Dispersal and management of invasive aquatic plants in Mississippi waterways

By

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To understand the flow of water as a factor that influences aquatic vegetation communities and aquatic plant dispersal, custom-made Global Positioning System (GPS) drones were used to monitor the movement of water in Aliceville Lake, Columbus Lake, and Ross Barnett Reservoir, MS. In each reservoir, the drones drifted in the wind-generated surface current. Analysis of wind speeds suggests that a certain wind speed may be necessary to overcome gradient flow. Wind direction and wind speed should be incorporated in future spatial simulation models for aquatic plant dispersal and distribution. An herbicide evaluation on Cuban bulrush (*Oxycaryum cubenese*) was conducted to determine what herbicides would effectively control the invasive species. Applications made pre-flowering were more successful than post-flowering applications for all herbicides tested with glyphosate, 2,4-D, triclopyr, diquat, imazamox, and imazapyr resulting in 100% mean biomass reduction. For post-flowering applications, glyphosate, triclopyr, and diquat are recommended.

DEDICATION

This thesis is dedicated to Victor and Peggy, my parents, and Scott, my husband. They provided endless love and support which I often needed as I tried to achieve this goal. Their complete confidence in my abilities kept me motivated, and I cannot express how truly grateful I am that God blessed me with such an amazing family.

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CHAPTER I

INTRODUCTION: EARLY DETECTION AND RAPID RESPONSE FOR EFFECTIVELY MANAGING INVASIVE AQUATIC WEEDS

Nonindigenous invasive species are one of the leading causes of decline in biodiversity leading to losses in ecosystem services and aesthetics (Allendorf and Lundquist 2003; Pimental et al. 2005). For invasive plants to severely impact an ecosystem, the invaders must survive the phases of the invasion process (Lockwood et al. 2007). First the invader must survive transport from elsewhere and be introduced in to a new area. Then, it must establish in new areas which is typically followed by a lag phase. Finally, the newly established population must spread to other areas, where if left uncontrolled, the weeds can have ecological and human impacts (Lockwood et al. 2007). To minimize their negative impact, in the U.S.A., $100 million is annually spent on managing nonnative aquatic weeds (Pimental et al. 2005). Many managers aim for early detection and rapid response to new invaders because it is most cost effective while populations are small and invaded sites minimal (Westbrook 2004). In the aquatic ecosystem, this means understanding water flow dynamics such as current velocity and flow direction because these affects aquatic plant dispersal, distribution, and growth (Madsen et al. 2001).

Flow of water

Localized spread of aquatic plants is typically due to dispersal of seed and vegetative fragments by water (Johansson and Nilsson 1993). Plant propagules either float on the water surface or are carried along the water surface (van der Maarel 2005). Plants that travel on the water surface often travel long distances due to specialized structures that allow them to float while plants with specialized organs for asexual perennation are usually heavier than water, so they travel shorter distances (Santamaria 2002). In lotic systems, the directional flow of water is usually constant, except under very strong wind conditions (Downing-Kunz and Stacey 2011). In lentic systems, the directional flow of the surface water current is driven by the wind (Downing-Kunz and Stacey 2011). Once propagules are carried to an area, successful establishment is influenced by current velocity.

Current velocity influences aquatic plant growth by altering the habitat, photosynthesis rates, and the physical features of the plant itself (Madsen et al. 2001; Downing-Kunz and Stacey 2011). High velocity currents increase erosion of the sediment (Madsen et al. 2001). The heavier, less nutrient rich sand substrate remains, while the lighter organic matter and clay particles are swept away. Furthermore, higher velocity currents can increase turbidity of the water, thereby preventing light from reaching submersed aquatic vegetation (Madsen et al. 2001). In slow moving water, as current velocity increased, nutrient uptake and photosynthesis rates increased as the thickness of the diffusive boundary layer at the plant's surface decreased. However, if the velocity of the current is too fast, the mechanical stress acting on the plant can inhibit photosynthesis (Koch 1994; Madsen et al. 2001). To deal with the physical force acting upon the plant from the current, plants will grow at an angle to the sediment, bend in the direction of the current, and modify their support structures as necessary to counter the demands of the current (Madsen et. 2001).

Methods for Tracking Movement

Monitoring drift in the water has been studied using many different methods. To identify potential infestation sites of *Spartina* spp., Howard et al. (2006) released drift cards from source populations. Individuals that found the cards weeks or months later would report the collection location and date. Merrit and Wohl (2002) released color-coded *Betula fontinalis* seed in flumes for 10 minutes to investigate the relationship between flow regime, channel morphology, dispersal phenology, and seed deposition patterns. Johansson and Nilsson (1993) studied the dispersal extent and range of *Ranunculus lingua* over two years by marking *R. lingua* fragments with a red thread. Nilsson et al. (1991) used red-painted pine cubes to mimic seed difference is deposition along a riverbank. They found that cube deposition was related to species composition of the riparian vegetation. Drift bottles are another common tool used for tracking surface currents in waterbodies; however, unless continuously monitored, the only definite information obtained is the time and place of release and recovery (Chew et al. 1962).

Tracking movement reaches beyond the water. Radio telemetry, which uses Global Positioning Systems (GPS) technology, is a popular choice in collecting data on animal movement and habitat uses (Ropert-Coudert and Wilson 2005). A remote tracking device is attached to the animal and the animal's movement as well as environmental data can be recorded. While plant-attached devices have not yet been developed, this technology could eventually help predict how aquatic vegetation will move in water systems.

Invasive Species in Mississippi

Alternanthera philoxeroides

Alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.) is an amphibious weed belonging to the family Amaranthaceae (Julien et al. 1995). It originated in South America and spread to North America, Asia, and Australia. Alligatorweed rarely sets seed and reproduces vegetatively from apical buds, axillary stem and root buds. In aquatic ecosystems it can produce dense mats or root into the bank (Julien et al. 1995). Alligatorweed interferes with fishing, irrigation, agricultural drainage, and flood control programs, and it invades cultivated fields such as soybean fields near alligator weed infested lakes (Kay and Haller 1982). Alligatorweed also provides habitat for mosquitoes to breed (Sainty et al. 1998). Biological control of alligatorweed in the U.S.A. began in the 1960's with the introduction of a flea beetle (*Agasicles hygrophila*), moth (*Vogtia malloi*), and thrips (*Amynothrips andersoni*) (Julien et al. 1995). In the southern United States, the flea beetle has controlled alligatorweed, while in the northern most areas, biological control was unsuccessful (Julien et al. 1995). Herbicides that have been used to control alligatorweed include 2,4-D [(2,4-dichlorophenoxyacetic) acid], imazapyr [2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-3-pyridinecarboxylic acid] and triclopyr {[(3,5,6-trichloro-2-pyridinyl)oxy]-acetic acid} which have demonstrated success in both immediate and longer term control (Allen et al. 2007; Cox et al. 2010), glyphosate [N-(phosphonomethyl)glycine] which has provided control in alligatorweed over water, and metsulfuron and dichlobenil for control on land and in shallow water (Sainty et al. 1998).

Eichhornia crassipes

Waterhyacinth is a free floating, mat-forming, perennial aquatic plant in the Pontederiaceae family (Penfound and Earle 1948; Barrett and Forno 1982). It is native to South America and is now distributed throughout the world (Barrett and Forno 1982). Waterhyacinth reproduces through clonal propagation and sexual reproduction. Its ability to clone itself has allowed it to take over lakes, reservoirs, and canals. Waterhyacinth has one of the fastest growth rates of any plant (Madsen et al. 1993). In areas where there are frequent changes in water level, the seeds from water hyacinth have the ecological conditions necessary for germination and establishment (Barrett and Forno 1982). Waterhyacinth impedes navigation in waterways, destroys wildlife resources, and prevents runoff from streams; consequently, increasing back water and flooding conditions (Penfound and Earle 1948). Integrating herbicide management with the waterhyacinth weevils (*Neochentina eichhorniae* Warner and *N. bruchi* Hustache) have been successful in managing the weed (Haag 1986; Haag and Habeck 1991). On the Ross Barnett Reservoir located in Mississippi, 2,4-D has been used for the control of waterhyacinth (Cox et al. 2010). 2,4-D is also the most common herbicide applied to waterhyacinth in Florida waterbodies (Ramey and Hassell 2009).

Hydrilla verticillata

Hydrilla (*Hydrilla verticillata* (L.F.) Royle) is a submersed aquatic plant from Southeast Asia and is currently found in Europe, Asia, Australia, New Zealand, the

Pacific Islands, Africa, North America, and South America (Langeland 1996). It has both monoecious and dioecious biotypes. The monoecious biotypes produce viable seeds while the dioecious biotypes do not (Langeland and Smith 1984). Hydrilla reproduces four ways including fragmentation, tubers, turions, and seed (Langeland 1996). Usually, hydrilla roots into the bottom of the waterbody, but fragments that break loose can survive free-floating. It can grow up to 1 inch per day (Langeland 1996). Hydrilla often out competes native vegetation because it can multiply rapidly, photosynthesize at low light levels, and it is unaffected by water quality (Shearer and Nelson 2002). Problems caused by hydrilla include flow reduction in drainage canals resulting in flooding and damage to canal banks and structures, clogging intakes of pumps used for irrigation water, and impeding navigation for recreational and commercial boats (Langeland 1996). Control of hydrilla has been somewhat successful using the herbicide active ingredients copper chelate [7-oxabicyclo (2.2.1) heptanes-2,3-dicarboxylic acid], diquat [6,7-dihydrodipyrido(1, 2-a:2', 1'-c) pyrazinediium], endothall [7-oxabicyclo (2.2.1) heptanes-2,3-dicarboxylic acid], and fluridone [1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl] -4(1H)-pyridinone] (Langeland 1996). An integration of fungal pathogens with endothall or fluridone has also been studied as control mechanisms for hydrilla (Shearer and Nelson 2002). The efficacy of fluridone has recently decreased in some populations due to a mutation in hydrilla causing resistance (Michel et al. 2004).

Oxycaryum cubense

Cuban bulrush (*Oxycaryum cubense* (Poepp& Kunth) Palla) is the only species in the genus *Oxycaryum* of the family Cyperaceae (Bruhl 2002). There are two forms of Cuban bulrush that are different due to inflorescence features. *Oxycaryum. cubense* forma c*ubense* has an umbellate inflorescence, while *O. cubense* forma *paraguayens* has monocephalous inflorescence (Barros 1960).

Cuban bulrush is found in the West Indies, South and Central America, tropical Africa, and the southeastern United States. In the southeastern United States, it is found in Florida (Anderson 2007), southern Georgia (Bryson et al. 1996), southern Alabama (Lelong 1988), Louisiana (Thomas and Allen 1993), coastal Texas (Turner et al. 2003) and Mississippi (Cox et al. 2010). Cuban bulrush was likely carried to the United States by migratory birds or ship ballast (Bryson et al. 1996).

Cuban bulrush is epiphytic so it depends on other aquatic species for structure (Tur 1971). It has been found in association with waterhyacinth, water spingles (*Salvinia minima* Baker), hydrilla, water pennywort (*Hydrocotyle ranunculoides* L.f.), angelstem primrose-willow (*Ludwigia leptocarpa* (Nutt.) H. Harra), parrotfeather (*Myriophyllum aquaticum* (Vell.) Verdc.), Eurasian watermilfoil, longleaf pondweed (*Potamogeton nodsus* Poir.), marsh mermaidweed (*Proserpinaca palustris* L.) and humped bladderwort (*Utricularia gibba* L.) (Bryson and et al. 2008). The large floating rafts impede navigation in rivers, lakes, ponds, ditches, and impounded swamps and can displace native organisms (Mallison et al. 2001). Robles et al. (2007) noted Cuban bulrush outcompeting waterhyacinth on Columbus Lake in Mississippi.

Cuban bulrush is believed to be dispersed by water and animals (Bryson et al. 1996). The spongy suberized pericarp of the achenes helps with flotation and dispersal by moving water, while the large mats allow for asexual reproduction as fragments are broken from the rafts and carried by water (Haines and Lye 1983). It is suggested that

Cuban bulrush may be in a lag phase or the sporadic distribution suggests low fertility of achenes (Bryson and Carter 2008).

Currently, no studies have been published documenting effective management techniques or herbicide rates for control of Cuban bulrush.

Early Detection and Rapid Response to Aquatic Weeds Using Herbicides

After finding new populations, the next step is implementing a control or eradication plan. There are several types of control options available including mechanical, cultural, biological and chemical; however, herbicides are usually the most cost effective tool for managers (Getsinger et al. 2008). There are currently 13 herbicides labeled for use in aquatics.

Penoxsulam [2-(2,2-difuoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazol[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide], a triazolopyrimidine sulfonamide herbicide, is an acetohydroxyacid synthase (AHAS) or acetolactate (ALS) inhibitor that has been used in the post emergent control of annual grasses, broadleaf weeds and sedges in rice fields (Jabusch and Tjeerdema 2006). As plants are starved of the branched-chain amino acids, they exhibit rapid growth inhibition which is followed by chlorosis, vein reddening and eventually deathb (Stidham 1991).

Flumioxazin [2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2h)-dione] is a N-phenylphthalimide herbicide that inhibit protophorphyrinogen oxidase (PPO) (Hess 2000). The first visible signs of membrane disruption include darkening of treated leaf tissue, followed by dessication and tissue necrosis (Hess 2000). When applying flumioxazin, water pH is important to consider because the half-life ($t_{1/2}$) for flumioxazin decreases as pH increases due to pH dependent hydrolysis. At a pH of 5, 7, and 9, the hydrolytic $t_{1/2}$ is 16.4, 9.1, and 0.25 h, respectively (Kwon et al. 2004).

Glyphosate is an organophosphorus herbicide that is absorbed by the foliage and transported via the phloem throughout the plant (Roberts 1998). The herbicide inhibits is 5-enolpyruvylshikimate-3 phosphate synthase (EPSPS) leading to a deficiency of tryptophan, phenylalanine, and tyrosine and an accumulation of shikimate (Tan et al. 2006). Glyphosate is a non-selective herbicide that is effective against giant salvinia (*Salvinia molesta*; Nelson et al. 2001), wild taro (*Colocasia esculenta*; Nelson and Getsinger 2000), and purple loosestrife (*Lythrum salicaria*). Rodeo® (Dow Agrosciences, Indianapolis, IN) is a glyphosate herbicide that is registered for use in and around aquatic systems (Paveglio et al. 1996). Unlike many of the glyphosate herbicides, Rodeo® does not contain a surfactant so during application a non-ionic surfactant must be added (Major et al. 2003).

2,4-D is in the phenoxy group of herbicides and affects plants by mimicking plant hormones known as auxins resulting in unregulated growth (Grossman 2010). 2,4-D is known for its selectivity for dicots (Grossman 2010). It has been used for the control of Eurasian watermilfoil (Parsons et al. 2003), wild taro (Nelson et al. 2001), alligatorweed (Maddox et al. 1971), and waterhyacinth (Center et al. 1999).

Triclopyr, a pyridinecarboxylic acid, is an auxim mimic like 2,4-D (Getsinger et al. 2003; Petty et al. 2003). Triclopyr is selective for dicots and has worked effectively on aquatic weeds such as Eurasian watermilfoil, purple loosestrife, waterhyacinth, and alligatorweed (Gardner and Grue 1996; Getsinger et al. 2003; Petty et al. 2003; Allen et al. 2007).

Diquat is a photosystem I inhibiting herbicide of the bipyridilum family (Ahrens 1994; Hess 2000). As a contact herbicide, it tightly adsorbs to the leaf surface and then rapidly absorbs into the leaves. Injury symptoms develop within a few hours and include darkening of treated leaves due to plasma membrane disruption. Within 1 to 2 days, the tissue becomes necrotic (Hess 2000). The herbicide has been used in canals, lakes, ponds, and drainage ditches for control of submersed and floating weed species such as hydrilla, common duckweed (*Lemna minor* L.), watermeal (*Wolffia* spp.), water lettuce (*Pistia stratiotes* L.), pondweeds (*Potamogeton* spp.) and giant salvinia ((*Salvinia molesta* D.S. Mitchell) (Nelson et al. 2001; Puri et al. 2008; Wersal and Madsen 2009).

Bispyribac-sodium [sodium 2,6-bis[(4,6-dimethoxy-2-pyrimidinyl)oxy]benzoate] is a pyrimidinyl carboxy herbicide that is an AHAS inhibitor (Lycan and Hart 2006). It has been used for control of annual bluegrass (*Poa annua* L.) and roughstalk bluegrass (*Poa trivialis* L.) on golf courses and sod farms, and for control of rice barnyardgrass (*Echinochloa phyllopogon* (Staph) Koso-Pol) and variable flatsedge (*Cyperus difformis* L.) in rice fields (Osuna et al. 2002; Park et al. 2002; Lycan and Hart 2006). In Experimental Use Permit (EUP) studies, the herbicide provided 70% or better control of weeds such as Eurasian watermilfoil, sago pondweed (*Potamogeton pectinatus* L.), waterhyacinth, and water lettuce (ValentCorp 2010).

Imazapyr is a systemic, AHAS inhibiting herbicide belonging to the imidazolinone herbicide family (Stidham 1991). It has been shown to control smooth cordgrass (*Spartina alterniflora* Loisel), torpedograss (*Panicum repens* L.), parrotfeather, and common reed (Patten 2002; Wersal and Madsen 2007; Mozdzer et al. 2008).

Imazamox [2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-5-(methoxymethyl)3-pyridinecarboxylic acid-3-pyridinecarboxylic acid], like imazapyr, is in the imidazoline family of herbicides and is an AHAS inhibitor (Quivet et al. 2006). Imazamox has been used in aquatic system for control of submersed, floating, and emergent aquatic weeds (Richardson 2008).

Carfentrazone [ethyl α, 2-dichloro-5-(4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl)-4-fluorobenzenepropanoate] is an PPO inhibitor belonging to the aryltriazolinone herbicides (Ngim and Crosby 2001; Ramsdale and Messersmith 2001). It has been used to control morningglories (*Ipomea* spp.) velvetleaf (*Abutilon theophrasti* Medik), wild buckwheat (*Polygonum convolvulus* L.), and common lambsquarter (*Chenopodium album* L.) that grow in soybeans, rice, corn and other crops (Dayan et al. 1997; Ngim and Crosby 2001; Ramsdale and Messersmith 2001). Water pH is important to consider when using carfentrazone because at pH 9, the $t_{½}$ is 3.36 hours, but increases to 131 hours at pH 7 (Ngim and Crosby 2001).

Endothall is a contact herbicide most commonly used on submersed species, such as Eurasian watermilfoil and hydrilla (Skogerboe and Getsinger 2001). The concentration and exposure time of endothall to the target plant is important when using this herbicide since plant sensitivity varies. Consequently, endothall is useful in areas where nuisance plants are mixed with native or desirable plants (Skogerboe and Getsinger 2001).

Fluridone is carotenoid biosynthesis inhibitor. Without carotenoids, plants treated with PDS inhibitors exhibit characteristic bleaching (Arias et al. 2005). The chlorosis symptoms appear 3 to 6 days after treatment (McCowen et al. 1979). The exposure time between fluridone and the target plants is important for control (Macdonald et al. 1996). For control of Eurasian watermilfoil and hydrilla, an exposure time of 60 or more days is often necessary. Microbial degradation and photolysis are the two main degradation pathways for the herbicide. The $t_{½}$ of fluridone in water ranges from 5 to 90 days and depends on factors such as water depth, plant coverage, water clarity (MacDonald et al. 1996).

Management objectives for invasive plants align with phases of weed invasion. When the weed is not present, managers aim to prevent its introduction (Lockwood et al. 2007). If prevention fails, the next objective is to find a new population as early as possible and implement management techniques quickly to minimize their impact. By identifying new populations early, eradication is more likely and control methods are less costly. Once the invaders reach the latter stages of invasion where they are now impacting the ecosystem and human activities, the management objective is typically to control and contain the species, which is often a time consuming and costly process (Hobbs and Humphries 1995; Lockwood et al. 2007). Two studies were conducted to provide managers with tools that will hopefully prevent invasive plants from reaching the latter stage of invasion. First, a GPS tracking device was developed to monitor drift in three Mississippi reservoirs. These devices would provide information on drift that could be used in monitoring plant spread. A study to evaluate the efficacy of several herbicide treatments on Cuban bulrush, an invasive plant currently contained to the southeastern United States, was also conducted. By identifying those herbicides that effectively manage Cuban bulrush, new found populations can be quickly managed and spread will be minimized.

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CHAPTER II

TRACKING THE MOVEMENT OF WATER IN THREE MISSISSIPPI RESERVOIRS

Abstract

From current velocity impacting substrate composition and water turbidity to the physical movement of plant propagules, the movement of water plays an important role in shaping aquatic vegetation communities. To monitor the movement of water, custom-made GPS drones were released in Aliceville Lake, Columbus Lake, and Ross Barnett Reservoir, MS. In 2011, the drones were released at least four times in each reservoir during June, August, September, and October. In 2012, the drones were released at least seven times in each reservoir as an attempt was made to collect data under normal conditions and after a disturbance. For each year, the drones were released near a plant bed and allowed to drift for two to six hours. While the drones drifted, plant fragments were sampled for 100m with a dip net at 50, 100, and 250m from the plant bed to monitor fragment quantities throughout the growing season and under normal and disturbed conditions. Data collected by the drones were exported into ArcMap10™. The drones drift paths were compared to the expected flow direction as mapped by the National Hydrography Dataset. Wind data from nearby weather station were used to analyze patterns in drone drift that were not described by the expected flow direction. A significant difference in wind speed was detected between drone drift that was considered wind-driven and drift that was not considered wind-driven. Future spatial simulation

models dealing with aquatic vegetation distribution should incorporate wind direction and speed into consideration especially in fragmented ecosystems such as reservoirs. Lastly, results from the vegetation fragment sampling indicated a significant difference in mean fragment presence between disturbed and normal conditions in Aliceville Lake and Columbus Lake. Management strategies that may act as a disturbance such a mechanical removal are not recommended in these systems.

Introduction

The importance of flowing water on aquatic plant communities ranges from current velocity dictating sediment deposition, water turbidity, photosynthesis rates and physical growth characteristics of the plant to hydrochory, dispersal by water (Madsen et al. 2001). Dispersal of vegetative fragments is common reproductive method used by many aquatic plant species (Santamaria 2002). River discharge rates and mechanical breakage due to human activities or weather events can increase the amount of fragment being dispersed (Madsen et al. 2001). Plant spread rates are important for maintaining biodiversity and determining invasive success of alien organisms (Higgins et al. 2001). Many aquatic plants also have other vegetative means of dispersal that float at varying depths in the water column (Santamaria 2002). As buoyancy varies among these structures, studies have suggested that flowing water may play a role in structuring aquatic and riparian plant communities (Nilsson et al. 1991; Brown and Chenoweth 2008). The ability to monitor the flow of water could provide water-body specific insight on current velocity and commonly forming currents which can help explain how flowing water aides in aquatic plant distribution patterns.

Many different methods have been utilized for monitoring drift in water. Drift cards were used to identify potential infestation sites of *Spartina* spp. (Howard et al. 2006). Drift bottles were used to monitor surface wind-generated drift currents (Shulman and Bryson 1961). Individual *Ranunculus lingua* fragments were marked with a red thread to evaluate dispersal extent and range (Johansson and Nilsson 1993). To mimic seed deposition along a riverbank, red-painted pine cubes were released in river (Nilsson et al. 2001). For all of these methods, a commonly cited problem is that the only definite information obtained is the time and location of release and the location of collection for each entity (Shulman and Bryson 1961). Using GPS technology can help amend this problem.

In recent years, GPS technology has been a popular choice for tracking animal movement (Ropert-Coudert and Wilson 2005). A remote tracking device is attached to the animal and the animal's movement as well as environmental data can be recorded. While plant-attached devices have not yet been developed, for this study a waterproof, floating GPS tracking device was developed to monitor drift in water. The objectives of the study were to identify patterns in water movement using the GPS tracking device. Since the bulk flow of water follows an elevational gradient flow direction, I hypothesized that the drones will drift in the same direction as the mass water movement. Because an increase in the amount of plant fragments increases the chance for successful establishment of new plant populations, patterns in plant fragments quantities were monitored across the growing season and in the presence and absence of events that might generate propagules. I expected to see differences in plant fragment quantities throughout the growing season as plant biomass varies with time. Lastly, I predicted more plant fragments would be collected after a disturbance than in the absence of a disturbance.

Materials and Methods

Study Sites

The study was conducted in 2011 and 2012 in three Mississippi water bodies (Fig. 2.1). Ross Barnett Reservoir (32.4571°N, 90.0179° W) is a 13,354 ha reservoir managed and built by the Pearl River Valley Water Supply District. The reservoir provides water for Jackson, MS, as well as recreational opportunities for 2.5 million annual visitors (RossBarnettReservoir.org 2001). Since 2005, the reservoir's aquatic plant populations have been surveyed using a pointintercept method (Sartain et al. 2012). American lotus (*Nelumbo lutea*) is the most dominant species with a 27% frequency in 2009 (Cox et al. 2010). Nonnative invasive species found in the reservoir include alligatorweed (*Alternanthera philoxeroides*), waterhyacinth (*Eichhornia crassipies*), hydrilla (*Hydrilla verticillata*), parrotfeather (*Myriophyllum aquaticum*), brittle naiad (*Najas minor*), Cuban bulrush (*Oxycaryum cubense*), and water lettuce (*Pistia stratiotes*). Aliceville Lake (33.2389° N, 88.2847° W) and Columbus Lake (33.5341° N, 88.4910° W) are part of the Tennessee-Tombigbee system. The Tennessee-Tombigbee waterway was completed in 1984 by the U.S. Army Corps of Engineers and connects the Tennessee River in Northeast Mississippi with the Tombigbee River near Amory, MS (USACE 2010). The waterway is 377 km long and has ten lakes made by a series of locks and dams that allow for shorter routes for boats from the interior United States to the Gulf of Mexico (USACE 2010). Aliceville Lake and Columbus Lake encompass 3,320 and 1,208 ha respectively (Miranda and Pugh 1997; Robles et al. 2011). In both of reservoirs, hydrilla, waterhyacinth, Eurasian watermilfoil and Cuban bulrush have been found (Robles et al. 2011).

Drone Drift Study

To monitor water movement, a custom built GPS tracking device called a drone was created (Fig. 2.2). The drones are equipped with a GSM/GPS wireless communication devices that use a SIM card to communicate with the receiver's cellular phone via text message, or the user can receive an email. The drones also contain an on-board USB flash drive for back up storage of data in case the emails or text messages are not successfully sent. The drones relay the latitude and longitude of their location within 2.5 meters of the correct position along with the time at which the position was recorded. The frequency of position recordings is programmable. For this study, the drones recoded their position every fifteen minutes.

Much of the first year of this study was spent learning to properly handle the drones and how to use them in the most efficacious manner. In 2011, drone sampling occurred in June, August, September, and October. During a sampling trip, drones were released at two sites within the reservoir. Sites were chosen based on the presence of invasive species such as alligatorweed, hydrilla, Eurasian watermilfoil, waterhyacinth, or Cuban bulrush. At the chosen site, the two drones were released at the edge of the plant bed. Within a minute of being started, the drone recorded its position, time and date. The release location of the drone was manually recorded onto a Yuma™ using FarmWorks Site Mate® software. Water quality and light readings were also measured at each release location. Once the drones reached land, they were collected. At the collection site, the collection position was manually recorded on the Yuma, and water quality and light meter readings were again measured.

By the end of the 2011 sampling period, waterproofing and operation of the drones greatly improved with one set of drones typically lasting at least eight trips. The September and October sampling trips also had better drift results after learning that if the drones were placed too close to the plant beds, little movement occurred. This was especially true for days when the wind was blowing into the release site. The information learned during the 2011 season was then used to optimize drone drift sampling during 2012.

For the 2012 sampling period, drones were deployed in April, June, August, and October to sample throughout the growing season. In Aliceville and Columbus Lake, three sites were chosen within each lake and one drone was released at each site. The release location in relation to plant bed was dependent on the direction of the wind so that a drift path was generated. In Ross Barnett Reservoir, due to heavy boating traffic, only two sites were used so that the drones could be monitored at all times. At each of these two sites, two drones were deployed. As in 2011, release and collection locations were recorded with a Yuma®, water quality and light meter readings were collected at both spots, and plant fragment collection procedures remained the same.

Due to the observed importance of wind on drone movement, additional information was collected in 2012 included field recordings of wind direction based on personal observation and wind speed determined with a Kestrel® pocket wind meter. Wind readings were made approximately every hour; however, for use in the data analysis, wind information from nearby weather station was utilized because the weather station collected wind readings every 15 minutes. Wind data for Aliceville Lake and Columbus Lake was obtained from the Columbus Airforce Base (KCBM, 33.6439° N, 88.4439° W) and Ross Barnett Reservoir wind data was collected from Turtle Ridge, Brandon (32.3682° N, 90.0327° W). Data for these stations was accessed via the National Weather Service Climate Data Center.

River discharge data were collected from the USGS streamflow gauges. The streamflow gauges were near the study sites for both Aliceville Lake (Tom Bevil Lock and Dam 33.° N, 88.° W) and Columbus Lake (Stennis Lock and Dam 33.° N 88.° W). Discharge data for Ross Barnett Reservoir was not available, so discharge rates for the Pearl River at Jackson (32.2352° N, 90.0353° W) were used. Flow direction from the USGS National Hydrography Dataset (NHD) was used to establish the predicted direction in which the drones were expected to drift.

Plant Fragment Sampling

Using a 0.64 cm mesh sampling net with an area of 0.27 m^2, plant fragments were sampled at plant beds containing invasive species. Sampling occurred by dragging the net in the water across 100 m transects located 50, 100, and 250 m from the chosen plants beds. Each transect was sampled three times. After each 100 m run, all plant fragments were removed from the net, the species present were recorded and placed in labeled plastic bags. After a sampling trip, the plant fragments were dried for at least 48 hours at 70°C and then weighed.

An attempt to sample disturbed and undisturbed conditions was made. For the purposes of this study, the term “disturbance” is used to indicate an event in which plant fragments are generated (Riis and Sand-Jensen 2002). In most cases, weather events with rain accumulations of at least 0.76 cm were considered a disturbance and changes in discharge amounts were noticeable on hydrographs. Sampling occurred at most two days after the event. Undisturbed conditions indicate no obvious fragment generating event occurred.

In 2011, an attempt was made to sample under disturbed and undisturbed conditions. But, due to malfunction of the drones and subsequent replacement, sampling after a disturbance occurred only in June. For the 2012 sampling trips, Aliceville and Columbus Lake were sampled under both undisturbed and disturbed conditions for all months except October. For Ross Barnett Reservoir, sampling occurred under both disturbance conditions only in April and June.

Data Analysis

Plant Fragment Sampling

Due to inconsistencies during the 2011 plant fragment sampling, only data from 2012 was used for the data analysis. The proportion of transects in which a plant fragment was present was calculated for all sampling trips. Plant fragment presence, was fitted with a mixed model using the Mixed Procedure in SAS® (Wersal et al. In press). Fragment presence was included as the dependent variable while month and disturbance condition were included as independent variable. The transect (month by disturbance condition) interaction term was included as a random effect to account for its influence on the results. Each reservoir was analyzed independently.

A significant disturbance condition effect was observed in Aliceville (p=0.0035), and Columbus (p=0.0002), but not in Ross Barnett (p=0.1649). All analyses were conducted at the p=0.05 significance level.

Drone Drift Study

Drone drift paths that contained more than two points were used from both years of the study. The locations the drones recorded were exported into Excel from the text files saved onto the drone's USB. The points were then converted into decimal degrees and added to a map layer in ArcMap 10™ (ESRI). The points were converted to line shape files and then split at the vertices so that each line segment between two vertices represents 15 minutes of drone travel. Using the linear directional mean tool in the Spatial Statistics Toolbox, the average compass angle (clockwise from due North) and linear directional mean (counterclockwise from due East) were generated for each line. When wind creates surface drift currents, the direction the surface current travels deflects to some degree from the direction of the wind (Shulman and Bryson 1961). The amount of deflection varies with wind speed, Coriolis Effect, and water surface slope; however, at the scale of this study, the latter two are negligible (Shulman and Bryson 1961; Downing- Kunz and Stacey 2011).   To categorize drone drift as wind driven or not wind driven, two categories were created: deflection ≤45°and deflection >45° The deflection angle from the wind was calculated by subtracting the wind direction from the drone direction and taking the absolute value. If the angle was ≤45° movement was considered similar to the wind; however if drone movement was >°, drift was not considered wind driven. This allowed a southerly wind to blow a drone anywhere from the northeast to the northwest, and the drift path would be considered wind driven. Although mathematically the deflection angle could be greater than 180°, deflection angles were capped at 180° because at this angle, the drone would drift toward the direction from which the wind originated.

The Kruskali-Wallis rank sum test software system within $SAS^{®}$ was used to assess differences of rank means of wind speed and river discharge data for the two drift categories (McDonald 2009). River discharge was not significantly different between the two categories so it is not discussed any further. All analyses was conducted at the p=0.05 significance level.

Results and Discussion

Plant Fragment Sampling

In Aliceville, ten aquatic plant species were collected compared to seven species in Columbus, and four in Ross Barnett (Table 2.1). In Aliceville and Columbus, waterhyacinth was collected most frequently. In Ross Barnett, American lotus was the most frequently collected plant species. Disturbed and undisturbed sampling conditions were marked by an increase in river discharge rates (Table 2.2). No significant difference in mean fragment presence among sampling month was found for any of the reservoirs; however, a significant difference in mean fragment presence between disturbed and undisturbed conditions was detected in Aliceville and Columbus, but not Ross Barnett (Table 2.3). In Aliceville, waterhyacinth was the most frequently collected plant. This reservoir is off of the main river channel, so the increase in fragments is likely the result of mechanical wave action. Columbus is the most river-like of all the study sites and an increase in discharge, which would carry more fragments, in addition to mechanical-wave action, probably generated the plant fragments. Ross Barnett is a heavily managed system with routine herbicide applications throughout the growing season (Sartain et al. 2012). Small patches of alligatorweed, spotted on the first sampling trip on April 1, 2012, showed signs of herbicide injury on the subsequent trip in April 26, 2012. By

August, these alligatorweed patches were gone. Throughout the study, American lotus was the only plant to visually increase in the sampling area. Since the plant is native, it is not a target of herbicide treatments. The active management strategy for Ross Barnett may be the reason so few plant fragments were collected throughout the study period (Sartain et al. 2012). Seasonal patterns in fragment loads are often expected as plant biomass increases throughout the growing season. Owens et al. (2001), detected a seasonal pattern in biomass loading rates in the San Marcos River; however, in these three Mississippi Reservoirs, no seasonal pattern was detected. In Aliceville and Columbus, disturbance did affect mean plant fragment presence. In these reservoirs, plants that reproduce via stem fragments may be favored since stem fragments are often continuously available while seeds and turions are confined to seasons (Santamaria 2002). Furthermore, stem fragments provide a low production cost means of reproducing for aquatic plants and are believed to establish more successfully than other means (Johansson and Nilsson 1993). The ability to reproduce via vegetative fragments is a well-documented characteristic of invasive plants (Rejmanek 2000, Kolar and Lodge 2001). Since an increase in fragment quantities is believed to increase the chance for successful establishment, management efforts that may increase fragment quantities should be avoided, or at least minimized, in these systems (Rejmanek 2000).

Drone Drift Study

A total of 104 drone releases were made 2011 and 2012. The initial drone prototype required a series of modifications to improve reliability. Several malfunctions that had to be corrected were issues, such as a decline in the frequency of successful text messages transmissions. Initially, cell phone signal was thought to be the problem;

however, after testing the drones on land, it was ruled not to be the problem. The onboard USB always recorded the drone location, even in the absence of a text message, as long as the drones were properly functioning. The on-board thermometer recorded the temperature within the drone instead of the outside temperature. The highest temperature recorded was 57°C.

In 2011, twelve drones were used throughout the sampling period for a total of five trips. Usually during any given sampling trip, at least one drone would not correctly collect data. In 2012, after a series of attempted improvements to drone construction, the drones worked until the final trips in October when data were sporadically collected. Improved performance in 2012 was likely due to better construction and waterproofing.

On the June 21, 2011, collection date in Aliceville, an unexpected storm forced a quick exit of the reservoir without two drones. During this trip, text messages were not being sent, but the next day, one drone was recovered after searching for it in the reservoir. The drone had a hole in the side from repeated knocking against a log. The drone recorded its location onto the USB until water entered into the drone.

The second drone drifted to a location where it sat for nine days while continuously recording its position every 15 minutes (Fig. 2.3). On June 29, 2011, the drone began to drift again moving along a similar path in which it previously traveled (Fig. 2.4).

The Aliceville example showcases the advantage GPS tracking devices provide over drift card, drift bottles, and other drift monitoring methods. Without the GPS track, the collection location would have been marked and a path inferred. However, the drones showed an intermediate stop before reaching the final collection location.

For the drone drift analysis, in Aliceville, 22 paths were used, and in Columbus and Ross Barnett, 25 and 20, respectively. Drone drift occurred in several directions in all of the reservoirs (Fig. 2.5-2.7). For Aliceville, water flows out of the study sites toward the east (Fig. 2.8). It then moves south joining the main Tenn-Tom river channel. Therefore, the drones were expected to drift toward the southeastern waters as those currents pulled water from the study site. Conversely, the drones most commonly drifted toward the west, followed by the southeast and then northwest (Fig. 2.11). In Columbus Lake, the water flow in the study site is also toward the southeast (Fig. 2.9). In this reservoir, the most common drift direction was the southeast followed by south and then northeast (Fig. 2.11). For Ross Barnett, expected drift path was west (Fig. 2.10). However, most drift was toward the southeast followed by northeast, south, and southwest (Fig. 2.11).

To account for the departure from the expected drift path, wind direction was considered. Deflection from moving exactly with the wind is expected, but assuming perfect movement with the wind allowed for an idea of which drone drift paths were moving in the general direction of the wind (Fig. 2.12; George 1981). Allowing for at most 45° deflection, in Aliceville, Columbus, and Ross Barnett, 50, 52, and 48% of drone drift paths moved with the wind, respectively.

Since not all drift paths could be described by the wind direction alone, wind speed was considered. For all of the reservoirs, median wind speeds for drones that moved with the wind were significantly higher than for drones that deflected >45° (Table 2.4). This suggests that there may be a threshold wind speed necessary to overcome gradient flow. The factors influencing the drift direction of the drones with >45°

deflection seems to vary by reservoir. In Aliceville, the drones drifted toward two directions which can be explained by the NHD prescribed flow direction. First, most of the drones drifted towards the west (Fig. 2.13a). This directional movement maybe explained by inflow water that is directed into the study site from the northeast. The other common drift direction was eastward which would be expected as the water is pulled toward the southeast to rejoin the main Tombigbee channel. When the wind was not the main driving drift factor in Columbus, there was an easterly pattern to the drone drift which matches the flow direction assigned to the study site by the NHD (Fig. 2.13b). In Ross Barnett, the majority of drone drift that was not well described by wind, drifted southward; however, 42% actually drifted toward the north (Fig. 2.13c). The lack of explanation for this movement is likely due to using off-site weather stations. Using wind data from off-site stations presents some challenges especially when wind speeds were low. Field observations often noted variable wind directions that were not recorded in the off-site weather data. Using an on-site weather station that documents wind conditions in ten minute intervals would provide a better understanding of the forces acting on the drones.

The drones identified a gap in aquatic plant research that impacts the statistical methods used for mapping plant dispersal and surface water movements in reservoirs. At the landscape scale, water flows in one direction from areas of higher elevation to lower elevation, but when looking at an individual reservoir, wind becomes a major driver of currents that are multi-directional (Merrit and Wohl 2006). Since direction is a circular variable that is represented as a point on a circle, the standard linear methods of analysis are not applicable (Jammalamadaka and Lund 2006). Instead statistical analyses have to consider the values being used as the zero-direction and whether the rotation is clockwise or counter-clockwise. Different measures of correlation are necessary depending on if the analysis involves two circular variables, or one circular variable and one linear variable. In the case of the drones, wind direction and drone drift direction would involve circular-circular correlation while drift direction and drift rate, would require circular-linear regression (Jammalamadaka and Lund 2006). The use of circular statistics should be considered for any sort of water movement studies in lakes or reservoirs where transport may be in multiple directions.

The directional data provided by the drone would be useful in defining dispersal direction parameters in spatial simulation models built for predicting rates and patterns of invasive plant spread (Higgins et al. 2001). Currently, programs such as ArcHydro or data from the NHD build flow models based on elevational gradients, so using these to model water movement or drift in reservoirs may not always be the best approach if the area of concern experiences strong seasonal or diurnal wind patterns which may alter local dispersal direction.

Several authors have documented the impacts of river fragmentation on propagule dispersal (Johansson and Nilsson 1993; Merrit and Wohl 2002; Merrit and Wohl 2006; Brown and Chenoweth 2008; Rood et al. 2010). The reservoir that is created acts as a propagule trap limiting downstream dispersal as gradient flow diminishes and wind currents are generated. Although observations of wind effects on seed and plant fragment transport in reservoirs have been noted by these researchers, the drones have quantified the effects of wind on plant movement in water by suggesting that a threshold wind speed needs to be reached in order to overcome gradient flow. Furthermore, results from the lab drift study suggest that the morphology of the plant may influence the susceptibility of the plant to movement by wind (Appendix). Floating plants such as waterhyacinth have a leaf canopy that acts like a sail catching the wind. Qualitative descriptions of wind directed waterhyacinth movement support the observation of wind directing waterhyacinth against gradient flow (Penfound and Earle 1948; Gay 1960; Bock 1969). However, in a lab study by Downing-Kunz and Stacey (2011) water currents were found to be a greater driving force on waterhyacinth mat movement than wind, so evaluating drones in rivers and streams with defined flow is necessary.

The susceptibility of plant fragments to wind movement depends on the buoyancy of the plant tissue. Plant fragments, seeds and other vegetative propagules, may float on the water surface or may be suspended in water column (Riis and Sand-Jensen 2006). Vegetative propagules floating on the current surface may not have as much surface area catching the wind, but they will still move with the wind-driven surface currents. For propagules suspended in the water column, drift direction will depend on reservoir characteristics such as width and depth as well as the duration, speed, and direction of the wind (Zyryanov and Frolov 2006). In wide, deep areas of a reservoir, a two layer current can be created based on the direction and strength of the wind. When the wind blows opposite to the gradient current, wind-driven surface currents move against the gradient current. When the wind blows with the gradient current, the combination causes a tilting of the surface and a countercurrent develops at greater depths. Zyranov and Frolov (2006), found at winds of 3 ms^{-1} (6.7 mph), a countercurrent began to develop 2 m below the surface. In both of these scenarios, floating propagules or aquatic plants could possibly drift opposite of propagules suspended at greater depths. Based on these observations, future studies on plant community patterns downstream from a reservoir could consider species susceptibility to wind-generated surface currents as an explanatory variable to plant community composition.

For use in future research, the reliability of the drone to send text messages needs to be improved. On-site weather stations or drone-attached wind devices that are a better representative of the wind conditions actually experienced by a particular area in a lake or reservoir should also be utilized. To decrease the amount of surface area in contact with the wind the drones should be engineered to sit lower in the water or a weight should be added to the bottom of the drones to simulate root drag. Evaluating the drones in systems with more defined flows is necessary to identify how much flow is needed to overcome wind movement. Lastly, studies of drift that expand more than a few hours would identify further advantages and disadvantages to using a GPS device in place of previous methods used to monitor drift.

Table 2.1 Percent frequency of occurrence for species collected under normal (N) or disturbed (D) conditions in 2012.

PLANT	COLUMBUS						ALICEVILLE						ROSS BARNETT			
	Apr		Jun		Aug		Apr		Jun		Aug		Apr		Jun	
	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D
Eichhornia crassipies	0	11	7	30	0	7	22	33	19	55	15	22	*	*	*	*
Ceratophyllum demersum	0	15	0	0	0	0	4	0	0	4	0	7	0	4	0	0
Alternanthera philoxeroides	0	26	0	0	0	0	14	11	4	4	0	7	0	7	0	0
Salvinia minima	*	*	*	*	*	*	0	0	0	7	19	30	*	*	*	*
Hydrilla verticillata	0	7	0	0	0	0	7	11	0	0	0	0	*	*	*	*
Oxycaryum cubense	*	*	*	*	*	*	0	4	11	0	0	4	*	*	*	*
Hydrocotyle ranunculoides	*	*	*	*	*	*	0	0	0	0	0	4	*	*	*	*
Limnobium spongia	*	*	*	*	*	*	0	0	0	0	0	4	*	*	*	*
Utricularia sp.	0	4	0	0	0	4	0	0	4	0	0	37	*	*	*	*
Lemna minor	0	0	0	0	0	4	0	0	0	0	0	4	*	*	*	*
Potamogeton nodusus	0	0	0	0	0	4	*	*	*	*	*	*	*	*	*	*
Nelumbo lutea	*	*	*	*	*	*	*	*	*	*	*	*	4	22	6	11
Eleocharis tenuis	*	*	*	*	*	*	*	*	*	*	*	*	0	0	6	6

*Species were not collected in reservoir during sampling.

Table 2.2 River discharge rates for sampling days in 2012.

Aliceville	Discharge (cfs)	Columbus	Discharge (cfs)	Ross Barnett	Discharge (cfs)
19-Apr	12,947*	**19-Apr**	8,150*	**1-Apr**	12,704*
30-Apr	2,071	**30-Apr**	2,050	**26-Apr**	1,243
5-Jun	,*	**5-Jun**	3,960*	**6-Jun**	2,922*
21-Jun	1,559	**20-Jun**	1,890	**27-Jun**	222
1-Aug	1,295	**31-Jul**	655		
14-Aug	1589*	**14-Aug**	1590*		

*Indicates after a disturbance.

Table 2.3 Results of the mixed model and mean fragment presence in 2012.

Condition	**Aliceville**			**Columbus**			**Ross Barnett Reservoir**	
	April	June	August	April	June	August	April	June
Normal	0.25	0.19	0.19	0	0.07	0	0.05	0.11
Disturbed	0.33	0.59	0.37	0.26	0.3	0.07	0.3	.05

* In Aliceville and Columbus Lakes a significant difference was observed between normal and disturbed conditions (p=0.0035, p=0.0002, respectively).

*In Ross Barnett Reservoir no difference (p=0.1649) was observed between normal and disturbed conditions.

Table 2.4 Wind speeds for drones categorized as moving with the wind (≤45°) and not being wind-driven (>45°) in 2012.

	Wind speed (kph)		Kruskali-Wallis test statistic
	≤45° deflection	>45° deflection	
Aliceville	6.68	3.19	H=10.31, P=.0013
Ross Barnett	10.77	3.59	H=14.18, P=.0002
Columbus	6.31	2.85	H=7.33, P=0.0006

* A significant difference was detected between the wind speeds within each reservoir at p=0.05 significance level.

Figure 2.1 Locations of drone drift studies in 2011 and 2012.

Figure 2.2 The interior circuit board of the GPS drone.

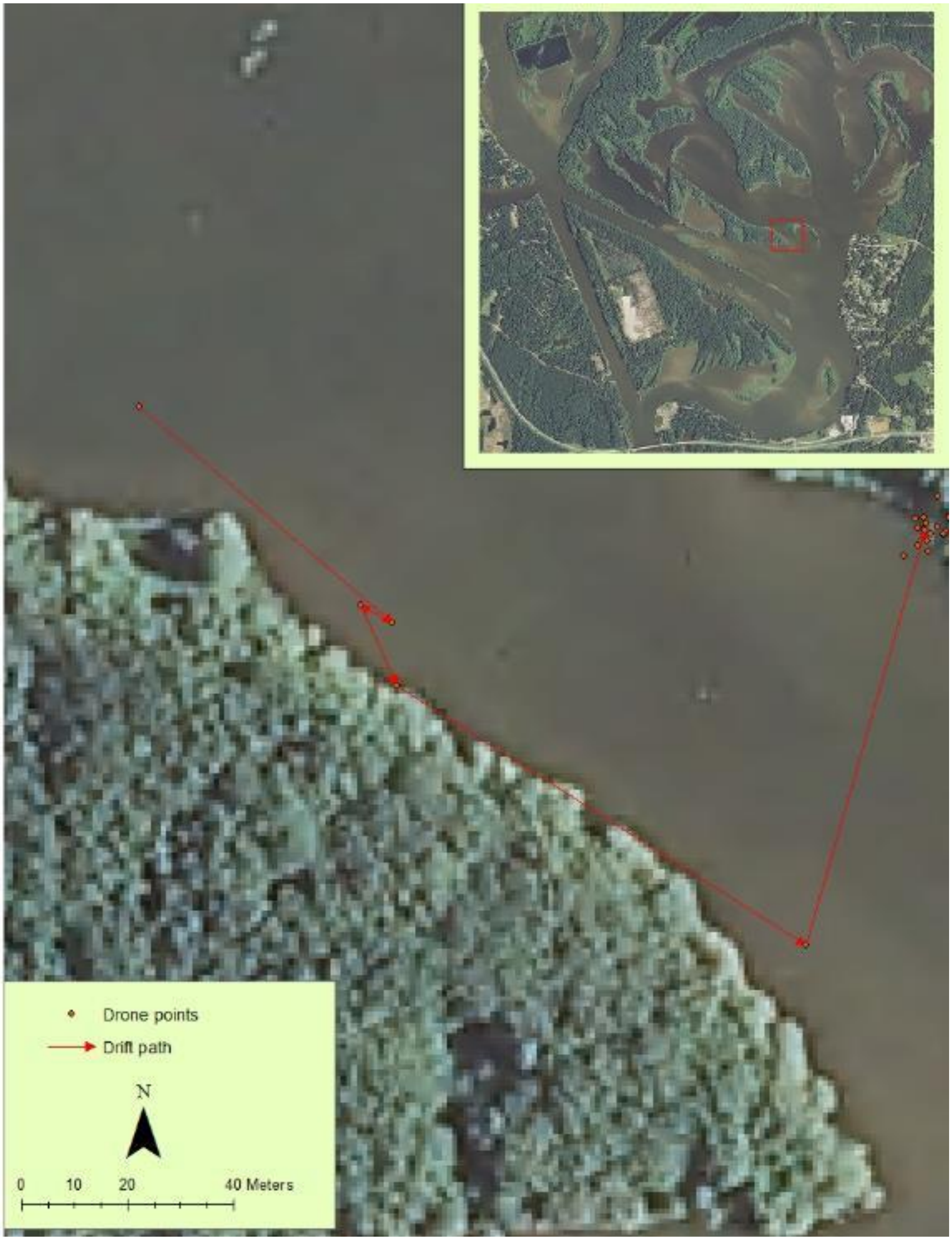

Figure 2.3 June 21, 2011 drone drift track in Aliceville Lake, Mississippi.

Figure 2.4 Drone drift path from June 21-29, 2011 in Aliceville Lake, Mississippi

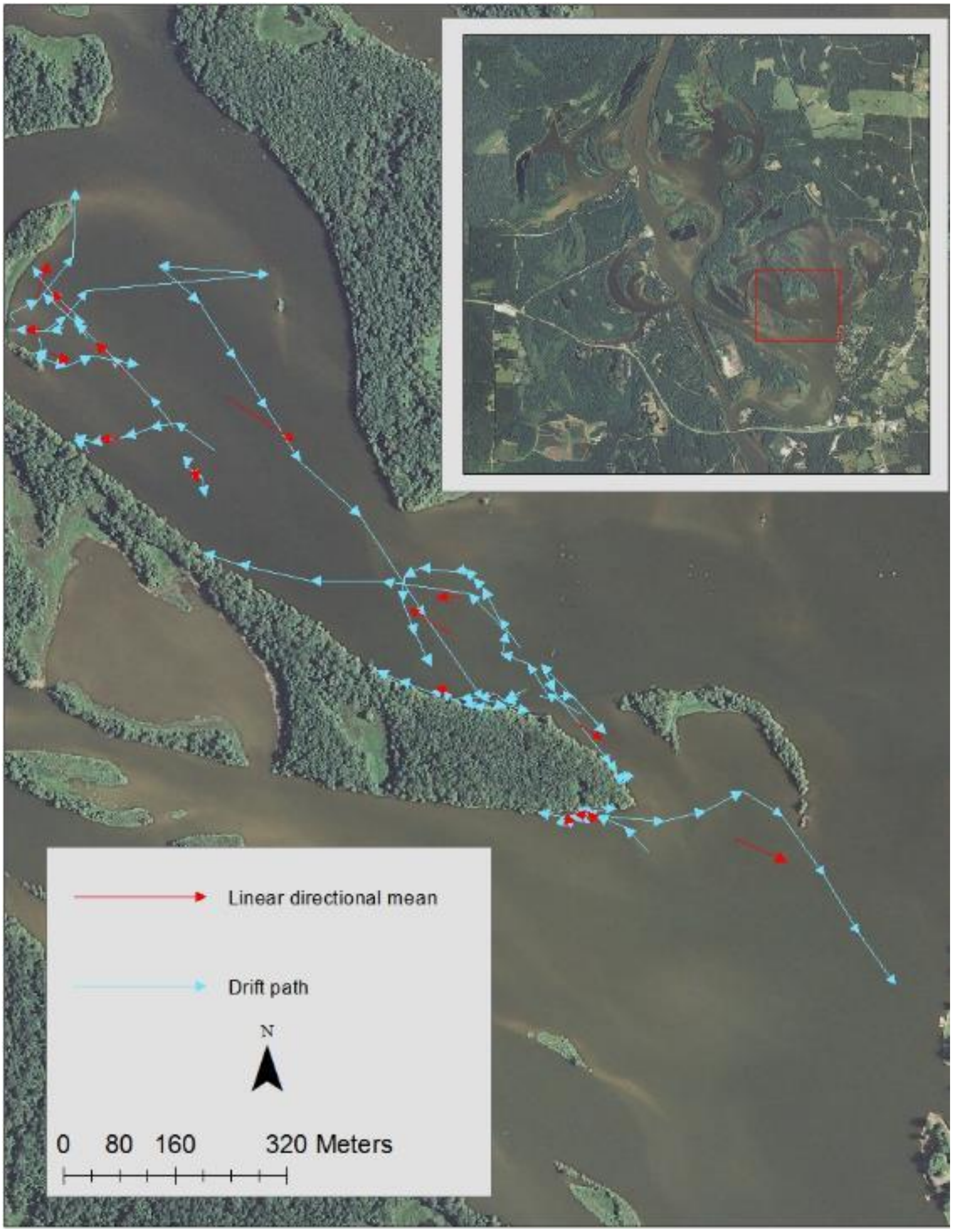

Figure 2.5 Drone drift paths and linear directional means in Aliceville Lake, Mississippi, 2012.

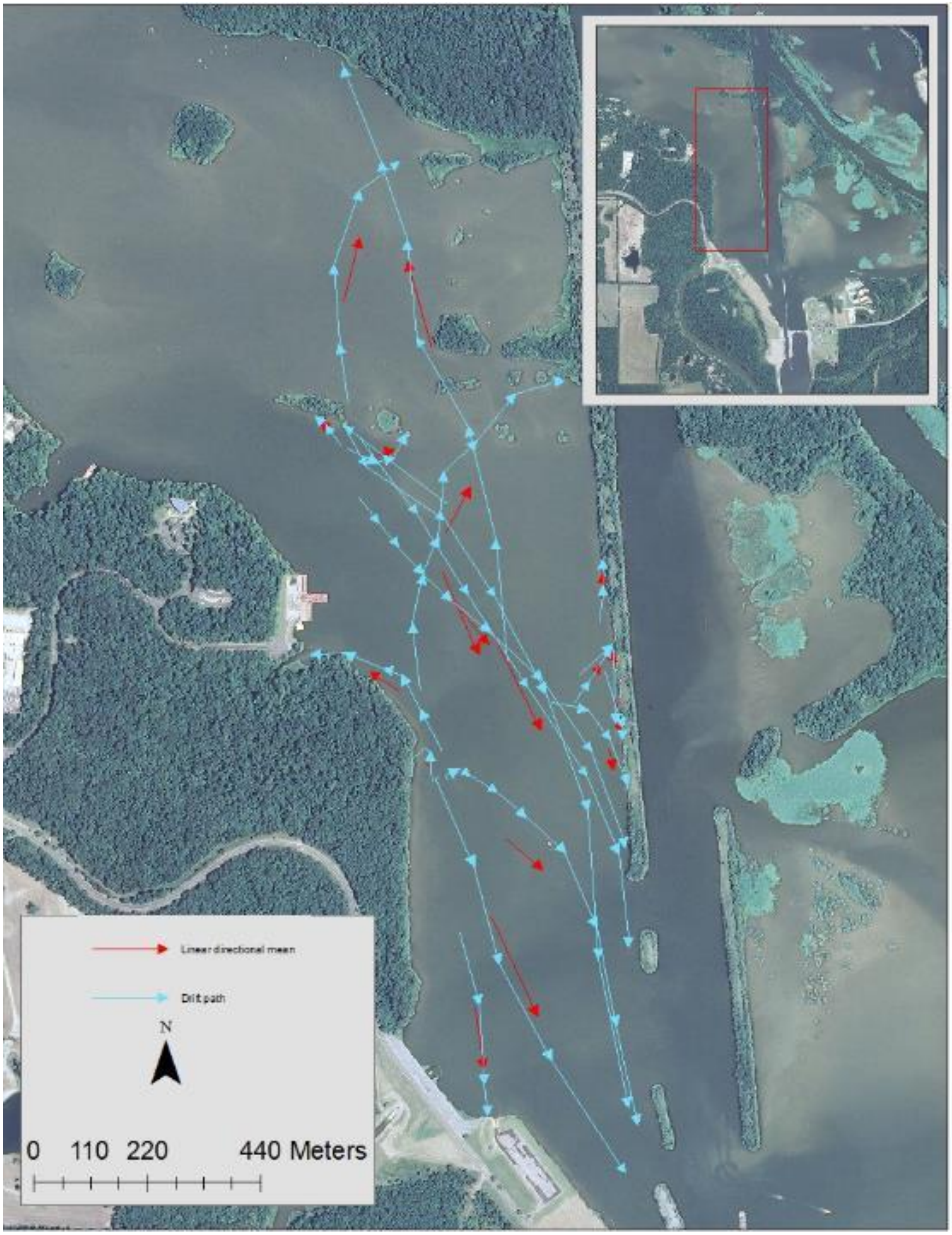

Figure 2.6 Drone drift paths and linear directional means for drones released in Columbus Lake, Mississippi, 2012.

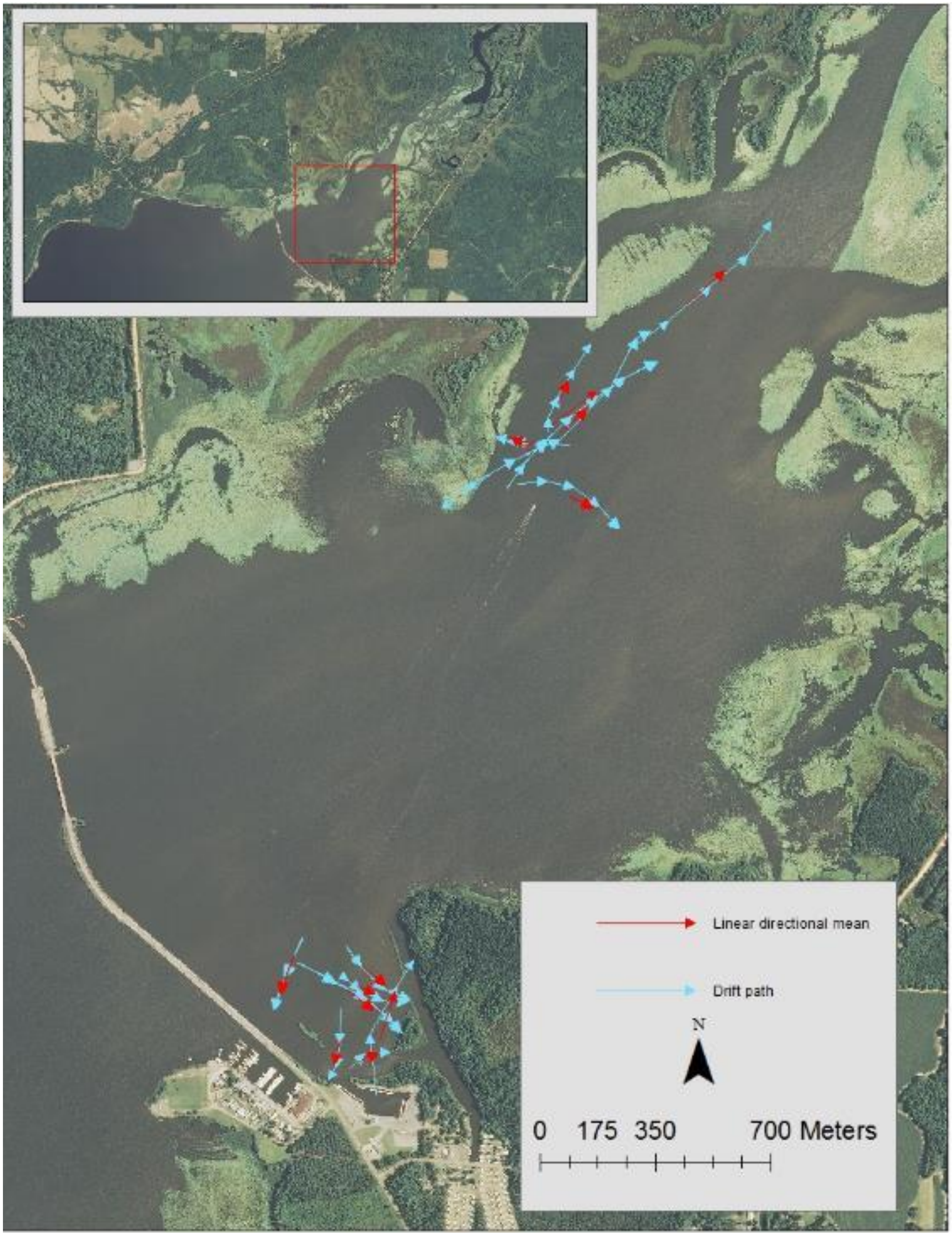

Figure 2.7 Drone drift paths and the linear directional means for drones released in Ross Barnett Reservoir, Mississippi, 2012

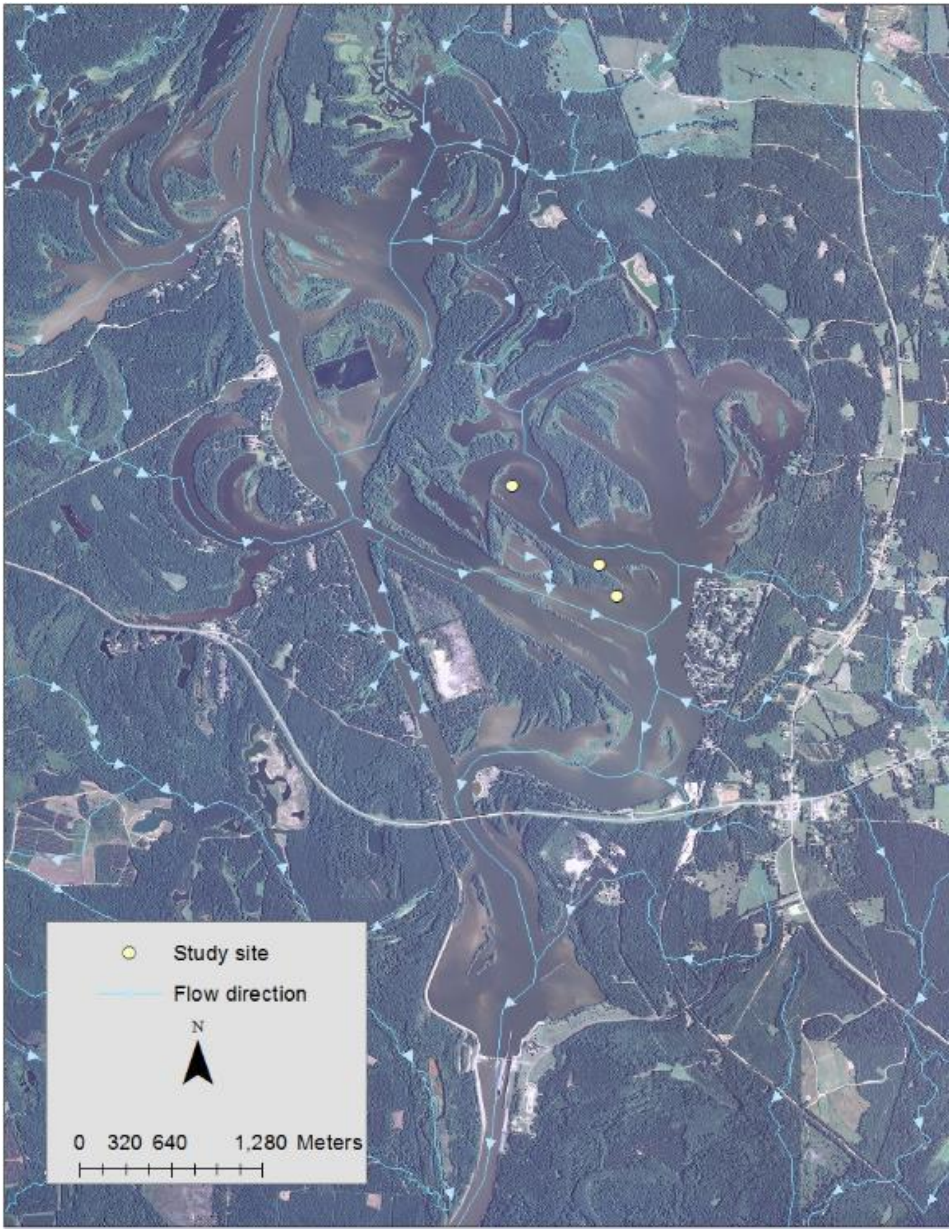

Figure 2.8 USGS National Hydrography Dataset flow direction for Aliceville Lake, Mississippi.

Figure 2.9 USGS National Hydrography Dataset flow direction for Columbus Lake, Mississippi.

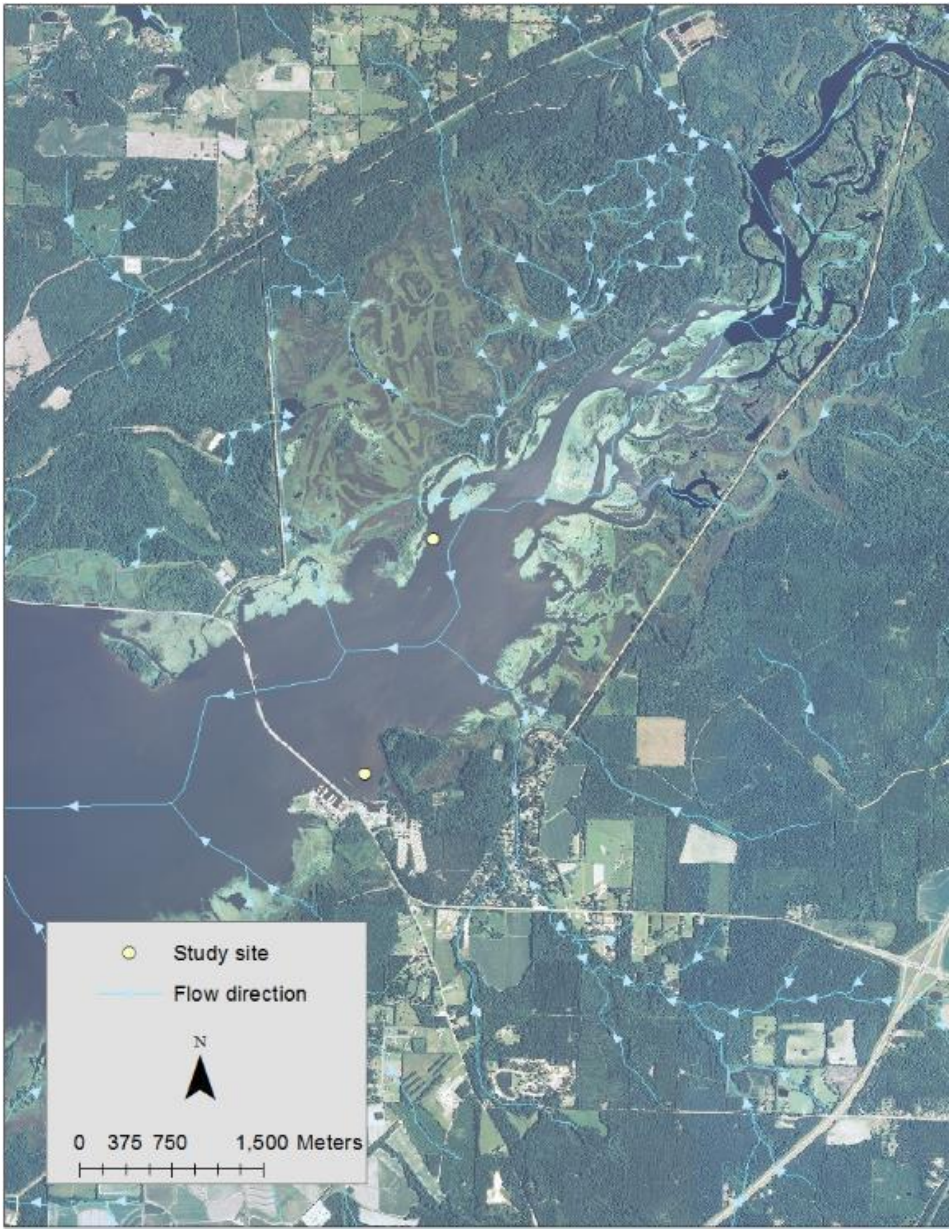

Figure 2.10 USGS National Hydrography Dataset flow direction for Ross Barnett Reservoir, Mississppi

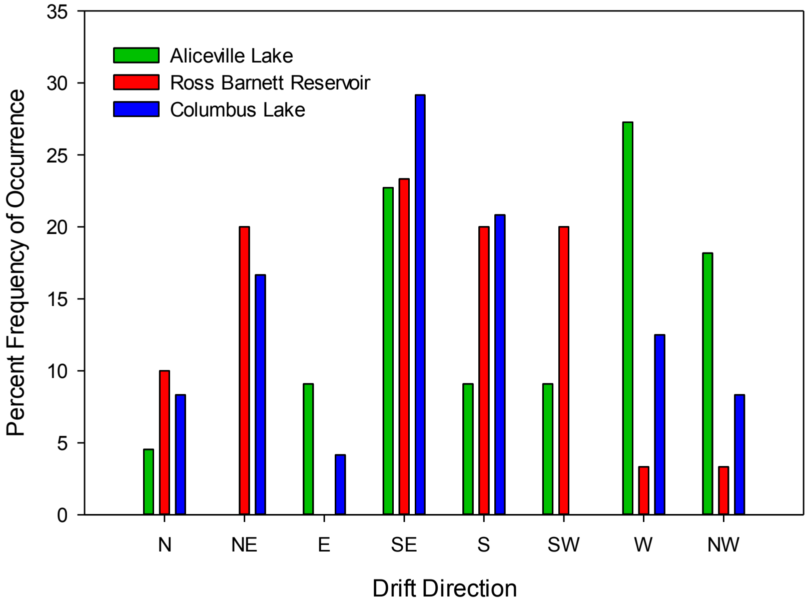

Figure 2.11 Percent frequency of occurrence in which a drone drifted in a particular direction, 2011 and 2012

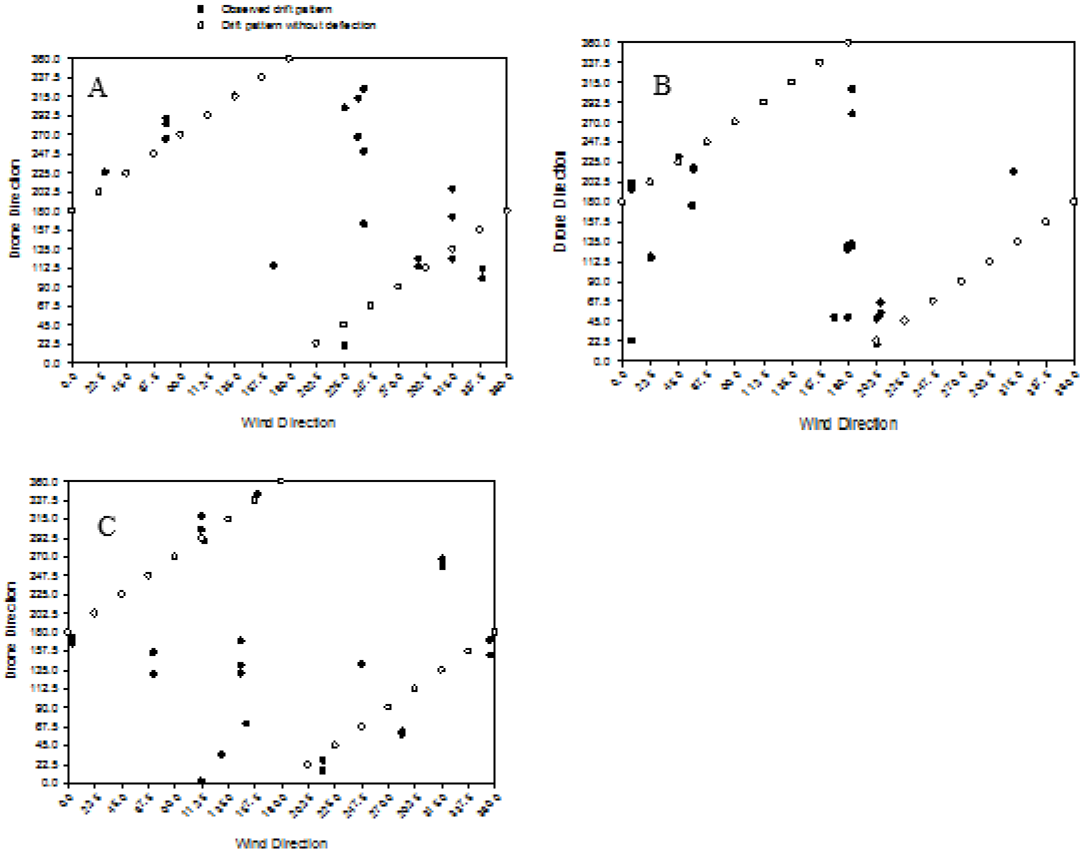

Figure 2.12 Drone drift direction plotted against wind direction

for A) Columbus Lake, B) Aliceville Lake, and C) Ross Barnett Reservoir, 2011 and 2012.
*Open circles show expected drift direction without deflection from wind.

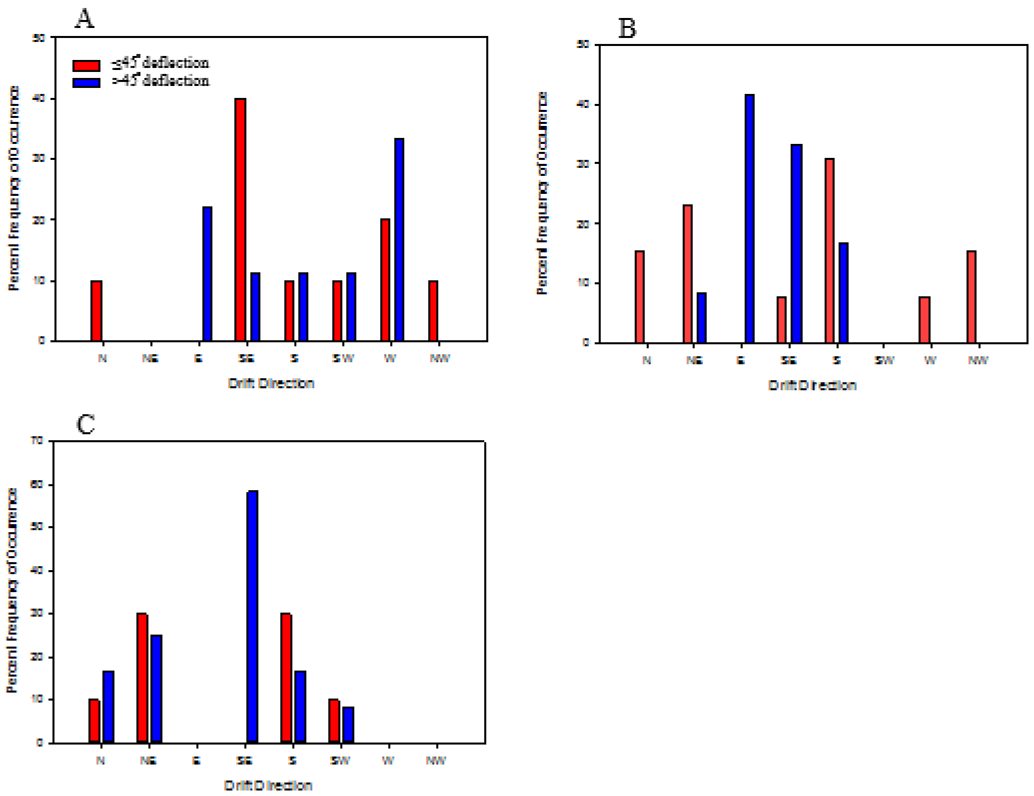

Figure 2.13 Percent frequency of drift path directions by 45° deflection categories

for A) Aliceville Lake, B) Columbus Lake, and C) Ross Barnett Reservoir, 2011 and 2012.

*Deflection category is based on the amount of deflection between the drone drift path and observed wind direction.

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CHAPTER III

THE EFFECT OF HERBICIDE AND GROWTH STAGE ON CUBAN BULRUSH CONTROL

Abstract

Cuban bulrush (*Oxycaryum cubense* (Poepp. & Kunth) Palla) is a floating, epiphytic, perennial aquatic plant from South America and the West Indies sporadically distributed in Florida, Louisiana, southern Georgia, southern Alabama, Mississippi, and coastal Texas. To date, there are no published studies documenting management techniques for Cuban bulrush. The objectives of this study were to determine the efficacy of ten aquatic-labeled herbicides for Cuban bulrush management, and to determine if there is a difference in efficacy between pre and post flowering herbicide applications. Foliar applications of glyphosate (4.54 kg ae ha^{-1}), carfentrazone (0.22 kg ai ha^{-1}), flumioxazin (0.42 kg ai ha^{-1}), 2,4-D (4.26 kg ae ha^{-1}), triclopyr (6.72 kg ae ha^{-1}), imazamox (0.56 kg ai ha^{-1}), imazapyr (1.68 kg ai ha^{-1}), penoxsulam (0.10 kg ai ha^{-1}), bispyribac-sodium (0.448 kg ai ha^{-1}) and diquat (4.48 kg ai ha^{-1}) were made to Cuban bulrush grown in 151 L tanks fitted with a plastic mesh fence to simulate the epiphytic growth pattern of Cuban bulrush. Each herbicide and an untreated reference were replicated four times for a total of 44 tanks per study. A significant interaction between herbicide and growth stage was detected ($p=0.0048$). Mean biomass of Cuban bulrush treated pre-flowering was lower than the post-flowering herbicide applications for all of

the herbicides. For bispyribac-sodium, carfentrazone, imazamox, imazapyr, and penoxsulam, the difference in mean biomass reduction for the two growth stages was significant. All herbicides applied pre-flowering achieved >80% biomass reduction. For the post flowering application, >80% biomass reduction was achieved only by glyphosate, diquat, and triclopyr. Future studies should assess herbicide tank mixes on Cuban bulrush control and Cuban bulrush mat thickness on herbicide efficacy.

Introduction

Cuban bulrush (*Oxycaryum cubense* (Poepp& Kunth) Palla) is an aquatic, invasive plant that is spreading in the southeastern United States. It is an emergent, rhizomatous, perennial epiphyte with triangular stems that grow 0.3 to 0.9 m in height (Godfrey and Wooten 1979, Robles et al. 2007, Bryson et al. 2008). Two forms of Cuban bulrush are found in the United States that can be differentiated by their inflorescence features. *Oxycaryum cubense* forma *cubense* has an umbellate inflorescence while *O. cubense* forma *paraguayense* has monocephalous inflorescence (Fig. 3.1; Barros 1960). The root and rhizomes of Cuban bulrush intertwine with the roots of other plants to create dense floating mats. It is often found in association with plants such as waterhyacinth (*Eichhornia crassipies*), water springles (*Salvinia minima*), hydrilla (*Hydrilla verticillata*), water pennywort (*Hydrocotyl ranunculoides*), angelstem primrose-willow (*Ludwigia leptocarpta*), parrotfeather (*Myriophyllum aquaticum*), Eurasian watermilfoil (*Myriophyllum spicatum*), longleaf pondweed (*Potamogeton nodosus*), marsh mermaidweed (*Proserpinaca palustris*) and humped bladderwort (*Utricularia gibba;* Bryson and Carter 2008).

Cuban bulrush is adapted to dispersal by water (Haines and Lye 1983). It reproduces via buoyant vegetative fragments that break from the floating mats, and by achenes which have a spongy suberized pericarp allowing them to float (Haines and Lye 1983). Seed placement may play an important role in the establishment of Cuban bulrush since germination in the leaf axils of waterhyacinth has been observed (Tur 1971).

Cuban bulrush is thought to be native to South America and the West Indies. It was likely introduced into the United States by migratory birds or ship ballast from these areas (Bryson et al. 1996). Cuban bulrush is now found throughout Central America, tropical Africa, and the southeastern United States including Florida (Anderson 2007), southern Georgia (Bryson et al. 1996), Alabama (Lelong 1988), Louisiana (Thomas and Allen 1993), coastal Texas (Turner et al. 2003), and Mississippi (Cox et al. 2010). Bryson and Carter (2008) suggest that the sporadic distribution of Cuban bulrush in North America could be is due to a lag phase or the low fertility of achenes.

The highly aggressive nature of Cuban bulrush allows it to exclude other vegetation, including the ability to outcompete and overtake waterhyacinth (Robles et al. 2007). In many countries the greatest problem associated with Cuban bulrush is the extensive floating mats that it forms. These rafts block access points to waterways, impede recreation and navigation, and create poor fisheries habitat as the water below the mats is often low in dissolved oxygen and high in organic matter (Mallison et al. 2001). To date, there are no studies published documenting effective management techniques for control of Cuban bulrush.

For many areas of the southeastern United States, Cuban bulrush is not widespread, so there is greater opportunity for preventing new invasions and possibly eradicating new, smaller populations. As part of the early detection and rapid response strategy, tools to control these small populations are needed. An important part of effective control involves implementing the management technique when the greatest chance for success is most likely. Success often depends on the phenology of the plant (Madsen and Owens 1998). Management studies were conducted in a mescosm to evaluate 10 foliar active herbicides labeled for use in aquatic systems applied to Cuban bulrush at two different growth stages, i.e., before and after flowering. The objectives were to (1) identify the most efficacious herbicides for Cuban bulrush control and (2) determine if a difference in herbicide efficacy existed for these two growth stages. The hypotheses tested were aquatic foliar herbicides differed in efficacy on Cuban bulrush control and there are differences in herbicide efficacy between plants treated before and after inflorescence emergence.

Materials and Methods

The studies were conducted in 2011 and 2012 at an outdoor mesocosm at the R. R. Foil Plant Science Research Center, Mississippi State University, Starkville, MS. For both 2011 and 2012, the pre-flowering study began in August and ended in October. In 2011, the pre-flowering study ran for eight weeks, while the 2012 study was harvested nine weeks after treatment due to travel requirements. The six week, post-flowering study began in September and ended in November for both years.

Eighty-eight, 151 L tanks were set up and covered with a plastic mesh netting with 1.9 cm^2 openings. The tanks were filled with water and amended with 30 mg L^{-1} of 24-8-16 Miracle Gro® fertilizer and 0.5 ml L^{-1} of Aquashade® water dye each week throughout the study (Cheshier et al. 2011). Cuban bulrush that was 15.24 to 25.40 cm tall was harvested from Ross Barnett Reservoir in Jackson, MS, and Columbus Lake in Columbus, MS beginning in June for both years. Ten plants (stem and rhizome) were placed through the holes of the mesh netting. Several plantings were required before transplants became established. The final planting occurred four weeks prior to herbicide application.

Eleven treatments which included 10 herbicides and an untreated reference were assigned to the tanks in a completely randomized design. Each treatment was replicated four times for a total of 44 tanks per study. The 44 tanks used in the post-flowering study were not sprayed during the pre-flowering study.

For both pre- and post-flowering studies, prior to treatment, Cuban bulrush was sampled by taking all of the plant biomass above and below the mesh within a 0.01 m^{-2} quadrat. Plants were dried at 70°C for at least four days, and then weighed to assess the pre-treatment biomass.

Foliar applications of the 10 herbicides were made to Cuban bulrush using a CO_2 pressurized sprayer at a spray volume of 468 L ha^{-1} with 0.1% v/v non-ionic surfactant (Dyne-Amic®) added. Herbicides were applied at the maximum labeled rate, with the exception of flumioxazin, which was applied at one-tenth of the maximum rate. Herbicides used included diquat (Reward®, 4.48 kg ai ha^{-1}), imazapyr (Habitat®, 1.68 kg ai ha^{-1}), imazamox (Clearcast®, 0.56 kg ai ha^{-1}), glyphosate (Rodeo®, 4.54 kg ai ha^{-1}), penoxsulum (Galleon SC®, 0.10 kg ai ha^{-1}), 2,4-D (DMA IV-IVM®, 4.26 kg ai ha^{-1}), triclopyr (Renovate 3®, 6.72 kg ai ha^{-1}), bispyribac-sodium (Tradewind®, 0.448 kg ai ha^{-1}), flumioxazin (Clipper®, 0.42 kg ai ha^{-1}), and carfentrazone (Stringray®, 0.22 kg ai

ha^{-1}). A plastic barrier was placed around the tanks during treatment to prevent herbicide spray drift.

Every week after the initial treatment, plants were visually rated for percent control. Cuban bulrush control was assessed on a scale of 0 to 100 where 0 = no control and 100 = complete plant mortality. At the end of each study, all of the living plant biomass above and below the mesh within a 0.01 m^{-2} quadrat was removed, oven-dried at 70°C, and weighed.

A mixed procedures model of SAS using year as a random effect was used to evaluate the effects of herbicide, growth stage, and potential interactions between these two on mean biomass reduction of Cuban bulrush (Littell et al. 2006). If a main effect was significant, means were separated by least square means and grouped using the least square differences procedure. All analyses were conducted at a $p=0.05$ level of significance in SAS®. The visual ratings were not statistically analyzed, but will be used in the discussion.

Results and Discussion

A significant interaction between herbicide and growth stage was detected ($p=0.0048$). Except for the post flowering application of carfentrazone, each herbicide achieved statistically significant mean biomass reduction compared to the untreated reference at both growth stages (Fig. 3.2). For imazapyr, imazamox, penoxsulam, bispyribac-sodium, and carfentrazone, a significant difference in mean biomass reduction was detected between the two growth stages. All of the herbicides applied to Cuban bulrush prior to inflorescence emergence reduced biomass ≥85%. For the post flowering application, only diquat, triclopyr and glyphosate provided ≥85% biomass reduction.

Imazapyr, imazamox, penoxsulam, bispyribac-sodium are ALS inhibitors. ALS compounds inhibit the production of amino acids which are necessary for protein production. As plants approach maturity and the onset of seed production, protein production is already reduced which likely contributed to reduced control observed between growth stages (Koschnick et al. 2007). These herbicides are also systemic, so the decrease in biomass reduction during the post flowering application could be due to the leaf senescence and corresponding reduced photosynthesis rates due to reduced leaf mass resulting in limited translocation outside the treated leaves to the roots (Bussan and Dyer 1999). When applied to the pre-flowering Cuban bulrush, the visual ratings for the systemic herbicide imazapyr showed 80 to 90% control of Cuban bulrush by two weeks after treatment and 100% was reached by four weeks after treatment. Conversely, the post flowering application did not show 80 to 90% control until four weeks after treatment, and never reached 100% control.

Another possible explanation for the differences measured in control between growth stages could be due to differences in the amount of biomass present during the pre-flowering and post flowering herbicide applications. For these studies, the average biomass in the pre- and post-flowering tanks was 400 and 1,480 g dry weight (DW) m^{-2}, respectively. The greater amount of bulrush biomass during the post flowering herbicide application made it difficult to get complete coverage of all the plants growing in the tank. This factor could explain why carfentrazone, a contact herbicide, did not significantly reduce mean Cuban bulrush biomass during the post flowering application.

The results of this study show that both pre- and post-flowering herbicide applications can effectively reduce Cuban bulrush biomass; however, the herbicide used should be dependent on the growth stage of the plant. For pre-flowering herbicide applications, all of the herbicides provided adequate control of Cuban bulrush; however, for post-flowering herbicide applications, bulrush control with carfentrazone did not differ significantly from the untreated. Furthermore, triclopyr, diquat, and glyphosate were the only herbicides to achieve ≥85% biomass reduction. Since this is the first documented study of herbicide activity on Cuban bulrush, this information will be useful for rapid response to new populations.

During the course of the study, we discovered that the two reservoirs from which we harvested the bulrush had the two different biotypes. Ross Barnett Reservoir had *O.cubense* forma *cubense*, and Columbus Lake had *O.cubense* forma *paraguayense.* At the time of the first harvest, neither population had flowered, so the differences in the two forms were not known. Samples from the two populations were sent Dr. Ryan Thum at Grand Valley State for genetic analysis. Although more samples are needed, the preliminary works suggests there may be a genetic difference between the two biotypes (R.Thum and T. Pashnick unpubl. data).

Future work on Cuban bulrush should include further genetic testing since herbicide efficacy may be different for the two biotypes. With the increasing issue of herbicide resistance, herbicide tank mixtures and various herbicide application rates should be evaluated as well. Since information is very limited on the growth and reproduction of Cuban bulrush, biological and ecological studies are necessary for better management of the species.

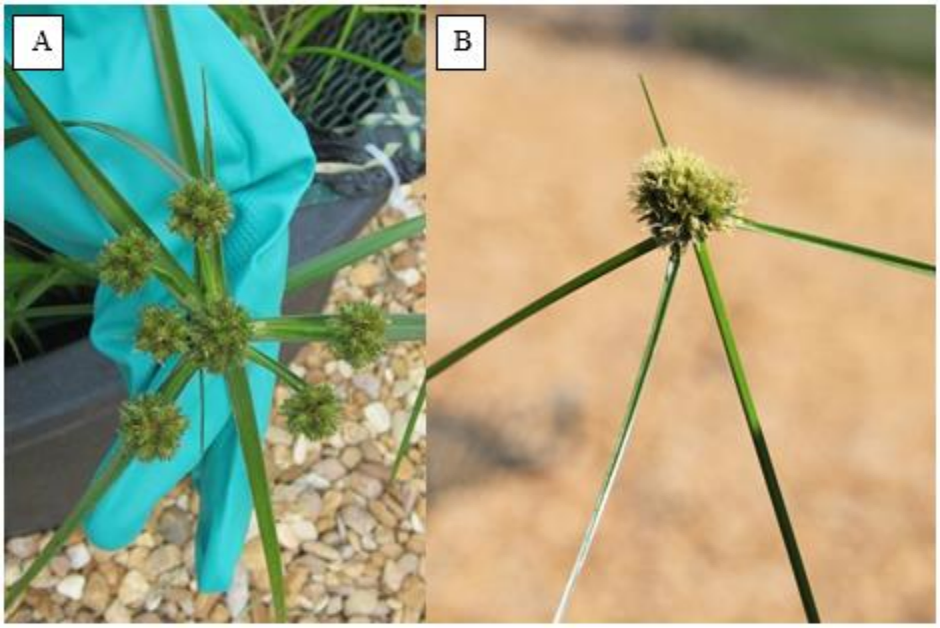

Figure 3.1 Inflorescences of A) *O. cubense* forma *cubense*, B) *O. cubense* forma *paraguayense*.

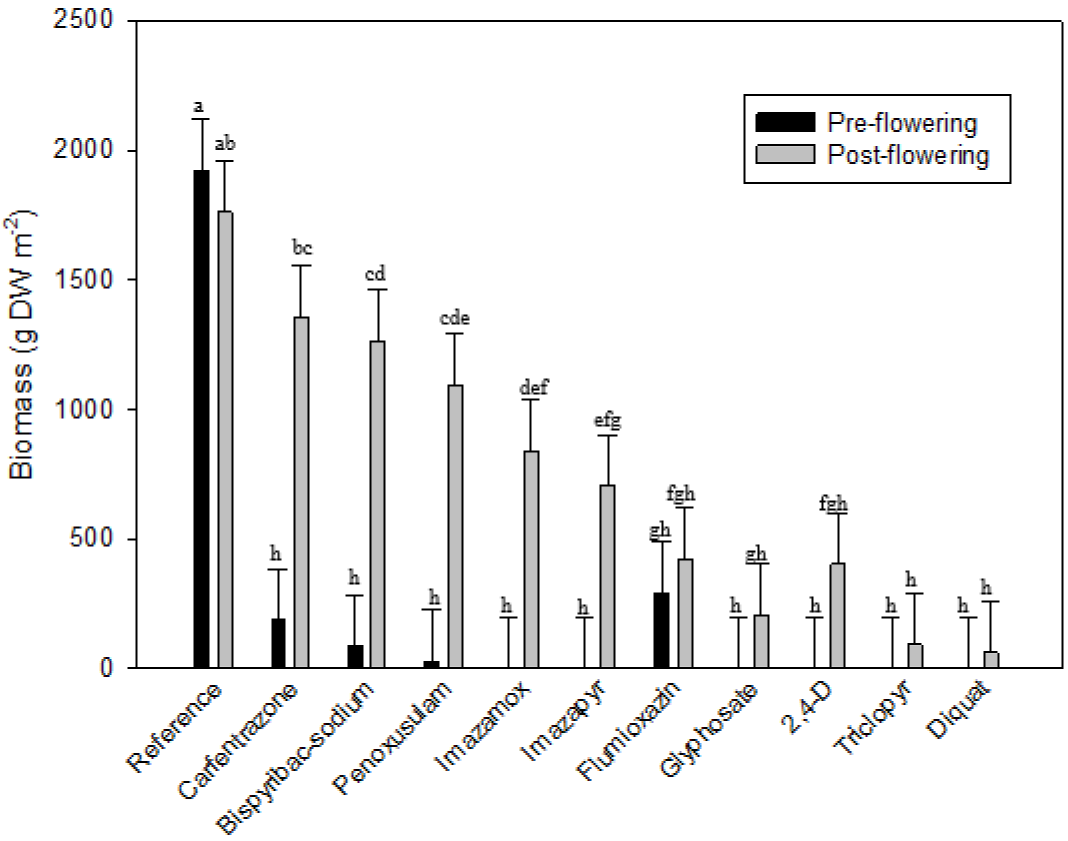

Figure 3.2 Mean (±1SE) Cuban bulrush biomass (g DW m-2) for pre-flowering and post-flowering studies.

*Mean biomass was combined for 2011 and 2012.

* Bars sharing the same letter are not significantly different at the p=0.05 level of significance according to least square means.

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CHAPTER IV

PREDICTABILITY AND MANAGEMENT OF NEW INVADERS

Due to the negative ecological and economic impacts invasive species can have on their environment, predicting invasive species spread has been a recent focus of many studies (Dodds 2009). Kolar and Lodge (2001) identified that large number of releases or repeated releases of organisms increased the probability of establishment. In Aliceville and Columbus Lakes, there were significant increases in plant fragments after disturbance which could increase the chance for establishment if other necessary conditions are met, such as site availability. Consequently, management strategies that act as a disturbance, such as mechanical removal, are not recommended.

The most predictable entities move in the assumed material flow direction that is gravity flow (Dodds 2009). For watersheds, the assumed flow direction is therefore based on elevational gradients. However, the drones showed that surface flow in reservoirs is often dictated my wind . When predicting spread of an invasive species on fragmented rivers, wind patterns of the area of concern should be included as a parameter in future spatial simulation models to better represent local dispersal direction. Use of wind data to identify surface drift currents could also provide and explanation to plant community patterns since species susceptibility to wind will vary.

The ability to predict invasive plant spread will help alert managers to potential new invaders so efforts to monitor for these species can increase and management strategies implemented (Downing-Kunz and Stacey 2011). Cuban bulrush is currently contained to the southeast United States where it is patchily distributed, so managers have the opportunity to prevent Cuban bulrush from having a widespread negative impact. For best results, pre-flowering herbicide applications are recommended because all of the aquatic-labeled herbicides achieved at least 85% control with 2,4-D, diquat, glyphosate, imazamox, imazapyr, and triclopyr achieving 100% control. For applications to populations after inflorescence emergence, ALS inhibitor and carfentrazone are not recommended. Diquat, triclopyr, and glyphosate achieved 97, 95 and 88% respectively and are recommended. By managing small populations, the control cost and negative ecological impacts are reduced.

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APPENDIX A

COMPARING DRONE DRIFT TO WATERHYACINTH, ALLIGATORWEED, AND HYDRILLA

Introduction

Plants drift at different levels of the water column depending on the growth form of the plant and the buoyance of the plant tissue (Riis and Sand-Jansen 2006). For floating species, there is often more surface area above the water in comparison to emergent and submersed species. Consequently, drift may differ among these types due to varying susceptibility to wind.

The objective of this study was to determine how the drones drift in comparison to aquatic plants waterhyacinth (*Eichhornia crassipies*), alligatorweed (*Alternanthera philoxeroides*), and hydrilla (*Hydrilla verticillata*). Waterhyacinth and alligatorweed are free-floating species, while hydrilla grows rooted and submersed.

Materials and Methods

To compare the movement of the drones to the movement of waterhyacinth, alligatorweed and hydrilla, a study was conducted using a 1.6 m diameter tank. Next to the tank, an electric fan that generated 4.8 kph winds was placed. Three mats with surface areas similar to the drones were collected for both alligatorweed and waterhyacinth. Three fragments were used to track hydrilla movement. To avoid interference between plants and drones, subjects were floated separately. The movement of each drone, plant mat, or fragment was monitored three times. Each movement was recorded on a mounted video camera. Drift angle was calculated by measuring the angle formed between the expected drift path (direct movement with the wind), and the actual drift path. Rate (cm/min) for each object was also measured. The drift angle and rate for each object were then analyzed in SAS using a general linear model. A significant difference among objects was detected for mean drift angle (p<0.0001), and mean drift rate (p=.0011) due to differences in wind susceptibility among the different plant species and drones . Means were then separated using the least squares method and grouped using the least significant difference.

Results and Discussion

With respect to mean drift angle, no significant difference was detected among the drones, waterhyacinth, and alligatorweed; however, a significant difference was detected for hydrilla's mean drift angle when compared to the other three surface floaters (Table 1). Hydrilla is a submersed species with little surface area above the water. The drones drift is likely more comparable to surface floating plants like alligatorweed and waterhyacinth due to the surface area that comes in direct contact with wind.

The drones moved significantly faster than all of the plants studied (Table 1). The spherical shape makes the drones more aerodynamic, and the drones lack root drag which also allows them to move faster in the water.

Future studies should engineer the drones to sit lower in the water to reduce surface area exposure. The additional weight could be added below the circuit board in the drone; however, increased waterproofing may be necessary where the top and bottom halves of the drone meet. To better represent root drag, securing dangling weights to the outside bottom of the drone would likely simulate resistance.

Table A.1 Drift angle and rate for the drones, waterhyacinth, alligatorweed, and hydrilla.

	Drift Angle	**Drift Rate (cm/min)**
Drone	37.44^a	302.80^a
Waterhyacinth	48.94^a	102.44^b
Alligatorweed	39.44^a	67.96^c
Hydrilla	21.00^b	58.63^c

* Letters in a column denote a significant difference among study subjects at a P=0.05 significance level.

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