White Water Lily	P = 0.050	-42 ± 24 (P = 0.048)	-36 ± 22 (P = 0.153)
American Pondweed	P = 0.008	-60 ± 26 (P = 0.004)	-24 ± 22 (P = 0.842)
Illinois Pondweed	P = 0.000	-86 ± 18 (P = 0.000)	-0.7 ± 0.9 (P = 0.475)
Water Stargrass	P = 0.000	-82 ± 2 (P = 0.000)	-18 ± 20 (P = 0.493)
Yellow Water Lily	P = 0.001	-70 ± 24 (P = 0.001)	-30 ± 24 (P = 0.544)
Pickerel Weed	P = 0.347	0 -	-2 ± 6 (P = 0.347)

Table 2- Continued

Plant Species	**ANOVA Statistic Prob > F**	**Percent Δ in plant abundance associated with Herbicide**	**Percent Δ in plant abundance associated with Herbivory**
Water Pennywort	P = 0.743	-4 ± 12 (*P* = 0.782)	-14 ± 26 (*P* = 0.570)
American Bulrush	-	0 -	0 -
American Waterwillow	P = 0.393	0 -	-12 ± 14 (*P* = 0.393)
Bull Tongue	P = 0.354	-6 ± 18 (*P* = 0.487)	-20 ± 18 (*P* = 0.328)
Squarestem Spikerush	-	0 -	0 -

Table 3- ANCOVA results for the percent change in plant abundance associated with herbicide application and herbivory for each plant type.

Plant Type	**ANOVA Statistic Prob > F**	**Percent Δ in plant abundance associated with Herbicide**	**Percent Δ in plant abundance associated with Herbivory**
Deep Water	P = 0.000	-61 ± 31.4 (*P* = 0.000)	-28.4 ± 23.8 (*P* = 0.464)
Shallow Water	P = 0.372	-1.6 ± 9.2 (*P* = 0.195)	-8 ± 15.8 (*P* = 0.770)

Photographs

Over the one month time period, 47,779 pictures were taken within the exclosures. There were no pictures of the water stargrass exclosure due to a camera malfunction and no remaining plants after the first week of observation. Due to camera problems such as inconsistent battery life, the number of pictures differed for each plant species. The underwater pictures indicated the American pondweed exclosure was utilized most frequently by aquatic animals followed by pickerel weed, water stargrass, and yellow water lily (Table 4). Aquatic animals observed included largemouth bass, *Lepomis* spp., grass pickerel *Esox americanus*, *Cyprinidae* spp., *Ictalurus* sp., spotted gar *Lepisosteus oculatus*, red-eared slider turtle and an unidentifiable mammal.

Overall frequency of herbivores present in the exclosures was low. Herbivory pressure was indicated by the underwater pictures for wild celery, American pondweed and water pennywort. One red-eared slider was observed in the wild celery exclosure, three red-eared sliders in the American pondweed exclosure and one mammal and two red-eared sliders in the water pennywort exclosure.

Table 4- Utilization of exclosures by aquatic animals shown by "number of pictures with aquatic animals" /"number of pictures for each exclosure" . Utilization of exclosures by aquatic herbivores shown by "number of pictures with aquatic herbivores" / "number of pictures with aquatic animals"

Exclosure	Plant Species	Aquatic Animal Utilization	Utilization by Herbivores
1	Wild Celery	$\frac{105}{6041}$	$\frac{1}{105}$
11	Yellow Water Lily	$\frac{244}{4315}$	$\frac{0}{244}$
27	White Water Lily	$\frac{49}{1268}$	$\frac{0}{49}$
29	American Pondweed	$\frac{1424}{8069}$	$\frac{3}{1424}$
31	Illinois Pondweed	$\frac{254}{3433}$	$\frac{0}{254}$
73	Pickerel Weed	$\frac{418}{4856}$	$\frac{0}{418}$
95	Squarestem Spikerush	$\frac{139}{6716}$	$\frac{0}{139}$
99	Water Pennywort	$\frac{196}{6550}$	$\frac{1}{196}$
117	Bull Tongue	$\frac{36}{4380}$	$\frac{0}{34}$

Statement of Effect

There was no significant interaction between herbicide and herbivory in the study. A significant effect from herbicide was found for some of the native aquatic plant species. Herbivory did not play a significant role in plant abundance.

CHAPTER IV

DISCUSSION AND CONCLUSION

Herbicide

Hydrilla biomass did not have a noticeable decrease during the study as a result of the fluridone application. It is possible that the hydrilla in Lake Raven developed fluridone resistance or a strain of fluridone resistant hydrilla was introduced. Fluridone is a slow-acting herbicide, which limits new plant growth before necrosis and a decrease in plant biomass can occur (MacDonald et al., 1993). Fluridone is lethal to hydrilla at a concentration as low as 5 ppb with higher application rates resulting in faster control (McCowen et al., 1979; MacDonald et al., 1993). At an application rate of 5 ppb, it has been reported that vegetative hydrilla growth may continue for six weeks after fluridone treatment (MacDonald et al., 1993). Noticeable hydrilla control can begin around three weeks depending on the application rate (Arnold, 1979; McCowen et al., 1979). An application rate of one pound per acre with an average water depth of 5.3 feet achieves complete control of hydrilla by 81 days (Arnold, 1979). At an application rate of 30 ppb, symptoms of fluridone exposure occur around 6 days followed by the vegetation gradually sinking to the bottom and complete control achieved by 8 weeks after treatment (McCowen et al., 1979). The first indicator of development of fluridone resistance is the reduction in effectiveness of fluridone reducing the amount of hydrilla (Michel et al., 2004; Netherland and Jones, 2015). Hydrilla resistance to fluridone varies and biotypes can be two to seven times less resistant to fluridone than susceptible hydrilla (Michel et al., 2004; Puri et al., 2007). Gene sequencing would be required to determine if hydrilla has developed a mutation at the target site of fluridone and therefore, resistant to fluridone (Arias et al., 2005; Dayan and Netherland, 2005).

The concentration and effect of fluridone on the deep water native plant species was greater than anticipated for this study with no significant plant loss of any shallow water species. Wild celery and water pennywort had the least amount of chlorosis throughout the study making them suitable plants for utilizing in water bodies treated with fluridone. White water lily, American pondweed and American waterwillow sustained the highest level of chlorosis. There were not Yellow water lily, water stargrass or Illinois ponweed plants surviving at the end of the study. Deep water plants, American pondweed, Illinois pondweed, white water lily and wild celery can recover from a fluridone application rate of 5 ppb (MacDonald et al., 1993; Netherland et al., 1997; Pedlow et al., 2006). During the second month of establishment, the chlorosis levels of some native plant species decreased. Chlorosis from fluridone treatment is generally first exhibited in new growth and apical meristems, followed by tissue deterioration and plant death (McCowen et al., 1979; Doong et al., 1993). New plant growth was not affected as the fluridone concentration likely continued to decrease during the second month of the study.

It is likely that the elevated concentration of fluridone played a role in plant mortality by means of chlorosis. The reason fluridone concentrations were greater than expected in the Prairie Branch eight weeks after treatment is unclear. The amount of fluridone used in Lake Raven should have resulted in a maximum initial concentration of 60 ppb. Fluridone application in June was expected to have a rapid dissipation rate as degradation of fluridone is associated with ultraviolet radiation and temperature (West et al., 1979; Muir and Grift, 1982). Studies have shown that 21 days and 60 days after treatment, fluridone levels are less than 30 percent and 5 percent of the original concentration, respectively (West et al., 1979; Muir et al., 1980; Muir and Grift, 1982; West et al., 1983; Osborne et al., 1989).

The significant decrease in plant abundance prior to herbivory exposure indicates that the fluridone treatment negatively impacted plant establishment; consequently, delaying planting time longer after treatment would have likely changed the outcome of the study. The effects that fluridone treatment had on plant survival shed light on the need to utilize plant species that are not sensitive to fluridone levels of 9 ppb including white water lily, yellow water lily, water stargrass, American pondweed and Illinois pondweed. As well as, consider other methods of herbicide treatment when incorporating an ecological approach into the integrated pest management program.

Herbivory

There was no evidence to indicate herbivory significantly impacted plant survival during the study. The game camera pictures indicated that herbivores did not frequently come into any of the exclosures. Due to the hydrilla biomass remaining constant in the Prairie Branch, it is suspected that herbivores had a sufficient amount of food which created a hesitancy to enter the open exclosures with native plants. Hydrilla is the aquatic plant species most preferred by grass carp which are unlikely to consume other aquatic plants when hydrilla is present (Hestand and Carter, 1978; Masser, 2002). If the hydrilla biomass had decreased, it is possible that herbivores would have significantly impacted the native vegetation. The low density of grass carp in Lake Raven may explain their absence from the exclosures. TPWD recommends stocking 5 to 10 grass carp per acre of water body (Chilton II, 1998). Grass carp were most recently stocked in 2009 at a rate of 2 adult grass carp per acre of water body and likely sustained mortality over 5 years. In a system that does not have a high density of grass carp, such as Lake Raven, red-eared sliders will be the primary herbivore challenging native plant establishment. Red-eared sliders will consume large amounts of wild celery when it is available (Dick et al., 1995). However, hydrilla is also a desired aquatic plant limiting the need for red-eared sliders to seek out the preferred wild celery planted in Lake Raven (Dick et al., 1995). Due to the density of grass carp not significantly impacting the establishment of native plants, incorporating an ecological approach with the existing biological approaches in Lake Raven is feasible.

Future Research

Continued researched is needed to assess the success of incorporating an ecological approach into an existing IPM. Lake Raven would again be suitable as the study site to conduct another study on establishing native aquatic vegetation. Upon completion of this study, exclosures were left in place and remaining vegetation given the opportunity to continue growing. A new study could utilize fencing materials to reform exclosures for deep water native aquatic plants as the loss of deep water plants prior to herbivory exposure limited data of herbivory impacts. The shallow water vegetation had overall successful establishment and the exclosures could be moved for replanting.

Herbicide treatment should again occur prior to native aquatic vegetation planting to allow for minimal plant damage and opportunity to study herbivory impacts. Native plant survival may be more likely if the herbicide has a faster dissipation rate or given enough time to reduce to a tolerable concentration. Herbicides recommended for the control of hydrilla are diquat, endothall, copper and fluridone (Blackburn and Weldon, 1970; Sutton et al., 1971; Van et al., 1987; MacDonald et al., 1993; Chilton II, 1998). Diquat is a fast-acting herbicide that is most suitable for use in flowing water systems (Van et al., 1987; Chilton II, 1998). Endothall has a short half-life in water and requires little exposure time (Langeland and Warner, 1986; Chilton II, 1998). Native plants including wild celery, soft-stem bulrush *Scirpus validus*, water stargrass and Illinois pondweed have shown some resistance to endothall application (Skogerboe and Getsinger, 2001). However, American pondweed, arrowhead *Sagittaria latifolia*, and white water lily were susceptible to endothall application rates high enough for effective hydrilla control (Skogerboe and Getsinger, 2001). Copper requires a short contact time and can be used in combination with other herbicides such as endothall (Sutton et al., 1971; Pennington et al., 2001). Combining chelated copper with endothall is effective at controlling hydrilla at even the lowest application rates (Pennington et al., 2001).

Fluridone or an endothall and copper combination would be suitable for a future study. An endothall and copper treatment could be used throughout the study area and native aquatic vegetation could still be planted shortly after treatment. Fluridone would require a longer dissipation period and treatment should not be within the study area. Monitoring of the fluridone concentration before planting should also help to minimize plant loss from chlorotic vegetation.

Additional methods of plant monitoring could be used for more comparisons throughout a study. When planting only a few plants in a pattern, plants can be counted by the number of surviving clumps until growth prevents distinguishing between plants (Smart et al., 1996; Doyle et al., 1997). Percent cover can be determined within the exclosure as a whole or from dividing the exclosure into sections (Hestand and Carter, 1978; Smart et al., 1996; Doyle et al., 1997). A combination of these methods would allow for analysis of plant survival as well as growth and establishment success.

Conclusion

Fluridone levels greater than 5.0 ppb prevented the submersed species, white water lily, American pondweed, Illinois pondweed, water stargrass and yellow water lily to successfully establish. Determining herbivory impacts on the native aquatic vegetation was limited due to herbicide induced plant loss. It is likely that the abundance of hydrilla in Prairie Branch allowed for sufficient forage for herbivores making them reluctant to enter the open exclosures to graze on the newly established native plants. Emergent plants, pickerel weed, water pennywort, American bulrush, American waterwillow, bull tongue and squarestem spikerush established and overall continued to thrive through herbicide and herbivory exposure. Future research will conclude if there is an ideal combination of using biological, ecological, mechanical and chemical approaches together in an IPM.

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