DISTRIBUTION AND MANAGEMENT OF INVASIVE PLANT SPECIES IN THE ROSS BARNETT RESERVOIR

By

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A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Agriculture
in the Department of Plant and Soil Sciences

Mississippi State, Mississippi

April 2011

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A point intercept survey was conducted from 2005 to 2010 on the Ross Barnett Reservoir near Jackson, MS to calculate the frequency of occurrence of all aquatic plant species in the Reservoir. Water lotus (*Nelumbo lutea* Willd.) was the native species that occurred most often, while alligatorweed (*Alternanthera philoxeroides* [Mart.] Griseb.) occurred most often with regard to non-natives. A logistic regression model indicated that as species richness increases, the probability of observing a non-native species also increases. Herbicide evaluations implied that the chemical imazapyr provided the largest biomass reduction in alligatorweed over a twelve week period; however, 2,4-D would be the most economical option for long-term control. A pathogen study on alligatorweed revealed the presence of the fungus (*Ceratorhiza hydrophilum* [Xu, Harrington, Gleason, Et Batzer, Comb., Nov. (*Sclerotium hydrophilum* [Sacc.]). Future studies should verify the potential or lack thereof of this fungus being a biological control agent on alligatorweed.

DEDICATION

I dedicate this thesis to my Lord and Savior Jesus Christ who makes all things possible by his love and grace, and to my parents Wayne and Diane. Their unending love and support for me during my many endeavors in my college career has been the foundation and motivation of my work. Through their encouragement, understanding, patience, and unselfishness, I was able to complete this thesis and degree. Words cannot express how thankful I am to be blessed with such wonderful parents and family who encouraged me at a young age to use all of my God-given abilities and fully develop my potential. My prayer is that one day I will be able to return to them at least a small portion of their gifts as thanks for their support and sacrifices.

ACKNOWLEDGEMENTS

I would like to especially thank Dr. John D. Madsen for guiding me and giving me the opportunity to further my education. I also owe a tremendous thanks to Dr. Ryan Wersal. Words cannot express how patient, understanding, and generous he was during my graduate research. For the never-ending questions that you so patiently answered Ryan, thank you. I would also like to thank Joshua Cheshier for assisting me with field trial setups, and providing me with the many gadgets that made my field work easier. Special thanks go to the Pearl River Valley Water Supply District for providing the funding and support that made this research possible. To Dr. Wilfredo Robles, Dr. Victor Maddox, Dr. Patrick Gerard, Dr. Jonathan Huff, Dr. Tim Schauwecker, my friends and family, and the many others who gave me direction and support during my years at Mississippi State University, thank you.

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Biology and Ecology of *Eichhornia crassipes*

Waterhyacinth (*Eichhornia crassipes* [Mart.] Solms) is a mat-forming, floating aquatic plant of the Pontederiaceae family, introduced into the United States before 1890 from South America. It can currently be found in Central America, North America (predominately southern states and California), Africa, India, Asia, and Australia. Waterhyacinth is adapted to a broad range of aquatic environments including lakes, ponds, rivers, ditches, and backwater areas. High nutrient availability in the water provides waterhyacinth with an environment optimal to its spread and growth (Aquatic Ecosystem Restoration Foundation 2005). Waterhyacinth can double its population in under a month due to its vigorous vegetative growth and has one of the highest growth rates of any known plant (Madsen et al. 1993). Problems associated with waterhyacinth include: decrease in water quality, mosquito control, and waterflow impediment (Owens and Madsen 1995). Navigation interference, fish and native plant mortality, and water loss from evapotranspiration are also problems attributed to waterhyacinth and its growth habit (Timmer and Weldon 1967). For energy reserves in times of stress, waterhyacinth stores carbohydrates in the stem base during the fall. However, due to the lack of mechanisms necessary for survival during cold temperatures, air temperatures below 0°C significantly decrease the survival rate of the plant (Owens and Madsen 1995).

Management of *Eichhornia crassipes*

Chemical Control

Control of waterhyacinth is mainly performed by chemical methods. Small and limited applications of herbicides such as 2,4-D [(2,4-dichlorophenoxy)acetic acid], diquat [6,7-dihydrodipyrido(1, 2-a:2', 1'-c) pyrazinediium] , and glyphosate [N-(phosphonomethyl)glycine] have been utilized in previous studies to decrease the surface cover of waterhyacinth. For each herbicide, only multiple applications were successful (Haag 1986; Haag and Habeck 1991; Lopez 1993). Although chemical control will suppress waterhyacinth distribution and densities, pollution of groundwater and health hazards of humans and wildlife are concerns (Haag 1986). According to Sacher (1978), glyphosate degrades in water and does not limit irrigation timing. Bronstad and Friestand (1985) also stated that glyphosate does not normally affect aquatic organisms or fish at the rates applied. The mode of action of glyphosate may be ideal for control of waterhyacinth since it is easily absorbed and translocated in broadleaf weeds, and waterhyacinth links itself by way of stolons (Lopez 1993). 2,4-D, however, is a more preferred choice in the U.S. for waterhyacinth control because of its selectivity, effectiveness, and low cost (Madsen 2004; AERF 2005).

Biological Control

Several insects have been introduced into the United States for biological control of waterhyacinth, and some insects are still presently being studied. *Neochetina eichhorniae* Warner and *N. bruchi* Hustache are two host-specific phytophagous weevils that were released in Florida after being imported from Argentina in 1972 and 1974. Other insects, particularly several arthropod species, have been investigated for possible effective control agents of waterhyacinth. Some of these include: an oribatid mite (*Orthogalumna terebrantis* Wallwork), a crambine moth (*Acigona ingusella* Walker), and an acridid grasshopper (*Cornops aquaticum* Bruner; Coulson 1971). Noted by Madsen (2006), suppression of waterhyacinth by indicated insect predators has predominantly been found only in reduction of flowering and biomass.

Biology and Ecology of *Hydrilla verticillata*

Hydrilla (*Hydrilla verticillata* [L.f.] Royle) is a submersed aquatic macrophyte that belongs to the family *Hydrocharitaceae*. It has been referred to as "the perfect aquatic weed" because of its adaptive characteristics that allow it to survive in many aquatic situations (Langeland 1996). A native of warmer areas in Asia, hydrilla was first discovered in the United States in 1958 on the west coast of Florida (Yeo et al. 1984). Over the next 25 years, hydrilla's presence was reported in 13 more states of the United States. Hydrilla populations can impose serious problems on waterflow and recreational activities including: filter clogging in irrigation pumps, boating, water skiing, fishing, swimming, and other water navigation activities (Yeo et al. 1984). Hydrilla also displaces native aquatic plants while becoming established. Because of its adaptive characteristics, hydrilla can out-compete other neighboring aquatic species for sunlight and nutrients, enabling it to take over the area. A very fast growth rate of up to one inch per day allows hydrilla to reach the water surface very quickly and then profusely branch out and produce a dense mat of stems (Haller and Sutton 1975). It will also tolerate a wide range of pH levels, nutrient levels, low light levels during photosynthesis (Van et al. 1976; Bowes et al. 1977), and can grow in water depths up to 15 meters (Steward 1991). The very efficient reproductive structures and methods of hydrilla (fragmentation, tubers, turions, and seeds) are conducive for surviving adverse conditions and continual distribution (Langeland 1996).

Management of *Hydrilla verticillata*

Chemical Control

Because hydrilla is very resistant to most aquatic herbicides (Blackburn and Weldon 1970), chemical control options are somewhat limited. Copper chelate [7-oxabicyclo (2.2.1) heptanes-2,3-dicarboxylic acid], diquat, endothall [7-oxabicyclo (2.2.1) heptanes-2,3-dicarboxylic acid], and fluridone [1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl] -4(1*H*)-pyridinone] are active ingredients that are effective for controlling hydrilla (Langeland 1996); however, resistance to fluridone has been detected (Michel et al. 2004). Several factors have been accredited to this developed resistance including hydrilla's fast growth and multiple means of propagation, favorable gene expression allowing adaptation in suppressed environments, and the use of a single herbicide which exposes the species to low doses over a long period of time (Arias et al. 2005). Blackburn and Weldon (1970) reported that low concentrations of copper added to diquat and endothall greatly increased control of hydrilla, with the most effective combination being copper and diquat. Though normally used for control of phytoplankton and algae, chelated copper compounds successfully control hydrilla (Madsen 2000).

Biological Control

Grass carp (*Ctenopharyngodon idealla* Val.) was introduced in 1970 in Florida for a potential biological control agent of hydrilla (Osborne and Sassic 1979). Although an effective control agent of hydrilla, grass carp is a non-specific herbivore and rarely used in multi-purpose water bodies that utilize aquatic vegetation for fishing and waterfowl habitat (Langeland 1996).

Over 40 species of insects in the United States have been studied and found to suppress hydrilla. Some of these include a weevil (*Bagous affinis* Hustache), a leaf mining fly (*Hydrellia pakistanae* Deonier), and an aquatic moth (*Parapoynx diminutalis* Snellen). The most damage of hydrilla observed by an insect was from the larvae of aquatic moths (Lepidoptera: Pyralidae; Balciunas and Minno 1985). However, factors such as predation, damage vs. hydrilla growth and reproduction ability, and timing of damage have prevented most of these insects from being favorable hydrilla control options (Langeland 1996).

Biology and Ecology of *Alternanthera philoxeroides*

Alligatorweed (*Alternanthera philoxeroides* [Mart.] Griseb.) is an aquatic, mat-forming weed introduced from South America into the United States in 1897, and has rapidly spread across the southern portion of the nation (Kay and Haller 1982). It is a member of the dicotyledon family Amaranthaceae, and has the ability to grow in a variety of conditions including conservation and agricultural systems of tropical, subtropical, and temperate climates (Julien and Stanley 1999). Described by Vogt and others (1979) as an amphibious plant because of its ability to grow in terrestrial or aquatic conditions, alligatorweed can adapt in many different environmental conditions and moisture levels. It is very likely that alligatorweed can grow under a broader spectrum of soil and water conditions than any other aquatic plant species (Wain et al. 1984). A perennial plant that rarely produces viable seed, alligatorweed reproduces by vegetative structures (Julien et al. 1995). It exhibits two distinctive morphological variations, attributed to different environmental conditions (Kay and Haller 1982). Alligatorweed in aquatic habitats has larger hollow stems, which provide buoyancy and gives them a free-floating mat-like habit. Terrestrial-growing alligatorweed has smaller diameter stems lacking aerenchyma (Julien and Chan 1992). Variances in response to herbicides suggest that one alligatorweed biotype may be more tolerant to some herbicides than the others (Kay 1992).

Management of *Alternanthera philoxeroides*

Chemical Control

Many techniques and procedures have been and are currently being used for the control of alligatorweed. Chemical control methods, such as applications of 2,4-D and glyphosate, are used on populations of alligatorweed. Glyphosate tolerance and control ineffectiveness in alligatorweed may be caused by poor translocation to roots and rhizomes, dilution by underground biomass, metabolism to nontoxic metabolites, and exudation from the roots (Eberbach and Bowmer 1995). In addition, high concentrations, multiple applications, and high cost associated with retreatments of herbicides in general for control of alligatorweed have made chemical control methods quite limited (Gangstad et al. 1975).

Biological Control

Biological control of alligatorweed was undertaken in the United States in the 1960s by introduction of a flea beetle, *Agasicles hygrophila* Selman and Vogt, a moth, *Vogtia malloi* Pastrana, and a thrips, *Amynothrips andersoni* O'Neill, from South America (Spencer and Coulson 1976). Control of alligatorweed predominately from damage done by the flea beetle and sometimes by a combination of the beetle and moth were observed in various locations. However, the flea beetle is only effective on the aquatic form and has no effect on terrestrial alligatorweed (Julien et al. 1995). The flea beetle also has a more limited survival zone than alligatorweed due to climate (temperature and altitude) restrictions (Buckingham et al. 1983). Few fungal species have been reported to be pathogenic on alligatorweed; although infections of *Alternaria alternantherae* Holcomb & Antonopoulos (Holcomb 1977) and *Cercospora alternantherae* (Barreto and Torres 1999; Xiang et al. 1998) have been documented to have a pathogenic response on alligatorweed. According to Gangstad and others (1975), integrating chemical and biological control methods provides the most effective and cost efficient control of alligatorweed.

Point Intercept Survey

The point-intercept method of gathering data is a relatively simple technique that records measurements at strategically spaced, defined locations over a preselected grid system. Having been broadly used in terrestrial plant and animal ecology surveys, it has also been adapted to use in aquatic plant ecology, and provides the alternative to randomly selecting research locations in the field (Madsen 1999). Finding these points in the field may be done manually, with a GPS (Global Positioning System), a GIS (geographic information system), or a mapping software package. After determining the distance between the points on the grid, environmental data can be entered into the system to provide additional information about the area (i.e. water depth, bottom type, etc.), with water depth being the most critical in all surveys dealing with aquatic plant occurrence. The presence/absence technique of recording species is used to calculate percent frequency of the species. A “1” indicates species present, and a “0” indicates the absence of a species. Observations of the species can be made from the surface using a bathyscope or by deploying a weighted rake to collect submersed plants. All observations taken at each point in a relative grid system should be done consistently by the same method. Percent frequency is then calculated by dividing the number of present marks by the total number of points on the grid and multiplied by 100. This gives a percentage of how often an aquatic plant species occurs in that area (Madsen 1999). This technique has been previously used in data analyses of invasive species population occurrences in the Ross Barnett Reservoir on a yearly basis since 2005 (Wersal et al. 2007). By using this analysis method in correlation with point intercept surveys, present management practices can be evaluated for their efficiency.

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CHAPTER II

ASSESSING THE AQUATIC PLANT COMMUNITY COMPOSITION WITHIN THE LITTORAL ZONE OF THE ROSS BARNETT RESERVOIR, MS: A SIX YEAR EVALUATION

Abstract

Alligatorweed (*Alternanthera philoxeroides* [Mart.] Griseb.), waterhyacinth (*Eichhornia crassipes* [Mart.] Solms), and hydrilla (*Hydrilla verticillata* [L.f.] Royle) are three non-native species of concern in the Ross Barnett Reservoir near Jackson, MS. Herbicide treatments have been performed over the last six years to suppress these species and prevent their spread. Point intercept surveys have been conducted on the Reservoir from 2005 to 2010 to monitor native and non-native species' distributions and assess treatment efficacy across the reservoir. American lotus (*Nelumbo lutea* Willd.) is the native species which has been observed the most throughout the survey years, with occurrence frequencies averaging between 17 and 27%. Alligatorweed populations significantly decreased from 21% in 2005 to 4% in 2006 due to rigorous herbicide applications; however, it has consistently increased in the last 4 years to 12% occurrence in 2010. Waterhyacinth occurrence has remained relatively constant over the study period, averaging below 10% occurrence. Hydrilla was discovered in the Reservoir in late 2005 and has remained below 2% in frequency of occurrence since 2006. Suppression of these non-native species is attributed to rigorous monitoring and herbicide applications conducted on the Reservoir since 2005. A logistic regression model

indicated that as native species richness increased, the likelihood of a non-native species occurring also increased; likewise, the occurrence of alligatorweed, an emergent non-native species, increased with increasing native species richness.

Introduction

The Ross Barnett Reservoir, located near Jackson, MS, is the state's largest surface water impoundment. This 13,355 hectare water body provides the city of Jackson with potable water, fishing, recreational opportunities, and wildlife habitat. The introduction of non-native plant species in the Reservoir has threatened its biodiversity and natural processes (Madsen 2004). Not only can multiple non-native plants do extreme harm to an area, but just one exotic species can alter an entire ecosystem if not controlled properly (Pimental et al. 2000). The exotic invasive plant hydrilla, was observed in the Reservoir in 2005 (Wersal et al. 2006a). This submersed aquatic plant is on the State and Federal Noxious Weed Lists and has been nicknamed "the perfect aquatic weed" due to its aggressive growth habit and adaptive morphological characteristics (Langeland 1996). Alligatorweed and waterhyacinth are also species of concern that have spread to a large degree and negatively impacted the Reservoir's services and available recreational opportunities. Impacts from these plant species, as well as other aquatic invasives, prompted the Pearl River Valley Water Supply District to create a long-term management plan to strategically monitor these plants and assess control methods to suppress their spread.

Systemic herbicide applications have primarily been the management technique used for alligatorweed and waterhyacinth over the last decade (Wersal et al. 2009). Hydrilla has been managed over the last six years by the combinations of the contact herbicides copper [7-oxabicyclo (2.2.1) heptanes-2,3-dicarboxylic acid] and diquat [6,7-dihydrodipyrido(1, 2-a:2', 1'-c) pyrazinediium] and the systemic herbicide fluridone [1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl] -4(1*H*)-pyridinone]. Applications of fluridone have proven successful, greatly reducing the populations of hydrilla in the Reservoir. However, fragmentation of hydrilla and water movement aid in dispersing this species and allow for new populations to develop. Alligatorweed and waterhyacinth populations have been greatly suppressed by applications of glyphosate [N-(phosphonomethyl)glycine] and 2,4-D [(2,4-dichlorophenoxy)acetic acid] since 2005; however, fluctuating water levels and varying plant densities throughout the Reservoir have made treatment efforts difficult at times. To ensure that the current management techniques are effective, intensive surveying and regular assessments are imperative to the success of any long-term management maintenance program (Madsen 2007). The objectives of this study were to 1) quantify changes in the aquatic plant community composition over time; 2) identify major factors that influence changes in plant community composition; 3) develop a simple model to predict areas within the reservoir that are more likely to promote the growth of hydrilla, alligatorweed, and waterhyacinth based on total species richness throughout the reservoir; and 4) assess the management strategies that are ongoing in the Ross Barnett Reservoir with respect to hydrilla, waterhyacinth, and alligatorweed.

Materials and Methods

Vegetation Survey

Surveys were conducted using a 300 meter grid of points (Madsen 1999; Wersal et al. 2009) in the summers of 2005 to 2010 to evaluate aquatic plant distribution in the

Reservoir. Only points located in the littoral zone (water depths of 3 meters or less) were surveyed. Light extinction coefficients were utilized to determine the optimal water depth(s) for rooted submersed macrophyte growth in the Reservoir (Wersal et al. 2006a). The maximum depth of macrophyte colonization in the Reservoir was estimated to be 2.2 m; therefore, the littoral zone was assigned depths of 3 m or less to ensure efficiency of sampling. Sampling of the littoral zone allowed for a more rigorous survey on the Reservoir at locations most favorable for plant growth (Fig. 2.1). Sampling of the same points from 2005 to 2010 allows changes in the plant community to be statistically quantified over time.

Survey accuracy of 1-3 meters (m) was achieved by using a Trimble AgGPS106™ receiver (Sunnyvale, California) coupled with a Panasonic C-29 Toughbook™ computer (Secaucus, New Jersey). A total of 677 points were surveyed in 2005, 508 in 2006, 423 in 2007, 627 in 2008, 695 in 2009, and 620 points in 2010. Variations in total sample locations between years resulted from water level fluctuations and plant population densities that inhibited or prohibited boat accessibility to the sampling locations. At each survey point, a weighted plant rake with an attached rope was deployed and pulled in to determine the presence or absence of plant species. Depth was recorded at each point with a Lowrance LCX-28C depth finder (Tulsa, Oklahoma) or with a sounding rod at depths less than 3 m. Navigation to survey points, the display and collection of geographic and attribute data while afield and spatial data were recorded electronically using FarmWorks Site Mate® software version 11.4 (Hamilton, Indiana) using templates and pick lists created for this project. Collecting survey data in this manner decreases the likelihood of errors in data entry and post processing time.

Non-Native Species Assessment

Data obtained from the point intercept surveys conducted on the Reservoir were used to assess management efficacy on hydrilla, waterhyacinth, and alligatorweed. A quantitative comparison was then made by the analysis of changes in the frequency of occurrence of each species between years. Spring applications of the herbicide fluridone and summer combination applications of chelated copper and diquat were implemented for hydrilla management on the Reservoir from 2005 to 2010.

Tuber surveys were conducted in the early springs of 2006 to 2010 to assess the current density of hydrilla tubers in the Ross Barnett Reservoir. Sampling the sediment for tubers in areas of known hydrilla occurrence allows for estimation of future hydrilla populations. A PVC coring device was used to collect 20 sediment samples at each site (Madsen et al. 2007). The sediment was sieved through a pail with a wire mesh bottom to separate the sediment from any plant material. Any tubers found were transported to Mississippi State University where they were sorted, dried, and weighed to calculate tuber biomass and density.

Foliar applications of the herbicides glyphosate (diammonium salt formulation), 2,4-D, or imazapyr [2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl)-3-pyridinecarboxylic acid] were applied to waterhyacinth and alligatorweed populations in the Reservoir from 2005 to 2010. Cuban bulrush (*Oxycaryum cubense* [Poepp. & Kunth] Lye) was treated with combination applications of 2,4-D and diquat in 2009 and 2010. The small population of waterlettuce (*Pistia stratiotes* L.) observed in 2009 was also treated with a combination of 2,4-D and diquat in the fall of that year and has not been observed in the Reservoir since those treatments. All herbicides were applied by licensed applicators in compliance with state and federal regulations. The authors of this paper did not administer any herbicides in this study.

Data Analysis

Plant species presence was averaged over all points sampled and multiplied by 100 to obtain percent frequency. Total species richness was calculated and presented as the mean (± 1 SE) of all species observed at each point. Mean species richness was compared between years using a general linear model. Changes in the occurrence of plant species was determined using McNemar's Test for dichotomous response variables that assesses differences in the correlated proportions within a given data set between variables that are not independent (Stokes et al. 2000; Wersal et al. 2006a; Wersal et al. 2008). Only points that were sampled in consecutive years were included in the analysis. A pairwise comparison of species occurrences was made between years using the Cochran-Mantel-Haenszel statistic (Stokes et al. 2000; Wersal et al. 2006b; Wersal et al. 2009; Cox et al. 2010). All analyses were conducted at the $P \leq 0.05$ significance level.

Logistic Model

A logistic regression model was developed using SAS® to determine the relationship between the presences of non-native species and increasing native species richness (Stokes et al. 2000). Only native species richness values that had more than 30 observations were used in the model; therefore, values ranged from one to five. The predictor variable (Native Species Richness) was transformed using the X + 1 procedure to eliminate zeros in the data range (Quinn and Keough 2002). The natural log was then calculated for the new range of values to reduce variability within the model. Logistic regression estimates the probability that a defined set of variables accurately predicts dichotomous or categorical variables (Trexler and Travis 1993; Buchan and Padilla 2000). The use of logistic regression is useful because it provides a measure of association (Buchan and Padilla 2000); in the case of this study, it was used to approximate the probability of observing a non-native species and alligatorweed alone given increasing native species richness. A similar model was used to determine the probability of observing alligatorweed as native species richness increases. Although data were transformed for analysis purposes, non-transformed probabilities are given for ease of interpretation when comparing to vegetation survey frequencies for a given species.

Results

Vegetation Survey

Surveys conducted on the Ross Barnett Reservoir from 2005 to 2010 resulted in 28 aquatic or riparian plant species (Table 2.1). The native plant American lotus was the most abundant species across all years. American lotus increased in occurrence from 17% in 2005 to 27% in 2010 (Table 2.1). The presence of white waterlily (*Nymphae odorata* Aiton) remained constant over time, while coontail (*Ceratophyllum demersum* L.) significantly decreased from 8% in 2008 to 4% in 2010. Other native species that commonly occurred were waterprimrose (*Ludwigia peploides* [Kunth] P.H. Raven) and giant cutgrass (*Zizaniopsis miliacea* [Michx.] Doll & Asch.). Species richness was significantly greater in 2009 than in 2008 where on average 1 plant species was observed per point (Fig. 2.2). Species richness was lower in 2006 than all other years with approximately 0.6 plant species observed per point. Water depth was a key determinant in species richness at each point during the year of the survey. Low water levels in 2005, 2006, and 2007 resulted in lower plant species occurrence per point. Increased water depth in 2008, 2009, and 2010 resulted in higher species richness per point; however, accessibility to some points was limited as plant community densities increased over time (Fig. 2.3).

Non-Native Species Assessment

Hydrilla and waterhyacinth had occurrences below 10% for all survey years (Table 2.1). The frequency of occurrence for alligatorweed decreased significantly ($P \leq 0.01$) from 21% in 2005 to 7% in 2008, increased to 15% in 2009, and decreased to 12% in 2010. Waterhyacinth decreased in frequency of occurrence from 2005 to 2007, while significantly increasing from 1% in 2007 to 4% in 2008 and 9% in 2009. Alligatorweed was the non-native species observed most often in all years, followed by waterhyacinth, hydrilla, brittle naiad (*Najas minor* All.), and parrotfeather (*Myriophyllum aquaticum* Vell. Verdc.) (Table 2.1). To date, a total of 16 hydrilla populations have been observed throughout the Reservoir; however, many of these populations have been eradicated by herbicide treatments and are still being monitored. Generally, the occurrence of all aquatic plant species was in Pelahatchie Bay and the northern portion of the Reservoir where water levels and environmental conditions favor plant growth.

Waterlettuce was not found during the 2010 survey after being observed in 2009 along a small channel in Pelahatchie Bay. Several small pockets (< 0.10 hectares) of waterlettuce were discovered in the early fall of 2009 and included into the management scheme of the Reservoir. It appears that the early detection and immediate combination applications of 2,4-D and diquat were successful at eradicating this species. Cuban bulrush was also discovered in Pelahatchie Bay in 2009 and is still well established there. Combination applications of 2,4-D and diquat were made in 2009 and 2010, but efficacy of those treatments is currently not attainable because the extent of its establishment is still being determined.

Logistic Model

This model was tested against the conceptual idea that greater native species richness inhibits non-native species occurrence and establishment (Hobbs and Huenneke 1992). We developed a logistic regression model to determine the relationship of increasing native species richness (i.e. increased growth) to observation of non-native plant species (i.e. resisting invasion), and to predict the probability of observing a non-native species or alligatorweed alone at given point. Our model found a significant positive relationship ($P \leq 0.01$) between the presence of a non-native species or alligatorweed alone and increasing native species richness. Based on our model, as species richness increases there is a greater likelihood of invasion by non-native species and in particular alligatorweed. For example, when native species were present at a sample point there was a probability of 0.14 of observing a non-native species (Fig. 2.4). However, as species richness increased to 5 (the maximum richness observed in this lake) the probability of observing a non-native species increased to 0.88. Similar to these results, the probability of observing alligatorweed at a sample point when native species were present at mean richness values of 1 and 5 were 0.10 and 0.81, respectively (Fig. 2.5).

Discussion

Vegetation Survey

Non-Native Species Assessment

The estimated coverage of alligatorweed had more than doubled from 2008 to 2009, according to survey data from 2009. This tremendous increase in occurrence may be attributed to the increase in water level of the Reservoir in 2009 and the addition of approximately 25 locations of alligatorweed observed up the Pearl River that were not surveyed in 2008 and cover an estimated 225 hectares (8.9 hectares per point). The estimated coverage of alligatorweed for 2010 decreased by approximately 283 hectares; this reduction is attributed to low water levels, rigorous herbicide applications, and a fluctuation in the number of surveyed locations between years. Small, existing alligatorweed populations along the river are likely responsible for supplying propagules and establishing new populations in the Reservoir. Dense pockets and pools of vegetation that are not accessible by boat may also provide plant propagules to the Reservoir. The significant increase in occurrence is most likely due to higher water levels and the ability to access more of the survey points to find these populations. The significant decrease in waterhyacinth from 2009 to 2010 is due to successful herbicide applications and low water levels making many infested areas inaccessible to survey. "Hidden" plants among dense stands of other plant species may also make surveying and treatment difficult.

Hydrilla Assessment

The suppression of hydrilla distribution in the Reservoir over the last 6 years is attributed to intensive management strategies. Approximately 5 of 16 total hydrilla populations have been eradicated on the Reservoir since 2005. Some of these populations have just recently been discovered; therefore, herbicide treatments have not had adequate time to become effective. Despite rigorous herbicide applications, 3 hydrilla populations have consistently persisted. These reoccurrences may be attributed to the inhibition of herbicidal activity on hydrilla due to water movement limiting chemical-plant contact times in these particular locations. Still, hydrilla has consistently reoccurred throughout the Reservoir in untreated locations. Fragmentation and transportation of hydrilla by mechanical boat parts is likely the cause of these new populations sporadically occurring throughout the Reservoir.

Subterranean turions, or tubers, produced by hydrilla are vital to the life cycle of this plant and may remain viable in undisturbed sediment for up to 4 years (Netherland 1997). Tuber surveys conducted on the Ross Barnett Reservoir since 2006 have yielded very few hydrilla tubers. Tubers were found in one location in the Reservoir in 2006, which explained the presence of new hydrilla plants in that location in 2008. Although no other tubers have been found, it is possible that hydrilla plants may be overwintering and re-growing from healthy root crowns with very little tuber production. Low tuber densities may decrease the year to year recruitment of hydrilla and possibly the number of herbicide treatments necessary for eradication. Fluridone treatments at 5.0 to 50 parts per billion (ppb) have been documented to inhibit tuber production as well as remove standing biomass (MacDonald et al. 1993). If herbicide treatments are reduced, minimizing fragmentation and transport of hydrilla within the Reservoir would become more critical.

Logistic Model

The logistic regression approach to predicting invasion success has been used for non-native species in lakes in Connecticut and Wisconsin (Buchan and Padilla 2000; Capers et al. 2007). Buchan and Padilla (2000) utilized water quality data to predict the invasion of Wisconsin lakes by Eurasian watermilfoil (*Myriophyllum spicatum* L.). Our data corroborates those reported by Capers et al. (2007) where increasing native species richness did not resist invasion by non-native species as spatial scales increase. In most of the lakes the authors investigated, a positive relationship was found, indicating that native and non-native species have an affinity for the same abiotic resources. Although studies on invasibility and invasion success are variable in their conclusions, data from this study support the claim that the "rich get richer" (Stohlgren et al. 1999; Stohlgren et al. 2003). This means that areas already rich in total species will be invaded more often than areas of low species richness and, at least over short time periods, have a net increase in total species richness.

Although native species richness does not impede invasion, native plant density was shown to have a negative effect on the presence of non-native species (Capers et al. 2007). Dense native plant beds are presumably better able to prevent the colonization and establishment of non-native propagules thus reducing the invasibility of non-native species (Capers et al. 2007). This typically only occurs at very high plant densities and high densities may not be achievable due to re-occurring disturbance (Shea and Chesson 2002; Capers et al. 2007) or frequent re-introduction of non-native propagules by humans. There are many factors that determine the invasibility of a habitat, such as species richness, plant density, inter and intraspecific interactions, habitat complexity, resource availability, and abiotic factors; and many of these are interconnected and difficult in separating their direct influences.

The use of the point intercept survey facilitated the quantitative assessment of a lake-wide non-native plant control program for the Ross Barnett Reservoir. The use of herbicides resulted in the suppression of hydrilla, alligatorweed, and waterhyacinth with no significant long-term impact to the native plant community. Our logistic regression model indicated that areas of high species richness could be used to predict the probability of invasion by non-native species. Therefore, existing mixed plant species communities are more likely to be invaded by non-native plants than areas without native plants. The addition of other variables in the model would increase the predictive power and aid in further identifying specific areas of the lake that are more susceptible to invasion. Monitoring can then be focused more intensely in these areas making early detection and rapid response feasible.

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Table 2.1 Percent frequency of occurrence for aquatic plant species observed in the littoral zone during the Ross Barnett Reservoir Surveys 2005-2010. The letter 'n' refers to the total number of points sampled in a given year. Letters in a row for a given species denotes a significant difference among years at a $P \leq 0.05$ level of significance.

Species Name	Common Name	Native (N) or Exotic (E), Invasive (I)	2005 % Frequency (n=677)	2006 % Frequency (n=508)	2007 % Frequency (n=423)	2008 % Frequency (n=627)	2009 % Frequency (n=695)	2010 % Frequency (n=620)
Alternanthera philoxeroides	alligatorweed	E I	21	4	4	7	15a	12
Azolla caroliniana	mosquito fern	N	0	0	0	0	1	0
Cabomba caroliniana	fanwort	N	2	0	1	1a	1	0
Ceratophyllum demersum	coontail	N	4	5	4	8a	4a	4
Colocasia esculenta	wild taro	E I	0	1	1	2a	2	2
Eichhornia crassipes	waterhyacinth	E I	5	3	1	4a	9a	5a
Hydrilla verticillata	hydrilla	E I	0	1a	1a	1a	1	1
Hydrocotyle ranunculoides	pennywort	N	6	1	1	3a	1a	0
Juncus effusus	common rush	N	0	0	0	0	2	2
Lemna minor	common duckweed	N	3	3	2	1a	1	2
Limnobium spongia	American frogbit	N	2	1	1	1	0	0
Ludwigia peploides	waterprimrose	N	5	7	4	10a	15a	12
Myriophyllum aquaticum	parrotfeather	E I	1	0	0	1a	0	0
Najas minor	brittle naiad	E I	0	0	2a	1a	0	0
Nelumbo lutea	American lotus	N	17	18	21	25a	27	27
Nitella sp.	stonewort	N	0	0	0	0	0	0
Nymphaea odorata	white waterlily	N	4	3	5	5	6	5
Oxycaryum cubense	Cuban bulrush	E I	-	-	-	-	-	0
Pistia stratiotes	waterlettuce	E I	-	-	-	-	-	0
Potamogeton foliosus	leafy pondweed	N	0	0	0	1	0	0
Potamogeton nodosus	American pondweed	N	3	3	2	3	3	1
Sagittaria latifolia	broadleaf arrowhead	N	1	1	0a	1	1	1

Table 2.1 (continued)

Species Name	Common Name	Native (N) or Exotic (E), Invasive (I)	2005 % Frequency (n=677)	2006 % Frequency (n=508)	2007 % Frequency (n=423)	2008 % Frequency (n=627)	2009 % Frequency (n=695)	2010 % Frequency (n=620)
Sagittaria platyphylla	delta arrowhead	N	0	2	1	0a	2a	1
Scirpus validus	softstem bulrush	N	1	0	0	0	0	0
Spirodella polyrhiza	giant duckweed	N	0	0	0	0	1	1
Typha sp.	cattail	N	1	2a	1	1	7a	6
Utricularia vulgaris	bladderwort	N	0	0	0	1	0	0
Zizaniopsis miliacea	giant cutgrass	N I	2	4	2a	4	10a	9

Note: An "a" indicates a statistically significant change in frequency of occurrence from the previous year for the indicated plant species.

Table 2.2 Estimated and treated surface hectares of non-native plant species in the Ross Barnett Reservoir from 2005 to 2010.

Species	2005 Estimated Hectares	2006 Estimated Hectares	2007 Estimated Hectares	2008 Estimated Hectares	2008 Hectares Treated[1]	2009 Estimated Hectares	2009 Hectares Treated[1]	2010 Estimated Hectares	2010 Hectares Treated[1]
Alligatorweed	1285	179	153	413	137	934	124	665	263
Brittle naiad	0	0	72	45		18		9	
Hydrilla	49	27	45	36	111	54	63	54	32
Parrotfeather	45	45	9	54		27		9	
Waterhyacinth	297	135	45	225	68	539	227	287	166
Waterlettuce*	-	-	-	-	-	-	2*	0	0.2
Cuban bulrush*	-	-	-	-	-	-	21*	9	53

Notes: [1]Hectares treated refers to the total surface area of water treated, not necessarily to the point of plant infestation.
*Denotes first observation in 2009 of the indicated plant species

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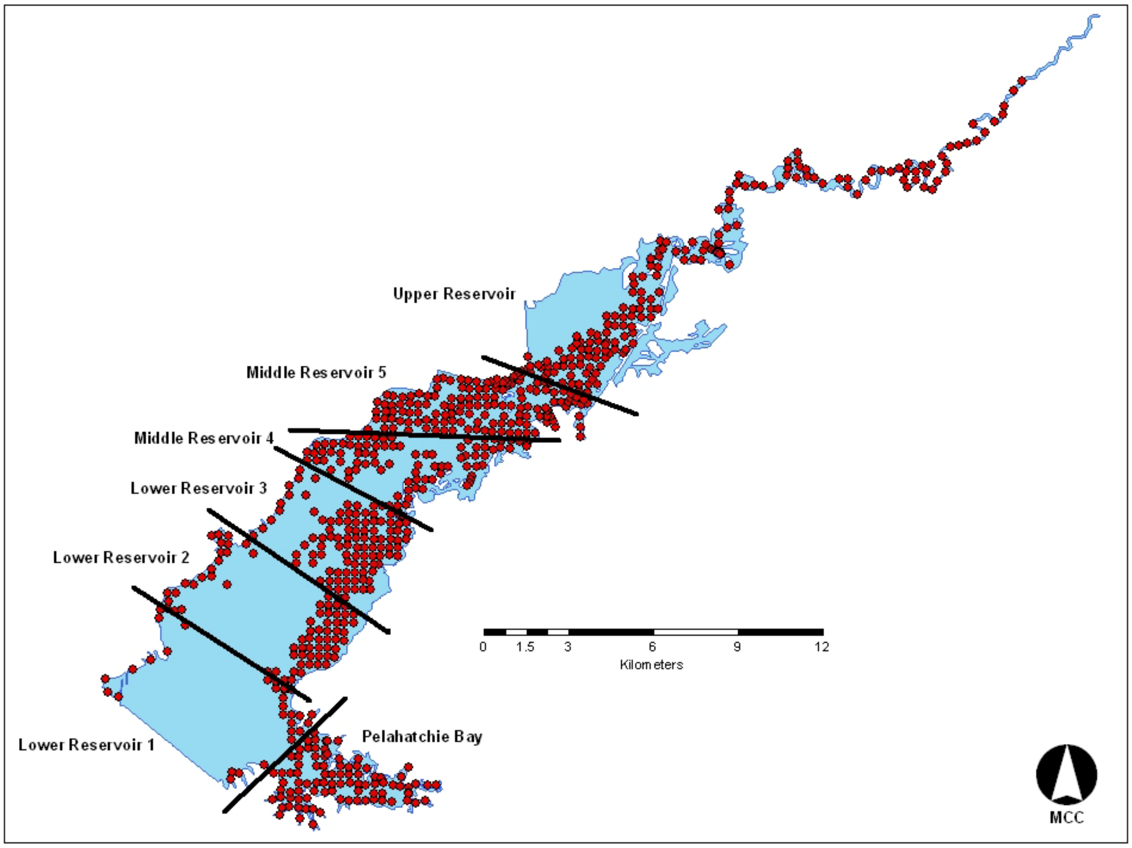

Figure 2.1 Sampling locations within the littoral zone of the Ross Barnett Reservoir from 2005 to 2010.

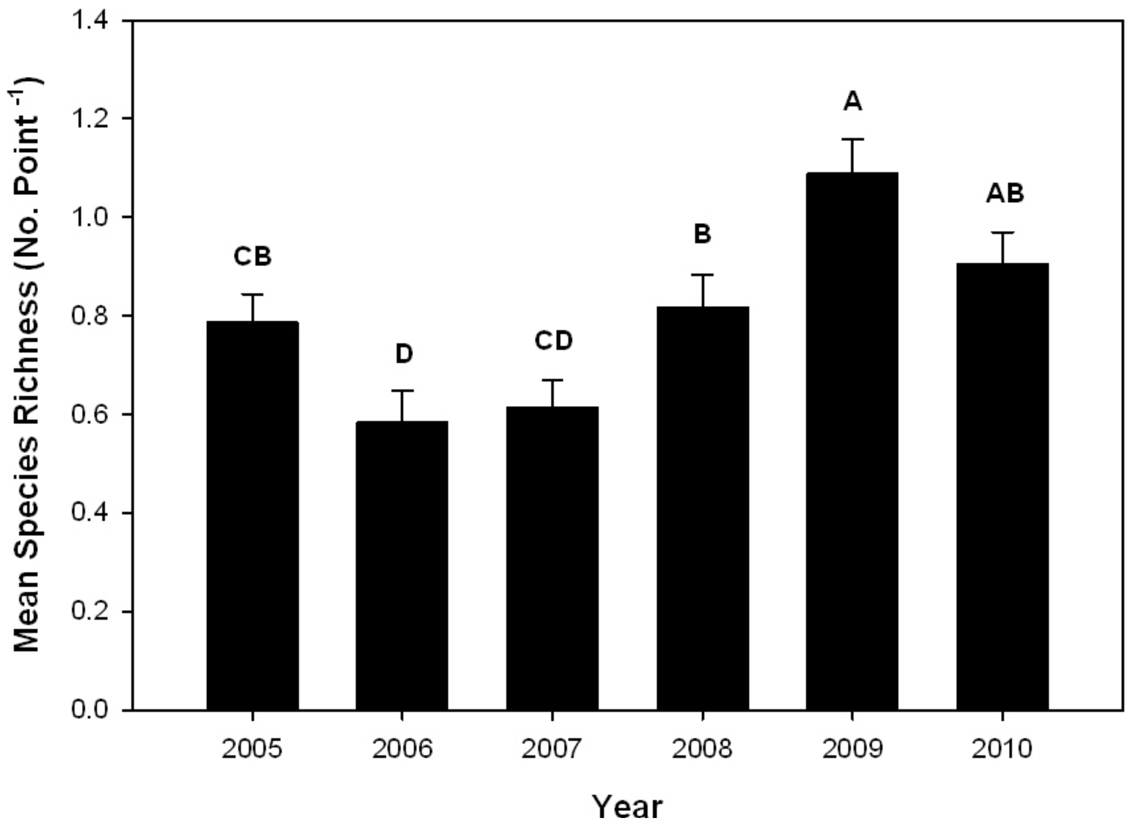

Figure 2.2 Mean plant species (number of species observed per point) at each sampled location on the Ross Barnett Reservoir from 2005 to 2010.

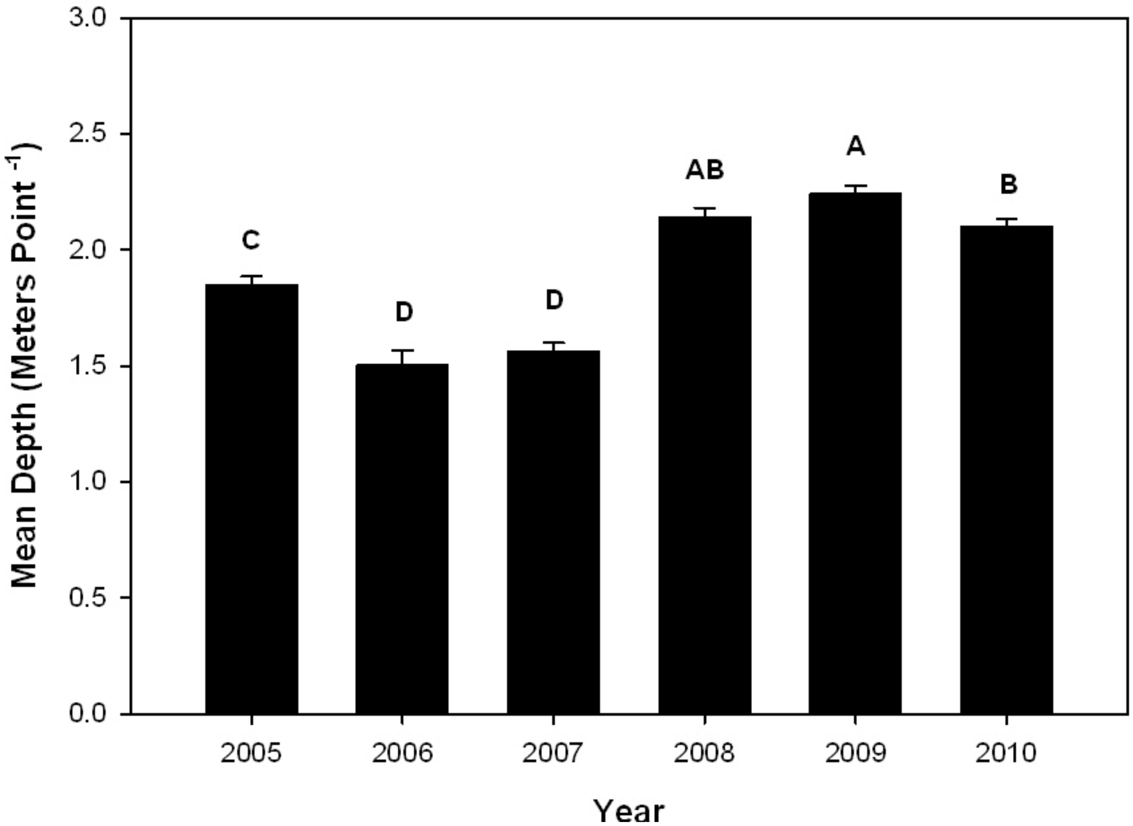

Figure 2.3 Mean water depth (meters per point) at each sampled location on the Ross Barnett Reservoir from 2005 to 2010.

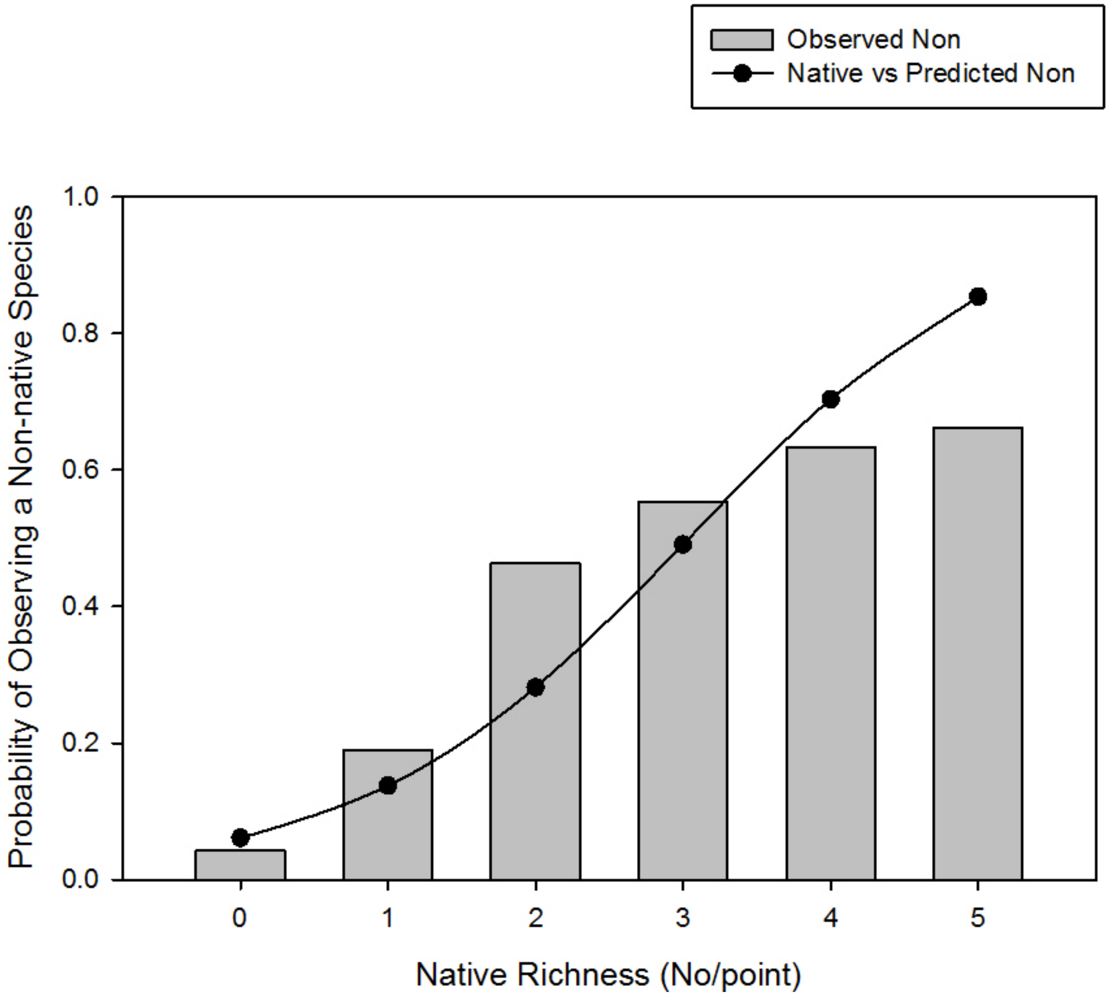

Figure 2.4 The probability of observing a non-native species in the presence/absence of a native species.

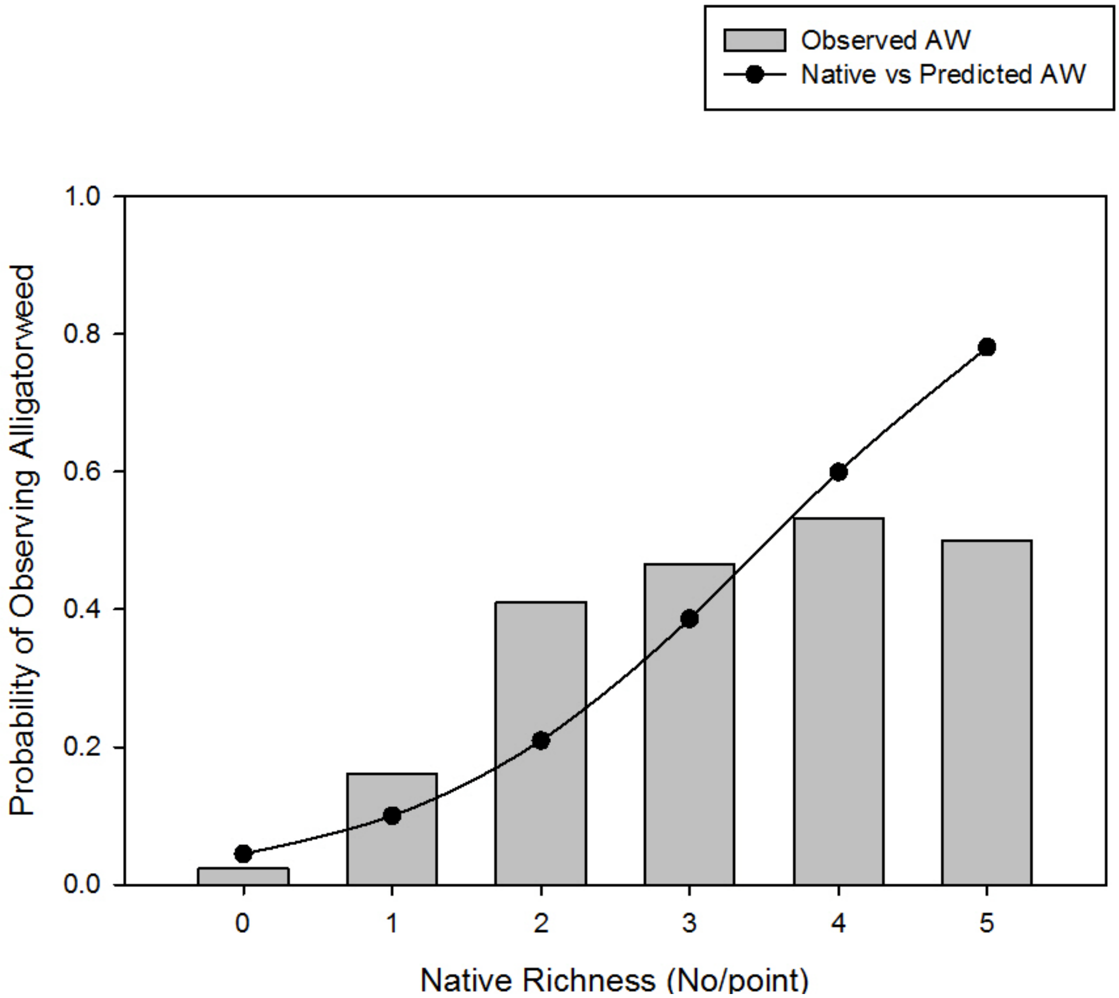

Figure 2.5 The probability of observing alligatorweed in the presence/absence of a native species.

CHAPTER III

EVALUATION OF FOLIAR APPLIED HERBICIDES FOR ALLIGATORWEED (*ALTERNANTHERA PHILOXEROIDES*) CONTROL

Abstract

Alligatorweed (*Alternanthera philoxeroides* [Mart.] Griseb.) is an invasive, aquatic plant native to South America that has invaded the United States and over 32 countries around the world. Alligatorweed forms hollow stems that produce adventitious roots at the nodes and anchor into nearby sediment or organic matter. This aquatic invasive plant is capable of forming dense, floating mats that may impede boat traffic, harbor insects carrying pathogens, cause flooding, and reduce overall water quality. The objective of this study was to evaluate the control of alligatorweed with eight different herbicides applied at both half and the maximum label rate: diquat (2.24 and 4.48 kg ai/ha), glyphosate (isopropylamine salt at 2.27 and 4.54 kg ae/ha), 2,4-D (1.06 and 2.13 kg ae/ha), carfentrazone-ethyl (0.11 and 0.22 kg ai/ha), penoxsulam (0.05 and 0.101 kg ai/ha), imazamox (0.28 and 0.56 kg ae/ha), imazapyr (0.56 and 1.12 kg ae/ha), and triclopyr (3.36 and 6.72 kg ae/ha). Visual control ratings (0-100%) were taken every 7 days, beginning after treatment. At 28, 56, and 84 days after treatment (DAT) plant tissue was harvested and weighed to determine biomass. Carfentrazone-ethyl applied at both rates did not effectively control alligatorweed 1 to 12 weeks after treatment (WAT). Applications of glyphosate, 2,4-D, imazamox, imazapyr, triclopyr, and the maximum label rates of diquat and penoxsulam did not significantly differ 12 WAT with respect to

dry weight. The application of imazapy at 0.56 kg ae/ha resulted in 99% biomass reduction with no regrowth to 12 WAT.

Introduction

Alligatorweed (*Alternanthera philoxeroides* [Mart.] Griseb.) is an invasive aquatic plant native to South America (Vogt et al. 1979) that has become a nuisance in the United States, particularly in the southern states (Kay and Haller 1982). Alligatorweed can be characterized by its oppositely arranged, lanceolate leaves, white flowers with a prominent style arranged in a globular spike supported by hollow stems (Buckingham 2002). Alligatorweed reproduces primarily by vegetative means in the United States, although reproduction by seed has been documented in South America (Holm et al. 1997; Julien et al. 1995). Often referred to as an amphibious plant (Vogt et al. 1979) because of its ability to exhibit two distinctive morphological variations, alligatorweed can be found in an aquatic or terrestrial form (Kay and Haller 1982). One morphological form of alligatorweed produces long leaves and large, hollow stems that provide buoyancy in aquatic settings (Wain et al. 1984). The terrestrial variation has shorter leaves and more lignified stems that are smaller in diameter and lack aerenchyma (Julien and Bourne 1988; Julien and Chan 1992).

As stems and stolons mature, they form impenetrable mats that may extend several meters from shorelines into waterways (Spencer and Coulson 1976). When stems become fragmented, floating sections of alligatorweed may drift to new locations and root in available substrate (Sainty et al. 1998). Dense populations of alligatorweed provide favorable habitat to many harmful insects that are vectors of disease (Ferguson 1968). As with other invasive aquatic plants, the presence of alligatorweed also increases flood risk, reduces water quality, clogs irrigation canals, and increases water loss due to evapotranspiration, resulting in increased production costs for agricultural systems and reductions in property values (Carpenter 1980; Gangstad et al. 1975; James et al. 2001; Rockwell 2003). Wetland and marsh habitat that provide refuge to many animal species and a rich diversity of native plant species are negatively impacted by alligatorweed through reductions in light penetration, a decrease in dissolved oxygen, competition for nutrients, and reductions in habitat complexity (Quimby and Kay 1977; Vogt et al. 1992; Buckingham 1996; Holm et al. 1997).

Various techniques have been used for controlling alligatorweed. Physical control methods have proven to be unsuccessful at controlling alligatorweed due to fragmentation of the plant that leads to redistribution and further spread (Holm et al. 1997). The alligatorweed flea beetle (*Agasicles hygrophila* Selman and Vogt) has been successful at controlling alligatorweed in temperate climates but not in northern locations where mean winter temperatures fall below 11.1 C (Coulson 1977; Vogt et al. 1992). Herbicides have also been widely used for management of this invasive species. Penoxsulam [2-(2,2-difluoroethoxy)--6-(trifluoromethyl-N-(5,8-dimethoxy[1,2,4] triazolo[1,5-c]pyrimidin-2-yl))benzenesulfonamide], an ALS inhibiting herbicide registered for aquatic use in 2009, applied at 0.035 kg ha^{-1} provided biomass reductions of alligatorweed greater than 70% 42 days after treatment (DAT); though control decreased as temperatures increased (Willingham et al. 2008).

Applications of 2,4-D [(2,4-dichlorophenoxy)acetic acid] and glyphosate [N-(phosphonomethyl)glycine] are currently the most used herbicides for control of alligatorweed due to their consistent suppression of the species (Eberbach and Bowmer 1995; Earle et al. 1951; Eggler 1953; Kay 1999). 2,4-D has been a preferred choice in the U.S. for alligatorweed control because of its effectiveness and low cost (Madsen 2004; AERF 2005). Although glyphosate is commonly used for alligatorweed control, tolerances of glyphosate in alligatorweed may be caused by poor translocation to roots and rhizomes, dilution, metabolism, and exudation by roots (Eberbach and Bowmer 1995). Imazapyr [2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl)-3-pyridinecarboxylic acid] applied at 1.04 kg ae ha^{-1} provided approximately twice the amount of control of *A. philoxeroides* than triclopyr amine [(3,5,6-trichloro-2-pyridinyl)oxy]-acetic acid) at 5.18 kg ae ha^{-1} in April of the treatment year; however, control of *A. philoxeroides* did not significantly differ in July of the same year using either herbicide (Allen et al. 2007).

Due to the unreliable control of alligatorweed with some herbicides, research needs to identify additional options for effective control. Relying only on 2,4-D may result in herbicide resistance in the future; therefore, options need to be in place for herbicide stewardship. The objective of this study was to screen available aquatic labeled herbicides that can be applied to the foliage of alligatorweed. These data will provide recommendations for herbicide alternatives to 2,4-D and glyphosate.

Materials and Methods

Planting

The study was conducted in 76, 240-L mesocosms at the R. R. Foil Plant Science Research Facility, Mississippi State University, for 12 weeks from June to August 2009 and repeated again in 2010. Alligatorweed samples were obtained from a pond on the campus of Mississippi State University. Two stems, approximately 20 cm in length, were planted into each of 760, 4.2-L poly-cel bags containing a top soil, loam, and sand mixture. Soil was amended with 2 g L^{-1} (0.27 oz gal^{-1}) of Osmocote fertilizer (24-8-16) (Scotts-Sierra Horticultural Products Company, Marysville, OH) to maintain growth throughout the 12 week time span. Ten bags of planted alligatorweed were placed into each of the 76 mesocosms. Water levels in each mesocosm were maintained at approximately 8 cm above the soil line. Plants were allowed 3 weeks to acclimate and grow in their respective mesocosms prior to herbicide treatment. A single pretreatment biomass sample was collected from every mesocosm on the day of herbicide application by cutting plant biomass at the sediment surface. Plants were dried for at least 7 days at 70 C and weighed for pretreatment biomass.

Treatment Methods

Foliar applications of the following herbicides at maximum and half-maximum label rate were made: diquat (Reward®, Syngenta Professional Products, Greensboro, NC) (2.24 and 4.48 kg ai/ha), glyphosate (Rodeo®, Dow Agrosciences, Indianapolis, IN) (isopropylamine salt at 2.27 and 4.54 kg ae/ha), 2,4-D (DMA 4-IVM®, Dow Agrosciences, Indianapolis, IN) (1.06 and 2.13 kg ae/ ha), carfentrazone-ethyl [ethyl α,2-dichloro-5-(4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl)-4-

fluorobenzenepropanoate] (Stingray™, FMC Corporation, Philadelphia, PA) (0.11 and 0.22 kg ai/ha), penoxsulam (Galleon SC®, SePRO Corporation, Carmel, IN) (0.05 and 0.101 kg ai/ha), imazamox [2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl)-5-(methoxymethyl)3-pyridinecarboxylic acid-3-pyridinecarboxylic acid] (Clearcast®, BASF Corporation, Research Triangle Park, NC) (0.28 and 0.56 kg ae/ha), imazapyr (Habitat®, BASF Corporation, Research Triangle Park, NC) (0.56 and 1.12 kg ae/ha), and triclopyr (Renovate® 3, SePro Corporation, Carmel, IN) (3.36 and 6.72 kg ae/ha). Herbicides were applied to plant foliage at a spray volume of 468 L ha^{-1} using a CO_2-pressurized, single-nozzle (8002 flat fan (TeeJet Technologies, Wheaton, IL)) spray system (R&D Sprayers, Opelousas, LA). A nonionic surfactant (Dyne-Amic®, Helena Chemical Company, Collierville, TN) was added to the spray solution at a rate of 0.5% vol:vol. All foliar herbicide treatments were replicated in four mesocosms.

Data Analysis

Alligatorweed was visually rated weekly from 0 to 100% control (0, no control; 100, complete control) for 12 weeks. Visual ratings are reported, however, these data were not subjected to statistical analyses. Twelve weeks after treatment, live plant material was harvested at the soil surface, dried for at least 7 days at 70 C, and weighed to determine plant biomass. Pretreatment biomass was 7.54 g dry weight (DW) pot^{-1}, and by 12 WAT, the untreated control plant biomass had increased to 78.21 g DW pot^{-1} indicating plants were actively growing throughout the study. A mixed procedures model was developed in SAS® using treatment as the main effect and year as a random effect to determine difference in plant biomass at 4, 8, and 12 WAT. If a significant main effect was observed, means were separated by least square means and grouped using the

Fisher's LSD procedure. Analyses were conducted within WAT at a $P \leq 0.05$ significance level.

Results

Carfentrazone-ethyl and the half maximum label rate of diquat resulted in significantly less control 12 WAT (Table 3.1). Maximum label rate applications of penoxsulam, glyphosate (IPA salt formulation), 2,4-D, triclopyr, imazamox, imazapyr, and diquat resulted in biomass reductions of 87%, 95%, 94%, 95%, 96%, 99%, and 94%, respectively, but did not significantly differ 12 WAT with respect to dry weight (Table 3.1). However, the use of imazapyr resulted in almost 100% biomass reduction from 1 to 12 WAT.

Discussion

Although carfentrazone-ethyl did not control alligatorweed 12 WAT, the herbicide showed excellent initial control (80-90%) with regrowth occurring approximately 2 to 3 WAT. This suggests that combinations of carfentrazone-ethyl or diquat with a systemic herbicide such as 2,4-D or glyphosate may increase control of alligatorweed by utilizing the initial control of a contact herbicide with the long-term control usually exemplified by a systemic herbicide. Still, some herbicide combinations may exhibit antagonistic effects. Wersal and Madsen (2010) reported evidence of antagonism with combinations of penoxsulam and diquat when applied to the foliage of waterhyacinth and common salvinia.

Similar to results observed by Willingham et al. (2008) except for the 0.035 kg ai ha^{-1} concentration, penoxsulam provided excellent control of alligatorweed 6 WAT. However, by 12 WAT a decrease in the efficacy of penoxsulam was observed, suggesting that foliar applications of penoxsulam do not provide long term control of alligatorweed. Increases in temperature have been documented to reduce efficacy of penoxsulam on alligatorweed (Willingham et al. 2008). Pursuant to this, spring applications of penoxsulam may provide significantly better control of alligatorweed than summer treatments when temperatures are normally highest. Biomass is typically lower during the spring, suggesting that herbicide treatments should provide greater control when applied during this time. Results from a study by Allen et al. (2007) showed that applications of imazapyr at rates of 0.29-1.04 kg ae ha^{-1} gave better control of alligatorweed in April of the treatment year than triclopy applied at 1.73-5.18 kg ae ha^{-1}, while there was no significant difference in control between the herbicide treatments in July.

Greater biomass densities in the summer, as well as low movement of the herbicide within the plant, may reduce herbicide efficacy and overall control. As documented by Bowmer et al. (1993) and Tucker et al. (1994), limited efficacy of glyphosate in alligatorweed has been attributed to a low rate of translocation to roots. Glyphosate provided excellent control (80-100%) of alligatorweed 4 to 8 WAT. However, control of alligatorweed decreased by 12 WAT when biomass increased by 43% over plants harvested during the 4 WAT harvest. Overall, glyphosate still provided good control (70-90%) 12 WAT, indicating that it is an option for longer-term control of alligatorweed.

The use of imazapyr visually resulted in 100% control, though biomass reductions were similar to glyphosate (IPA salt), imazamox, triclopyr, 2,4-D, and the maximum label rates of diquat and penoxsulam 12 WAT. Alligatorweed control with applications of imazamox was not significantly different 12 WAT than imazapy, triclopyr, glyphosate

(IPA salt), or penoxsulam and diquat at the maximum label rate, with respect to biomass. Although imazamox is somewhat of a new aquatic herbicide, like imazapyr, it is a member of the imidazolinone family (ALS or AHAS inhibitors) (Senseman 2007) and shows excellent long-term control of alligatorweed (Table 3.1).

Applications of triclopyr provided very good control (80-100%) of alligatorweed 1 to 12 WAT in this study but did not significantly differ to glyphosate (IPA salt), imazamox, imazapyr, 2,4-D, or the maximum label rates of diquat and penoxsulam 12 WAT, with respect to biomass.

Applications of 2,4-D provided excellent control (80-100%) of alligatorweed 6 WAT in this study, while some re-growth began to appear approximately 7 WAT and control began to slightly decline (70-90%) to 12 WAT. These results directly correspond with Eggler's (1953) work and indicate that repeated applications of 2,4-D would increase treatment efficacy.

Based on the results of this study, foliar applications of imazapyr, imazamox, triclopyr, glyphosate (IPA salt formulation), or 2,4-D would provide similar control of alligatorweed resulting in > 90% biomass reductions . Though when considering the industry standards, imazapyr, triclopyr, and glyphosate are two to five times the cost per liter of the herbicide 2,4-D. When the cost per liter is applied to the maximum label rate of the herbicide, application of imazapyr is approximately twice the cost per hectare of 2,4-D. The cost per liter of imazamox greatly exceeds the unit cost of imazapyr, resulting in over seven times the application cost of 2,4-D. Triclopyr is generally less expensive per liter of herbicide than imazapyr and imazamox but significantly more expensive per hectare than imazapyr, glyphosate, and 2,4-D due to high label rates of application. This cost comparison between the suggested herbicides shows 2,4-D to be the most economical choice for control of alligatorweed. Future work should evaluate combinations with low use rates, herbicide timing with plant phenology, and developing an Integrated Pest Management strategy consisting of integrating the alligatorweed flea beetle and herbicides.

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Table 3.1 Mean dry weight (g) of alligatorweed following foliar aquatic herbicide applications.

	Weeks after treatment[a,b]			
Herbicide Treatment	4	8	12	Biomass Reduction 12 WAT (%)
Carfentrazone-ethyl 0.11 kg ai ha^{-1}	11.1 bc	19.2 b	34.8 c	56
Carfentrazone-ethyl 0.22 kg ai ha-1	14.0 b	17.7 b	51.3 b	35
Diquat 2.24 kg ai ha-1	6.7 cde	12.0 c	45.1 bc	42
Diquat 4.48 kg ai ha-1	4.4 de	3.6 de	4.7 de	94
Glyphosate (IPA salt) 2.27 kg ae ha-1	3.4 e	3.6 de	7.5 de	90
Glyphosate (IPA salt) 4.54 kg ae ha-1	2.4 e	2.8 de	4.2 e	95
Imazamox 0.28 kg ae ha-1	2.0 e	0.3 e	2.0 e	98
Imazamox 0.56 kg ae ha-1	2.7 e	1.3 de	3.1 e	96
Imazapyr 0.56 kg ae ha-1	1.3 e	1.8 de	0.0 e	99
Imazapyr 1.12 kg ae ha-1	1.3 e	0.1 e	0.3 e	99
Penoxsulam 0.05 kg ai ha-1	9.8 bcd	10.1 c	17.5 d	78
Penoxsulam 0.10 kg ai ha-1	10.5 bc	6.9 cd	9.8 de	87
Triclopyr 3.36 kg ae ha-1	2.0 e	3.3 de	4.2 e	95
Triclopyr 6.72 kg ae ha-1	4.0 de	0.8 e	3.8 e	95
2,4-D 1.06 kg ae ha-1	3.7 e	1.0 de	7.0 de	91

Table 3.1 (continued)

	Weeks after treatment[a,b]			
Herbicide Treatment	4	8	12	Biomass Reduction 12 WAT (%)
2,4-D 2.13 kg ae ha	12.6 e	1.9 de	4.7 de	94
Untreated reference	35.5 a	27.9 a	78.2 a	0

[a] Means in a column followed by the same letter are not statistically different according to a Fisher's Protected LSD test at a $P \leq 0.05$ level of significance.

[b] Analyses were conducted within weeks not across weeks, therefore comparisons can only be made within a given column.

CHAPTER IV

IDENTIFICATION OF THE AQUATIC PLANT PATHOGEN, *CERATORHIZA HYDROPHILUM*, [XU, HARRINGTON, GLEASON, ET BATZER, COMB., NOV. (*SCLEROTIUM HYDROPHILUM* [SACC.]), ISOLATED FROM ALLIGATORWEED (*ALTERNANTHERA PHILOXEROIDES* [MART]. GRISEB.)

Abstract

Few biological control agents have proven to be successful at controlling alligatorweed (*Alternanthera philoxeroides* [Mart.] Griseb.). Some fungi species such as *Nimbya alternantherae* (=*Alternaria alternantherae*), and *Cercospora alternantherae* have been documented to be pathogenic on alligatorweed. The objective of this study was to determine if any pathogenic fungal species were present in the Ross Barnett Reservoir, near Jackson, MS, that may have potential biocontrol abilities for use on alligatorweed. Nine fungal species were identified from the alligatorweed tissue samples. Of these nine species, 5 fungal isolates illustrating *Rhizoctonia*-like characteristics were furthered studied due to the history of plant-pathogenic properties associated with *Rhizoctonia spp*. The pathogenic fungus *Ceratorhiza hydrophilum* was identified from several alligatorweed tissue samples. *Ceratorhiza hydrophilum* has been observed on other aquatic or semi-aquatic plant species; however, there is no indication that *C. hydrophilum* may be a potential biocontrol agent for use on alligatorweed at this time.

Introduction

Alligatorweed is an emergent, perennial plant native to South America. It is a nuisance species in aquatic and riparian regions of temperate to tropical climates of the world (Kay and Haller 1982; Madsen 2004; Sculthorpe 1967). Pathogenic responses on alligatorweed have been documented through infections of the fungi *Alternaria alternantherae* Holcomb & Antonopoulos (Holcomb 1977), *Nimbya alternantherae*, and *Cercospora alternantherae* (Barreto and Torres 1999; Xiang et al. 1998). Injury associated with *Alternaria alternantherae* is minimal and does not provide long-term control of alligatorweed (Holcomb 1977). Symptoms of *Nimbya* species on alligatorweed consist of purple/red stem lesions, chlorosis, leaf damage, and stem fragmentation. Research on *Nimbya* species for potential biocontrol of weed species is currently being conducted (Gilbert et al. 2004).

The objectives of this study were to isolate any fungal species present on alligatorweed tissue samples taken from the Ross Barnett Reservoir and accurately identify the fungal species to determine if they are pathogenic on alligatorweed based on previous research and documentation.

Materials and Methods

Sixty alligatorweed samples were collected in Pelahatchie Bay and the upper lake portion of the Ross Barnett Reservoir in September 2009. Plants slightly damaged by herbivory of the alligatorweed flea beetle (*Agasicles hygrophila*) (Figure 4.1) were selectively chosen for the study, due to the injury providing favorable pathogen entrances into plant tissues. A weighted rake was deployed and used to gather plant samples from the water. Plants were then dried thoroughly with towels, placed in labeled plastic bags, and stored in coolers for transport.

Once transported to the R. R. Foil Plant Science Research Center at Mississippi State University in Starkville, MS, the samples were refrigerated for approximately two weeks. Fungal isolation was implemented to obtain pure cultures of associated pathogens present on foliar tissues. Water agar (WA; 12 g/L) was poured into petri plates and solidified. Two nodes and three leaf bases with petioles attached were included for isolation. Each sample was washed once in 70% ethanol solution, once in 10% clorox solution, and three times in sterile micropure water for one minute. Plant samples were then placed on filter paper to dry in a sterile laminar flow hood. After samples were dried, they were plated onto WA and petri plates were placed in plastic bags and incubated on a laboratory bench top for three days. Hyphal tips of fungal colonies growing from plant tissues were transferred using a heated needle. Fungal colony transfers were incubated in the laboratory as previously described for approximately two months. Following the fungi maturation period, each pure culture colony was placed under a microscope for identification.

After identifying a potential pathogenic *Rhizoctonia*-like fungus on approximately five of the plant tissue samples, further research was conducted to determine fungal identity. The isolate was transferred to potato dextrose agar for enhanced vegetative production. Mycelium was collected and lypholized for DNA extraction. Following genomic DNA extraction using the DNEasy Plant Mini Kit (Valencia, CA), the internal transcribed spacer region of ribosomal DNA was amplified by PCR using ITS1 and ITS4 primers (White et al. 1990). The resultant 665-bp was sequenced for the unknown isolate. Automated sequencing was performed by Eurofins MWG Operon (Huntsville, AL). The resultant sequence was submitted to NCBI BLAST (Bethesda, MD) to search the data base for a biological sequence of nucleotides similar to the *Rhizoctonia*-like fungal sample.

Results and Discussion

Several fungal species were identified from alligatorweed. *Alternaria spp*., *Fusarium spp*., and *Penicillium spp*. were some of the most common. A *Rhizoctonia*-like fungus was isolated from a few of the plant samples, based on hyphal characteristics that include constricted, right angle branching, bulbils, and binucleate hyphal cells (Figures 4.2 and 4.3) (Donk 1962). Results of the sequence BLAST of the *Rhizoctonia*-like fungus were 98% similar to the sequence of *Sclerotium hydrophilum* (GenBank FJ231396) which has been previously reported on aquatic or semi-aquatic plants in marshy areas such as wild rice (*Zizania aquatic* L.), rice (*Oryza sativa* L.), white waterlilies (*Nymphaea odorata* Aiton), Eurasian watermilfoil (*Myriophyllum spicatum* L.), cattails (*Typha spp*. L.), barnyardgrass (*Echinocloa crus-galli* [L.] P. Beauv), and others (Farr et al. 1995). A phylogenetic placement of *S. hydrophilum* conducted by Xu et al. (2009), resulted in a taxonomic change to *Ceratorhiza hydrophilum* (Sacc.) Xu, Harrington, Gleason, et Batzer, comb. nov. ≡ *Sclerotium hydrophilum* Saccardo. Currently, there is no experimental data that proves *C. hydrophilum* may be a potential agent for biocontrol use on alligatorweed. This is a first report however, of *C. hydrophilum* isolated from alligatorweed in the United States or world-wide.

Future work should be conducted to determine if *C. hydrophilum* is pathogenic on alligatorweed or any other invasive, aquatic plant species. Biocontrol agents are favorable options for weed control in most settings; however, future research should ascertain the host range of this fungus to prevent harm of non-target plant and animal species if utilized as a biocontrol agent. When incorporated into an Integrated Pest Management plan, biocontrol agents may lower the risk of herbicide resistance, control costs, and application time.

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Figure 4.1 Alligatorweed leaf damaged by herbivory from the alligatorweed flea beetle (*Agasicles hygrophila*).

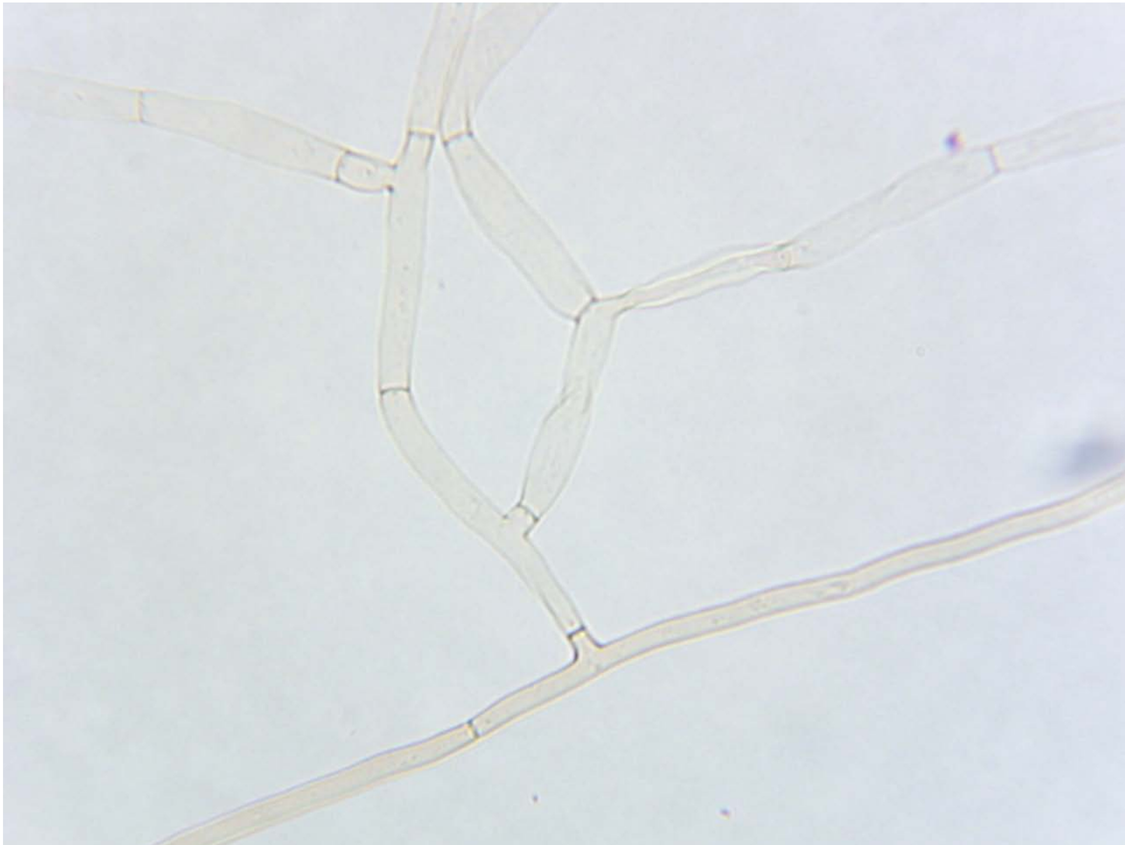

Figure 4.2 Hyphae of the fungus displaying constricted, right-angle branching.

Figure 4.3 A binucleate cell in the hyphae of the fungal culture.

APPENDIX A

SAMPLING LOCATIONS WITHIN THE ROSS BARNETT RESERVOIR FROM 2005 TO 2010

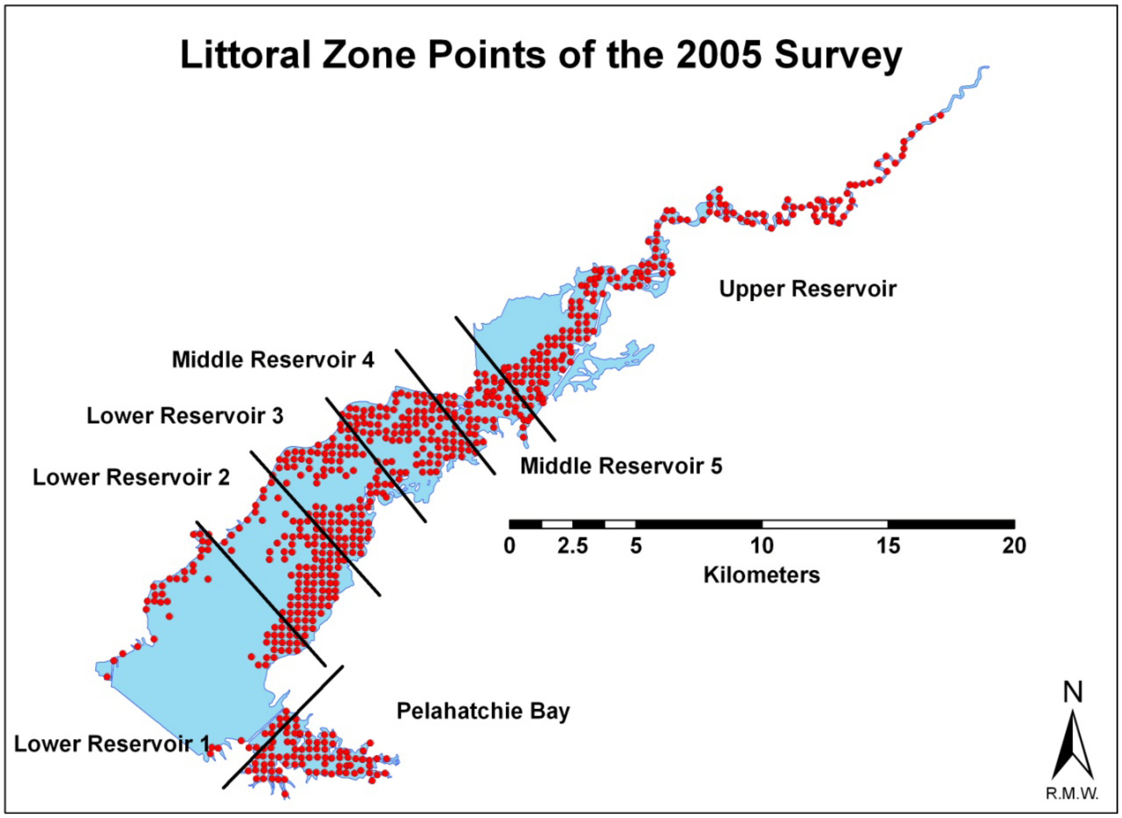

Figure A.1 Sampling locations for the 2005 littoral zone survey of the Ross Barnett Reservoir (Wersal et al. 2006a).

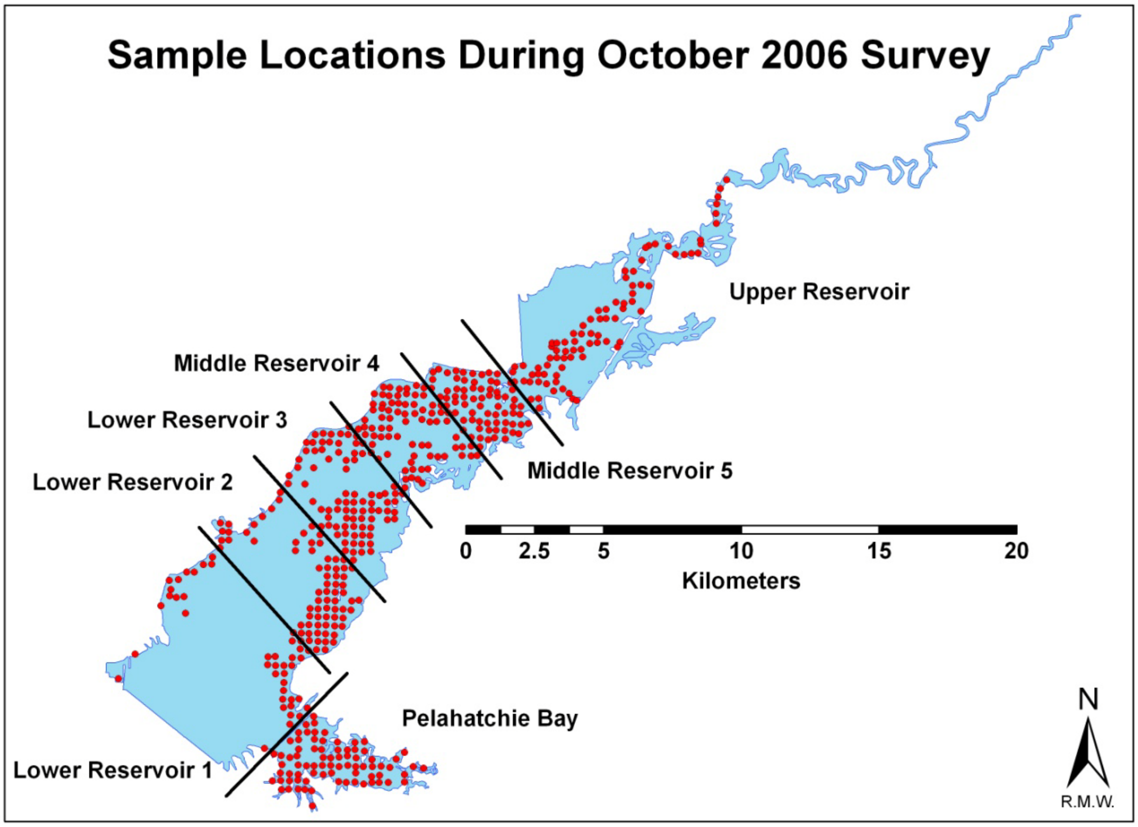

Figure A.2 Sampling locations for the 2006 littoral zone survey of the Ross Barnett Reservoir (Wersal et al. 2007).

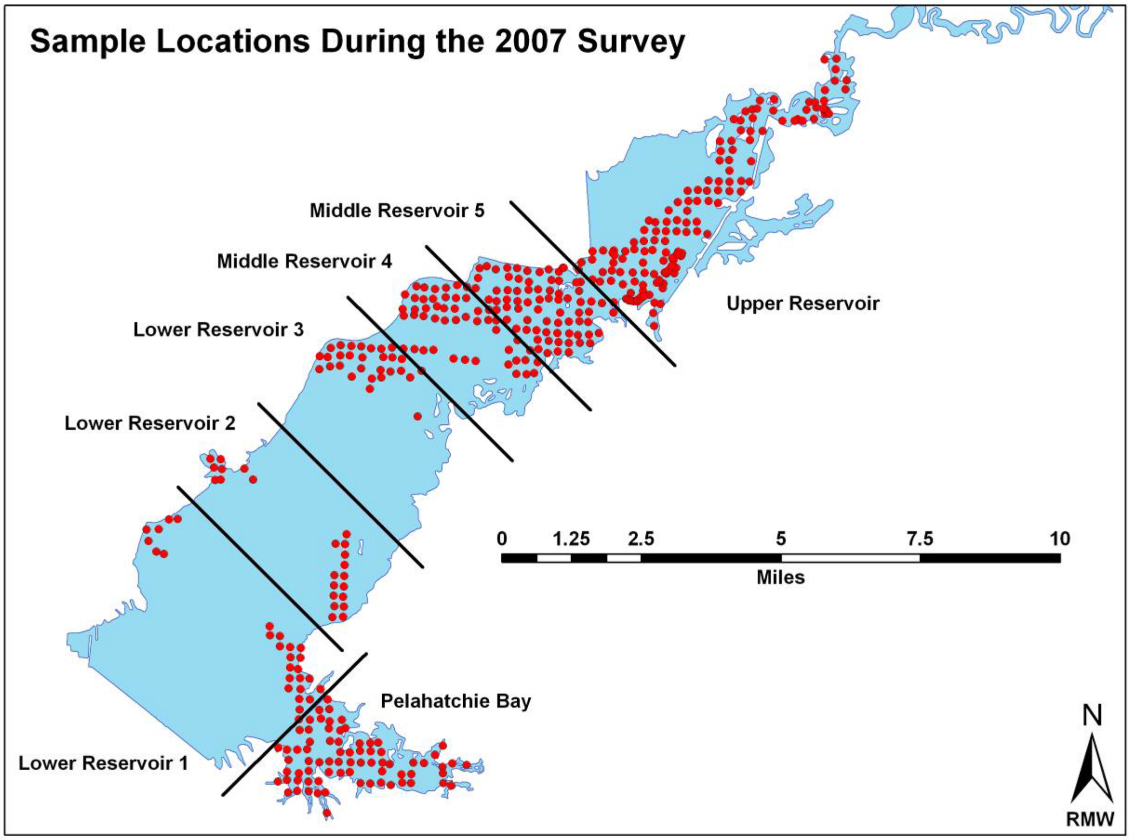

Figure A.3 Sampling locations for the 2007 littoral zone survey of the Ross Barnett Reservoir (Wersal et al. 2008).

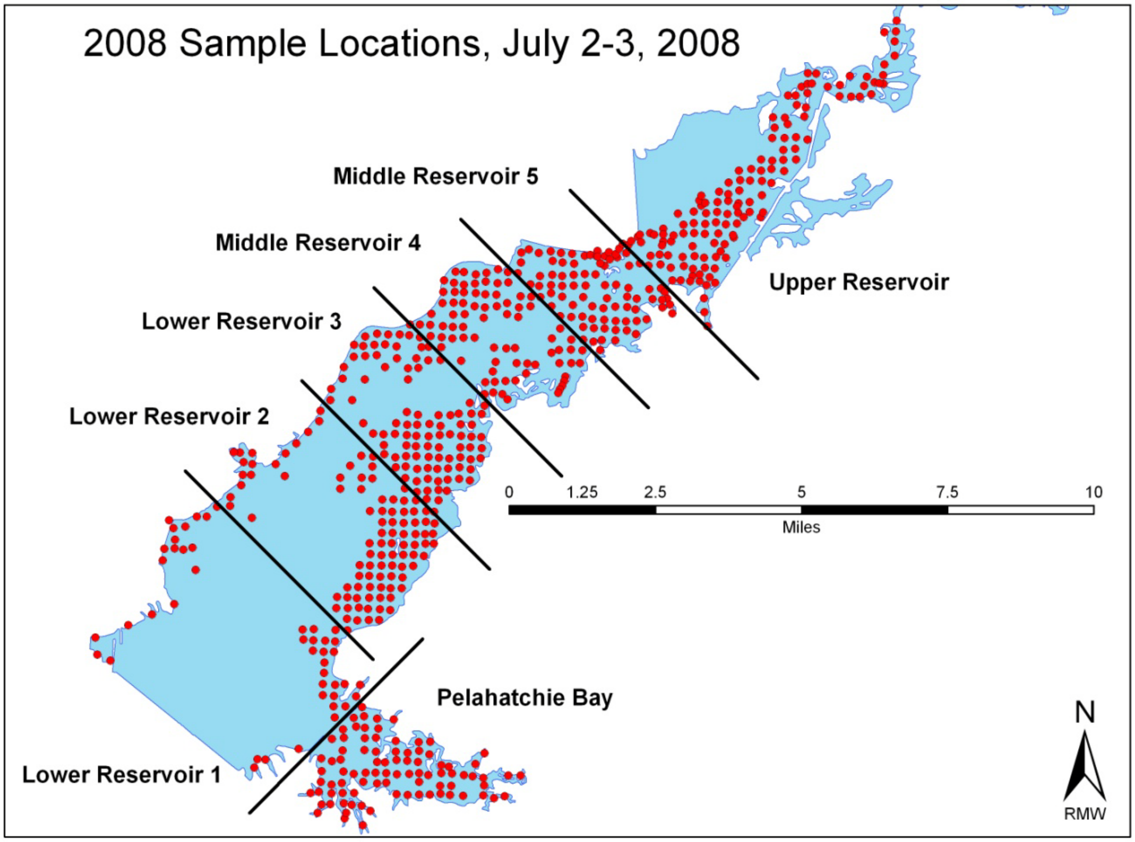

Figure A.4 Sampling locations for the 2008 littoral zone survey of the Ross Barnett Reservoir (Wersal et al. 2009).

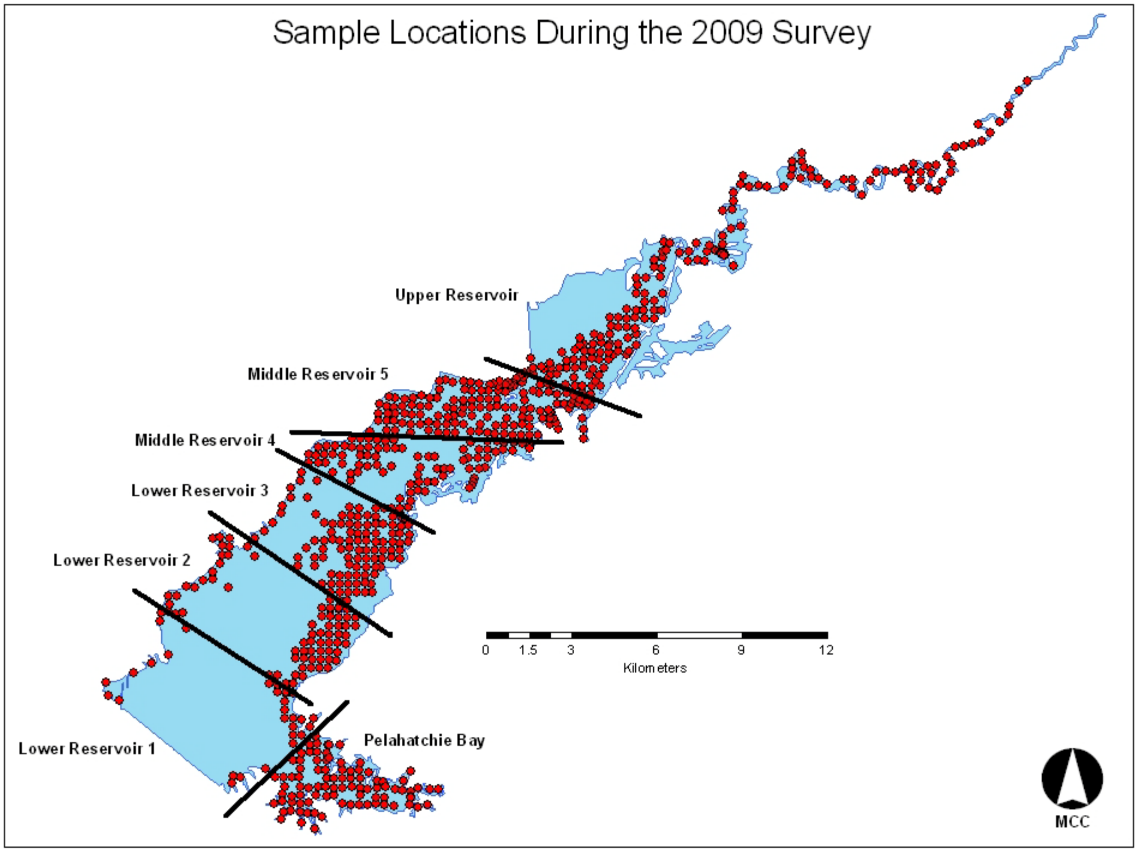

Figure A.5 Sampling locations for the 2009 littoral zone survey of the Ross Barnett Reservoir (Cox et al. 2010).

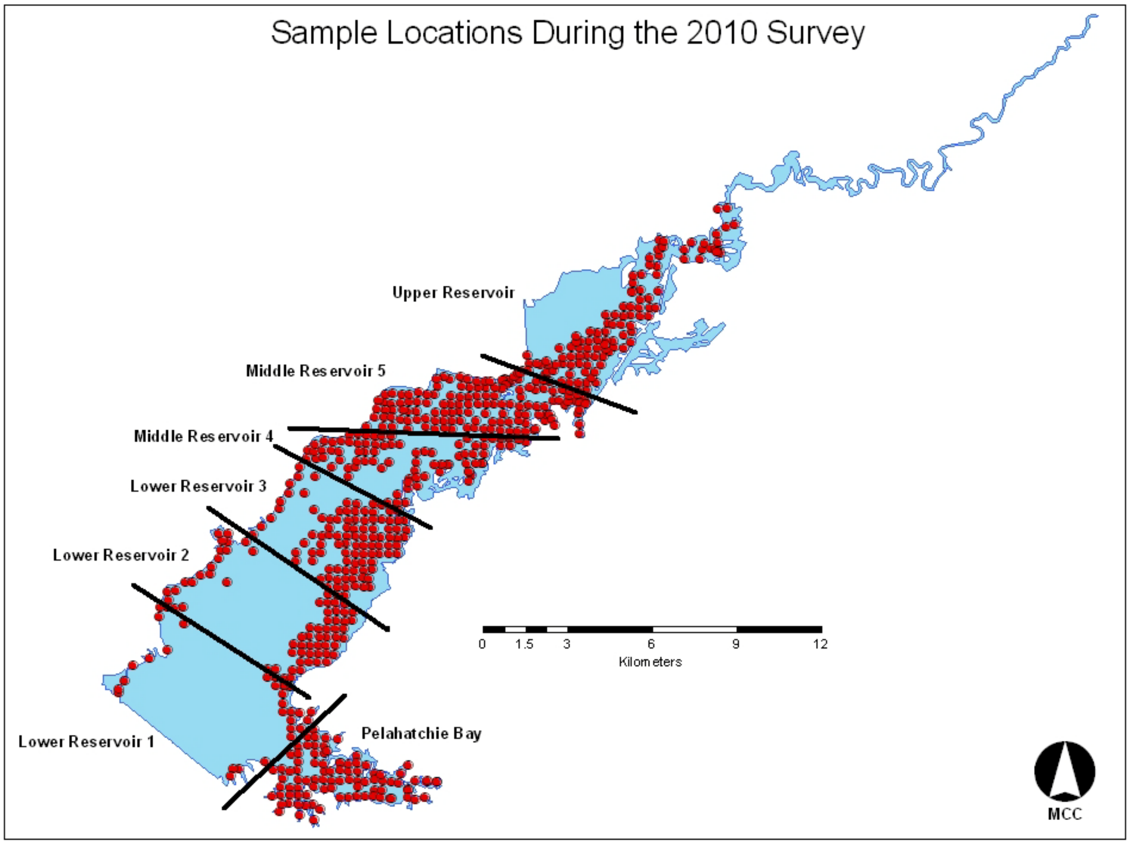

Figure A.6 Sampling locations for the 2010 littoral zone survey of the Ross Barnett Reservoir.

APPENDIX B

HYDRILLA TREATMENT DATA WITHIN THE ROSS BARNETT RESERVOIR

FROM 2005 TO 2010

Table B.1 Hydrilla treatment records on the Ross Barnett Reservoir from 2005 to 2010.

		Treatment Records					
Hydrilla Site	**Year Discovered**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
1	2005	1	1-F (April)	1-F	1	1-FC	C (Jun, Aug, & Oct)
2	2005	1	1-F (April)	F			
3	2006		1-F (April)	F			
4	2006		1-F (April)	1-F	1	F	
5	2006		1-F (April)	F	?	1-FC	1-C (Jun, Aug, & Oct)
5b[1]	2009					1	
6	2007			1-F	?	1	1-C (Jun, Aug, &Oct)
7	2007			1	?	F	
8	2007			1	?		
9	2007			1	?		
10	2007			1	?		
11	2007			1-F	1-F	1-F	C (Jun, Aug, & Oct)
12	2009					1	F (June)
13	2009					1	F (June)
14	2010						1-C (Aug & Oct)
15	2010						1-C (August)
16	2010						1

"1" indicates observation of hydrilla
"F" indicates fluridone treatment
"C" indicates copper & diquat treatment
"?" indicates that treatment status is unknown
[1] Site 5b was discovered in 2009 and recently merged with Site 5

APPENDIX C

AQUATIC PLANT SPECIES LOCATIONS WITHIN THE ROSS BARNETT RESERVOIR FROM 2005 TO 2010

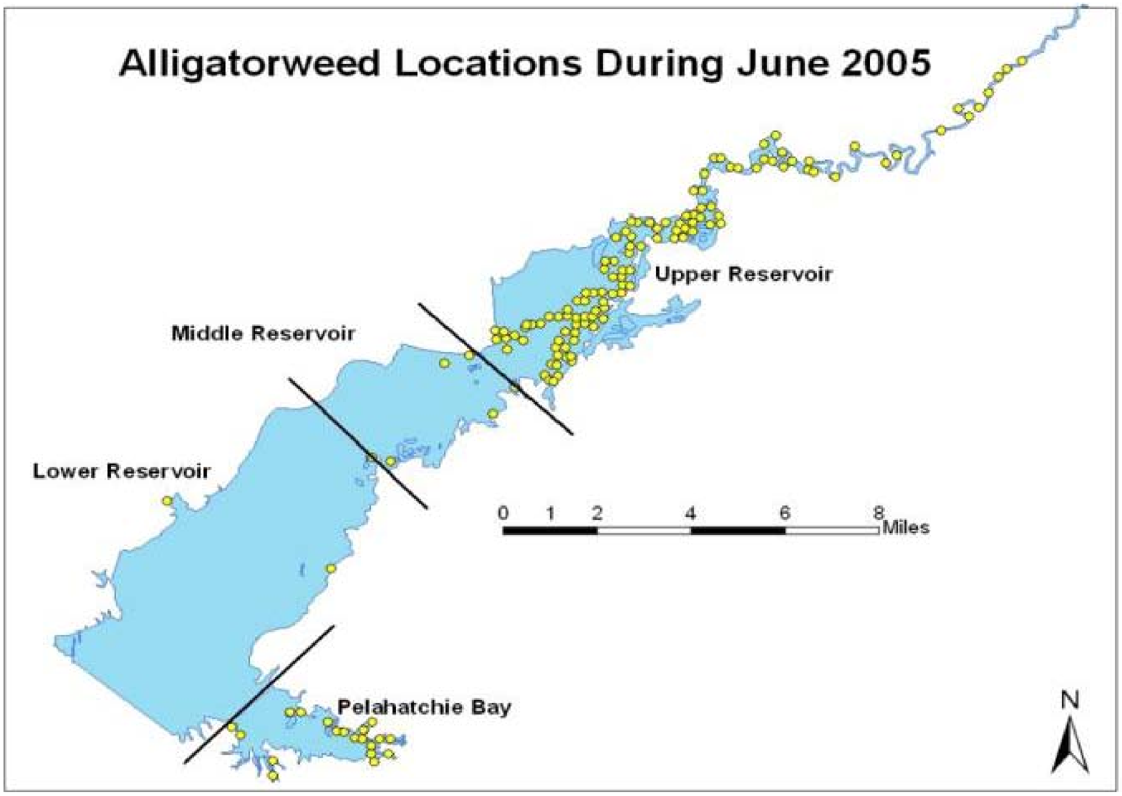

Figure C.1 Locations of alligatorweed within the Ross Barnett Reservoir in 2005 (Wersal et al. 2006a).

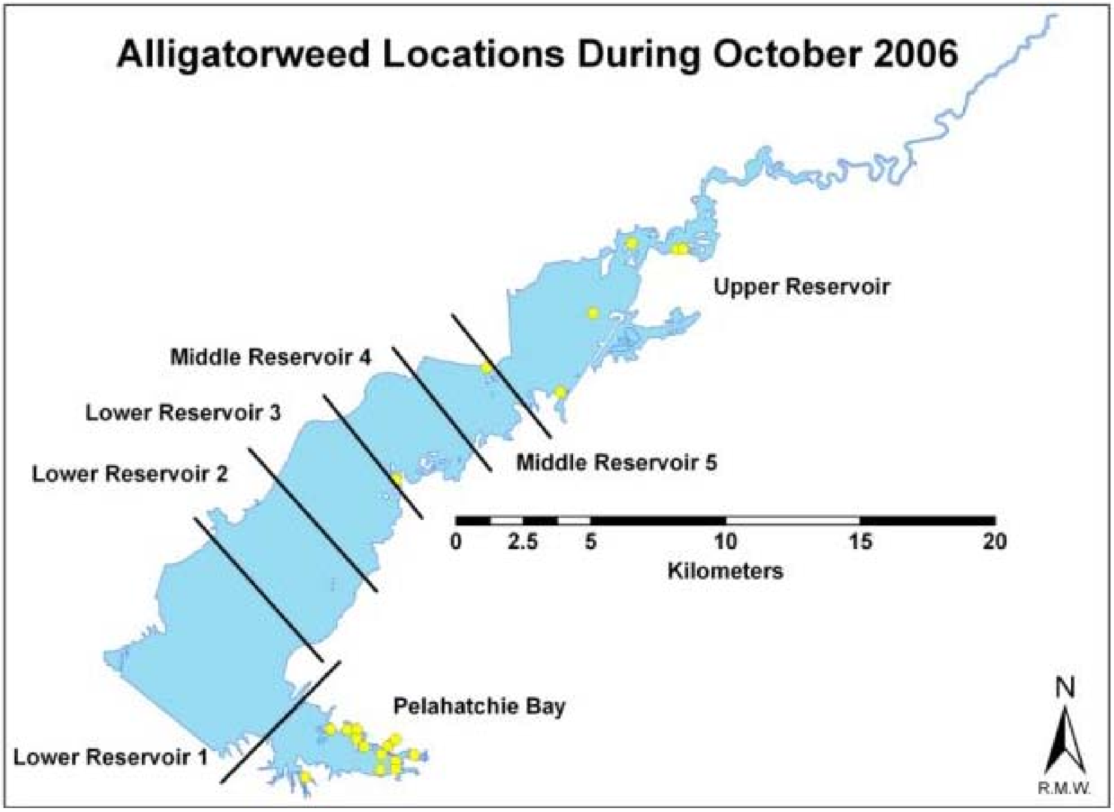

Figure C.2 Locations of alligatorweed within the Ross Barnett Reservoir in 2006 (Wersal et al. 2007).

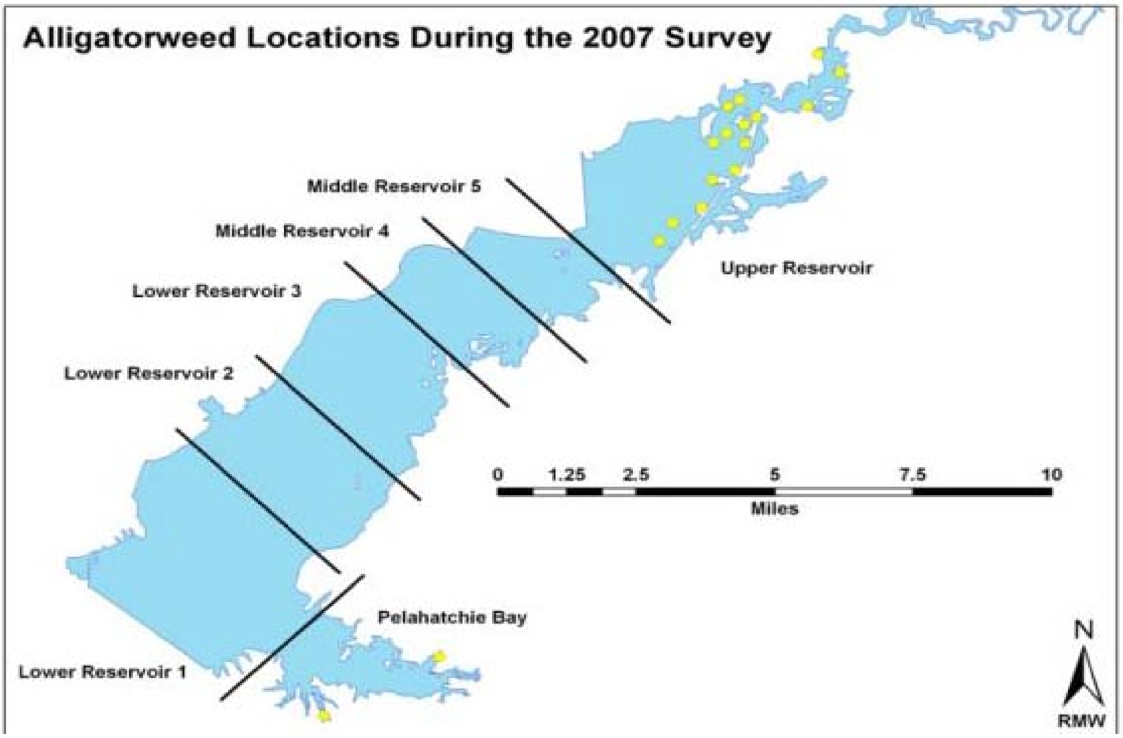

Figure C.3 Locations of alligatorweed within the Ross Barnett Reservoir in 2007 (Wersal et al. 2008).

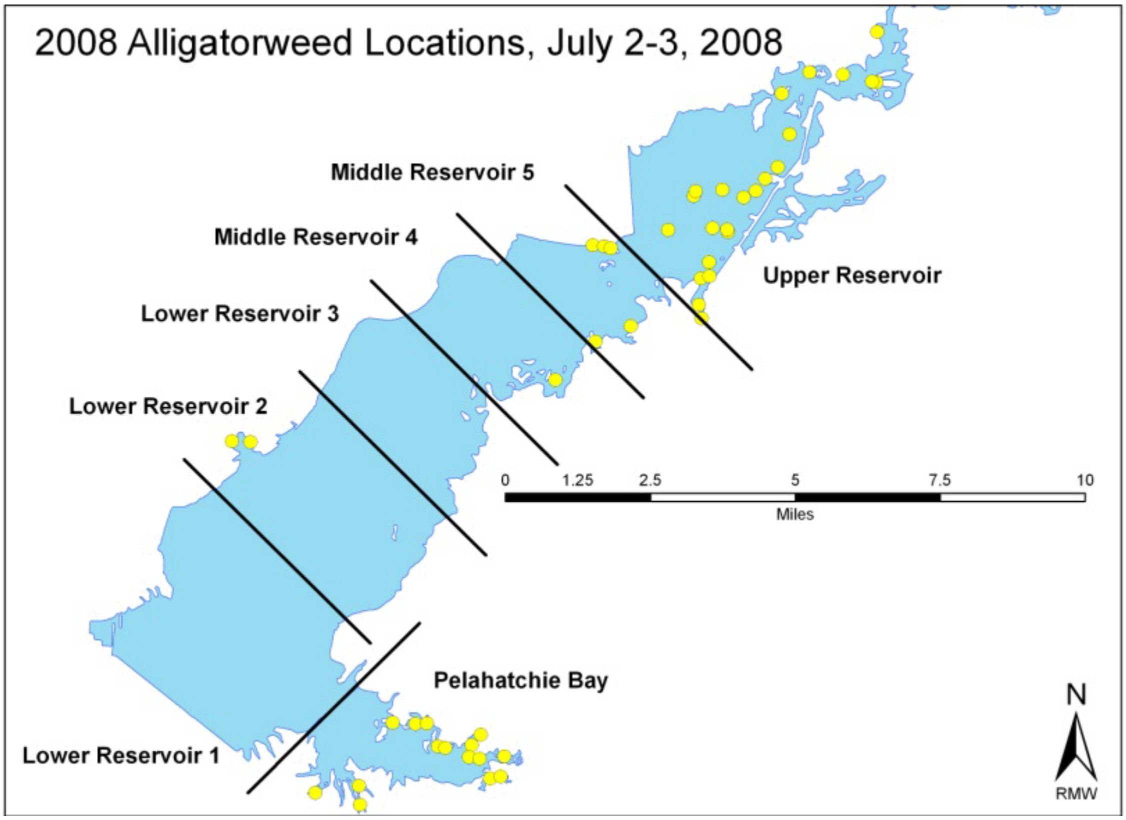

Figure C.4 Locations of alligatorweed within the Ross Barnett Reservoir in 2008 (Wersal et al. 2009).

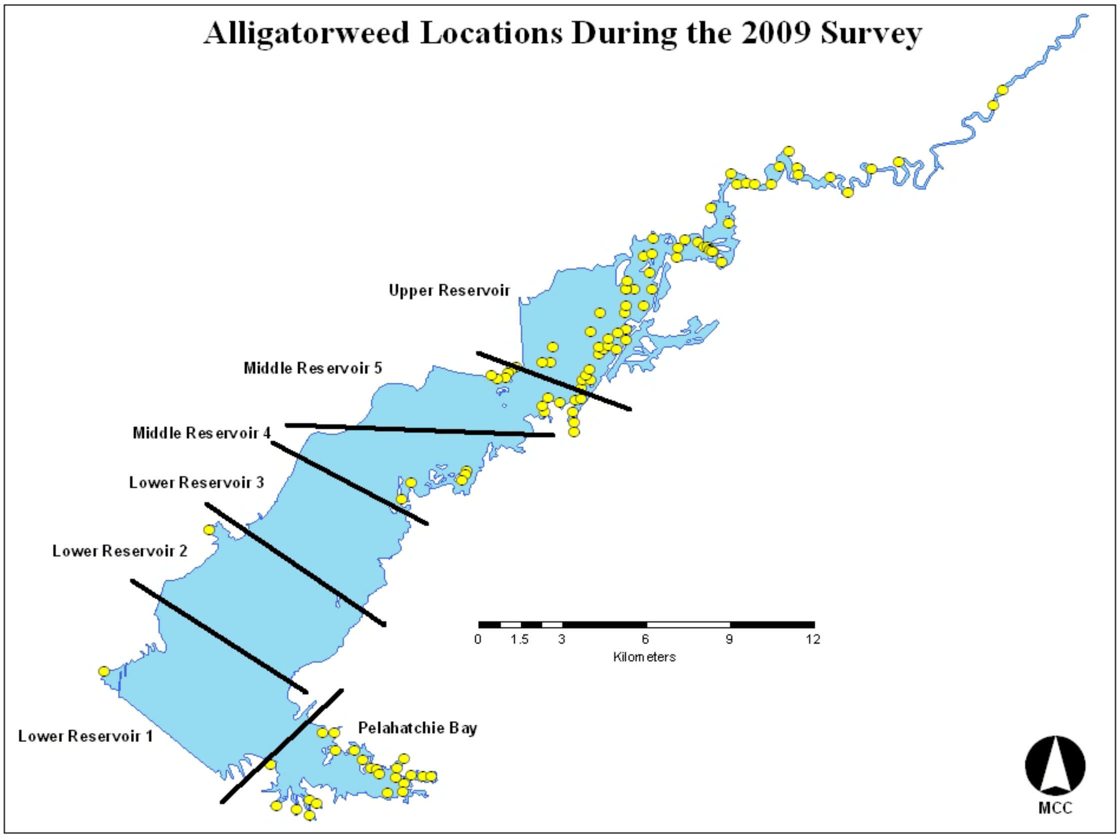

Figure C.5 Locations of alligatorweed within the Ross Barnett Reservoir in 2009 (Cox et al. 2010).

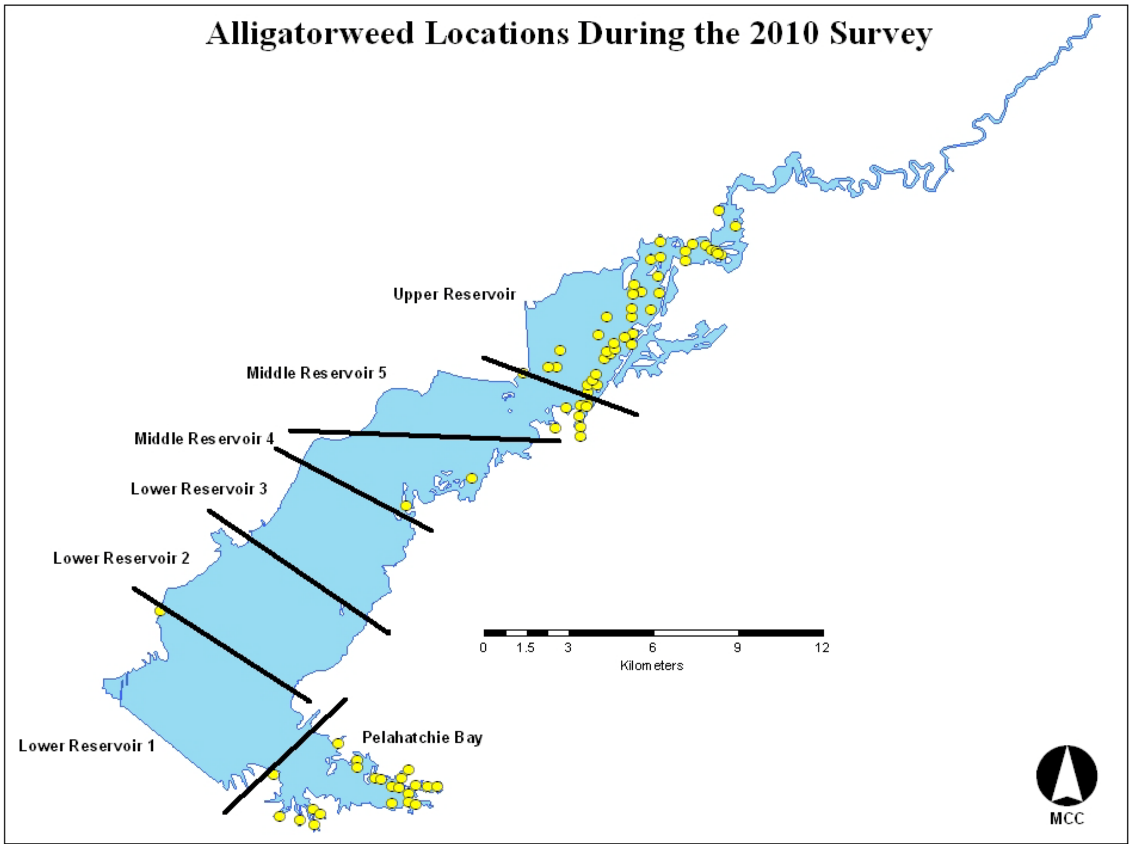

Figure C.6 Locations of alligatorweed within the Ross Barnett Reservoir in 2010.

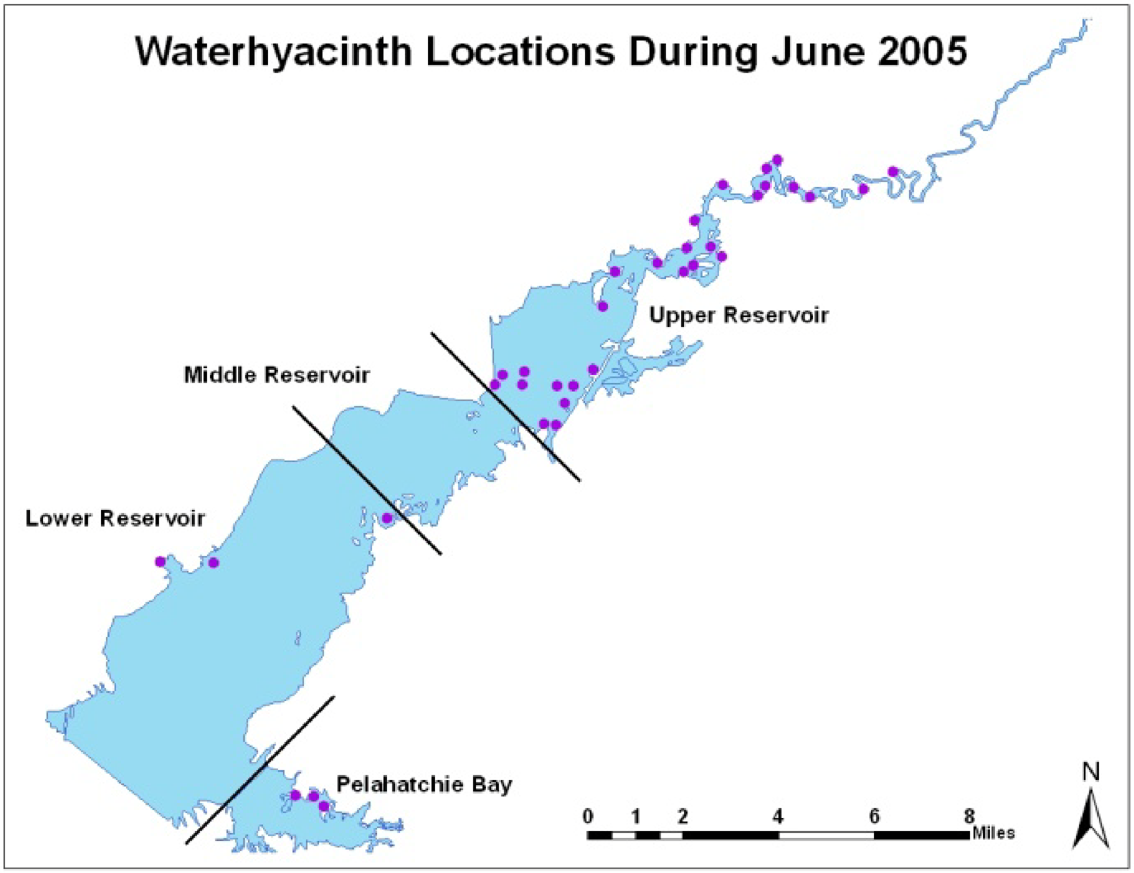

Figure C.7 Locations of waterhyacinth within the Ross Barnett Reservoir in 2005 (Wersal et al. 2006a).

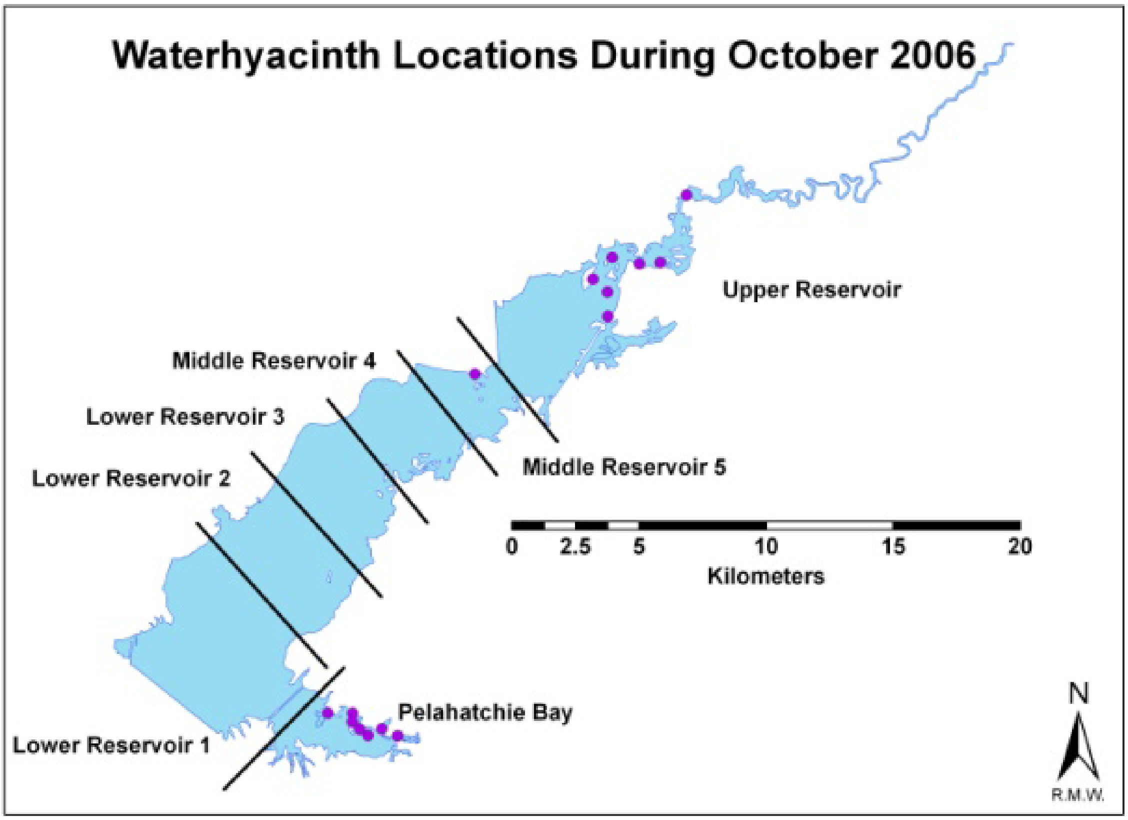

Figure C.8 Locations of waterhyacinth within the Ross Barnett Reservoir in 2006 (Wersal et al. 2007).

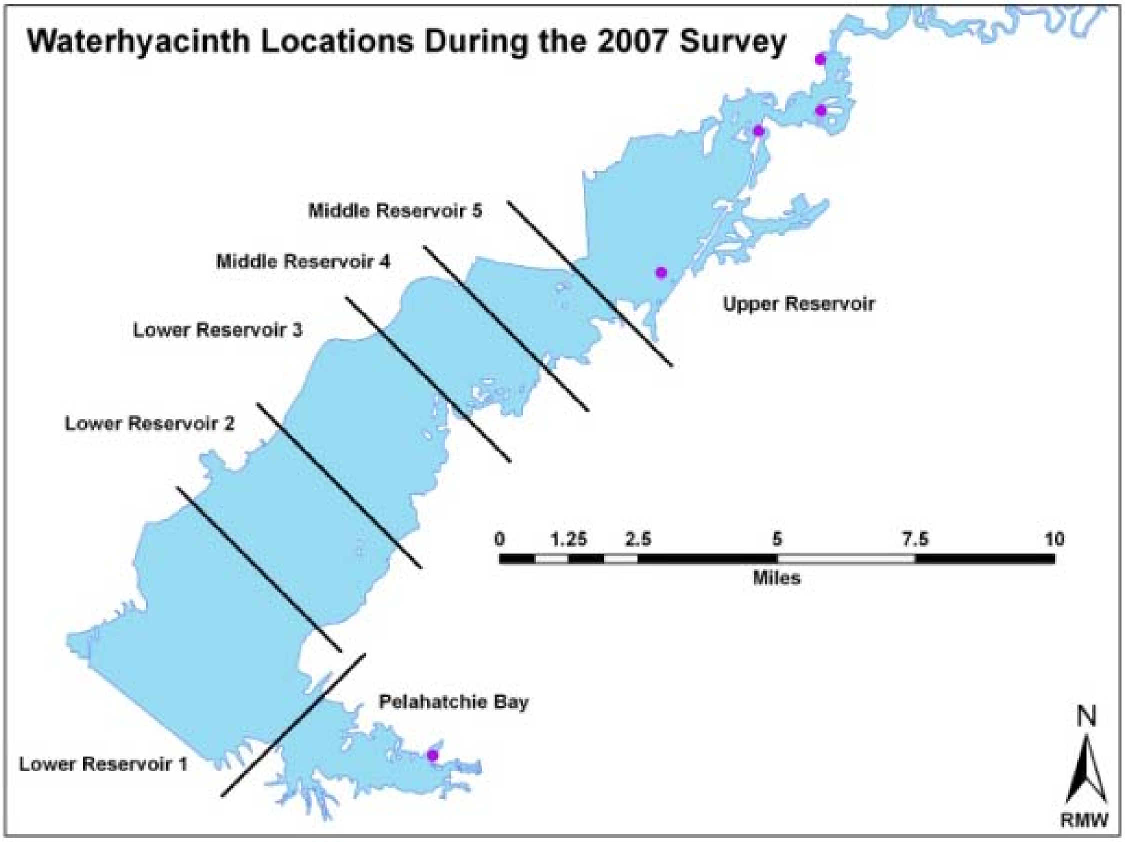

Figure C.9 Locations of waterhyacinth within the Ross Barnett Reservoir in 2007 (Wersal et al. 2008).

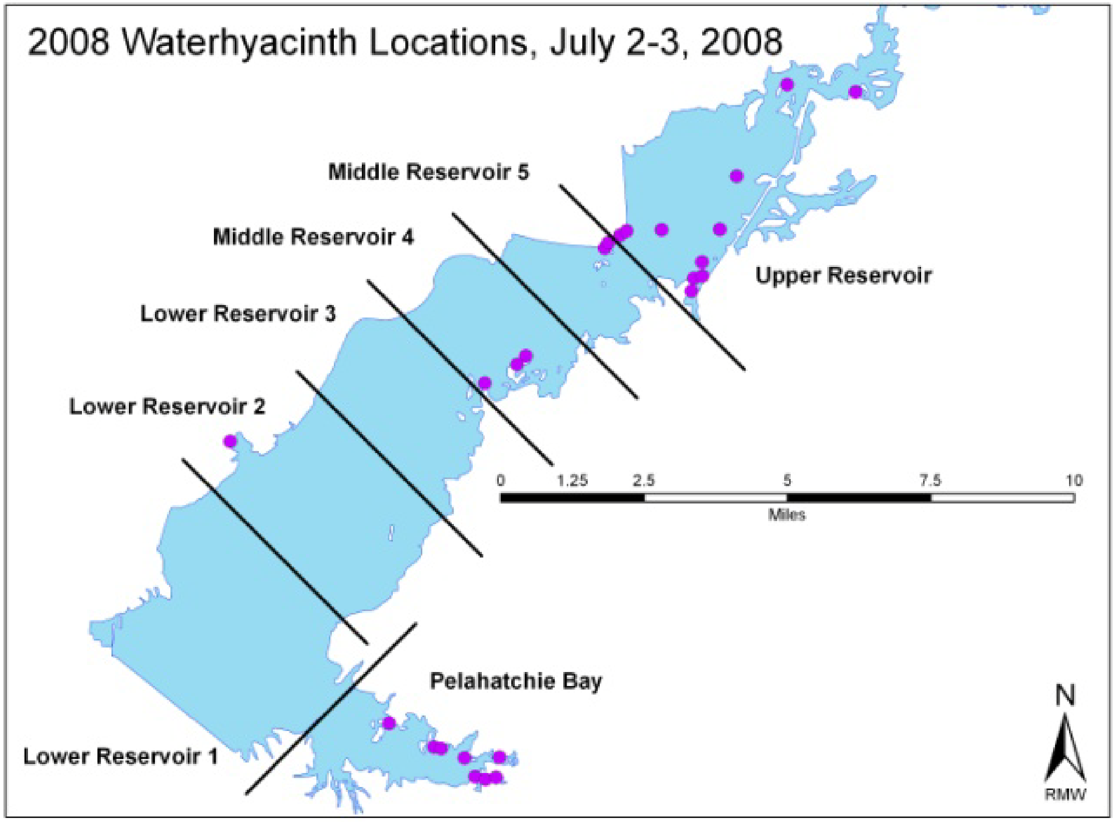

Figure C.10 Locations of waterhyacinth within the Ross Barnett Reservoir in 2008 (Wersal et al. 2009).

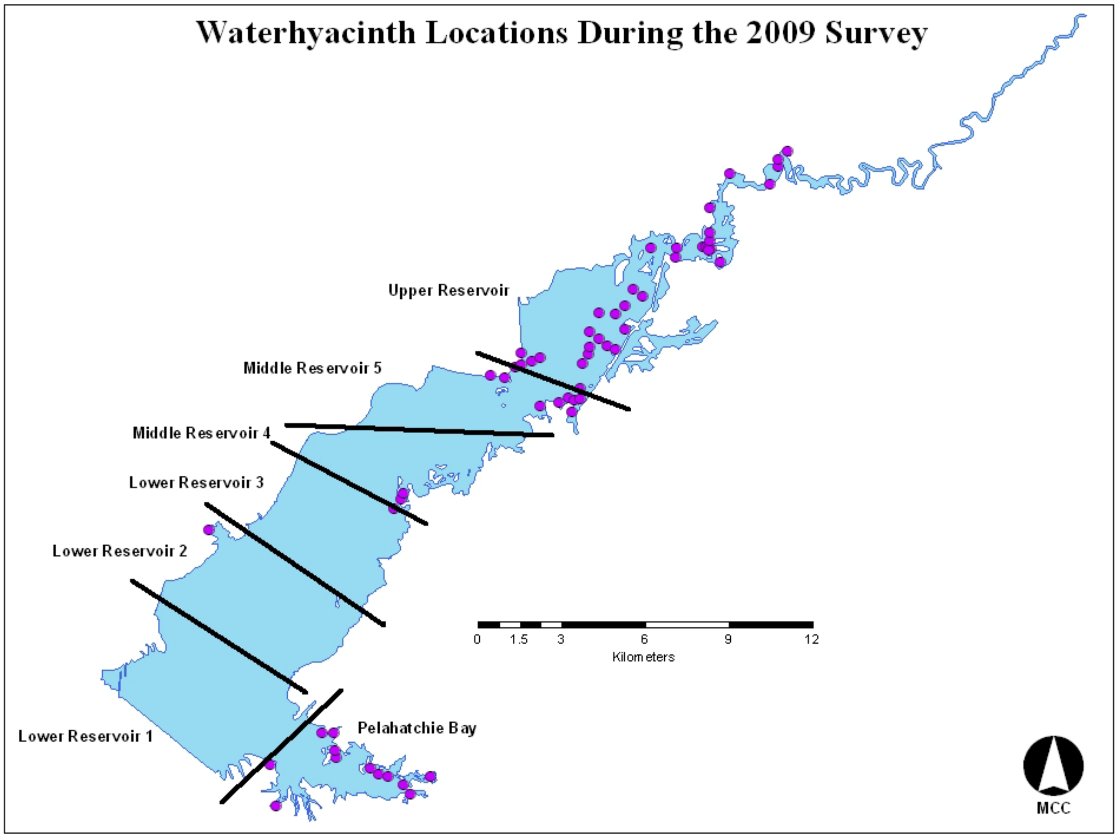

Figure C.11 Locations of waterhyacinth within the Ross Barnett Reservoir in 2009 (Cox et al. 2010).

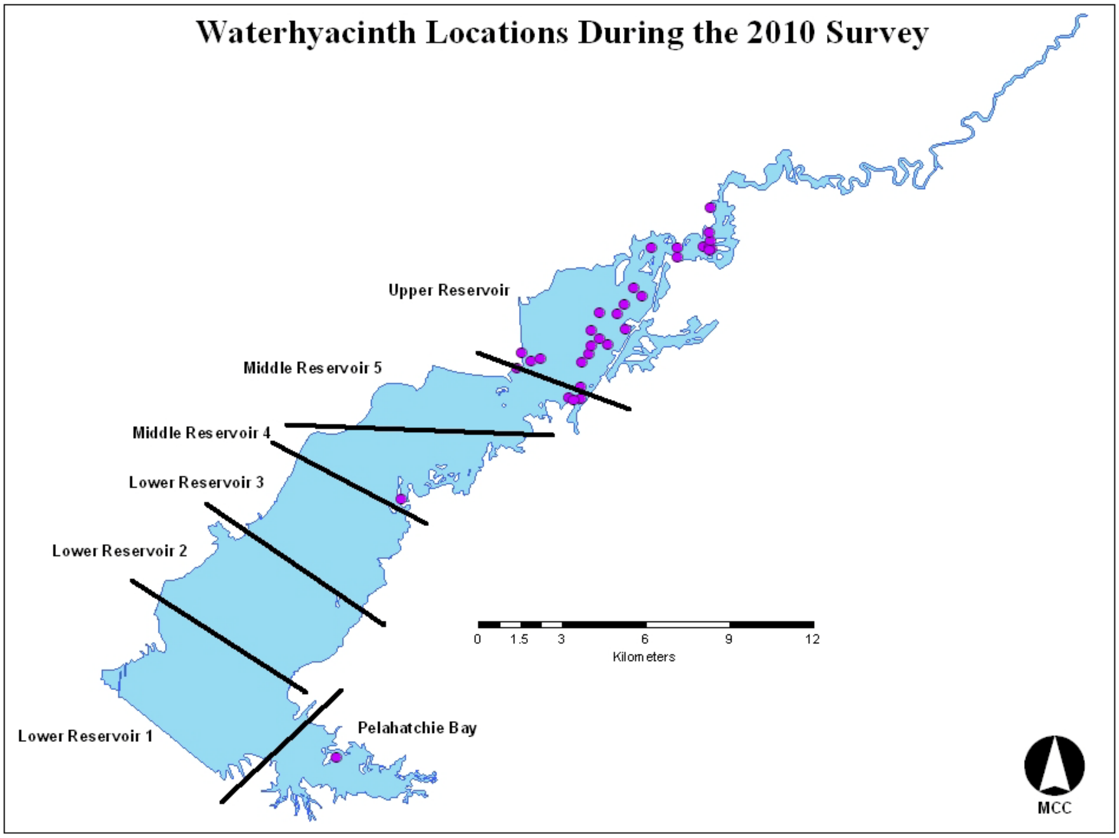

Figure C.12 Locations of waterhyacinth within the Ross Barnett Reservoir in 2010.

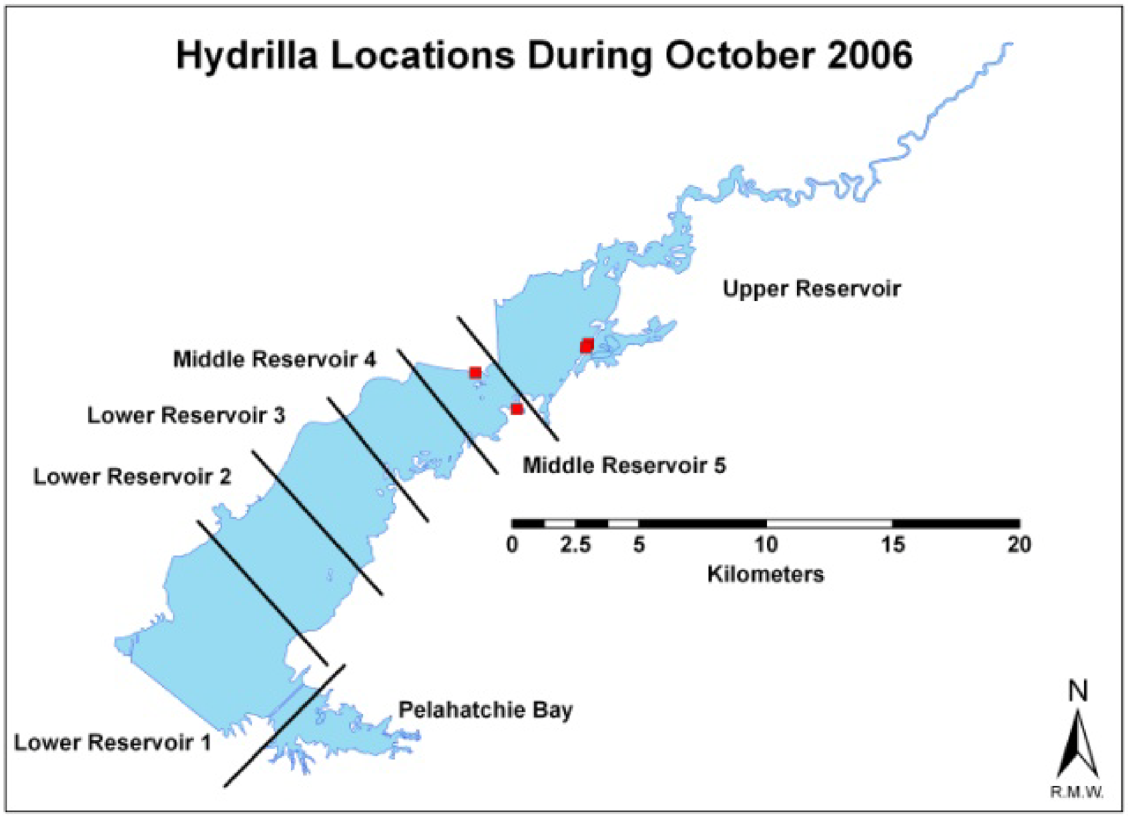

Figure C.13 Locations of hydrilla within the Ross Barnett Reservoir in 2006 (Wersal et al. 2007).

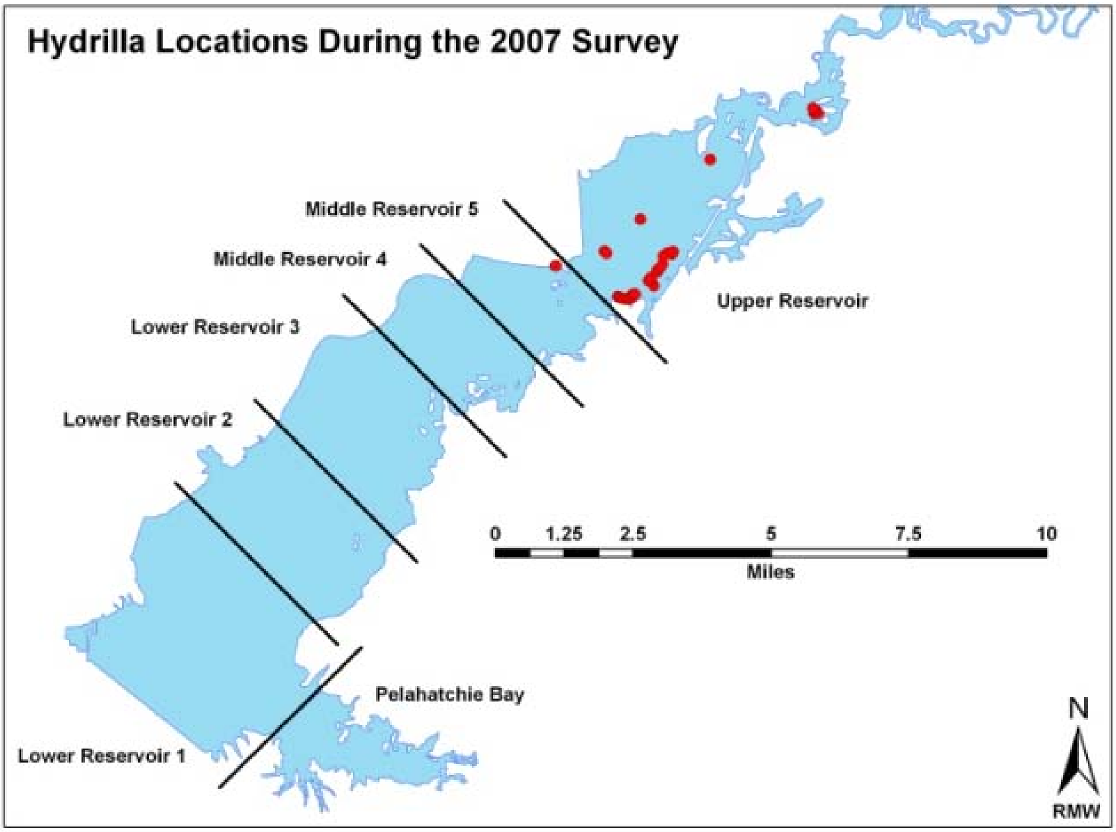

Figure C.14 Locations of hydrilla within the Ross Barnett Reservoir in 2007 (Wersal et al. 2008).

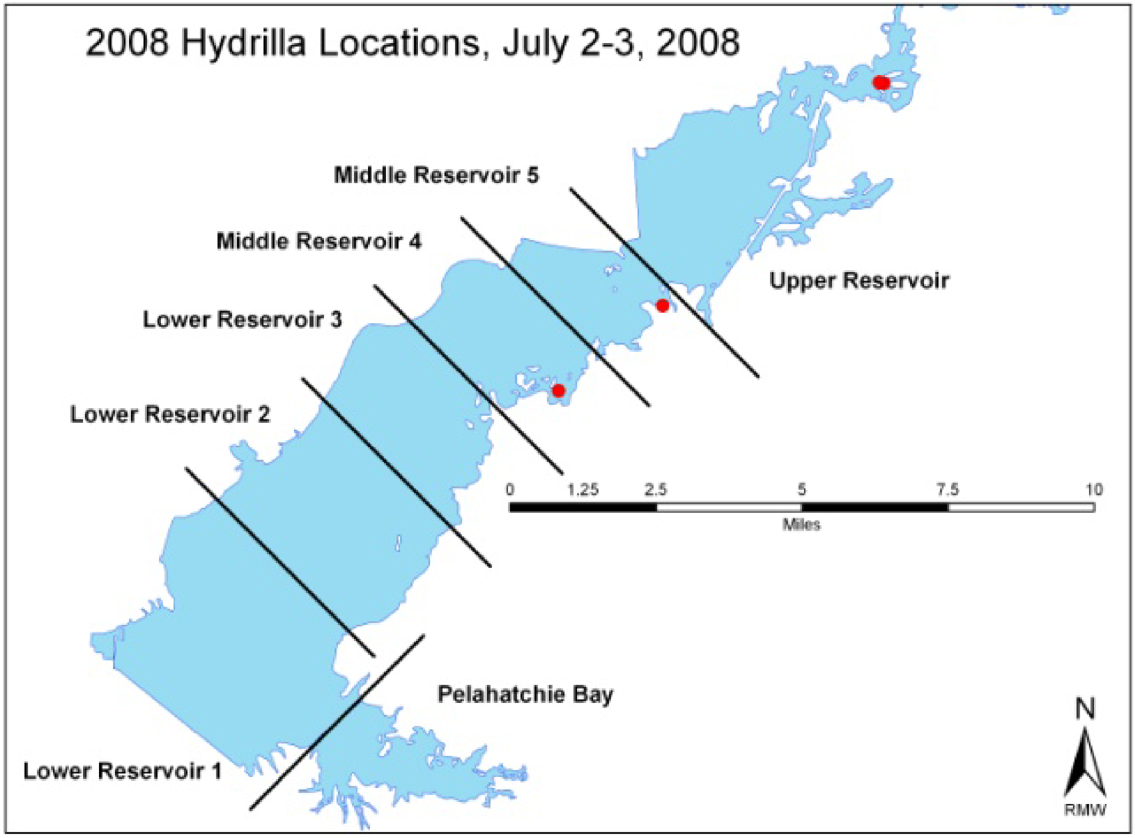

Figure C.15 Locations of hydrilla within the Ross Barnett Reservoir in 2008 (Wersal et al. 2009).

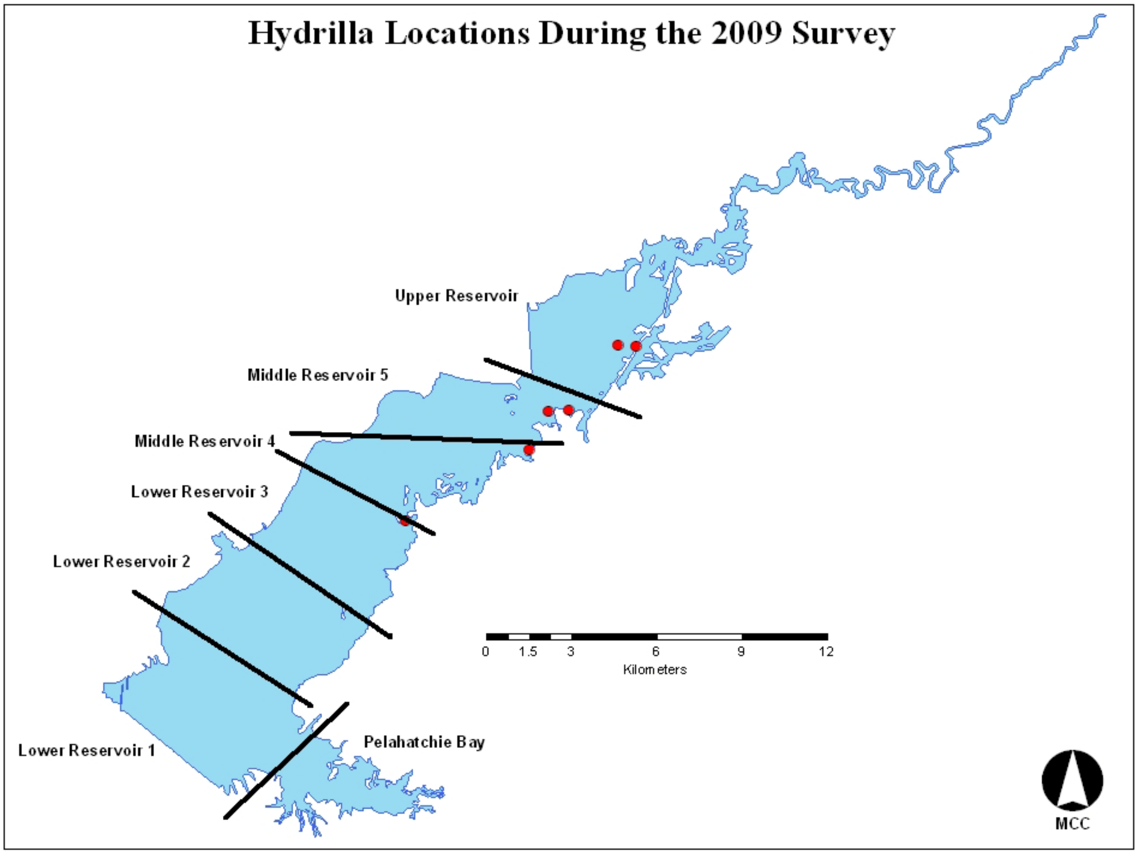

Figure C.16 Locations of hydrilla within the Ross Barnett Reservoir in 2009 (Cox et al. 2010).

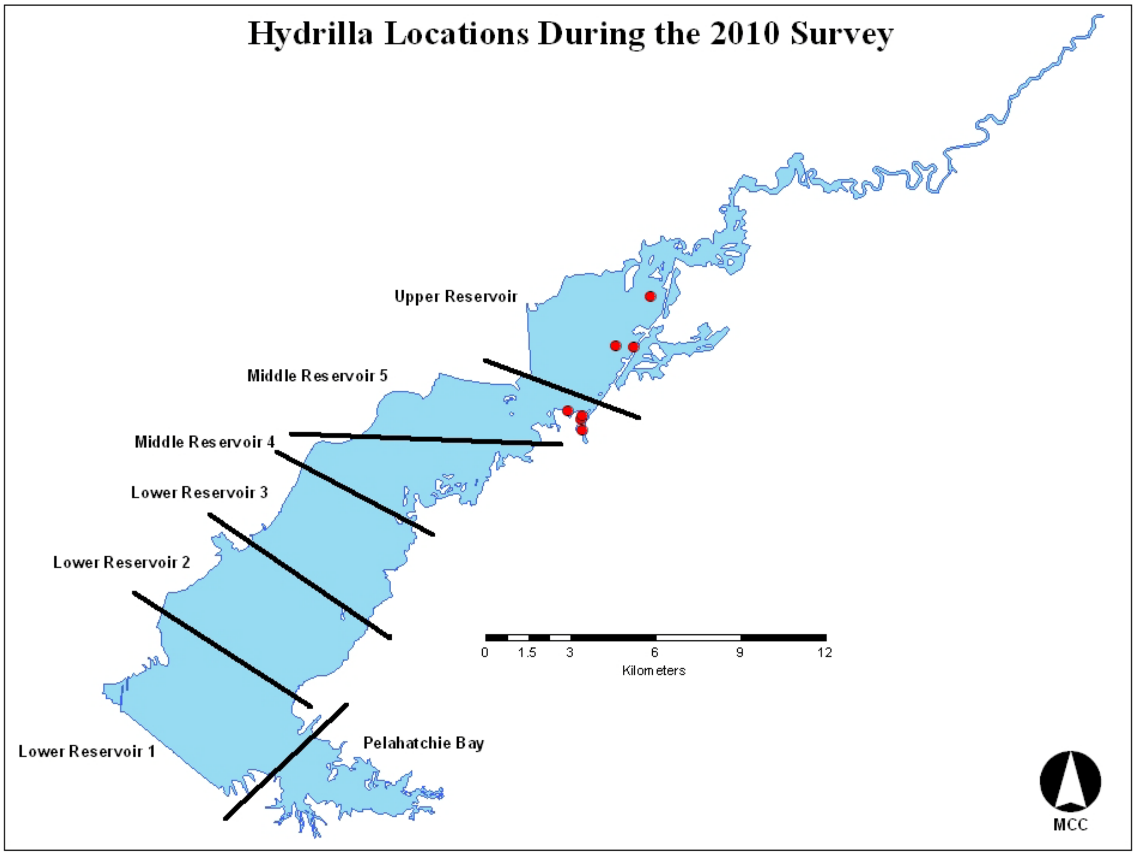

Figure C.17 Locations of hydrilla within the Ross Barnett Reservoir in 2010.

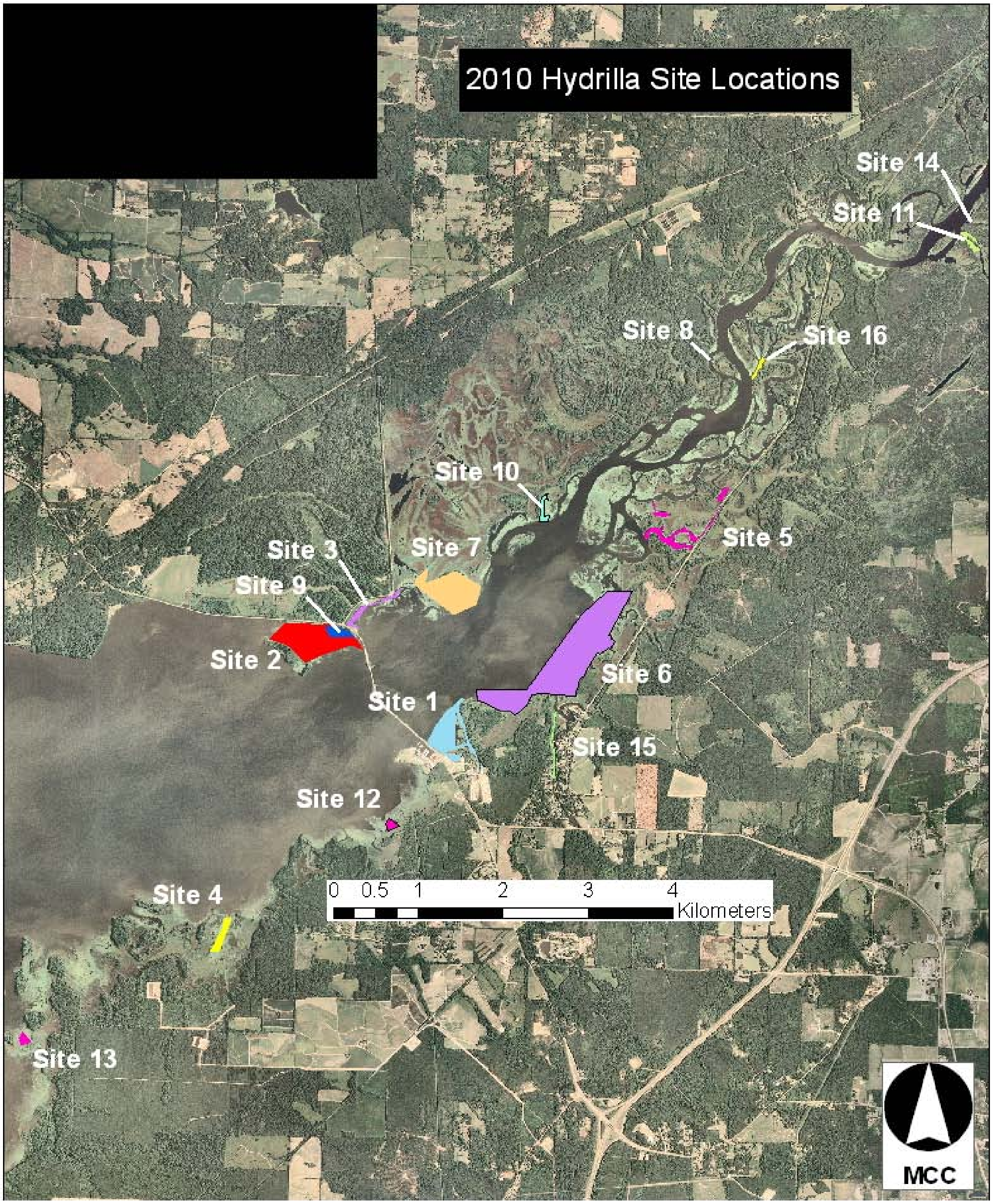

Figure C.18 Hydrilla site locations on the Ross Barnett Reservoir as of 2010.