ABSTRACT


Invasive macrophytes, such as non-native *Hydrilla verticillata*, negatively impact lentic systems of the southeastern United States through impediment of recreational activities, power generation, and disruption of indigenous ecological function. Expenditures associated with aquatic weed management include costs accompanied with monitoring, mapping and implementing control measures. Prompt aquatic vegetation detection and mapping are critical factors when formulating management decisions and practices. Management protocols are often reliant upon the perceived extent of invasive vegetation incursion. Point-intercept methods have traditionally persisted as industry standards for accessing lakewide macrophyte presence, abundance, and distribution. Although point-intercept survey methods are essential for lake managers, these surveys are prone to subjectivity in vegetation richness, spatial estimation, and are exceptionally inefficient. Traditional sampling techniques often require significant labor inputs, which limits repeatability, the scale of sampling, and the rapidness of processing. Advances in commercially available optic, hydroacoustic, and post-processing technologies, provide lake managers an ability to passively evaluate spatial elements of aquatic vegetation while conducting point-intercept surveys methods with reduced labor and economic requirements. This research focuses on several survey techniques for mapping lakewide vegetation with less financial input of commercial satellite imagery, and greater objectivity than point-intercept methods alone. Implications from this study supports the comparison of seasonal
Global demands for reduced greenhouse gas emissions have prompted research of alternative energy sources from second-generation biofuel crops. *Arundo donax* (giant reed), a high biomass yielding cane species, continues to gain acknowledgment for lignocellulosic biofuel production. However, the physiological characteristics which make giant reed a leading candidate for biofuel production may also promote invasiveness. Nevertheless, bio-energy advocates support planting giant reed in regions east of the Mississippi River. Our objective was to evaluate techniques for eliminating established giant reed populations. Based upon results, well-established giant reed may be readily controlled with glyphosate to eliminate escapes or allow crop rotation.

by
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A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the degree of Master of Science in Crop Science

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DEDICATION

“The conservation of natural resources is the fundamental problem. Unless we solve that problem, it will avail us little to solve all others.” – (Theodore Roosevelt). This thesis is dedicated to the stewards of our natural resources and in remembrance of my hunting buddy, Zachary McNeill.
BIOGRAPHY

Andrew W. Howell was born and raised in Sanford, NC. He became interested in agriculture and environmental management at an early age which lead him to obtain his Eagle Scout Award. He received his B.S. degree in Crop Production with a Minor in Horticulture at North Carolina State University. During Andrew’s undergraduate career, he saw first-hand the risks posed by exotic, invasive plant species on native ecosystems and agronomic settings while interning at several research farms. Andrew decided to pursue a M.S. in Aquatic Weed Science and a Graduate Certificate in Geographic Information Systems to assist others in combating the issues of invasive species. During his graduate career, Andrew remained active in several professional societies and conservation organizations. Andrew foresees himself continuing invasive species research and strives to provide environmental stewardship of native plant ranges. In his free time, Andrew enjoys spending his time outdoors fly fishing, bowhunting, and hiking.
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CHAPTER 1

Literature Review and Rationale
MACROPHYTES OVERVIEW

Freshwater aquatic vegetation is extensively recognized for contributing to the ecological structure of lentic, lotic, and wetland systems (Likens 1975; Madsen et al. 2001; Valley et al. 2004). As primary producers (Lehmann et al. 1994), macrophytes assist in the overall net primary production of aquatic environments (Likens 1975; Boston et al. 1989; Madsen and Sand-Jensen 1991; Dodson et al. 2000; Takamura et al. 2003; Brothers et al. 2013), and remain fundamental constituents of the global food web (Hanson and Butler 1994; Havens et al. 1996; Zeigler et al. 2015). Consequently, macrophytes act as trophic indicators of ecosystem quality (Lehmann et al. 1994) while offering habitat for macroinvertebrates, littoral fish species, and waterfowl (Heck and Crowder 1991; Petr 2000; Schmieder et al. 2006). Furthermore, macrophytes provide critical niches within aquatic assemblies which support lakewide sustainability and development. One class of macrophyte in particular, submersed aquatic vegetation (SAV), plays an essential role in the maintenance of aquatic environments by reducing shoreline erosion and stabilizing sediment loads for reduced turbidity (Carpenter and Lodge 1986; Barko et al. 1991; Madsen et al. 2001), operating as eutrophic sinks from anthropogenic impacts (Kufel and Kufel 2002; Gao et al. 2009), functioning in seasonal nutrient cycling (Twilley et al. 1985; Carpenter and Lodge 1986; Granéli and Solander 1988), and preserving fluvial regimes (Dodds and Biggs 2002; Doering et al. 2002). However, the absence of SAV within littoral regions may hinder the environmental advantages formerly defined, decrease sport fish opportunities (Ploskey 1981;
Valley et al. 2004; Durocher et al. 2011), and ultimately reduce the ecologic and economic value of a waterbody.

The evolution and development of macrophytes relies upon a conditional mixture of physiological processes and abiotic parameters (Coops et al. 1996; Lehmann et al. 1997; Herb and Stefan 2003). Aquatic ecologists have acknowledged various limiting factors effecting submersed macrophyte growth and spatial occurrence, including: littoral slope and depth zonation (Daurte and Kalff 1986; Middelboe and Markager 1997; Sousa et al. 2011), thermal variability (Barko et al. 1982), nutrient availability (including P sources and C:N ratios) (Hough et al. 1989; Moss et al. 2012 ; Kovalenko and Dibble 2014), sediment suspension (Barko et al. 1991; Lauridsen et al. 1993), turbidity and light attenuation (Barko et al. 1982; Madsen et al. 2001; Hansel-Welch et al. 2003; Havens 2003), disturbance (Lehmann et al. 1997; Valley and Drake 2005; Cao et al. 2012), and interspecific competition (Haller and Sutton 1975; Capers et al. 2007; Shekede et al. 2008; Meadows and Richardson 2012). Of these confines, carbon and light availability provide the greatest influence of submersed macrophyte occurrence (Hough and Fornwall 1988; Barko et al. 1991; Madsen and Sand-Jensen 1994; Sidorkewicj et al. 2004); however, this is highly dependent upon the waterbody of interest.

Community assemblages of SAV have been documented to indicate ecological diversity (Carpenter and Lodge 1986) and support the intricate constraints involved in aquatic ecosystem sustainability and efficiency (Hough et al. 1989; Egertson 2004; Capers et al. 2007; Brothers et al. 2013; Simon et al. 2015). Nevertheless, community assemblages
which lack heterogeneity, or which have low species richness, are recognized for reducing the intrinsic biodiversity of aquatic environments (Kovalenko and Dibble 2014), which is often an adverse response among waterbodies dominated with exotic, invasive species.

INVASIVE AND AQUATIC SPECIES MANAGEMENT

Overview:
Invasive exotic species (IES) are non-native plants or fauna which have unfavorable effects upon native ecologic function and commonly generate economic hindrance among invaded areas (Keast 1984; Gordon 1998; Pimentel et al 2000; Santos et al. 2011; Gallardo et al. 2016). One reason IES tend to thrive among new ranges is the lack of predation and incapacity of native species opposition (Keane and Crawley 2002; Callaway and Ridenour 2004). Lacking predation, IES reduce innate ecosystem biodiversity through resource competition (Madsen 1991; Zhang and Boyle 2010), dominance (Houlahan and Findlay 2004), and habitat disruption (Thum and Lennon 2009; Schultz and Dibble 2012). Although IES threaten ecosystem processes (Wilcove et al. 1998), the expenditures associated with IES in the United States are of equal importance; costing stakeholders around $120 billion annually (Pimental et al. 2005), which fiscally complicates the evolutionary issue of invasion.

While terrestrial and riparian incursions in the United States can be ecologically and economically severe, none are more important than those which affect our waterways and threaten the basic necessity of life—water. Exotic SAV, such as Hydrilla verticillata (L.f) Royle and Myriophyllum spicatum L., inhibit native macrophyte maturity by establishing
dense, monoculture stands (Langeland 1996; Eiswerth et al. 2000), which increase chemical and biological competition, while excluding light interception from dense canopies (Smart et al. 1994; Spencer and Ksander 2000). In the case of *H. verticillata*, this competition often displaces native SAV beds (Madsen et al. 1991; Valley et al. 2004; True-Meadows et al. 2016), and can host cyanobacteria which create illness for fauna in some instances (Wilde et al. 2005). Likewise, exotic plant beds pose human health risks when surface mats provide breeding opportunities for mosquitos (eg. *Aedes* spp.) which vector disease (Hearnden and Kay 1997; Wersal et al. 2007; Cuda 2009). Moreover, these aquatic IES may reduce lakefront property values (Zhang and Boyle 2010), inhibit recreational activities and navigation, obstruct water intakes, and hinder power generation (Langeland 1996), which concurrently costs the United States over $100 million dollars annually towards macrophyte management (Rockwell 2003).

To reduce the ecologic and economic downfalls associated with IES’s, aquatic plant managers develop sound management tactics which sustain and encourage the stewardship of native ecosystems while warranting prevention, providing early detection and rapid response of infested areas (Westbrooks 2004; Davies and Johnson 2011). One of the most basic methods to moderate the spread of IES’s is averting an incursion. As a cost-effective tool, prevention programs like Stop Aquatic Hitchhikers!™, provide public outreach among recreational groups through raising awareness of aquatic IES’s to reduce the risk of spread (Seekamp et al. 2016). Furthermore, regions which receive early detection lessen the financial and environmental costs associated with invasions (Rejmànek and Pitcairn 2002;
Hestir et al. 2008; Thum and Lennon 2009; Lambert et al. 2010). However, when SAV incursions arise, aquatic ecologists and plant managers must provide integrated weed management measures. Physical control methods, which often apply the use of benthic barriers, drawdown programs, and mechanical removal are effective actions in several situations (Murphy 1988; Madsen 1997; Shaw et al. 2016). However, destructive harvests of SAV are rarely applicable in southeastern U.S. reservoirs because of complex littoral profiles, longer growing seasons, and SAV physiological properties (ie. *H. verticillata* can reproduce via fragmentation) (Haller 2009). Among many scenarios, managers will implement biological control methods to provide long-term management (Cuda 2009) whether it be with arthropods (Reeves and Lorch 2012) or herbivorous fish species like grass carp (*Ctenopharyngodon idella*) (Van Dyke et al. 1984; Bonar et al. 1993; Manuel et al. 2013). Still, these methods lack a comprehensive management regime due to biological agent mortality, semi-selective feeding (ie. can harm native vegetation), and regulation (Richardson 2008). Nevertheless, chemical control methods, which use registered aquatic herbicide formulations (Parochetti et al 2008; Richardson 2008), provide an opportunity to treat regions of infestation and minimize further IES persistence to meet specific management goals (ie. weed suppression or eradication). Conversely, herbicide formulations and treatment applications are often mandated on a state-by-states basis, require knowledge of species selectivity to minimize off-target effects (ie. harm native species), and are often an expensive option in highly invaded systems (Netherland 2009). Therefore, to acquire a holistic management approach, managers often employ the support of cultural, physical, and chemical control options once an invasion is evaluated.
The benefits of early detection require water resource managers to conduct waterbody-wide surveys which support the management opportunities previously described. Timely monitoring and proficient mapping tactics have continued as essential tools for evaluating native and exotic aquatic vegetation (Maceina et al. 1984; Lehmann & Lachavanne 1997; Valley and Drake 2005; Hestir et al. 2008; Santos et al. 2011; Husson et al. 2016). While several macrophyte surveying techniques have been recognized (eg. Ackleson and Klemas 1987; Schneider et al. 2004; Gunn et al. 2010), in situ point-intercept sampling procedures have become industry standard for describing spatial patterns in SAV presence, distribution, and richness (Madsen 1999). However, there are several drawbacks to the point-intercept methodology, as assessments require a skilled workforce (ie. correctly identify species; boating proficiency) and there is often subjectivity among surveyors, which can reduce survey accuracy. Likewise, these methods demand extensive labor and time commitments as the extent of lakewide evaluations are highly correlated to the accuracy, coverage, and time spent appraising each sampling location. Therefore, researchers and lake managers seek to engage recent advances in remote sensing technologies to reduce the labor and economic expenditures involved in macrophyte surveys.

*Hydrilla verticillata* (L.f.) Royle:

*H. verticillata* (hydrilla or waterthyme), is an invasive, exotic submersed macrophyte within the *Hydrocharitaceae* family, which has been coined the “perfect aquatic weed” (Langeland 1996). Native to Asia (Cook and Lound 1982), *H. verticillata* was first introduced in the United States sometime around 1960 though the aquarium trade (Wilde et
al. 2005). Since introduction, *H. verticillata* has expanded its range from Florida (Blackburn et al. 1969), northwest to Washington, and as far north as Maine (Spencer and Ksander 2000). Two *H. verticillata* biotypes occur in the United States, monoecious and dioecious (Cook and Lound 1982). Monoecious *H. verticillata* was first discovered in North Carolina in 1981 at Lake Umstead in Wake Co. (Harlan et al. 1985), and to date monoecious *H. verticillata* has spread to nearly half of the 100 counties within the state (Haug et al. 2016).

*H. verticillata* has developed into an ecologic and economic epidemic. Nationwide, millions of dollars are spent annually towards *H. verticillata* management, with $14.5 million spent in Florida alone every year (Center et al. 1997). Given the invasive nature and burden of *H. verticillata*, the United States government has declared *H. verticillata* a federal noxious weed (USDA 2010), with state noxious listings occurring on a state-by-state basis.

Although morphologically similar to *Egeria densa* (exotic) and *Elodea canadensis* (native), *H. verticillata* is distinguishable; having profound leaves which are whorled (4-8 leaves per whorl) with visible serrated margins (True-Meadows et al. 2016). As a rooted, annual species in North Carolina, propagation may occur via fragmentation (Langeland and Sutton 1980), stolons, tubers (subterranean), and turions (axillary) which sum to many of the competitive advantages of *H. verticillata* (Harlan et al. 1985; Smart et al. 1994). Of these asexual methods, over-wintering tuber structures provide the greatest spatiotemporal persistence (Steward and Van 1987), as benthic tubers have been shown to remain viable four to six years in undisturbed sediment (Van and Steward 1990; unpublished data NCSU). Additionally, Nawrocki (2011) studied tuber densities among several North Carolina
waterbodies to find more than 1,700 tubers m$^{-2}$ may be present within a given location, which further defines the temporal longevity and competitive advantage *H. verticillata* encompasses for developing monoculture stands (Havel et al. 2015).

Evolutionary adaptions to environmental factors also provide *H. verticillata* with competitive physiological leads that offer an ability to survive the harshest freshwater systems. *H. verticillata* has the capacity to intercept light in photic regions as low as one percent (Langeland 1996), which permits a multitude of depth, shading, and turbidity tolerances (Canfield et al. 1985). Similarly, *H. verticillata* has developed a lower CO$_2$ compensation point due to its capability of C$_3$ and C$_4$-like production modification (Holaday and Bowes 1980; Ginkel et al. 2001), which allows *H. verticillata* to photosynthesize earlier than most other submersed angiosperms (Langeland 1996). Likewise, *H. verticillata* has adapted to pH, salinity, temperature, and nutrient tolerances (Haller et al. 1974; Van et al. 1976; Cook and Luond 1982; Steward and Van 1987; Steward 1991).

*H. verticillata* inhibits native macrophyte development and reduces ecosystem biodiversity through establishment of dense, monoculture stands (Langeland 1996; Eiswerth et al. 2000). Furthermore, *H. verticillata* produces more than 50% of standing biomass in the top 0.5 m$^{-1}$ of the water column (Haller and Sutton 1975) which prohibits sunlight to neighboring SAV and ultimately reduces interspecific competition (Smart et al. 1994; Langeland 1996; Spencer and Ksander 2000). Competition by *H. verticillata* often displaces native SAV beds (Madsen et al. 1991; Valley et al. 2004; True-Meadows et al. 2016), and can promote cyanobacteria which produce neurotoxic effects among waterfowl and bald

*Arundo donax* L.:  

*A. donax* (giant reed), is a robust perennial cane species within the *Poaceae* family which prefers riparian areas (Wijte et al. 2005; Tarin et al. 2013; Corno et al. 2014). Closely resembling the phenotypic and morphologic growth patterns of *Phragmites* spp. and *Bambusa* spp., *A. donax* has an erect growth stature of 7-10 m\(^{-1}\) (Coffman et al. 2010; Lambert et al. 2010; Saltonstall et al. 2010). Furthermore, the clump-forming *A. donax* (Lambert et al. 2010), follows a C\(_3\) photosynthetic pathway, though more comparable to the prolific growth patterns of C\(_4\) plants (Rossa et al. 1998). Once established, annual yield potential has been documented to be > 20 t/ha\(^{-1}\) (Perdue 1958; Angelini et al. 2009; Impagliazzo et al. 2016). Generating high seasonal biomass, *A. donax* continues to gain recognition among researchers examining second-generation biofuels (Birur et al. 2008; Mantineo et al. 2009; Baldini et al. 2016; Corno et al. 2016). Furthermore, some environmental groups support the planting of *A. donax* to reduce anthropogenic nutrient impacts (Quinn et al. 2007; Zucaro et al. 2016), moderate soil erosion (Pilu et al. 2012; Fagnano et al. 2015), add income to marginal agronomic land (Nassi o Di Nasso et al. 2013), and increase soil carbon (Williams et al. 2008).
For thousands of years, *A. donax* has been cultivated for its immense utilitarian potential. Modern usages are documented in paper pulp production (Shatalov and Pereira 2006, Ge et al. 2016), erosion control and fodder (Hardion et al. 2014), construction material (Lambert et al. 2010; Karahancer et al. 2016), and reeds for musical instruments (Perdue 1958). From these practical characteristics, anthropologists and plant geneticists suggest human activity most likely contributed to the spread of *A. donax* from its origin in either Asia (Polunin and Huxley 1987; Angelini et al. 2009) or the Mediterranean (Tarin et al. 2013; Hardion et al. 2014), to eventually California, where it was planted for erosion control in the early 1800’s (Mariani et al. 2010).

Since first introduced into the United States, *A. donax* has expanded its range longitudinally from California to Maryland (Bell 1997), and most recently, Delaware (USDA 2017). *A. donax* reproduction in North American occurs asexually through clonal fragmentation of stems (apical internodes), and rhizomatous structures, since inflorescences are unable to produce viable seed (Wijte et al. 2005; Santín-Montanyá et al. 2013). Unwarranted colonies of *A. donax*, from cultivated areas, have exhibited invasive properties in aquatic habitats within the Southwestern United States (Rieger and Kreager 1989; Tarin et al. 2013), and along the Rio Grande from Texas into Mexico (Everitt et al. 2005), with recent estimates of intrusive *A. donax* stands comprising ~6,000 ha\(^{-1}\) along the river (Yang et al. 2011). Among locations experiencing *A. donax* invasion, monoculture expanses have been recognized for displacing native flora (Koop et al. 2012), altering hydrologic morphology (Bell 1997), and increasing flood risk (Coffman et al. 2004; Spencer et al. 2013) and wildfire
potential (Coffman et al. 2010). Therefore, officials have declared giant reed a state noxious weed in California, Nevada, and Texas (Koop et al. 2012).

**REMOTE SENSING OF AQUATIC PLANTS**

With the evolution of remote sensing platforms, monitoring and mapping strategies continually gain recognition for use among aquatic environments. Over the last several decades, the use of active hydroacoustic systems (Duarte 1987; Sabol et al. 2002; Valley and Drake 2005) and optical sensors (Ackleson and Klemas 1987; Sawaya et al. 2003; Nelson et al. 2006; Hestir et al. 2008), has forged important toolsets for aquatic plant scientists which aid in macrophyte survey procedures and support the classification of complex spatiotemporal dynamics found with aquatic vegetation. Furthermore, coupling remotely sensed data with computer based programs, like geographic information system (GIS) (eg. ArcGIS; ENVI; RStudio), has increased the performance of mapping techniques (Lehmann and Lachavanne 1997; Shaw 2005; Santos et al. 2016).

Implementing photosynthetic principles and spectral canopy radiance, optical remote sensing facilitates the identification and measurement of submersed and emergent macrophytes (Peñuelas et al. 1997; Silva 2008). Plant pigments chlorophyll $a$ & $b$ strongly absorb electromagnetic radiation (EMR) in the blue (400-500 nm) and red (600-700 nm) bands, with low absorption in the green (500-600 nm) band and high reflectance in the near-infrared (NIR) (700-950 nm) band due to light scattered by the spongy mesophyll cells (Knipling 1970; Everitt et al. 2009; Cho et al. 2012). Conversely, open water absorbs
strongly in the NIR region (Gao 1996). Exploiting these spectral features provides the groundwork for remote sensing of aquatic vegetation and supports the classification of plant stands (Gilmore et al. 2008; Husson et al. 2016), macrophyte distribution (Valta-Hulkkonen et al. 2004; Khanna et al. 2012), and examining species heterogeneity (Hestir et al. 2008). Furthermore, by comparing spectral regions, band reflectance and absorbance attributes, spectral indices may be used to summarize key reflectance features. One of the most popular and simplistic plant science indexes, designed by Rouse et al. (1974), is the normalized difference vegetation index (NDVI), which summarizes the strong absorption in red due to leaf chlorophyll and the strong reflectance in the NIR due to leaf structure:

\[
\text{Index equation: } NDVI = \frac{NIR - R}{NIR + R}.
\]

Remote sensing studies investigating macrophytes often use NDVI when distinguishing non-submersed vegetation against absorption features (ie. water or submersed vegetation) or quantifying vegetation across spatial expanses (Peñuelas et al. 1993; Cho et al. 2008; Shekede et al. 2008; Blanco et al. 2012). However, most aquatic studies implementing satellites lack the spatial resolution to describe communal dynamics, and are unable to differentiate among species using NDVI alone, since most macrophytes contain chlorophyll.

Conventionally, remote sensing of aquatic plants has applied spectral data collected from multispectral satellite scanners (eg. Landsat TM/ETM+, MODIS, and WorldView-2) and hyperspectral sensors (Alberotanza et al. 2006; Chipman et al. 2009), to investigate environments occupied with aquatic vegetation. Measurements of submersed and emergent vegetation have been successfully performed using multispectral imagery (Ackleson and
Klemas 1987; Peñuelas et al. 1997; Dogan et al. 2009; Martin et al. 2010). However, only hyperspectral units provide sufficient narrow spectral bandwidths for species differentiation (Williams et al. 2003; Khanna et al. 2012). Furthermore, the cost of repeated application over large spatial scales often limits the use of airborne hyperspectral sensors (Underwood 2003). However, multispectral open-source sensors, such as Landsat 7 TM/ETM+, provide the basic spectral ranges needed for accessing broad population estimates of emergent vegetation, but lack the spatial and temporal resolution (eg. ~ 30 m\(^2\) and ~15 days\(^{-1}\); respectively) to accurately represent SAV or between alike species. Some commercial multispectral images do provide the required spectral and spatial resolutions to discern community assemblages, but the economic hindrance associated with those images often financially excludes small-scale lake managers from this application (eg. WorldView-2 image). Still, there are several limitations for using multispectral platforms for acquisition of aquatic vegetation specifically, SAV.

Optical remote sensing of submersed vegetation varies greatly from that of emergent macrophytes. Both plant physiological and environmental factors create complications when evaluating SAV, especially when observing canopies above the water column. Issues include atmospheric properties (eg. cloud cover; scattering), water column interference (eg. light attenuation and absorption due to water and water quality constituents), and benthic composition (Ackleson and Klemas 1987; Blanco et al. 2012; Cho et al. 2012). For example, to simulate the spectral range of multispectral satellite sensors, Cho et al. (2008) conducted a mesocosm experiment to determine the limiting depth for detecting *Myriophyllum*
aquaticum, Cabomba caroliniana, and Ceratophyllum demersum was when submersed vegetation occurred < 0.3 m\(^{-1}\) from the water surface. Likewise, Hestir et al. (2008) noted the presence of algae also affected the accuracy of SAV detection using optical platforms. Furthermore, Nelson et al. (2006) studied the use of Landsat-5 TM images for modeling SAV abundance and distribution, but discovered high variability in estimation could be confounded by water clarity parameters (eg. secchi depth; chlorophyll \(a\); water color). Nevertheless, Hestir et al. (2012) showed detection of SAV despite high turbidity using airborne imaging spectroscopy.

A common method to remotely assess SAV is with the use of hydroacoustic systems. Several studies have operated boat-based echosounding arrangements to meet the aforementioned confines of optically sensed SAV (eg. Duarte and Kalff 1990; Lehmann 1998; Sabel et al. 2002; Valley et al. 2005). Using commercial grade, single-beam transducers, researchers have documented the capability to map and quantify waterbody parameters which include: bathymetry and littoral slope (Duarte and Kalff 1986; Reshitnyk et al. 2014), SAV biomass and biovolume (ie. percent of water column occupied) (Macéina and Shireman 1980; Thomas et al. 1990; Vis et al. 2003), spatial coverage and distribution (Haga et al. 2007; Valley and Drake 2005), and benthic composition (Lehmann et al. 1997). Most recently, the introduction of software programs and automated cloud-based systems (eg. EcoSAV and ciBioBase 5.2; respectively) further assist in acoustically derived post-processing procedures. For example, studies have coupled hydroacoustic surveys with point-intercept protocols to develop species-wise SAV abundance maps (Valley et al. 2015).
However, it is important to note that the ability to detect submersed vegetation using hydroacoustic systems does not replace the ability to classify SAV species. Furthermore, the accuracy of acoustic data is limited to areas > 0.76 m\(^{-1}\) in depth due to heavy noise and backscatter from the transducer in shallower littoral regions (Duarte 1987; Valley et al. 2015).

Much like the management of invasive species, remote sensing requires a culmination of techniques, in situ observation, and specialized knowledge to accurately characterize aquatic vegetation. However, the big-data and information gained from the application of remotely sensed surveys will likely contribute to the management, sustainability, and stewardship of our aquatic systems in the future (Adam et al. 2010).

**NOTEWORTHY STATISTICAL PROCEDURES**

*Generalized Additive Models (GAMs)*

Originally developed and described by Hastie and Tibshirani (1986), GAMs transform non-parametric environmental parameters using smoothing (splining) techniques (eg. penalized least squares, cubic regression, Gaussian models, etc.) to predict an ecological response variable through addition of each individual model predictors (Hastie and Tibshirani 1990; Yee and Mitchell 1991). This robust and modern statistical formula assists in accounting for environmental variations which do not behave in a linear fashion (Wood and Augustin 2002; Venables and Dichmont 2004; Ahmadi-Nedushan et al. 2006). Therefore, the benefit of GAM equations comes from being primarily data driven instead of
using predictors regressed from a fixed model like in the case of MLR predictions (Lehmann 1998; Guisan et al. 2002). Consequently, uneven data distributions and non-linear responses, may be explained or interpolated by expending any number of individual predictor variables to access responses of the dependent variable within an ecological limit (Yee and Mitchell 1991; Lehmann et al. 2002). An example of the base GAM function with covariate factors used for model considerations shown is derived from Hastie and Tibshirani (1990), Wood (2006), and Larson (2015):

\[
E(Y) = B0 + s1(x1) + s2(x2) + \ldots + s_p(x_p),
\]

where \(Y\) is the dependent variable (eg. biomass), \(E(Y)\) signifies the expected value, and the covariates \(s_1(x_1) + s_2(x_2) + \ldots + s_p(x_p)\) represent the smoothing terms (eg. depth; biovolume).

GAMs have become increasingly utilized by aquatic ecologists over the last three decades to describe significant environmental effects which explain prediction outcomes of response variables estimated from compilations of independent ecologic characteristics (Guisan et al. 2002; Wood and Augustin 2002). Aquatic studies applying GAMs have included: macrophyte distributions (Lamon et al. 1996; Lehmann 1998; Valley et al. 2005), abiotic influences on water quality parameters such as eutrophication and nutrient removal rates, fetch, and turbidity (Reckhow and Qian 1994; Morton and Henderson 2008; Bustamante et al. 2009; Tao et al. 2012), and fish dependency to macroinvertebrate assemblages (Milner et al. 2001; Brosse and Lek 2002; Jowett and Davey 2002) and macrophyte abundance (Conrad et al. 2016).
Kernel Density Estimators (KDEs)

Well described by Silverman (1986), KDEs are a robust, non-parametric spatial interpolation of locally weighted parameters which use a smoothing term (e.g. Gaussian) to reflect the concentration of neighboring regions within a defined unit area. The resulting output provides density estimations which delineates the breadth of input features to estimate zonal expanses (i.e. distribution) which are used to express regions of presence or likeness (Seaman and Powell 1996; Duong 2007; Bennion et al. 2011). One drawback to this method however, is the requirement of independence (Worton 1989; Fieberg 2007); as many ecological parameters are inherently spatially autocorrelated (i.e. dependent) (Legendre 1993). To reduce the probability of spatial autocorrelation, resampling and point elimination are needed to meet the requirement of independent observations and reduce the likelihood of over-estimating (i.e. Type I error) (Legendre 1993). However, removing data values ultimately reduces sampling size and ecologic significance (de Solla et al. 1999).

Nevertheless, utilizing tests, such as Moran’s I index (Moran 1950; Gibson 2014), assumptions may be met by outlining the degree of spatial autocorrelation effects. Spatial patterns, gauged from -1 (point scattering) to 1 (point grouping), represent positive and negative autocorrelation, while index values of 0 lack spatial autocorrelation (i.e. random pattern) (Mitchell 2005; Gibson 2014). While independent point selections are required, researchers continually employ KDEs for describing spatial trends within sampling regions. A fundamental univariate (i.e. binary) KDE function, with model factors used for point-data interpolation, is expressed using a derivative of Silverman (1986), Worton (1989), and Seaman and Powell (1996) parameters:
Model elements: \( \hat{f}(x) = \frac{1}{nh} \sum_{i=0}^{n} K \left( \frac{x-X_i}{h} \right) \),

where \( \hat{f}(x) \) is the estimated density outcome, \( n \) is the length of observations, \( h \) is the bandwidth (ie. search radius), \( x \) are the discrete spatial locations (ie. latitude, longitude), \( X_i \) define the input values (eg. binary terms for presence), and \( K(x) \) is the smoothing function (ie. Gaussian). Ecologically, fixed bandwidths are recognized for providing the greatest non-biased surface density estimations (Worton 1995; Seaman and Powell 1996; Vokoun 2003).

While not commonly used in macrophyte studies, several aquatic plant ecologists have employed the use of KDEs to correlate spatial trends of aquatic plant distribution to environmental elements (eg. Göktoğan et al. 2010; Santos et al. 2011; Compton et al. 2012; Olsen et al. 2016). Still, in ecology, KDEs are most often found in home range analysis studies, such as modeling faunal dispersal in lentic and lotic environments (Quinn et al. 1996; Sanvicente-Añorve et al. 2003; Vokoun 2003), and describing wildlife habitat heterogeneity, spatial zones and land preference, and seasonal travel (Chochrane et al. 2006; Robinson et al. 2010; Palminteri and Peres 2012; Immell et al. 2014).

**RESEARCH OBJECTIVES**

Chapter 2:

Submersed aquatic vegetation (SAV), contribute to whole-lake ecological assemblages by providing macroinvertebrate habitat (Strayer and Malcom 2007), physical structure and food for littoral fish species (Petr 2000), and other essential abiotic dynamics (Madsen et al. 2001). However, some freshwater macrophytes like the exotic species,
*Hydrilla verticillata* (L.f.) Royle, present negative impacts through competition and displacement of native SAV (Van et al. 1999; Spencer and Ksander 2000; Meadows and Richardson 2012), and the impediment of recreational activities and power generation (Langeland 1996). One measure of determining the severity of an exotic macrophyte invasion is through in situ biomass sampling over time (Madsen 1993). Numerous studies have revealed valuable implications for utilizing hydroacoustic technology for macrophyte estimation (Macéina et al. 1984; Daurte 1987; Thomas et al. 1990; Sabol and Melton 1995; Valley and Drake 2005). Current hydroacoustic equipment and procedures are exponentially superior to those units used nearly three decades ago. Recent advancements in commercial grade echosounders solve many of the constraints described by Macéina et al. (1984), Daurte (1987), and Thomas et al. (1990). Furthermore, advancements in GIS technology enable timely post-processing of survey data (Sabol et al. 2002; Valley and Drake 2005). The objective of this investigation was to document the use of a commercially available echosounders to: 1) delineate and characterize a relationship between hydroacoustic biovolume signature to in situ measured *H. verticillata* biomass; 2) develop an algorithm for the assessment of *H. verticillata* biomass from interpolated hydroacoustic biovolume records; 3) define seasonal *H. verticillata* growth patterns at two NC piedmont reservoirs; and 4) create a visual representation of temporal SAV development.

**Chapter 3:**

Macrophytes are extensively recognized for contributing to the overall net primary production of aquatic environments and aquatic ecosystem quality (Likens 1975; Boston et
al. 1989; Madsen and Sand-Jensen 1991; Dodson et al. 2000; Takamura et al. 2003; Brothers et al. 2013). However, alien plant invasions, like those caused by the submersed non-native species, *Hydrilla verticillata* (L.f.) Royle, often require water resource managers to conduct lakewide surveys to support management opportunities for ecologic and economic sustainability. Timely monitoring and efficient mapping strategies remain fundamental for assessing the abundance of native and exotic aquatic flora (Maceina et al. 1984; Lehmann & Lachavanne 1997; Hestir et al. 2008; Husson et al. 2016). To reduce labor, time, and alleviate some of the obstacles associated with in situ fixed-point sampling, research has focused on advances in commercially available remote sensing technologies to offer innovative approaches for assessing aquatic vegetation. Open source sensors, like Landsat 7 ETM+, provide coarse population dynamics, but lack the spatial and temporal resolution (eg. ~ 30 m$^2$ and ~15 days$^{-1}$; respectively) to accurately represent submersed and emergent vegetation. Conversely, commercial satellite images deliver the required spectral and spatial resolutions to discern community assemblages, but the economic hindrance associated with these images often prohibits small-scale application. Furthermore, satellite sensors have increased issues with atmospheric properties (eg. cloud cover; scattering), water column interference (eg. light attenuation and absorption due to water and water quality components), light penetration, and benthic substrate (Ackleson and Klemas 1987; Blanco et al. 2012; Cho et al. 2012). Therefore, the objective of this experimentation was to overcome atmospheric correction issues and develop an “on-demand” boat-based remote sensing platform to: 1) passively detect submersed, emergent, and floating vegetation while conducting point-intercept samplings; 2) increase in situ observation performance; and 3) offer innovative options for
mapping and post-processing for aquatic ecologists and lake managers through visual and statistical descriptions of macrophyte abundance levels linked to communal and temporal dynamics.

Chapter 4:

Macrophytes serve as fundamental trophic indicators of environmental quality and provide critical ecological niches among lentic, wetland, and riverine systems (Ackleson and Klemas 1987; Melzer 1999; Cho et al. 2012). Likewise, submersed aquatic vegetation (SAV) often aid in lakewide ecological sustainability (Twilley et al. 1985; Madsen et al. 2001; Kufel and Kufel 2002). However, introduction of non-native, invasive species such as Hydrilla verticillata (L.f.) Royle and Myriophyllum spicatum (L.) can impede native macrophyte and fauna conservancy (Langeland 1996; Eiswerth et al. 2000). As the final impoundment prior to the estuarine Albemarle Sound of North Carolina, Roanoke Rapids Lake has become part of a diverse and ecologically critical system for a multitude of aquatic flora and fauna (Meitzen 2016); most notably providing spawning opportunity for indigenous ray-finned species, such as striped bass (Morone saxatilis) and American eel (Anguilla rostrata) (Olsen and Rulifson 1992; Nelson 1998; USFWS 2015). Additionally, Roanoke Rapids Lake serves utilitarian purpose by providing power to surrounding basin districts, real estate, and recreation for water sport enthusiast and outdoorsmen. In 2006, Roanoke Rapids Lake was placed on the 303(d) List of Impaired Waters (Clean Water Act). However, there are limited quantitative assessments published discerning submersed plant dynamics at Roanoke Rapids Lake. Therefore, many SAV expanses remain unknown and lack appraisal of per annum or
decadal alteration among submersed macrophyte species. Our objective of this study was to investigate submersed macrophyte: 1) species-specific variations in occurrence, abundance, dominance, and spatial distributions among; 2) community richness, and co-occurrence; and 3) interspecific dynamics over time.

Chapter 5:

Over the last several decades, global demands for reduced carbon (CO\textsubscript{2}) and other greenhouse gas emissions due to fossil fuel consumption, have prompted research of alternative energy sources from second-generation bio-ethanol crops (Ragauskas et al. 2006; Hastings et al. 2008; Naik et al. 2010; Xu et al. 2015). Giant reed (\textit{Arundo donax} L.), continues to gain recognition among researchers examining second-generation raw materials for biofuel production (Birur et al. 2008; Mantineo et al. 2009; Baldini et al. 2016; Corno et al. 2016). In North Carolina, recent incentives from federal and states agencies has encouraged alternative fuel production to reduce the annual ecologic hindrance and economic dependence accompanying petroleum usage (Parcell and Westhoff 2005; Birur et al. 2008; Yacobucci 2012). While \textit{A. donax} phenological components support lignocellulosic biofuel production in North Carolina, there remains some uncertainty to the invasiveness of \textit{A. donax}. The objective of this study was to identify appropriate herbicides that may be used to control established \textit{A. donax} as part of crop rotations or control escapes from agronomic plantings.
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CHAPTER 2

Correlation of Hydroacoustic Signature to Submersed Plant Biomass
ABSTRACT

Invasive macrophytes, such as non-native *Hydrilla verticillata*, negatively impact lentic systems of the southeastern United States through impediment of recreational activities, power generation, and disruption of intrinsic ecological function. Expenditures associated with aquatic weed management include costs accompanied with monitoring, mapping and implementing control measures. Prompt SAV detection and accurate mapping are critical components when formulating management decisions and practices. Therefore, SAV management protocols are often reliant upon the perceived extent of incursion. Traditional biomass sampling techniques have been widely utilized, but often require significant labor inputs, which limits repeatability, the scale of sampling and the rapidness of processing. Advances in commercially available hydroacoustic technology and data post-processing offer the opportunity to estimate SAV biomass at scale with reduced labor and economic requirements. Research was conducted at two North Carolina reservoirs to compare cloud-based ciBioBase 5.2 biovolume estimations from a commercially available echosounder, to in situ hydrilla biomass measurements. Non-parametric analyses were used to describe acoustically derived biovolume to hydrilla biomass. Study locations were positively correlated at Shearon Harris (SH; Spearman rank $\rho$: 0.51, $P < 0.001$) and at Roanoke Rapids (RR; Spearman rank $\rho$: 0.73, $P < 0.001$). However, the pooled dataset provided the greatest explanation of association (Spearman rank $\rho$: 0.79, $P < 0.001$). Temporal patterns and spatial developments were also analyzed for all test sites, along with hydrilla biomass prediction equations. Our most robust predictive equation employed generalized additive models (GAMs) from the SH dataset ($\text{Dry Biomass} \cdot 25 m^3 = s_f(\text{SH})$...
\( Biovolume \) + \( s_2(\text{SH Depth}) + t_i(\text{SH Biovolume, SH Depth}) \) which, described environmental parameters with the lowest RMSE and excellent agreement (\( R^2_{\text{adj}} = 0.86, P < 0.001 \)). Each biovolume to biomass relationship supported the initial hypothesis that as biovolume increases, SAV biomass increases in a positive, non-linear trend. Implications from this study may prove extremely useful for comparing seasonal growth patterns, littoral occupancy, and herbicide treatment effects on a spatiotemporal level.

**INTRODUCTION**

Submersed aquatic vegetation (SAV), play a vital role in contributing to whole-lake ecological assemblages by providing macroinvertebrate habitat (Strayer and Malcom 2007), physical structure and food for shoreline fish species (Petr 2000), and other essential abiotic dynamics (Madsen et al. 2001). However, some freshwater macrophytes like the exotic species, *Hydrilla verticillata* (L.F.) Royle, present negative impacts through competition and displacement of native SAV (Van Dyke et al. 1999; Spencer and Ksander 2000; Meadows and Richardson 2012), and the impediment of recreational activities and power generation (Langeland 1996). Not only can invasive macrophytes create ecologic disturbance but also economic hindrance for stakeholders. In the United States, over $100 million are spent annually towards the management of aquatic plants (Rockwell 2003), thus signifying the importance of monitoring infested areas and timely management application.

One measure of determining the severity of an exotic macrophyte invasion is through in situ biomass sampling over time (Madsen 1993). Using this process, aquatic plant managers also have an opportunity to identify community taxa, estimate plant abundance
(Moore et al. 2000) and calculate stocking rates for grass carp (*Ctenopharyngodon idella*) (Van Dyke et al. 1984; Bonar et al. 1993). Conversely, there are several drawbacks to this method for accessing SAV. Destructive biomass sampling is an extremely laborious process (Madsen 1999) and it can be restrictive to evaluate expansion over large spatial scales (Duarte and Kalff 1990; Johnson and Newman 2011). Also, spatial and temporal development of littoral plant beds may be difficult to appraise from biomass point sampling alone. However, advances in hydroacoustic technology and geographic information systems (GIS) over the last several decades offer an opportunity to estimate SAV biomass at gamut with reduced labor and economic requirements.

Numerous studies have revealed valuable implications of utilizing hydroacoustic technology for macrophyte estimation (Macéina et al. 1984; Daurte 1987; Thomas et al. 1990; Sabol and Melton 1995; Valley and Drake 2005). Macéina and Shireman (1980) were the first investigators to recognize and document the performance of a recording fathometer (echosounder measuring depth in fathoms) to predict hydrilla biomass, although the accuracy of their regression equation was confounded by ecological growth patterns. To account for plant structure and littoral growth arrangement, Duarte (1987) successfully studied the form of macrophyte growth to create a comprehensive predictive model of submersed biomass. However, there were some limitations with this model as the echosounder transducer used was not capable of acquiring SAV less than 20 cm in height and the study site was comprised from a community of mixed species (Duarte 1987). To overcome the structurally derived component in biomass prediction from an echosounder, Thomas et al. (1990) used biovolume, or the quantity of the water-column occupied by SAV, to indicate spatial
abundance instead of complex models of plant height and form. Using biovolume as a predictor of plant structure was found to represent ecological growth habits more precise than plant bed height to quantify littoral zone development (Thomas et al. 1990). Although these researchers pioneered the use of hydroacoustic technology for predicting SAV biomass from acoustically derived signatures, there are several disadvantages from each sampling approach. Protocols commissioned by the previously described studies employed the use of scientific grade echosounders with narrow transducer beam angles (e.g. 6° to 15° beam angle) to achieve maximum SAV penetration to the benthic surface for clearer bottom detection (Macéina and Shireman 1980; Thomas et al. 1990) and commercially available echosounding deceives (e.g. 22° to 50° beam angle) to obtain a breadth of SAV profiles (Duarte 1987, Thomas et al. 1990). Implementation of transducer beam angle extremes (e.g. 6° or 50° beam angle) limits the scope of spatially available information, inhibits the detection of short vegetation, and increases plant bed saturation loss.

Another concern regards the use of mixed plant stands for the basis of regression analysis and biomass predictive models. Although Macéina and Shireman (1980) comprised their report around hydrilla, subsequent studies used a community of macrophytes to develop complex regressions between observed biomass to acoustically originated biomass projections. Therefore, a monospecific based equation should more accurately represent true biomass abundance and progression since not all SAV have the same structure to biomass ratio (Jørgensen 2013).

Current hydroacoustic equipment and procedures are more effective than those units used to study biomass and echosounder tracings near three decades ago. Recent
advancements in commercial level echosounders solve several constraints previously described, and advancements in GIS technology enable timely post-processing of survey data (Sabol et al. 2002). Modern echosounder units not only present a broader acoustic range, but also provide a cost-effective option for contiguous repeatability throughout the monitoring period (Valley et al. 2005).

The objectives of this research were to document the use of a commercially available echosounder to: 1) delineate and characterize a relationship between hydroacoustic biovolume signature to in situ measured hydrilla biomass; 2) develop an algorithm for the assessment of hydrilla biomass from interpolated hydroacoustic biovolume records; 3) define seasonal hydrilla growth patterns at two NC piedmont reservoirs; and 4) create a visual representation of temporal SAV development. From these objectives, the expected outcome was to describe a protocol for passive data acquisition while reducing the economic inputs associated with labor efforts involved in biomass collection and post-processing evaluation.

In our research, a commercially available echosounding unit was utilized to correlate biomass from monospecific stands of hydrilla within two different North Carolina piedmont reservoirs using a third-party, cloud-based algorithm to aid in post-processing.

**MATERIALS AND METHODS**

Between mid-June and late-October 2015, two NC piedmont reservoirs were sampled biweekly for hydrilla biomass and acoustically-derived SAV abundance. Timing of the boat-based sampling procedure conformed to measurements of hydrilla growth patterns from a previous report conducted on NC lakes (Harlan et al. 1985). The two NC piedmont water
bodies chosen for fixed sampling sites included Shearon Harris Reservoir (SH; Wake Co.; 35°38′0″N, 78°57′18″W) and Roanoke Rapids Lake (RR; Halifax Co.; 36°28′58.3″N, 77°43′38.7″W) (Figure 1). These locations were selected to simulate a range of ecological factors of bathymetric profile, littoral slope, water exchange frequency, macrophyte community assemblage, and seasonal SAV growth rates. Based on previously conducted surveys, hydrilla has been the dominant macrophyte in both reservoirs for over a decade (Nawrocki et al. 2016; NCDWQ 2006) thus, providing an optimal scenario for testing monospecific biomass correlation.

Biomass sampling

Predetermined hydrilla plots were georeferenced at both study locations prior to biomass sampling. Individual plots contained 60 sequentially numbered points (labeled: 1-60); comprising a surface area of 0.53 ha each (Figure 2). RR contained three plots while SH contained two plots (Figure 3). Points were loaded to the boat-based echosounding unit preceding the experimental period to reference biomass sampling positions.

SAV biomass was collected every two weeks at both study locations to ensure adequate temporal resolution within the sampling period for describing seasonal growth trends (Madsen 1993). For each of the sampling periods, four randomly selected points were selected from n=60 points per plot using a random number generator without replacement (R Core Team 2015). These sample points were then used for all plots at both lake locations for that sampling period (ie. SH = 8 points biweekly; RR = 12 points biweekly).

To sample SAV biomass at each of the study sites, a modified version of the boat-based vertical rake method, proposed by Johnson and Newman (2011) for macrophyte
biomass collection, was utilized. The sampling rake used in this study contained eight sampling tines covering a volumetric representation of 0.25 m$^3$, affixed to a 2.5 m$^{-1}$ pole. If depth of a given collection point was greater than 2.5 m$^{-1}$, a 3 m$^{-1}$ pole extension was added. To collect biomass within a test plot, the onboard global positioning system (GPS) from the echosounding unit was used to loiter the boat over designated random points. The rake was then lowered near the boat-based transducer, to increase true spatial proximity, into the water column and through any plant bed until lake bottom was reached. The rake was spun two full-rotations before slowly returning the rake back to the boat for analysis of above-ground plant material. At the time of rake retrieval, in situ estimates of rake coverage and average stem length were recorded (eg. one rake tine with macrophyte material = 12.5% coverage, two tines…= 25% coverage, etc.). If any other SAV species were detected within hydrilla biomass samples (< 5% occurrence of non-hydrilla), those macrophytes were separately bagged and analyzed. All representative hydrilla biomass collected were field washed of any detritus, individually bagged for dry weight analysis, and placed in a cooler until reaching the lab.

At the lab, samples were allowed to air dry on expanded metal tables for at least 24 h prior to oven drying to reduce dehydrating time and potential decay of wet plant material. Samples were then dried at 60°C for 48 h before biomass weights were recorded in dry biomass per unit rake volume (g dry wt·0.25 m$^3$).

**Hydroacoustic sampling**

A Lowrance HDS-7 Gen2$^1$ commercial grade fish-finding echosounder, with internal GPS capability of 5-Hz refresh rate and an accompanied 200-kHz single frequency
transducer with 20° beam angle at 10-15 pings s⁻¹, were used to log acoustically derived SAV signatures and corresponding spatial location.

Prior to biomass sampling, a hydroacoustic scan occurred at each discrete plot. Starting at the beginning of each plot, a serpentine transect with 7.5 m spacing occurred throughout the remaining length of the plot at a boat speed of ~8 km/h to determine bottom area interpolation of SAV abundance at each labeled biomass sampling point. The .sl2 sonar log representing a particular site location was given a unique identifier so future correlation could be prepared. All logged data were saved by the chartplotter to an internal 32GB SD memory card for further analysis and upload to ciBioBase 5.2 cloud-based software².

Data analysis

All hydroacoustic data uploaded to the ciBioBase 5.2 algorithm were exported as tabular ASCII-grid records of spatial location, biovolume percentage, and depth contour. Using similar methods to Valley et al. (2015), representative plots at all sites were imported into ESRI ArcGIS 10.2.2³ software for further post-processing and analysis. Biovolume grid-point features were transformed to shapefiles where the ArcGIS spatial analyst, feature-to-raster tool, was used to interpolate raster grids with 7.5 m² cell size. The resulting raster grids made it permissible to extract biovolume percentages to buffered hydrilla biomass point feature data collected throughout the sampling period. Using Hjmans (2015) RStudio⁴ raster package, a 2.5 m⁻¹ buffer was placed around each corresponding biomass point feature of interest. This buffer distance represented in situ spatial errors such as wind drift and GPS resolution while collecting biomass samples. The joined hydroacoustic and buffered biomass dataset attributes were then used to run correlation analysis and provide visual representation
in RStudio nonparametric and ggplot2 packages (Hayfield and Racine 2008; Wickham 2009). Digests such as percent area covered (PAC), bathymetry, and seasonal biovolume development among plots at both SH and RR could also be statistically associated using ArcGIS and RStudio platforms. A false-positive limiting depth of 0.76 m$^{-1}$ was assigned as the minimum depth used for correlation analysis due to heavy noise and backscatter from the transducer in shallow areas (Duarte 1987; Valley et al. 2015). Removing data points from the shallow regions in this study did not impede overall analysis as plot layouts for biomass harvesting were designed to exceed 0.76 m$^{-1}$ depths. Regression analysis and biomass prediction algorithms utilized RStudio base and non-parametric generalized additive models {mgcv} packages (Wood 2011; R Core Team 2015).

**RESULTS AND DISCUSSION**

**Study sites and plot characteristics**

Biomass was collected throughout the experimental period at 84 sampled points at SH and 71 sampled points at RR to represent a wide range of littoral features and seasonal variability among each test plot (Table 1). The mean seasonal biovolume percentages and biomass accumulation varied less at RR than at the SH test sites (Table 1). However, both locations had similar depth variability between individual test plots (Table 1). Illustration of depth contours at each given geographic location depict depth ranges ($\bar{x} = 1.56 – 2.4$ m$^{-1}$) representing plot profiles at SH and RR (Figure 4,5).

Hydrilla was the only macrophyte found at SH, however, *Ceratophyllum demersum* L. (coontail), *Cabomba caroliniana* A. Gray (cabomba), and *Myriophyllum spicatum*
(Eurasian watermilfoil) were found in 13 of the total number of samples at RR. Each of the three plots at RR had at least one non-hydrilla species sampled during this experiment; with plot number two containing the greatest number of non-hydrilla biomass samples. Samples which comprised >5% of non-hydrilla biomass were identified and tested in two separate correlation analyses at RR.

**Correlation of biovolume, biomass, depth and rake fullness estimations**

Non-linear regression analysis was used to explain non-parametric correlations among estimated biovolume percentages and observed hydrilla biomass (Figures 6 to 9). SH had lower agreement (Spearman rank $\rho$: 0.51, $P < 0.001$). However, there was a strong positive trend that indicated biomass would increase with biovolume (Figure 6). Hydrilla growth at both test plots at SH contained low biovolume to biomass ratios, which may explain why there was a low association when biovolume reached $\geq 25\%$ (Figure 6). Two correlations were comprised for RR; one with hydrilla only (Spearman rank $\rho$: 0.73, $P < 0.001$), and another with hydrilla in addition to non-hydrilla (Spearman rank $\rho$: 0.69, $P < 0.001$) (Figure 7,8). Both correlations from RR produced very strong agreement and represented high biovolume to biomass ratios well; conversely low biovolume estimates (ie. $\leq 25\%$) were not well represented. Independent correlations from both SH and RR study areas indicated strong, positive correlations that were highly significant, however the pooled data provided the greatest explanation of association (Spearman rank $\rho$: 0.79, $P < 0.001$) (Figure 9). A combination of both lake data sets characterized a wide range of biovolume (0 to 100%), depth (0 to 4.74 m$^{-1}$), and biomass (0 – 446.1 g dry·wt), that helped provide well
spread data and the most accurate representation of seasonal hydrilla growth. The entire range of biovolume estimates were represented at both lake locations (Figure 10). Among every correlation, hydrilla biomass was often found at maximum in shallow depth localities. Overall, each biovolume to biomass relationship supports the initial hypothesis that as biovolume increases, SAV biomass should increase in a positive trend, although alterable, since bathymetry aids in delineating biovolume estimations.

With both data sets pooled, hydrilla biomass was negatively correlated to bathymetric characteristics from both study sites (Spearman rank $\rho$: -0.59, $P < 0.001$). Depth profiles of SAV had a slightly right-skewed, semi-parabolic shape (Figure 11). Average depth occurred at $\bar{x} = 2.13 \pm .79$ m, with biomass decreasing to either side of the maximum observed biomass value. A comparable depth to biomass correlation has also been documented by Duarte and Kaliff (1990). Shallow depths limit the vertical expansion of SAV, while deeper depths do not provide as much light attenuation, due to suspended solids, to maximize SAV growth potential (Havens 2003). This supports our ecological trend where the majority of high biomass was arranged within one standard deviation of the mean depth zone.

Accessing trends in biovolume distribution against known depth profiles identified non-linear relationships among all observed datasets (Figure 12). Biovolume was influenced by depth range at both independent study sites when comparing repeated measurements of the mean biovolume percentages using Wilcoxon signed rank test (SH: $W = 48$, $P < 0.001$; RR: $W = 26$, $P < 0.001$) and also when both site datasets were combined and matched using Kruskal-Wallis rank sum test ($\chi^2 = 83.91$, $P < 0.001$) to access site specific biovolume differences. From these observations, it should be noted that SH had low biovolume
percentages at all depth bins while RR best fit the high range of biovolume percentages from 1.5 – 3.0 m\(^{-1}\). Therefore, the pooled data biovolume best represents depth bins for which water columns were fully comprised of SAV (ie. 100% biovolume). A tendency occurred where biovolume percentages rarely exceeded 15% at depths \(\geq 3.0 \text{ m}\). Valley et al. (2005) noticed an analogous trend shape in their study of depth to biovolume percentages, thus emphasizing the extent of SAV depth profiles in our experiment.

Employing non-linear modeling on rake fullness estimates, a high correlation was discovered between rake coverage and hydrilla biomass (Spearman rank \(\rho: 0.89\)). This is thought to be due to sampling points comprised of dense vegetation and low SAV height, or sparse vegetation with SAV capable of covering all rake tines. In situ plant height estimates were also found to be highly correlated to collected biomass (Spearman rank \(\rho: 0.89\)), which advocates that an increase in plant height may lead to an increase in SAV biomass even in less dense stands.

**Hydroacoustic biomass prediction algorithms**

Multiple linear regression (MLR) and non-parametric, generalized additive models (GAM), were utilized to provide prediction equations for SH, RR, and a pooled dataset of both test sites (Tables 2, 3). Using the correlation findings described previously of biomass and biovolume, individual study sites received predictive model parameters described by the ecological growth trends at all sampling locations. The MLR SH prediction equation (Table 2a), had the greatest projected coefficient of determination (\(R^2_{\text{adj}} = 0.71, \text{ P} < 0.001\)) and the greatest depth profile of the MLR models. Since depth has been shown to limit the extent of vertical hydrilla growth in this study, SH also received the highest maximum biomass
estimate. Similar results were obtained using GAMs; as SH provided the highest coefficient of determination ($R^2_{adj} = 0.86, P < 0.001$) and the lowest RMSE (129.72) of the GAM equations (Table 3). Biomass prediction for RR (Table 2b) did not have as high agreement ($R^2_{adj} = 0.34, P < 0.001$) as SH; possibly due to fewer samples in the MLR model and from depth parameter restrictions causing variation. Again, the GAM models reciprocated this trend as RR had the lowest agreement ($R^2_{adj} = 0.36, P < 0.001$) and deviance explained of the GAM models (Table 3). Our most demonstrative MLR model, from the perspective of environmental growth, was comprised from the pooled data (Table 2c). Although this predication equation ($Dry\ Biomass .25\ m^3 = 10.802 + 88.662\ (biovolume) + 5.622\ (depth)$) had moderate agreement ($R^2_{adj} = 0.47, P < 0.001$), it described the association of environmental parameters most similar to the non-parametric correlation values. However, the pooled data delivered the poorest model of the GAMs (Table 3) when cross-validated; with the highest RMSE (442.63) observed. This suggests that the utility of pooling data for the MLR dataset may not provide the best explanation of the environmental parameters formerly described. Therefore, the most robust model for this study was employed using GAMs from the SH dataset ($Dry\ Biomass = s_1(SH\ Biovolume) + s_2(SH\ Depth) + ti(SH\ Biovolume,\ SH\ Depth)$), which also provided the highest range of prediction values of all models tested (Figure 13; Tables 2,3).

One issue which arose from the SH algorithms was the lack of data values representing the upper scale of both biomass and biovolume. Conversely, the opposite issue occurred for the prediction equation of RR, where the low range of biomass and biovolume were not well embodied. Therefore, when utilizing MLR, data comprised of both sites not
only represented the full range of biomass, biovolume and depth, but also transformed the dataset from a non-linear trend to a more linear based trend to meet the assumptions of MLR analysis. Contrariwise, this method was rejected when employing GAMs on the alike dataset. Another problem which was faced during the composition of prediction equations involved the effect data points with low biomass and high biovolume had on the power of prediction. Again, with an increased number of sample points, the association of data points which lied outside the model parameters (ie. 95% confidence intervals) were not weighted as significant components of the model (Type II error) when using MLR. For both modeling techniques, implementing depth into the prediction equation reduced the effect of low biomass and high biovolume and accurately represented the predicted values by transforming the data into a unit of the water column occupied with SAV. Still, these issues were overcome when incorporating GAMs, as environmental parameters were weighted based upon field observed values and the non-parametric relationships among those variables, using smoothing splines to fit the data for prediction (Figure 13).

From our prediction equations, many of the restraints described from previous documents have been well satisfied. Following suggestions provided by Thomas et al. (1990), our algorithms used biovolume as a confounding predictor of future biomass estimates to spatially represent the percentage of the water column comprised of SAV. Using biovolume instead of plant height not only provided hydrilla littoral density, but also clearly characterized much of the ecological growth constrictions that occurred by Macéina and Shireman (1980); that is, standardizing the fluctuations in plant height and water depth. Also, by separating any significant (P > 0.05) non-hydrilla species in the prediction models, we
accounted for monospecific macrophyte signatures of biomass and structure. Doing this solidified the degree of projective accuracy since, SAV prediction equations involving mixed-stands have been prone to preeminent biomass inconsistency (Duarte 1987).

**Seasonal hydrilla growth and temporal development**

Acquiring trends in hydrilla growth is a vital component of timely management applications and for studying macrophyte composition levels within a water column (ie. biovolume). Box-whisker plots (Figures 14,15) show results of hydroacoustic scans occurring bi-weekly at SH and at RR along with each corresponding plot at individual test sites throughout this experiment. These figures were compiled using bi-linear interpolation to define temporal representations of biovolume accumulation throughout this study. At SH, the highest mean biovolume (Plot 1: $\bar{x} = 58.13\%$) occurred on sample date 9/30/2015. RR highest mean biovolume (Plot 1: $\bar{x} = 97.29\%$) fell on the 8/6/2015 sample date. The total seasonal biovolume accumulation plot means with corresponding C.V.’s may be viewed in Table 1. A baseline hydroacoustic scan, which occurred 3/18/16, represents both plots at SH with zero biovolume present.

To illustrate seasonal plot biovolume accumulation, (Figure 16,17) portray the ecological development pattern differences in biovolume over time by defining the transformations in biovolume at all test sites. SH hydrilla growth had the highest biovolume from early-September to mid-October 2015, while RR peak biovolume occurred early-July to early-September 2015. Sampling locations at RR contained plots with more shallow depths than at SH and therefore full SAV expansion of the water column developed quicker at RR. Plots at RR became topped-out with hydrilla (ie.100% biovolume) after the 9/2/16 sample
date and therefore sampling stopped beyond this date. This is also why some plots in Figure 16 do not show the full extent of the plot range. Senescence at either study location was not examined in the biovolume modeling components of this study.

Management implications

Our findings are consistent with those of Stent and Hanley (1985), Duarte and Kalff (1986), and Duarte and Kalff (1990), that biomass regression analysis is a site-specific procedure due to littoral slope, turbidity, water quality, and the presence of other macrophytes. We have also shown, that even when using monospecific stands of hydrilla, there is variation of SAV biomass among discrete waterbodies. However, using GAMs to engage vigorous statistical procedures, the power of obtaining a more precise prediction model has potential for explaining environmental factors causing deviation.

A few minor limitations involving the prediction of future hydrilla biomass were apparent in this study. These disadvantages were: 1) hydrilla biomass was highly variable as biovolume reached 100% water column occupancy; 2) once biovolume reached 100%, we were unable to predict future responses in our algorithms; 3) in areas where SAV height was at water surface, our boat was incapable of mapping those areas reliably without obstructing boat transects; and 4) we were not able to obtain biovolume estimates below 0.76 m$^{-1}$ due to transducer noise. On a cautionary note, all biomass estimations occurring when biovolume is at 100% should be double checked with depth parameters to ensure model elements are not extrapolated beyond the extent of the dataset. Also, to overcome unrepresented areas containing SAV at either depths $< 0.76$ m$^{-1}$, or areas containing 100% biovolume, spatial
interpolation techniques such as kriging, IDW, bilinear interpolation, or nearest neighbor may be utilized to define those regions (Valley et al. 2005).

Although some drawbacks were present with this research, the advantages of utilizing a commercially available echosounding unit for macrophyte biomass assessment far out compensated the obstacles formerly described. By exercising a third-party vendor for managing all data recorded by the echosounder, a major reduction in post-processing time was achieved. Furthermore, our methodology proved useful in both tracking and mapping temporal changes in biovolume and biomass accumulation over time. This not only offered a repeatable, non-destructive monitoring opportunity for ecological growth patterns, but also provided visual evidence for aquatic weed management applications. Aquatic plant managers may additionally want to employ the use of the algorithms previously designated in formulating proposals for herbicide treatments, grass carp stockings, or stakeholder reports. Since this study focused solely on hydilla, future studies may want to implicate validation of our models in similarly structured macrophytes for biomass estimation (eg. submersed Myriophyllum spp.).

In summary, our study defines the parallel between biovolume and hydilla biomass thus stipulating technological advances used by aquatic ecologists conducting fixed point-intercept sampling protocols, while also passively recoding hydilla biomass estimation using an over-the-counter commercial echosounder. One key advantage of this echosounding unit was the ease of operation, availability, cost (~$800), and minimal training requirements. Likewise, managers could easily train employees to use this system in only a few minutes. Our findings should offer noteworthy economic efficiency with reduction to the degree of
labor and time spent presenting spatiotemporal littoral zone dynamics as documented by Macéina et al. (1984). Following the results of this report, especially in lakewide survey scenarios, should offer improvements to traditional biomass sampling.

**SOURCES OF MATERIALS**

1. Lowrance HDS-7 Gen2 chartplotter, Navico Inc., 4500 South 129th East Avenue, Suite 200, Tulsa, OK 74134.

2. ciBioBase 5.2 cloud-based software, Contour Innovations, LLC, 1229 Tyler Street NE, Suite 120, Minneapolis, MN 55413.

3. ArcGIS 10.2.2, Environmental Systems Research Institute, 380 New York Street, Redlands, CA 92373.

LITERATURE CITED


Figure 1. Locations of two NC piedmont reservoirs sampled for hydilla biomass and hydroacoustic scans; Shearon Harris (bottom center) and Roanoke Rapids (top right).
Figure 2. Example of a singular plot used for seasonal biomass sampling and hydroacoustic scans, containing n=60 georeferenced points and representing a surface area of 0.53 ha$^{-2}$. In total, five identically designed plots were used in this study.
Figure 3. Sampling sites, shown in red, at each corresponding experimental lake location. Roanoke Rapids (A) contained three plots where Shearon Harris (B) encompassed two plots. Plot locations were visited bi-weekly for hydroacoustic scans and biomass sampling.
Figure 4. Map of raster cells (7.5 m$^2$) representing depth contours at each given geographic location at Shearon Harris characterizing Plot 1 and Plot 2 profiles. The grey shaded regions opposite the x and y axis signify littoral slope for each individual plot bathymetry.
Figure 5. Map of raster cells (7.5 m\(^2\)) representing depth contours at each given geographic location at Roanoke Rapids characterizing Plot 1, Plot 2, and Plot 3 profiles. The grey shaded regions opposite the x and y axis signify littoral slope for each individual plot bathymetry. *The plots in this map are to scalar extent so the size of the plots may not seem to represent the same spatial area but they all represent the same cell size and number.
Figure 6. Shearon Harris correlations among estimated biovolume percentages from ciBiobase 5.2 and observed hydrilla biomass (Spearman rank $\rho$ : 0.51, P < 0.001).
Figure 7. Roanoke Rapids correlations among estimated biovolume percentages from ciBiobase 5.2 and observed hydriilla biomass (Spearman rank $\rho$: 0.73, $P < 0.001$).
Figure 8. Roanoke Rapids correlations among estimated biovolume percentages from ciBiobase 5.2 and observed SAV biomass (Spearman rank $\rho: 0.69$, $P < 0.001$).
Figure 9. Pooled dataset correlations among estimated biovolume percentages from ciBiobase 5.2 and observed SAV biomass (Spearman rank $\rho: 0.79$, $P < 0.001$).
Figure 10. Pooled dataset correlations among estimated biovolume percentages from ciBiobase 5.2 and observed SAV biomass by sampling locations (Spearman rank $\rho$: 0.79, $P < 0.001$). From these observations, Shearon Harris best represents data points of low biovolume and low biomass where Roanoke Rapids more clearly depicts samples containing high biovolume and high biomass.
Figure 11. Depth profiles of SAV shown have a slightly right-skewed, semi-parabolic profile. Depth average occurred at \( \bar{x} = 2.13 \pm 0.79 \text{ m}^{-1} \), with biomass decreasing to either side of the maximum observed biomass value (446.10 g dry wt·0.25 m\(^{-3}\)).
Figure 12. Box-whisker plots of estimated biovolume detected at corresponding sampling locations at Shearon Harris, Roanoke Rapids, and a pooled dataset of both locations. Each plot depicts summary statistics of the median, the lower and upper quartiles, and the minimum and maximum biovolume values for each corresponding depth bin.
Figure 13. Three-dimensional, perspective plots used to represent site specific biomass (Z-axis) prediction values from respective generalized additive models (GAMs).
Figure 14. Box-whisker plots of estimated biovolume over time for Plot 1 and Plot 2 at Shearon Harris. Each plot represents summary statistics of the median, the lower and upper quartiles, and the minimum and maximum biovolume values for each corresponding sample date.
Figure 15. Box-whisker plots of estimated biovolume over time for Plot 1, Plot 2, and Plot 3 at Roanoke Rapids. Each plot represents summary statistics of the median, the lower and upper quartiles, and the minimum and maximum biovolume values for each corresponding sample date.
Figure 16. Map revealing plot specific development pattern differences in biovolume (Z-axis) over time at Shearon Harris.
Figure 17. Map revealing plot specific development pattern differences in biovolume (Z-axis) over time at Roanoke Rapids. Full scans of Plot 1 and Plot 3 on sample date 9/2/15 were not possible due to topped out SAV biomass interfering with our prescribed transect.
Table 1. Biomass and hydroacoustic sampling dynamics at each study location from 6/11/15 to 10/13/15.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Biomass Samples</th>
<th>Depth (m⁻¹)</th>
<th>Seasonal Biovolume (%)</th>
<th>Observed Biomass (g dry wt•0.25m⁻³)</th>
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<tr>
<td></td>
<td></td>
<td>N</td>
<td>Mean</td>
<td>C.V.</td>
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<td>Plot 1</td>
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<tr>
<td>Plot 3</td>
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<td>64.80</td>
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Table 2. Multiple linear regression equations comprised from either both study locations or the pooled dataset. *The highest biomass prediction at depth threshold of .76 m$^{-1}$ with a spatial representation of .25 m$^3$ using site specific training data.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Mean Depth (m$^{-1}$)</th>
<th>Regression Equations</th>
<th>DF</th>
<th>Prob &gt; F</th>
<th>R$^2_{adj}$</th>
<th>Maximum Biomass Estimate (g)*</th>
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</thead>
<tbody>
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<td>(a) Shearon Harris</td>
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<td>0.001</td>
<td>0.71</td>
<td>129.5</td>
</tr>
<tr>
<td>(b) Roanoke Rapids</td>
<td>1.69</td>
<td>DRY BIOMASS = -22.67 + 140.46 (BIOVOLUME) - 14.50 (DEPTH)</td>
<td>55</td>
<td>0.001</td>
<td>0.34</td>
<td>98.66</td>
</tr>
<tr>
<td>(a+b) Interaction</td>
<td>1.91</td>
<td>DRY BIOMASS = 10.802 + 88.662 (BIOVOLUME) - 5.622 (DEPTH)</td>
<td>139</td>
<td>0.001</td>
<td>0.47</td>
<td>95.33</td>
</tr>
</tbody>
</table>
Table 3. Generalized additive model (GAM) equations comprised from either both study locations or the pooled dataset. *The highest biomass prediction at depth threshold of .76 m$^{-1}$ with a spatial representation of .25 m$^3$ using site specific training data.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Mean Depth (m$^{-1}$)</th>
<th>Regression Equations</th>
<th>$R^2_{adj}$</th>
<th>Deviance Explained</th>
<th>RMSE</th>
<th>Maximum Biomass Estimate (g)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Shearon Harris (SH)</td>
<td>2.22</td>
<td>SH DRY BIOMASS = $s_1$(SH BIOVOLUME) + $s_2$(SH DEPTH) + $t_i$(SH BIOVOLUME, SH DEPTH)</td>
<td>0.86</td>
<td>88.1%</td>
<td>129.72</td>
<td>185.0</td>
</tr>
<tr>
<td>(b) Roanoke Rapids (RR)</td>
<td>1.69</td>
<td>RR DRY BIOMASS = $s_1$(RR BIOVOLUME) + $s_2$(RR DEPTH) + $t_i$(RR BIOVOLUME, RR DEPTH)</td>
<td>0.36</td>
<td>42.7%</td>
<td>372.74</td>
<td>106.9</td>
</tr>
<tr>
<td>(a+b) POOLED</td>
<td>1.91</td>
<td>POOLED DRY BIOMASS = $s_1$(POOLED BIOVOLUME) + $s_2$(POOLED DEPTH) + $t_i$(POOLED BIOVOLUME, POOLED DEPTH)</td>
<td>0.53</td>
<td>55.0%</td>
<td>442.63</td>
<td>111.8</td>
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</tbody>
</table>
CHAPTER 3

Combining Multiple Sensors to Acquire a Comprehensive Map of Lake Vegetation
ABSTRACT

In regions subject to diverse littoral characteristics, point-intercept methods have traditionally persisted as industry standards for accessing lakewide macrophyte presence, abundance, and distribution. Although point-intercept survey methods are essential for lake managers, these surveys are habitually prone to subjectivity in vegetation richness and spatial estimation, and are exceptionally inefficient while post-processing binary survey data. Advances in hydroacoustic technology provided lake managers an ability to passively evaluate spatial elements of submersed aquatic vegetation (SAV) while conducting point-intercept surveys methods; however, acoustic approaches are unable to simultaneously access vegetation above the water column. In fall 2015 and summer 2016, a GreenSeeker™ field crop sensor, Tetracam Agricultural Digital Camera multispectral imager, and two GoPro action cameras, were mounted in an array to a watercraft equipped with a hydroacoustic unit. An independent, supplementary study, occurred to distinguish the performance of merging transects from two boat-based echosounding units in fall 2015. Video recordings and images acquired from action cameras were processed in Canopeo, an application based program which estimates fractional green canopy cover (FGCC). All data collected from these sensors were geo-synchronized to the hydroacoustic unit for correlation while post-processing. With this equipment arrangement, we documented increased performance of survey vegetation analysis, through delivering quantitative biovolume estimates, video logs of submersed and emergent species, spectral canopy characteristics, and overall lake-wide macrophyte dynamics. Our survey technique postulates fundamental evidence for mapping lakewide
SAV and shoreline vegetation for a fraction of the economic input of commercial satellite imagery, and greater objectivity than point-intercept methods alone. Implications from this study may prove extremely useful for comparing seasonal growth patterns, littoral occupancy, and herbicide treatment effects on a spatiotemporal level.

INTRODUCTION

Macrophytes have been extensively recognized for contributing to the overall net primary production of aquatic environments and lentic ecosystem quality (Likens 1975; Boston et al. 1989; Madsen and Sand-Jensen 1991; Dodson et al. 2000; Takamura et al. 2003; Brothers et al. 2013). Aquatic vegetation often host predator-prey interactions by providing essential habitat for macroinvertebrates and fish structure (Heck and Crowder 1991), as well as supporting abiotic physiological properties such as reducing shoreline erosion (Coops et al. 1996) and seasonal nutrient cycling (Twilley et al. 1985; Granéli and Solander 1988). However, exotic macrophytes may pose threats to natural lake or reservoir systems by displacing endemic aquatic species with monospecific stands of dense biomass, thus reducing communal diversity. Alien plant invasions, like those caused by the submersed non-native species, Hydrilla verticillata (L.F.) Royle, often require water resource managers to conduct lakewide surveys to support management opportunities for ecologic and economic sustainability. Results from these quantitative surveys aid in producing comprehensive littoral vegetation maps which can be useful for environmental assessment, describing spatio-
temporal trends amongst plant species, or risk evaluation of anthropogenic impacts such as eutrophication (Sheffield 1970; Madsen 1999).

Timely monitoring and efficient mapping strategies have remained fundamental for assessing the abundance of native and exotic aquatic flora (Maceina et al. 1984; Lehmann and Lachavanne 1997; Hestir et al. 2008; Husson et al. 2016). Although several macrophyte surveying techniques are well documented (Ackleson and Klemas 1987; Schneider et al. 2004; Gunn et al. 2010), in situ fixed-point (point-intercept) sampling protocols have become widely accepted and utilized for accurately describing trends in macrophyte locale, coverage, and abundance (Madsen 1999). While point-intercept methods are conceivably proficient when coupled with robust statistical procedures (Bauer 1943; Valley et al. 2005; Gotelli and Ellison 2013), the level of ecological explanation for mapping and management is directly proportional to the accuracy, extent, and time spent appraising each sampled point. Since there is often some form of subjectivity between individual surveyors while estimating plant spread or abundance, we may often find this survey method inefficient as certain areas within the waterbody may need revisited. Unfortunately, anomalies found while post-processing data often go unresolved due to the inability to reprocess field records. Therefore, the underlying effectiveness of these binary surveys often remains dependent on the complete documentation of community dynamics and appropriate classification of aquatic plant species within the field recordings by the surveyor.

Other factors which may alter the precision of point-intercept evaluations include the spatial magnitude of the hydrologic system studied, since survey points are pre-determined
over spatial scale (Madsen 1999). For example, a 500 ha\(^{-1}\) whole-lake survey lacks the same precision as a 500 m\(^2\) study plot given the identical quantity of survey points. This is due to data-free-zones between points which may not explain potentially complex ecological parameters such as community assemblages, SAV occupancy within the water column (i.e. biovolume) or unidentified plant beds lying outside surveyed points. As previously noted, solid interpolation techniques have the capability of classifying areas which have not been sampled (Valley et al. 2005), however the actual representation of multifaceted characteristics remain exclusive during rendering without concern of extrapolation. Subsequently, survey protocols which commonly implement point-intercept methods alone only represent simple classes of aquatic species; whether it be submersed, floating or emergent vegetation. Nevertheless, point-intercept field surveys remain the industry standard in stipulating appropriate aquatic plant management strategies (Parsons et al. 2001; Nelson et al. 2006; Valley et al. 2015).

To reduce labor, time, and alleviate some of the obstacles associated with in situ sampling, research has focused on advances in commercially available remote sensing (RS) technologies to offer innovative approaches for assessing aquatic vegetation. Within the last several decades, RS techniques have become well refined and highly successful in identifying spatial and temporal dynamics of aquatic species using active hydroacoustic systems and passive optical sensors (Sabol et al. 2002; Silva et al. 2008). Additionally, pairing these RS programs with a geographic information system (GIS) has proven to further enhance the power of these RS technologies (Lehmann and Lachavanne 1997; Shaw 2005).
Numerous studies have utilized boat-based acoustic equipment to increase survey efficiency with reduced economic input. Traditionally, researchers have employed commercial grade, single-beam echosounders capable of mapping and examining lakewide factors including estimations of SAV biovolume and biomass (Macéina and Shireman 1980; Thomas et al. 1990), sediment types (Lehmann et al. 1997), percent area coverage (PAC) (Haga et al. 2007; Valley and Drake 2005), and bathymetry (Reshitnyk et al. 2014). Recently, the use of commercial echosounders has become further desirable for lake managers with the advent of software systems (eg. EcoSAV) and automated cloud-based algorithms (eg. ciBioBase 5.2) to aid in post-processing of sonar logs. With the multidimensional benefits of hydroacoustic assessments, Valley et al. (2015) set to homogenize the use of over-the-counter echosounders coupled with point-intercept methodology, to effectively gauge abundance patterns and community dominance over a range of spatial scales. Although the effectiveness of their boat-based acoustic system illustrated premise for describing SAV and other hydrologic parameters adequately, free-floating and shoreline vegetation remain undescribed with this enhanced method alone. Thus, when employing commercial echosounders for lakewide surveys, lake managers must consider other factors and options to fully explicate each macrophyte species class.

Plant physiological properties offer an opportunity to conduct methodical optical RS surveys through measuring macrophyte spectral radiances. Since plant pigments chlorophyll a & b absorb electromagnetic radiation (EMR) strongly in the blue (400- 500 nm) and red (600-700 nm) regions, with low absorption in the green (500-600 nm) region and high
reflectance in the near-infrared (NIR) (700-950 nm) wavelengths due to light scattering in the spongy mesophyll cells (Cho et al. 2012), EMR ratios can summarize key reflectance features of vegetation (Silva et al. 2008). Light attenuation of open-water systems absorbs strongly in the NIR band while healthy vegetation heavily reflects NIR wavelengths. Exploiting these photosensitive characteristics provides a foundation for optical RS of aquatic vegetation to support mapping of plant beds, macrophyte distribution, and accounting for interspecific heterogeneity (Hestir et al. 2008).

Conventionally, aquatic photosynthetic studies focus on multispectral satellite sensors (eg. Landsat TM/ETM+, MODIS, and WorldView-2) or fixed-wing aircraft scanners (Alberotanza et al. 2006), to quantify and analyze aquatic environments occupied with vegetation. Assertive measurements of SAV in shallow areas, and non-submersed vegetation have been successfully performed using multispectral imagery (Ackleson and Klemas 1987; Peñuelas et al. 1997; Dogan et al. 2009; Martin et al. 2010). However, there are substantial limitations for using satellite multispectral sensors for acquisition of macrophytes, especially SAV. Open source sensors, like Landsat 7, provide the basic spectral ranges for accessing coarse population dynamics, but lack the spatial and temporal resolution (eg. ~ 30 m⁻² and ~15 days⁻¹; respectively) to accurately represent non-binary properties. Conversely, commercial optical images deliver the required spectral and spatial resolutions to discern community assemblages, but the economic hindrance associated with these images often monetarily prohibits small-scale lake managers from this application (eg. WorldView-2 image). Other downfalls which can be attributed to RS in aquatic ecosystems include: cloud
cover, turbidity, solar radiance properties (sun glint), sensor angle geometry (BRDF), and inconvenient image capture timing.

Recent popularity and innovations of unmanned aerial vehicles (UAV’s) yield another RS platform for small visible and multispectral imagers. Many of the compact multispectral cameras used on UAV’s provide superior spatial resolution (< 0.5 m\(^2\)) and temporal jurisdiction with a fraction of the economic input of a single multispectral image from a commercial airborne sensor (Baluja et al. 2012; Turner et al. 2012; Candiago et al. 2015). Though UAV’s demonstrate potential for small-scale survey applications, they likely fall short of the necessary requirements for a complete lakewide analysis (ie. limited flight time and technical labor requirements). Implementing, a small multispectral sensor designed for UAV’s could, however, provide a foundation for enhancing boat-based surveys nonetheless.

Various spectral indices have been explored to detect unique characteristics among aquatic vegetation using data measurements of electromagnetic radiation (EMR) signals which have become reflected or absorbed via an objects surface. The normalized difference vegetation index (NDVI), designed by Rouse et al. (1974), has become a popular and simple index used to describe the relationship between NIR and visible bands for summarizing the strong absorption in red due to leaf chlorophyll and the strong reflectance in the NIR due to leaf structure where, \(\text{NDVI} = \frac{(\text{NIR}-R)}{(\text{NIR}+R)}\). Researchers studying aquatic vegetation often use NDVI when discerning among vegetation classes or quantifying vegetation densities over large spatial ranges (Peñuelas et al. 1993; Cho et al. 2008; Blanco et al. 2012).
Vegetation analyses outside of aquatic ecosystem management often implement field based units which provide instantaneous measurements of NDVI for canopy development and nitrogen use efficiency. GreenSeeker®, a prevalent handheld optical sensor established for agronomic scenarios, has proven useful in providing accurate and time-efficient representations of NDVI readings of crop canopies (Verhulst and Govaerts 2010; Kim et al. 2012). Studying several cereal grain crops, Ashraf et al. (2016) used the GreenSeeker® to monitor plant stress through heterogeneous spectral characteristics. With the active light source GreenSeeker® unit, geo-synchronized NDVI values are recorded via handheld computer to stipulate global positioning system (GPS) points for plotting site specific chlorophyll content. We hypothesize that paralleling this field crop sensor to an aquatic environment could provide a means of mapping emergent aquatic vegetation on-the-fly while conducting in situ littoral surveys.

Coupling the GreenSeeker® unit with digital action cameras may additionally provide greater detail of shoreline characteristics. An additional RS advancement designed for agronomic systems might enable other imaging capabilities for determining PAC in aquatic scenarios also. The imaging application, Canopeo, is a programing system which uses an automatic color threshold (ACT) to classify fractional green canopy cover (FGCC) to provide a systemic package for analytically computing digital photo and video recordings (Patrignani and Ochsner 2015). To test the validity of this software system to discern several cereal crops from bare soil, Patrignani and Ochsner (2015) discovered ≥ 91% classification accuracy of Canopeo’s FGCC estimates. Therefore, Canopeo software should also be capable
of discriminating healthy, green floating and emergent aquatic vegetation canopy cover from water by masking non-green absorption features, especially in highly turbid conditions. Using this system with video recordings could not only offer the capacity to measure FGCC, but denote data variances while post-processing as well.

Addressing the demands associated with conventional point-intercept and RS surveys, we tested a systematic protocol to deliver a comprehensive map of lakewide vegetation through incorporating a boat-based multiple sensor array (MSA) platform. In our research, two commercially available echosounders, a multispectral imager, an agronomic optical sensor, two action cameras, and several software packages were exploited to map regions of two separate North Carolina (NC) piedmont reservoirs. The aim of this study was to determine the effectiveness of the MSA platform to: 1) passively detect submersed, emergent, and floating vegetation while conducting fixed-point sampling requisites; 2) increase in situ observation performance; and 3) offer innovative options for mapping and post-processing for aquatic ecologists and lake managers through visual and statistical descriptions of macrophyte abundance levels linked to communal and temporal dynamics. From these objectives, we outline a practice for timely data attainment while reducing input errors associated with time and labor efforts involved in lakewide collection and post-processing calculations.
MATERIALS AND METHODS

Field-based sampling occurred mid-June to late-October in 2015 and 2016 at two NC piedmont reservoirs and one mesocosm experimental station. The timing of sampling procedures aligned with other SAV survey protocols and non-SAV growth patterns from previous whole-lake investigations conducted in the southeastern United States (Harlan et al. 1985; unpublished research from NC State University). Study sites selected for sampling included sections of Shearon Harris Reservoir (SH; Wake Co.; 35°38'0"N, 78°57'18"W), Roanoke Rapids Lake (RR; Halifax Co.; 36°28'58.3"N, 77°43'38.7"W), and the NC State University mesocosm research facility (MRF; Wake Co.; 35°46'0"N, 78°38'0"W). These locations were selected to represent a wide range of environmental elements including littoral profile, vegetation class, macrophyte assemblage, and growth patterns. At both reservoir locations, a series of sampling points and transects were designed to simulate point-intercept guidelines implemented from other macrophyte surveys (Madsen 1999; Valley et al. 2015).

MSA design

From previous survey experience, a boat-based platform was created to concurrently acquire numerous layers of vegetation data though incorporating a series of echosounders, optical sensors, and digital action cameras (Figure 1). A comparison of the sensors used for measuring macrophyte canopy NDVI values is shown in Table 1. All RS apparatus positions were constructed in relation to the conventional transducer location at the stern, to deduce a baseline fixed distance of the multiple data sources during analysis. To increase swath width of a single transducer, a 3.6 m⁻¹ collapsible extension arm fitted with an inflatable outrigger
float, was fabricated to provide a second transducer mounting location for representation of SAV inhabited areas often missed with a single transducer transect. All other sensor locations were intended to capture near surface SAV in areas of the waterbody \( \leq 0.76 \text{ m}^{-1} \) depth and non-SAV along the shoreline to allow for continually representation of littoral vegetation.

Two GoPro HERO4 action cameras\(^1\) were mounted at the bow of the watercraft to determine species composition and FGCC; one which faced the shoreline and another which digitally recorded the spatial extent of the optical sensors to provide control images during post-processing. Digital recording constraints for each GoPro were 1080p video resolution set at 60 fps\(^{-1}\) to reduce edge-blur effects in video frames used for evaluation.

**Hydroacoustic sampling**

To acoustically chart SAV profiles and corresponding spatial positions, a Lowrance HDS-7 Gen2\(^2\) commercial grade echosounder, with an internal GPS capability of 5-Hz refresh rate, was complemented to a 200-kHz single-beam transducer which produced a 20\(^\circ\) beam angle at 10-15 pings s\(^{-1}\). The testing of the dual transducer design, which occurred as a separate whole-lake survey at Roanoke Rapids Lake in October 2015, required a supplementary Lowrance HDS-7 Gen2 echosounder and auxiliary transducer for the outrigged float.

Throughout the optically derived in situ testing process, single hydroacoustic scans occurred simultaneously with other RS implements to provide a baseline of georeferenced GPS locations and time stamps to be referenced during post-processing. Beginning at each linear plot, transects paralleled the shoreline extending the length of each plot (\( \bar{x} \) plot length: 144 m)
~250 m⁻¹) at a boat speed of ~1.6–3.2 km/h or less to regulate benthic interpolation of SAV abundance at each prescribed fixed-point sampling. Echosounder .sl2 sonar logs at each locale were given distinctive identifiers so future analyses could be prepared with all records saved via the chartplotter to an internal 64GB SD memory card for further data analysis and upload to ciBioBase 5.2 cloud-based software³.

**Optical sampling**

Following analogous radiometric guidelines outlined by Mueller et al. (2003) for above-water RS, littoral canopy imaging and real-time photosynthetic readings of aquatic plant species were assimilated from a multispectral TetraCam Inc. Agricultural Digital Camera⁴ (ADC) and a Trimble® GreenSeeker⁵ crop sensing system. Using both instruments, nadir measurements occurred between the hours of 10:00-14:00 EST for all sampling periods. A white Teflon calibration plate was expended with the ADC, prior to image capture and in successive 15 minute intervals throughout the sampling regime, to provide instrument normalization of sun angle geometry (BRDF) and solar flux for all recoded images (Mueller et al. 2003). Site specific recordings of sensor height, boat course, imaging time, secchi depth, and further calculation information accompanied field observations (Table 2). Spectral and GPS information from the ADC were logged to an internal 2 GB CF card for access of .RAW images and metadata during analysis. Direct NDVI and GPS field recordings from the GreenSeeker were chronicled by a Trimble® Nomad 800 series handheld computer⁵ loaded with FarmWorks™ Mobile software⁶.

**Data analysis**
Hydroacoustic .sl2 data was uploaded to the cloud-based ciBioBase 5.2 algorithm then exported as tabular ASCII-grid records for further user defined analysis. Operating comparable methods outlined by Valley et al. (2015), descriptive plots were then imported into ESRI ArcGIS 10.2.2 software for further post-processing. Using the ArcGIS spatial analyst, feature-to-raster tool, biovolume grid-point features were converted to shapefiles and used to interpolate raster grids to a 7.5 m$^2$ cell dimension. To review the effectiveness of the dual transducer system, both transducer datasets were then joined to delineate acoustically derived data at Roanoke Rapids Lake. The subsequent raster grids permitted biovolume extractions at the predefined fixed-point locations collected throughout the sampling periods. Hydroacoustic and in situ dataset attributes such as PAC, bathymetry, and biovolume expansion within study sites were then statistically associated with correlation analysis, Fisher's LSD means separation, and visual representation provided by RStudio nonparametric and ggplot2 packages (Hayfield and Racine 2008; Wickham 2009; R Core Team 2015). A false-positive limiting depth of 0.76 m$^{-1}$ was assigned as the minimum depth used for all hydroacoustic evaluations due to heavy noise and backscatter from the transducers (Duarte 1987; Valley et al. 2015). Removing hydroacoustic data from shallow regions was not designed to hinder mapping, but provide georeferenced assessment of the optical RS platforms implemented at the Shearon Harris test location.

Imagery collected from the ADC sensor was pre-processed through PixelWrench2 (PW2) software$^4$ to permit association of embedded GPS logs to .RAW image files captured, and to calculate vegetation indices such as NDVI. Using PW2, the sensor instantaneous-
field-of-view (IFOV) was computed and a supervised selection of images were exported to TIFF format for further band manipulation and image statistics. These processes included filter window analysis, digital number statistics, and radiometric analyzation using Matlab R2016b computing software for image processing and RStudio raster packages (Hijmans 2015).

Georeferenced GreenSeeker data points were downloaded and analyzed in ArcGIS to attribute NDVI readings within each individual study plot. Associated NDVI values were then referenced to the identical locales of the hydroacoustic data and ADC imagery through offset GPS and timestamp correspondence. Validation of GreenSeeker NDVI responses were then analyzed, against the ADC sensor NDVI calculations, to make accuracy estimations from both RS platforms investigated.

For pixel-wise evaluations within .MOV video logs recorded by the GoPro action cameras, the Canopeo app-based program was expended with Matlab software, to produce a classification assessment of FGCC within each distinct video frame. User defined image noise reduction parameters were set at one to vindicate green pixels for each vegetation class (including surface level SAV) in paradox of the water column reflectance attributes, to differentiate vegetation pixels from absorption features (ie. open water). Hue and saturation controls persisted at default settings since sampling times were contiguous of the other optical RS systems thus, creating a reasonable spectral based comparison (ie. image capture timing of GoPro followed RS protocols). An average FGCC was calculated for each species.
present in this study to identify if FGCC contributed to inter- or intraspecific NDVI recordings. Canopeo computed FGCC was also compared to canopy cover estimates obtained during analysis of the ADC multispectral images, which occurred separately within Matlab image analysis and segmentation toolsets.

**RESULTS AND DISCUSSION**

**Study sites and design characteristics**

Optically derived recordings, from both the Tetracam ADC multispectral imager and the GreenSeeker sensor, were collected at Roanoke Rapids Lake and Shearon Harris Reservoir in 2015 (Table 3). However, in 2016, field based testing occurred exclusively at Shearon Harris Reservoir and the NCSU MRF (Table 4). The non-boat based testing platform implemented at the NCSU MRF provided another unique macrophyte for assessment not found at either lake location. Throughout the testing periods, a total of \( N = 1,410 \) images were collected in 2015 (RR: \( n = 275 \); SH: \( n = 1,135 \)) and \( N = 624 \) (SH: \( n = 579 \); MRF: \( n = 45 \)) images were collected in 2016. GreenSeeker recordings from the 2015 trials had corrupt GPS settings, therefore individual species associations from the SH and MRF test sites were only used in this study to gauge GreenSeeker performance. At SH, a total of \( N = 1,074 \text{ m}^{-1} \) (SD= 128.2 m\(^{-1}\)) of shoreline was recorded via GreenSeeker, among five separate plots (Plot 1: 198 m\(^{-1}\); Plot 2: 175 m\(^{-1}\); Plot 3: 211 m\(^{-1}\); Plot 4: 271 m\(^{-1}\); Plot 5: 219 m\(^{-1}\)), to investigate instantaneous NDVI readings of emergent macrophytes. Action camera recordings of shoreline and surface level vegetation provided a total of \( N = 40 \text{ min}^{-1} 32 \text{ s}^{-1} \).
of video logs for correlation procedures, macrophyte reference, and estimates of FGCC during post-processing utilizing Canopeo. During the fall of 2015, a lakewide survey of SAV was performed at RR, where the dual transducer system was implemented to map 91.16 km$^{-1}$ of littoral components. Site specific hydroacoustic data was used to provide baseline GPS locations during all other boat-based MSA testing of emergent aquatic vegetation.

At Roanoke Rapids Lake, *Hydrilla verticillata* (L.F.) Royal, *Justicia americana* (L.) Vahl, and *Typha* spp. represented the macrophytes studied in pre-defined test sites. However, at Shearon Harris Reservoir, *Ludwigia hexapetela* (H.&A.) and *Nelumbo lutea* Willd. were the only species present in our test plots. To provide additional dynamic spectral components in this investigation, *Myriophyllum aquaticum* (Vell.) Verdc. was also studied at the NCSU MRF (Table 3).

**Survey equipment analysis**

*Tetracam ADC*

Three discrete locations (RR; SH; MRF) were used to establish species specific NDVI values from six macrophytes, to provide data of canopy structure and radiometric properties, and to serve as a reference NDVI for appraisal of GreenSeeker assimilated NDVI recordings. To assess spectral and spatial canopy characteristics, a sensor head height of 2.59 m$^{-1}$ and 2.74 m$^{-1}$ (RR and SH; MRF, respectfully) was implemented to acquire fine spatial resolution in test plots comprised of high variability in submersed and emergent canopy coverage (0-100%) (Table. 2). This sensor head height was used to provide sufficient canopy
exposure from the horizontal 2.1 m\(^{-1}\) swath width and account for FGCC effects (Table 1). Using a filter window representative of the GreenSeeker FOV (0.61 m\(^{-1}\)), we computed average NDVI values, acquired from \(N=45\) stratified multispectral images, which were constructed from 3-band (NIR, R, G) composites captured from the ADC imager: NDVI= \(H.\) verticillata: \((n=5, \bar{x} = -0.067\pm0.058); H.\) verticillata (Surface): \((n=5, \bar{x} = 0.294\pm0.029); N.\) lutea: \((n=5, \bar{x} = 0.318\pm0.065); L.\) hexapetela: \((n=5, \bar{x} = 0.290\pm0.128); N.\) lutea: \(+ L.\) hexapetela: \((n=5, \bar{x} = 0.217\pm0.114); M.\) aquaticum: \((n=5, \bar{x} = 0.348\pm0.023); J.\) americana: \((n=5, \bar{x} = 0.628\pm0.024); Typha\) spp.: \((n=5, \bar{x} = 0.377\pm0.056);\) Open water: \((n=5, \bar{x} = -0.190\pm0.007).\) Interspecific NDVI variation was greatest among images which comprised \(L.\) hexapetela, \(N.\) lutea, and assemblages of \(N.\) lutea with \(L.\) hexapetela. The least variability was found among NDVI values calculated from images completely delimited of open water.

NDVI values calculated among the macrophytes tested in this study indicated significant interspecific differences \((P < 0.05)\) (Figure 2). Submersed \(H.\) verticillata was found statistically nondifferentiable from NDVI records of open water; however, all other species showed significantly different \((P > 0.05)\) NDVI values when compared to NDVI values of open water (Figure 2). \(J.\) americana was the only macrophyte tested which significantly differed from all other tested species.

To establish the extent to which canopy profiles and leaf configuration contribute to processed NDVI values from the ADC images, FGCC was calculated against open water using Matlab image analysis tools. We also used these FGCC estimates to simulate the outputs which could be achieved with RGB images from Canopeo. Submersed \(H.\) verticillata
and *M. aquaticum* were found to have the lowest FGCC, with *J. americana* and *L. hexapetala* having the highest FGCC (Table 4; Figure 3). Although the surface area on a per leaf basis is highest for *N. lutea*, the structural surface area of emergent plants with many leaves in their physical form, like *J. americana*, had higher levels of FGCC. Based on these results, we determined that high average canopy closure (FGCC ≥ 50%) provides appreciably to the overall estimation of NDVI; especially without removing background effects from absorption features among the images examined (water attenuation). To illustrate the relationship between canopy coverage and NDVI, we plotted average NDVI values against FGCC percentages (Figure 4). Linear regression was used to define interspecific FGCC variability and provide a threshold for employing NDVI as a tool for identifying aquatic vegetation used in this study. After eliminating outlying values, we set a NDVI threshold of NDVI = 0.15 as the lowermost value for emergent vegetation by comparing the group mean and standard deviation ($\mu = 0.301\pm0.193$) of emergent vegetation, to the lowest confirmed NDVI value. Our NDVI threshold closely resembles the thresholds used by Fusilli et al. (2011) for identifying emergent vegetation using NDVI. Using the ADC NDVI threshold value, a FGCC standard of FGCC = 30% was deliberated. From the subset images used to determine FGCC, all emergent macrophytes met this threshold. We admit that low NDVI values obtained for submersed *H. verticillata* were likely a result of low NIR reflectance from the water column rather than FGCC.

Accounting for intraspecific variability among images containing *N. lutea*, we investigated radiometric components to determine if plant physiological properties (ie.
cuticle reflectance and leaf surface scattering) produced irregularity among NDVI values. Figure 5 exhibits a histogram of brightness levels, with corresponding NDVI, comprised from the same multi-band image samples used for FGCC analysis. Emergent macrophytes reveal a right skewed distribution with considerable quantities of data values representing dark, green vegetation (values closer to 0). However, spectral response indicated from images comprised with *N. lutea* display a bimodal distribution, with a substantial portion of pixels in both the dark and bright tonal regions (values between 100-200). Thus, calculating NDVI from images containing *N. lutea* without further radiometric correction (additionally reduced surface reflectance effects) may explain the higher image brightness levels revealed when compared to the other macrophytes tested (El Hajj et al. 2008). Higher variation among samples containing *N. lutea* may therefore be attributed to the reflectance attributes of leaf surface components and explain NDVI variability.

ADC derived NDVI values from the macrophytes appraised in this study were comparable (group $\mu = \text{NDVI} \pm 0.2$) of the average NDVI values acquired from spectroradiometers and satellite multispectral imagers from previous reports (Peñuelas et al. 1993; Ghioca-Robrecht et al. 2008; Blanco et al. 2012). We hypothesize that high interspecific variability was likely due to macrophyte canopy form and structure; as canopy closure provides surface area to intercept incident light in photosynthetic regions and decrease spectral response from the absorption features of water. We also hypothesize that sensor distance to target (resulting FGCC), is responsible for the frequently lower NDVI values obtained for the macrophytes found in our ADC NDVI calculations. Although the
areas used for examining *H. verticillata* in this field study were less turbid than most proximal waterbodies, we recognize that turbidity could have greatly reduced the accuracy (Wolter et al. 2005).

To understand if NDVI had a predictive effect on SAV biovolume recordings, we regressed biovolume against ADC observed NDVI values for open water, submerged and surface *H. verticillata*. Locally weighted scatterplot smoothing (LOESS) was used as a non-parametric regression term to predict the trend of NDVI and estimated SAV biovolume. From model selection, LOESS provided excellent agreement ($R^2=0.97$; $P>0.001$) and explanation of prediction values ($RMSE=75.32$) for quantifying submerged vegetation from low NDVI values (Figure 6). Observing greater variation in both NDVI and biovolume near the surface of the water column (100% biovolume) is likely due to light scattering based on water depth and distance to plant canopy (decrease light penetration in the R and NIR). The results of Cho et al. (2008) showed similar findings of NDVI variation to near-surface measurements of SAV.

**GreenSeeker**

We investigated NDVI responses of target macrophytes at two separate locations (SH; MRF) at a sensor head height of 91 cm$^{-1}$ and in sites which had high variability in emergent leaf coverage (0-100%) (Table. 2). The purpose of using this sensor head height was to provide adequate canopy exposure from the horizontal 0.61 m$^{-1}$ FOV (Table 1). Species specific NDVI recordings were analyzed from two target macrophytes and one community of macrophytes at SH, and one target macrophyte at MRF (Table 3). Leaf surface
NDVI readings were correlated to fixed-point sampling conditions defined prior to implementation of GreenSeeker testing, based on GPS locales, for a total of $N=767$ georeferenced data points. An average NDVI was calculated for each species examined based on explicit spectral radiance characteristics recorded from the GreenSeeker unit:

- $N. lutea$: $(\bar{x} = 0.398 \pm 0.304)$;
- $L. hexapetela$: $(\bar{x} = 0.354 \pm 0.217)$;
- $N. lutea + L. hexapetela$: $(\bar{x} = 0.364 \pm 0.320)$;
- $M. aquaticum$: $(\bar{x} = 0.4018 \pm 0.155)$;
- Open water: $(\bar{x} = -0.068 \pm 0.020)$.

Intraspecific variation was greatest among samples comprised of $N. lutea$; with the least variation of NDVI in areas exclusively open water. We recognize that high variability of $N. lutea$ NDVI recordings is likely from plant physiological properties such as a thick leaf cuticle (increased albedo), and the presence of water droplets on the leaf surface (increased surface level moisture content). Observed NDVI responses denoted no significant interspecific difference among the macrophytes tested in this study ($P < 0.05$) (Figure 7). However, all detected species showed significantly different ($P > 0.05$) NDVI values when compared to NDVI readings of open water (Figure 7).

GreenSeeker NDVI values exhibited in this study were averaged recordings over a frequency range of $f$: ~1,000 readings/s. From previous research of canopy density effects on NDVI recordings, low vegetation density was found highly correlated to lower NDVI values obtained from a satellite platform (Holben 1986). However, an assumption was made that the demand for 100% canopy closure was not required to provide satisfactory NDVI evaluations in areas with healthy vegetation for our study. Kim et al. (2012) studied GreenSeeker NDVI performance of varying leaf coverage against a wood board and reported
30-100% leaf coverage sufficient for GreenSeeker application. Nevertheless, we are unsure of the canopy density level threshold needed to meet the assumption that NDVI readings obtained from GreenSeeker would not yield Type II errors in an aquatic scenario (ie. not observing plant material in littoral areas which are vegetated with low canopy coverage when compared to the surrounding absorption features). However, we can estimate that the threshold for emergent canopy assessment would likely follow the findings from the ADC imager (FGCC ~ 30%). To confidently presume the presence of emergent vegetation, regardless of canopy form and structure, we set a NDVI threshold of NDVI = 0.20 as the lowermost value for emergent vegetation. We obtained this threshold by comparing the group mean and standard deviation ($\mu = 0.380 \pm 0.237$) of emergent vegetation to the lowest confirmed NDVI value after the removal of outlying NDVI values. Since open water has a NDVI ≤ 0, sparse vegetation may not be represented if NDVI data returned are NDVI ≤ 0.20.

GreenSeeker NDVI values in this experiment were lower than values of healthy, green vegetation commonly obtained in agronomic settings (Kim et al. 2012; Ashraf et al. 2016). Although aquatic vegetation and terrestrial plants often differ greatly in spectral characteristics, non-vegetated areas (absorption and reflection features) which render low NDVI values in an agronomic setting should resemble the absorption and reflection attributes associated with the semi-turbid waters used in this study. We hypothesize that canopy closure, leaf surface characteristics, reflectance properties of the water column (eg. BRDF, sun glint, suspended solids), and sensor specifications for NDVI calculation likely contributed to the accuracy of our GreenSeeker NDVI readings.
**Dual transducers**

A systematic hydroacoustic survey occurred among three separate littoral (AOI) areas of interest (AOI 1, AOI 2, AOI 3), within RR lake, to discern the efficiency of simultaneously recording two transducer tracks (Figure 8). Transects logged from both transducers jointly collected $N = 17,646$ data points (transducer 1: $n = 9092$; transducer 2: $n = 8554$), which subtly varied in terms of mean biovolume percentages, PAC, and bathymetry (Table 5). Distribution plots of depth and biovolume mutually display a right skew spread; with transducer 1 exhibiting higher median depth and biovolume counts than transducer 2 (Figures 9 and 10). Overall, the AOI’s used in this separate experiment indicated a trend in which transducer 1 (closest to the shoreline) consistently produced higher estimations of biovolume and PAC, while transducer 2 (furthest from the shoreline) revealed greater depth profiles. Transect data merged from transducer 1 and 2 expanded the mapping capability of littoral components among all three AOI’s, and reduced the overall variance among biovolume, depth, and PAC (Table 5). Combining the georeferenced records from both transducers also provided an average increase of ~20% in swath coverage (Table 5). An example of improved precision of littoral characteristics and area coverage from the merged track of transducer 1 and transducer 2 may be viewed in (Figure 11).

Based on the limnologic properties of RR lake, our findings indicate improved performance of littoral estimates when incorporating two transducers. Extending sonar swath provided comprehensive estimates of water column dynamics, with greater spatial detail and ultimately reducing variation among littoral parameters compared to operating one transducer.
mounted to the stern. Although we saw minute deviation in terms of depth contours and biovolume in this experiment, we hypothesize that study sites containing irregular and patchy plant distributions would benefit from the dual transducer configuration; especially in scenarios with wide or erratic littoral gradients. Duarte and Kalff (1986) discovered littoral slope could account for 72% of the observed variability in SAV biomass. Thus, general SAV detection and quantification of intermittent macrophyte populations, within heterogeneous bathymetric contours and slopes, may often be overlooked from a solitary transect when employing a single transducer.

_Dual action cameras and Canopeo_

Implementing two action cameras, during in situ testing of the MSA apparatus, assisted in defining macrophyte presence, division, and assemblage. Using timestamp metadata, these recordings enhanced post-processing efficiency by providing video observation and image correlation for each set of the RS instruments deployed. Video and still frame recordings also provided quantitative assessment of overall macrophyte dynamics; as images comprising vegetation were processed using the Canopeo application to develop estimates of FGCC. From our observations, Canopeo canopy cover evaluations were comparable to the estimates of FGCC determined from the ADC multispectral images which were computed in Matlab image analysis software (Figure 12). Variation among acquired FGCC estimates from the vertically positioned action camera (angle 2) and the ADC were resemblant, since one action camera fully encompassed the ADC FOV (Figure 12). Action camera recordings explored from the horizontal viewing position (angle 1) of \( \angle \approx 25^\circ \) recorded higher FGCC estimates.
than images captured perpendicular (angle 2) to the water’s surface (Figure 12).

Subsequently, FGCC estimations from angle 1 camera viewings were incomparable to the ADC images due to BRDF and light interception from background terrestrial plant species. Images from angle 1 consequently required extensive post-processing to mask terrestrial plants from the shoreline aquatic plants. Thus, unlike image records from a nadir viewing angle, administering horizontal video records through Canopeo was determined deficient in describing shoreline canopy cover from a boat platform. However, we hypothesize utilizing the horizontal viewing angle would be beneficial for accessing binary factors of macrophyte presence and provide additional visual aid for analysis.

Since Canopeo FGCC classification of vegetated areas occurs via user applied settings in both the desktop and smartphone applications, we caution that a moderate degree of subjectivity may be involved in determining radiometric thresholds based on visual approximations alone when accessing canopy cover. Patrignani and Ochsner (2015) revealed several recommendations concerning noise reduction (ie. hue, saturation and brightness controls) and the importance of viewing angle for the application of Canopeo in agronomic scenarios. Our findings follow analogous suggestions for aquatic situations, with further emphasis on independent validation while assigning noise reduction levels. From our observations, acquiring estimates of FGCC from images which have not had proper radiometric correction may account for severe fluctuations in Type I sampling errors (over estimation of FGCC). For example, water columns with a high level of suspended solids (causing scattering) or excessive chlorophyte-algae communities should be determined on a
discrete site-by-site basis, as these biophysical properties will alter the FGCC accuracy of Canopeo, especially if the viewing angle is off nadir. Based on our findings, the Matlab Canopeo desktop application provides the greatest applicability, since users may adjust Red/Green and Blue/Green ratios, along with noise reduction criteria which may be saved for further assessment. This constituent is not provided with the current smartphone software version. Therefore, our recommendations are to either allow Canopeo to perform an unsupervised classification of FGCC to eliminate systemic error, or mangers should develop an orderly protocol for accessing FGCC thresholds to meet sampling error assumptions.

**Management implications**

The MSA design yielded spectral canopy responses similar to NDVI values reported from previous aquatic vegetation studies (Peñuelas et al. 1993; Ghioca-Robrecht et al. 2008; Fusilli et al. 2011; Blanco et al. 2012). Operating the GreenSeeker and echosounder concurrently, provided finite mapping options for distinguishing SAV from floating or emergent macrophytes. We recognize that the NDVI values obtained from GreenSeeker recordings averaged lower estimates in our aquatic scenario than would conventionally be found in a terrestrial system. However, we conducted a binary classification assessment comparing the detection accuracy of both the ADC multispectral imager and GreenSeeker unit for the ability to identify non-submersed macrophytes. Of the observed samples, both sensors met the industry standard of 80% detection accuracy (Figure 13). From our original hypothesis, these results parallel the findings of Peñuelas et al. (1993), that dense, emergent canopies will tend to have the highest NDVI values. For inclusive littoral hydroacoustic
observation, the dual transducer arrangement delivered an expansive swath width, which protracted water column parameters through improved precision of SAV detection. Furthermore, in scenarios where biovolume is near 100%, managers employing the MSA system or a multispectral imager may still be capable of recording biovolume estimations of SAV based upon results from our regression of NDVI and *H. verticillata* biovolume. Though not pertinent in this study, pairing the results of two simultaneous sonar tracks should be particularly relevant in littoral zones comprised of inconsistent SAV distributions or irregular depth contours. Likewise, operating digital action cameras provided a resource for passively accessing shoreline characteristics, FGCC appraisals using Canopeo, and an ability to reevaluate dataset anomalies without hindrance to standard point-intercept survey strategies. An illustration of what a comprehensive boat-based MSA mapping solution might address, in combination with point-intercept sampling, may be viewed in Figure 14.

We recognize underlying limitations transpired during the development and application of our MSA design, although determined minimal in the validity of application and proof-of-concept. These limitations included: 1) testing of the dual transducer arrangement occurred nonsynchronous of the optical RS unit’s deployment; 2) manual separation of sonar tracks during post-processing of the dual transducer design required pervasive GIS and labor requirements to ensure accurate spatial representation; 3) an inability to provide definitive results of NDVI recordings using GreenSeeker during the 2015 testing period which reduced our quantity of macrophyte class criterion; 4) assessment of diurnal radiance effects on GreenSeeker recordings in an aquatic environment; and 5) EMR
band specifications used to calculate NDVI from the GreenSeeker and TetraCam ADC differed slightly (Table 1). Although presumed nominal, we recommend light attenuation research from aquatic canopy reflectance recordings be evaluated with the GreenSeeker unit in conditions of low and high solar radiance. This additional research should focus on macrophytes with similar leaf surface characteristics to *N. lutea* (ie. *Nuphar* or *Nymphoides* spp.), as there remains a demand to investigate and determine if macrophytes with dense cuticles respond similarly in NDVI variability. However, to overcome at least one of the limitations, the dual transducer system accompanied a lakewide point-intercept survey in fall 2016, at Lake Gaston Reservoir (LKG; Halifax Co., NC; 36°31′08.3″N, 77°57′47.6″W), where an add-on GPS receiver was supplemented to the design. We connected a Lowrance Point-1 Antenna² to the outrigged transducer to provide defined GPS recordings of the auxiliary transducer readings; thus, reducing post-processing issues for accurate representation of sonar characteristics. This technique supports elimination of manual correction for the auxiliary transducer transects.

Despite the identified limitations, our methodology proved useful in mutually examining and mapping littoral variations in bathymetry, SAV abundance, and non-submersed macrophyte cover. The MSA system offers a passive and repeatable option for seasonal monitoring, recognizing ecological growth patterns, and providing visual evidence for aquatic weed management applications while conducting point-intercept provisions. Water resource managers may additionally want to employ the use of a boat-based pole or tethered balloon (Shaw et al. 2012), to increase FOV when applying a digital action camera
or accessing FGCC estimates with Canopeo. Furthermore, studies testing the applicability of the MSA system in a shallow waterbody scenario, with extensive plant cover or wide spatial distribution, may discover combining multiple GreenSeeker sensor heads on an airboat provides assessment of plant bed boundaries for floating (ie. *Nymphoides* spp. spatial distribution) or shoreline emergent species. Additionally, developers of multispectral sensors may consider incorporating a blue band in addition to the NIR and red bands since blue band indices, such as the water adjusted vegetation index (WAVI), have been shown to effectively map aquatic vegetation with less absorption feature interference among sparse plant canopies (Villa et al. 2014).

Littoral mapping is crucial for risk assessment of plant invaders, aquatic ecosystem changes, and providing sound parameters for conservation management and stewardship. Our conclusions delineate the performance of the MSA system for gauging lakewide macrophyte dynamics and supports adoption of a system aquatic ecologists should utilize while conducting point-intercept sampling protocols. Expending the RS instruments defined in this report offers the ability to passively record SAV and emergent canopy characteristics through exertion of an over-the-counter commercial echosounder, and optically originated RS devices. Several key advantages of the RS units expended in our experimentation include: ease of operation, availability, and minimal instructional requirements. Accordingly, managers could train unexperienced personnel to implement this system in a brief overview. Our findings should offer noteworthy economic and labor efficiency over time, with fewer post-processing issues for evaluating littoral macrophyte dynamics (Nelson et al. 2006;
Gilmore et al. 2008). Management protocols following these findings, particularly in lakewide survey situations, should offer valuable compliments to traditional fixed-point sampling.

**SOURCES OF MATERIALS**

1 GoPro HERO4 action camera, GoPro Inc., 3000 Clearview Way, San Mateo, CA 94402.

2 Lowrance HDS-7 Gen2 chartplotter and Point-1 antenna, Navico Inc., 4500 South 129th East Avenue, Suite 200, Tulsa, OK 74134.

3 ciBioBase 5.2 cloud-based software, Contour Innovations, LLC, 1229 Tyler Street NE, Suite 120, Minneapolis, MN 55413.

4 Agricultural Digital Camera and PixelWrench2 software, TetraCam Inc., 21601 Devonshire Street #310, Chatsworth, CA 91311.

5 GreenSeeker crop sensor and Nomad handheld computer, Trimble Inc., 935 Stewart Drive, Sunnyvale, CA 94085.

6 FarmWorks Mobile software, A Division of Trimble Inc., 6795 S. State Road 1, P.O. Box 250, Hamilton, IN 46742.

7 ArcGIS 10.2.2, Environmental Systems Research Institute, 380 New York Street, Redlands, CA 92373.

8 Matlab R2016b software, The MathWorks Inc., 1 Apple Hill Drive, Natick, MA 01760.

9 Canopeo application, Oklahoma State University, Department of Plant and Soil Sciences, 374 AGH, Stillwater, OK 74078.
LITERATURE CITED


Figure 1. Boat-based multiple sensor array design with respective remote sensing implement locations. A1) Traditional transducer position at stern; A2) auxiliary transducer with outrigged float; B1) corresponding echosounder for stern transducer; B2) corresponding echosounder for secondary transducer; C1) action camera angle 1 (horizontal); C2) action camera angle 2 (vertical); D) multispectral imager; and E) GreenSeeker® sensor.
Figure 2. Box-whisker plot of corresponding NDVI values obtained from ADC multispectral images, used to distinguish if the species presented in this study were significantly different than the average NDVI value obtained for open water. Red X’s represent mean NDVI values. Samples used to derive NDVI from false color composite images for each tested constituent are depicted under the corresponding species labels.
<table>
<thead>
<tr>
<th>Species</th>
<th>Canopy Cover</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. verticillata</em></td>
<td>21.0%</td>
</tr>
<tr>
<td><em>H. verticillata</em> (surf)</td>
<td>56.4%</td>
</tr>
<tr>
<td><em>H. luzula</em></td>
<td>69.0%</td>
</tr>
<tr>
<td><em>L. hexandra</em></td>
<td>82.1%</td>
</tr>
<tr>
<td><em>N. americanum</em></td>
<td>68.2%</td>
</tr>
<tr>
<td><em>M. auriculatum</em></td>
<td>37.7%</td>
</tr>
<tr>
<td><em>L. americanus</em></td>
<td>75.7%</td>
</tr>
<tr>
<td><em>Typha spp.</em></td>
<td>53.4%</td>
</tr>
</tbody>
</table>

Figure 3. Macrophyte canopy estimates from species specific 3-band (NIR,R,G) multispectral images captured with the Tetracam ADC sensor. Canopy segmentation analysis for this set of images calculated in the Matlab imaging application to mimic the Fractional Green Canopy Cover (FGCC) the Canopeo application would produce from an RGB image.
Figure 4. The relationship between Fractional Green Canopy Cover and NDVI used to define interspecific variability and provide a threshold for employing NDVI as a tool for identifying aquatic vegetation used in this study.
Figure 5. Radiometric brightness intensity responses for each species tested from the multispectral images. The x-axis scale represents tonal regions of pure black to pure white (0-255; respectively).
Figure 6. Line of fit for a non-parametric regression smoothing term (LOESS) portraying the relationship between biovolume and NDVI values calculated from the ADC imager for submersed *H. verticillata*, surface *H. verticillata*, and open water.
Figure 7. Box-whisker plot of corresponding NDVI values obtained from GreenSeeker recordings, used to distinguish if the species presented in this study were significantly different than the average NDVI value obtained for open water. Red X’s represent mean NDVI values.
Figure 8. Map of three separate littoral (AOI) areas of interest (AOI 1, AOI 2, AOI 3), within Roanoke Rapids Lake, to discern the efficiency of simultaneously recording two transducer tracks.
Figure 9. Distribution plots of depth profiles for transducers 1 and 2 during the dual transducer trial at Roanoke Rapids Lake. The red dashed lines depict the median depth range for each transducer.
Figure 10. Distribution plots of biovolume ranges for transducers 1 and 2 during the dual transducer trial at Roanoke Rapids Lake. The red dashed lines depict the median biovolume estimates for each transducer.
Figure 11. Mapping example of improved precision of littoral characteristics, such as biovolume, and swath area coverage increase from the merged track of transducer 1 and transducer 2 at Roanoke Rapids Lake. Using the dual transducer arrangement should ultimately decreases the time spent surveying areas with wide littoral zones and reduce benthic interpolation error in those scenarios.
Figure 12. Examples of resulting images processed through the Canopeo application from two action camera still frames. Vegetation cover appears as white pixels while masking all other absorption features shown as black pixels. Angle 1: Horizontal perspective. Angle 2: Nadir viewpoint. Processed images: a) uncorrected FGCC image containing background terrestrial plant species; b) the same image masked to remove background terrestrial plants; and c) FGCC estimates representative of the complete FOV of the multispectral imager and GreenSeeker unit.
Figure 13. Column chart used to depict detection accuracy for both the GreenSeeker and Tetracam ADC multispectral imager on a species-wise classification assessment.
Figure 14. Resulting output example from the multiple sensor array boat-based platform to assess lakewide vegetation. A.) Target waterbody (Shearon Harris Reservoir); B.) Target cove to analyze during post-processing; C.) Dual transducer recordings of biovolume, PAC, and bathymetric properties; D.) Action camera observation for identification of shoreline emergent vegetation; E.) Multispectral image false-color processed to compare optically sensed NDVI recordings from both the ADC imager and GreenSeeker sensor (GreenSeeker head pictured on the bottom portion of the image appearing white). The polygon area within this image represents the FOV of the GreenSeeker; F.) Sample of canopy segmentation derived from a multispectral image of *N. lutea* and *L. peploides*. 
<table>
<thead>
<tr>
<th>Sensor</th>
<th>Sensor type</th>
<th>Spectral band ranges (nm)</th>
<th>NDVI bandwidth (nm)</th>
<th>Spatial resolution</th>
<th>Radiometric resolution (bits)</th>
<th>Temporal resolution</th>
<th>Swath width</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Landsat 8 (OLI) &amp; (TIRS)</strong></td>
<td>Passive</td>
<td>0.43 - 0.45</td>
<td></td>
<td></td>
<td>12</td>
<td>16 days</td>
<td>183 km</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.45 - 0.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.53 - 0.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.64 - 0.67</td>
<td></td>
<td>30 m</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.85 - 0.88</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Landsat 7 (ETM+)</strong></td>
<td>Passive</td>
<td>0.45 - 0.52</td>
<td></td>
<td></td>
<td>8</td>
<td>16 days</td>
<td>183 km</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.52 - 0.60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.63 - 0.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.77 - 0.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADC</td>
<td>Passive</td>
<td>0.52 - 0.57</td>
<td>0.68 - 0.70</td>
<td>~1 mm</td>
<td>8</td>
<td>User defined</td>
<td>2.1 m</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.60 - 0.69</td>
<td>0.80</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.75 - 0.92</td>
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<td></td>
</tr>
<tr>
<td>GreenSeeker</td>
<td>Active</td>
<td>0.65 - 0.67</td>
<td>0.67</td>
<td>~2 mm</td>
<td>NA</td>
<td>User defined</td>
<td>0.61 m</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.76 - 0.78</td>
<td>0.78</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*a* Not used in this study, but provides a baseline multispectral sensor comparison of VIS-NIR bands.
Table 2. Site specific recordings which accompanied field observations during remote sensing equipment testing.

<table>
<thead>
<tr>
<th>Location</th>
<th>Acquisition time (EST)</th>
<th>Heading</th>
<th>Sensor head height (m)</th>
<th>Secchi depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roanoke Rapids</td>
<td>19 October 2015 11:20-11:38</td>
<td>321º</td>
<td>ADC:2.59 GS:0.91</td>
<td>1.3</td>
</tr>
<tr>
<td>Shearon Harris</td>
<td>20 October 2015 09:29-10:14</td>
<td>319º</td>
<td>ADC:2.59 GS:0.91</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>21 October 2015 10:09-11:08</td>
<td>318º</td>
<td>ADC:2.59 GS:0.91</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>22 June 2016 12:35-13:28</td>
<td>219º</td>
<td></td>
<td>1</td>
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<tr>
<td>Mesocosm Research Facility</td>
<td>23 June 2016 11:18-11:34</td>
<td>68º</td>
<td>ADC:2.74 GS:0.91</td>
<td>NA</td>
</tr>
</tbody>
</table>
Table 3. Outline of macrophytes studied; includes sensor used for each species and the respective location of the sensor trial.

<table>
<thead>
<tr>
<th>Species</th>
<th>Class</th>
<th>Status&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sensor&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Location&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. verticillata</em></td>
<td>Submersed</td>
<td>Exotic</td>
<td>ADC/DT</td>
<td>RR</td>
</tr>
<tr>
<td><em>M. aquaticum</em></td>
<td>Submersed/Emergent</td>
<td>Exotic</td>
<td>ADC/GS</td>
<td>MRF</td>
</tr>
<tr>
<td><em>N. lutea</em></td>
<td>Floating</td>
<td>Native</td>
<td>ADC/GS</td>
<td>SH</td>
</tr>
<tr>
<td><em>L. hexapetala</em></td>
<td>Floating/Emergent</td>
<td>Exotic</td>
<td>ADC/GS</td>
<td>SH</td>
</tr>
<tr>
<td><em>J. americana</em></td>
<td>Emergent</td>
<td>Native</td>
<td>ADC</td>
<td>RR</td>
</tr>
<tr>
<td><em>Typha spp.</em></td>
<td>Emergent</td>
<td>Native</td>
<td>ADC</td>
<td>RR</td>
</tr>
</tbody>
</table>

<sup>a</sup> Environmental status of aquatic species in the southeastern United States.

<sup>b</sup> Sensor used to study spectral and spatial components (ADC: Agricultural Digital Camera; GS: GreenSeeker; DT: Dual Transducer).

<sup>c</sup> Study sites used for each species listed (RR: Roanoke Rapids Lake; SH: Shearon Harris Reservoir; MRF: NC State Mesocosm Facility).
Table 4. Plant canopy structure separation analysis derived from ADC images using a filter window representing the field of view (FOV) of GreenSeeker. White pixels represent healthy green vegetation; black pixels define all other absorption features.

<table>
<thead>
<tr>
<th>Species</th>
<th>White Pixel</th>
<th>Black Pixel</th>
<th>Total</th>
<th>FGCC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>W:B Ratio&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. verticillata</em></td>
<td>12208</td>
<td>562392</td>
<td>574600</td>
<td>0.021</td>
<td>0.02:1</td>
</tr>
<tr>
<td><em>H. verticillata</em> (Surface)</td>
<td>324255</td>
<td>250345</td>
<td>574600</td>
<td>0.564</td>
<td>1.30:1</td>
</tr>
<tr>
<td><em>N. lutea</em></td>
<td>396203</td>
<td>178397</td>
<td>574600</td>
<td>0.690</td>
<td>2.22:1</td>
</tr>
<tr>
<td><em>N. lutea + L. hexapetela</em></td>
<td>391664</td>
<td>182936</td>
<td>574600</td>
<td>0.682</td>
<td>2.14:1</td>
</tr>
<tr>
<td><em>L. hexapetela</em></td>
<td>471903</td>
<td>102697</td>
<td>574600</td>
<td>0.821</td>
<td>4.60:1</td>
</tr>
<tr>
<td><em>M. aquaticum</em></td>
<td>181935</td>
<td>392665</td>
<td>574600</td>
<td>0.317</td>
<td>0.46:1</td>
</tr>
<tr>
<td><em>J. americana</em></td>
<td>434811</td>
<td>139789</td>
<td>574600</td>
<td>0.757</td>
<td>3.11:1</td>
</tr>
<tr>
<td><em>Typha spp.</em></td>
<td>307016</td>
<td>267584</td>
<td>574600</td>
<td>0.534</td>
<td>1.15:1</td>
</tr>
</tbody>
</table>

<sup>a</sup> FGCC = Fractional Green Canopy Cover.

<sup>b</sup> Calculated by dividing white from black pixels to convey canopy coverage likelihood.
Table 5. Dual transducer summary statistics for comparison of select area interactions of a whole-lake, hydroacoustic survey at Roanoke Rapids Lake fall 2015.

<table>
<thead>
<tr>
<th>AOI</th>
<th>Transducer</th>
<th>Biovolume (%)</th>
<th>Depth (m$^{-1}$)</th>
<th>PAC (%)</th>
<th>Area (ha$^{-1}$)</th>
<th>Swath Coverage</th>
<th>Distance (km$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
<td>Total</td>
<td>Total</td>
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<td>1</td>
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<td>0.500</td>
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<td></td>
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<td>0.237</td>
<td>3.156</td>
<td>1.002</td>
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<td>13.31</td>
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<tr>
<td></td>
<td>M</td>
<td>0.174</td>
<td>0.259</td>
<td>3.151</td>
<td>0.906</td>
<td>0.403</td>
<td>17.28</td>
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<tr>
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<td>1.761</td>
<td>0.614</td>
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<tr>
<td></td>
<td>2</td>
<td>0.175</td>
<td>0.216</td>
<td>2.738</td>
<td>2.058</td>
<td>0.550</td>
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<td>0.211</td>
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<tr>
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<td></td>
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<td>0.224</td>
<td>4.258</td>
<td>2.574</td>
<td>0.477</td>
<td>17.10</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>0.218</td>
<td>0.275</td>
<td>4.142</td>
<td>2.474</td>
<td>0.504</td>
<td>20.14</td>
</tr>
</tbody>
</table>

*a Used to determine increased swath area by comparing the area total from transducer 1 to the merged track area total.*
CHAPTER 4

Submersed Plant Population Dynamics Over Time at Roanoke Rapids Lake, NC
ABSTRACT

Constructed in 1955 for hydroelectric power, Roanoke Rapids Lake is the smallest of three successive reservoirs created mid-century along the Roanoke River. This waterbody has become part of a diverse and ecologically critical system for a multitude of aquatic flora and fauna, most notably offering spawning opportunity for striped bass (*Morone saxatilis*) and American eel (*Anguilla rostrata*). Roanoke Rapids Lake additionally serves utilitarian purpose by providing power to surrounding basin districts, real estate, and recreation for water sport enthusiast and outdoorsmen. Historical accounts depict the lake for hosting numerous native and exotic macrophytes and in 2006, Roanoke Rapids Lake was placed on the 303(d) List of Impaired Waters (*Clean Water Act*) concerning an incursion of aquatic weeds. To evaluate submersed aquatic vegetation (SAV) transformations over time, we conducted four whole-lake point-intercept surveys between 1999-2015. Two exotic species, *Hydrilla verticillata* and *Myriophyllum spicatum*, persisted as the most dominant species within the waterbody. Furthermore, exotic SAV had the greatest incidence of detection, and the highest observed frequency of co-occurrence. Between sampling years 1999 and 2012, species detections and community richness significantly changed with the introduction of *Cabomba caroliniana*, a native SAV species. Since detection, *C. caroliniana* has remained the most frequently detected native species in monoculture, and has co-occurred with greater incidence than other natives. We discovered exotic SAV co-occurred with other exotic species more often than with native species, and with exclusion to *H. verticillata*, both native and exotic submersed species co-occurred more often than in monoculture. Nevertheless, we
established that an increase in exotic species richness decreased the richness of native SAV communities. Furthermore, with exception of *M. spicatum* native species occurred at greater depths than exotic SAV. We suggest future interactions and competition among spatial ranges will be determined by the competiveness of *C. caroliniana*, and the persistence of *H. verticillata* and *M. spicatum*. Lacking a current management regime, we project littoral occupancy will remain above 70% in the future.

**INTRODUCTION**

Macrophytes serve as fundamental trophic indicators of environmental quality and provide critical ecological niches among lentic, wetland, and riverine systems (Ackleson and Klemas 1987; Melzer 1999; Cho et al. 2012; USEPA 2012). Submersed aquatic vegetation (SAV) often aid in lakewide improvement and sustainability by reducing shoreline erosion and stabilizing sediment loads for improved water clarity (Carpenter and Lodge 1986; Madsen et al. 2001), serving as eutrophic sinks (Kufel and Kufel 2002; Gao et al. 2009), operating as constituents of seasonal nutrient cycling (Twilley et al. 1985; Granéli and Solander 1988), and constructively amending fluvial patterns (Dodds and Biggs 2002). As primary producers (Likens 1975; Duarte 1987), SAV simultaneously provide essential architecture for macroinvertebrates, non-pelagic fish species, and waterfowl (Heck and Crowder 1991; Petr 2000; Schmieder et al. 2006), while also contributing to the aquatic food web (Hanson and Butler 1994; Havens et al. 1996). However, the absence of SAV can reduce the formerly described environmental assets and decrease sport fish opportunities (Ploskey
Communal assemblages of SAV frequently denote biological diversity through support of the multifaceted functions involved in aquatic habitat and ecosystem productivity (Hough et al. 1989; Egerton 2004; Capers et al. 2007; Brothers et al. 2013; Simon et al. 2015). Conversely, a lack of heterogeneity amid community assemblages, along with low species richness, has been recognized for reducing native biodiversity (Kovalenko and Dibble 2014). Often, this results as an adverse ecologic response commonly attributed to waterbodies dominated with exotic, invasive SAV species.

Nonindigenous, invasive species such as *Hydrilla verticillata* (L.F.) Royle and *Myriophyllum spicatum* (L.) impede native macrophyte conservancy (Langeland 1996; Eiswerth et al. 2000) establishing dense, monocultures which increase resource competition and form impenetrable canopies blocking sunlight (Smart et al. 1994; Spencer and Ksander 2000). Exotic SAV have been linked to displacement of native plants (Madsen et al. 1991; Valley et al. 2004; True-Meadows et al. 2016), and creating terminal illness for fauna in some instances (Wilde et al. 2005). Moreover, these non-native inhabitants impede recreational activities and power generation (Langeland 1996) which concurrently creates financial issues for stakeholders.

Within littoral areas, SAV occurrence, distribution, and community assemblage persist upon an assortment of physiological processes and abiotic parameters (Lehmann et al. 1997; Santos et al. 2011). Numerous studies have acknowledged various limiting factors affecting submersed macrophyte development and zonation, such as littoral slope and bathymetry (Daurte and Kalff 1986; Sousa et al. 2011), nutrient availability (*P* sources and...

Monitoring and mapping strategies remain fundamental for assessing native and exotic SAV occurrence, distribution, and richness within a waterway (Maceina et al. 1984; Lehmann and Lachavanne 1997; Madsen 1999; Hestir et al. 2008; Husson et al. 2016). The development and application of spatially driven systems (ie. GPS; GIS; remote sensing) (Peñuelas et al. 1993; Cho et al. 2012), robust interpolation procedures, such as kernel density estimators (Brunsdon 1995; Santos et al. 2011), mapping and modeling techniques (Lehmann 1998; Valley et al. 2004), and decision trees (Hestir et al. 2012; Liang et al. 2017), further implicates the effectiveness of lakewide evaluations. However, few quantitative assessments have been published observing submersed plant dynamics at Roanoke Rapids Lake. Therefore, many SAV expanses remain unknown and lack appraisal of per annum or decadal alteration among submersed macrophyte species.

Roanoke Rapids Lake was placed on the 303(d) List of Impaired Waters (Clean Water Act) in 2006 concerning an incursion of aquatic weeds (NCDWQ 2006). Waterbodies included in this list “…do not meet water quality use criteria, such as water supply, fishing, or propagation of aquatic life…”; a standard governed by the Environmental Protection
Agency. Traditionally, aquatic weed measures at the lake have been limited due to regulation by utility administration (Dominion 2010, 2015), fiscal prominence, and the economic breadth required for performing holistic management (Walton 2010). Commercial and residential herbicide treatments have occurred around docks and boat slips (unpublished data from NCSU); however, due to an intricate littoral zone, these treatments marginally represent lakewide management and supervision.

To evaluate SAV dynamics over time, we collated and analyzed four whole-lake point-intercept surveys between 1999-2015 to determine temporal SAV population transformations. Our objectives were to investigate: 1) species-specific variations in occurrence, abundance, dominance, and spatial distributions amongst survey years; 2) community richness, and co-occurrence; and 3) interspecific dynamics over time.

MATERIALS AND METHODS

Study site characteristics

Roanoke Rapids Lake (RRL; North Carolina; 36°28'58.3"N, 77°43'38.7"W) was constructed in 1955 for hydroelectric power and is the smallest (area: 1,737 ha\(^{-1}\); shoreline: 75 km\(^{-1}\)) of three consecutive reservoirs created mid-century along the Roanoke River chain (John H. Kerr, Lake Gaston, and Roanoke Rapids Lake; west to east respectively). The Roanoke River, which forms at the confluence of the Staunton and Dan rivers in southern Virginia (VA) connects the eastern face of the Appalachian Mountain range, to the Albemarle Sound of North Carolina (NC) (Figure 1.). As the final impoundment prior to the
estuarine Albemarle Sound, RRL has become part of a diverse and ecologically critical system for a multitude of aquatic flora and fauna (Meitzen 2016); most notably providing spawning opportunity for indigenous ray-finned species, such as striped bass (*Morone saxatilis*) and American eel (*Anguilla rostrata*) (Olsen and Rulifson 1992; Nelson 1998; USFWS 2015). In addition, RRL serves for power generation, recreational opportunities, and residential development.

Managed by Dominion Energy®, water levels frequently fluctuate from upstream dam release, satisfying power generation requirements and hydrologic administration from flood control efforts during precipitation events (USACEW 2015). Retention time at RRL is estimated 2-14 d⁻¹ (NCDENR 2000; Miller 2017). Thus, variation in lakewide water volumes likely contribute to the complex littoral composition effects produced from recurrent shoreline alterations (Madsen et al. 2001; Bornette and Puijalon 2011). A series of littoral nutrient samplings have classified the lake as maintaining moderately biologically productive (i.e. mesotrophic to meso-eutrophic status) over the last three decades (Walton 2010). A water quality report by NCDWQ (2006), documented average summer chlorophyll *a* concentrations at 6.0 ug/L⁻¹, and photic zone estimates ~3 m⁻¹ (turbidity); further confirming the previously recognized trophic assessments. While somewhat residentially developed, RRL rests as the primary drainage for several adjacent agricultural communities within the Roanoke River basin, though anthropogenic impacts have not been recorded to date.

**Point-intercept surveys**
Fixed-point sampling protocols were designed to align with seasonal SAV growth patterns noted from previous whole-lake investigations conducted in NC waterbodies (Harlan et al. 1985; unpublished research from NC State University). Following analogous protocols described by Madsen (1999), a series of point-intercept surveys were conducted in the spring of 1999, and fall of 2012, 2014, and 2015, to determine regional and temporal changes in SAV dynamics. Using a double-sided throw rake, points were sampled at ~90-500 m$^{-1}$ intervals transecting perpendicularly to the shoreline and Roanoke River. Two rake tosses occurred per predefined sampling point, one to either side of the boat, to ensure reliability in appraisal and coverage of each position sampled. Due to a complex littoral profile, points were additionally sampled in the center section of the waterbody. Field recordings from each survey followed a binary ranking system, where littoral plant presence or absence at each discrete point was assigned either a 0 (SAV not present) or a 1 (SAV present). Wherever plants were detected, records designated individual taxa occurrence and co-occurrence.

**Hydroacoustic surveys**

To passively record littoral features in survey years 2012 and 2015, a Lowrance HDS-7 Gen2$^1$ consumer grade echosounder, with an internal GPS capability of 5-Hz refresh rate, complemented a 200-kHz single frequency transducer with a 20° beam angle at 10-15 pings s$^{-1}$. Hydroacoustic transects between survey points were conducted at a boat speed of ~8 km/h to develop accurate benthic area interpolation, SAV biovolume percentages (ie. water column occupancy), and depth parameters. Logged .sl2 sonar files were given unique identifiers so future spatial correlations might be prepared based on point-intercept locales.
Hydroacoustic records were saved via the chartplotter to an internal 16GB SD memory card and uploaded to ciBioBase 5.2 cloud-based software\(^2\) for additional analysis. Processed hydroacoustic data, was expended for development of lakewide bathymetric and littoral profiles, which supplemented species-wise depth considerations defined during analysis (Figure 2).

**Data analyses**

*Survey records*

Percent occurrence and co-occurrence of each SAV species was analyzed from point-intercept data devising species-wise detections by the representative number of spatial locations visited among each respective survey year (Madsen 1999). Co-occurrence evaluations were designed to signify two-way assemblages among native and exotic macrophytes to provide evidence of species-by-species interactions (ie. frequency to mutually occur). Submersed species dominance was additionally explored to detect the time-based influence of native and exotic change in lakewide prevalence. Modifying the dominance index described by Valley et al. (2015), we defined dominance as:

Equation [1]:

\[
\text{Dominance per Survey} = \frac{\text{Sum of Species}(X) \text{ Occurrence}}{\text{Total Lakewide SAV Detected}}
\]

Furthermore, we assessed community richness based on depth parameters and frequency of community occurrence by species using RStudio nonparametric and ggplot2 packages (Hayfield and Racine 2008; R Core Team 2015; Wickham 2009). Individual species
monocultures were similarly gauged to determine average depth detection within and among survey years.

Post-processed hydroacoustic records downloaded from ciBioBase, were exported as tabular ASCII-grid files to produce lakewide depth profiles implemented during species distribution and persistence analysis. Following procedures by Valley et al. (2015), spatially referenced depth recordings were imported into ESRI ArcGIS 10.2.2\textsuperscript{3} software for analysis of lake bathymetry. Using the ArcGIS spatial analyst Inverse Distance Weighted (IDW) tool, depth grid-point features were transformed to raster grids of 18.6 m\textsuperscript{2} cell size to estimate depth regions not recorded by the echosounder. Geoprocessing environment settings limited the raster extent to the waterbody and Roanoke River boundaries during interpolation. The subsequent raster grid permitted species-wise depth extraction at each respective point-intercept feature visited among sampling years. Merged depth contours and point attributes were expended to develop associations of seasonal or temporal assessment of SAV richness, and depth distribution within the water column. A littoral depth threshold of 4.7 m\textsuperscript{1} was defined as the maximum extent each species would be masked to; a limit based on previously reported studies (Table 1).

_Calculating point dependence and spatial distribution_

Following procedures described by Santos et al. (2011), we examined SAV spatial range and temporal persistence among survey years by matching georeferenced point-intercept positions. Prior to assessing SAV spatial density and distribution, we separately
tested our dataset, by sampling year, for spatial autocorrelation using Moran’s (I) index to ensure assessments of SAV occurrences met the assumptions of independent observations (Moran 1950; Gibson 2014). Although spatial autocorrelation most often affects stream and river systems from regime alteration or minimal distance separation among survey data points (Ver Hoef et al. 2006; Dormann 2007; Detenbeck et al. 2016), we tested RRL point-intercept locations for spatial autocorrelation due to the intricate, riverine like littoral zone and adjacent point-by-point proximity.

Implementing the Spatial Autocorrelation (Global Moran’s I) tool from the ArcGIS spatial statistics toolbox, we calculated spatial autocorrelation among all four survey periods. This tool provides a statistical measure of point-feature spatial randomness, within each dataset, to determine neighboring point location effects (ie. test of independence). Results provide a z-score, p-value of autocorrelation significance, and Moran’s (I) coefficient. Spatial patterns, measured on a scale of -1 (point dispersion) to 1 (point clustering), represent positive and negative autocorrelation, while index values of 0 lack spatial autocorrelation (ie. random pattern) (Mitchell 2005; Gibson 2014). Autocorrelation results from our study may be viewed in Table 2. Survey years 1999 and 2015 lacked spatial autocorrelation when point locations were co-located (although resulting in a significant loss of point data in 1999); therefore, we accepted the null hypothesis that the points visited in these years exhibited a random distribution from independent observations. Survey years 2012 and 2014, were deficient in sampling size when data points were omitted to decrease spatial autocorrelation.
Estimation of macrophyte zonal densities and distribution have been successfully documented when applying kernel density functions (Göktoğan et al. 2010; Santos et al. 2011; Compton et al. 2012). Kernel density estimators are a robust, non-parametric model that interpolate locally weighted parameters using a smoothing function around each input point which, reflect regions concentrated around the corresponding input point observation (Silverman 1986). Kernel density estimations occurred exclusively among years 1999 and 2015 to evaluate the binary probability of spatiotemporal variances amid SAV distribution, community richness, and coverage. Utilizing the ArcGIS spatial analyst, Kernel Density tool, we estimated point-based littoral SAV coverage among each species-wise occurrence and pooled all occurrences to develop a total SAV coverage map among respective survey years. Estimate constraints were bound to a 250 m\(^{-1}\) search radius and masked by the lake boundary and Roanoke River, to produce a density distribution raster with a 7.5 m\(^2\) output grid cells. Resulting kernel records were output to Hijmans (2015) RStudio raster package for mapping options. Classification statistics were subsequently used to calculate areas occupied with submersed vegetation through summation of positive, non-zero pixel counts. Species specific littoral coverage estimates were additionally evaluated from the resulting estimated areas. To analyze seasonal and temporal spatial overlap among each representative macrophyte, the ArcGIS spatial analyst, Raster Calculator was used to determine communal density richness. Moreover, we computed the spatial overlap and temporal area persistence of monospecific SAV stands using equivalent methods.
RESULTS AND DISCUSSION

Submersed macrophytes were point sampled at \( N = 812 \) locations in spring of 1999. Fall sampling occurred at \( N = 263 \) locations in 2012, \( N = 123 \) locations in 2014, and \( N = 370 \) locations in 2015. Among sampling years 1999 and 2012, species detections and community richness critically transformed with the introduction of *Cabomba caroliniana* A. Gray; a native, submersed plant species.

**SAV detection, occurrence, and co-occurrence**

Within each sampling year, *H. verticillata* had the greatest incidence of detection, with 60-72% recognition among spatial locations visited (Table 3), and the highest observed frequency in monoculture at 28-52% occurrence (Table 4). *M. spicatum* had the second highest record of detection among years 1999, 2012, and 2015; while *C. caroliniana* had the second greatest frequency of detection in 2014 (+2% over *M. spicatum* in 2014) (Table 3). Among invaded systems containing *H. verticillata* and/or *M. spicatum*, researchers have reported similar trends of exotic SAV frequency (Lillie 1996; Rybicki et al. 2007). Excluding year 1999, *C. caroliniana* was the most frequent native species detected in monoculture within each survey year (Table 4). Furthermore, the unrooted native *C. demersum*, had more detections per survey than the exotic species *N. minor*, except for in 2015 (<1% difference) (Table 3). *C. demersum* also occurred in monoculture more often than *N. minor* (Table 4). Detections of the macroalgal *Chara* spp. reduced over time (Table 3), and was never observed in monoculture after the 1999 survey (Table 4). Throughout the study, *Potamogeton* spp. was the least detected submersed species; comprising ≤ 1% of total
detected detections per appraisal (Table 3) and was never discovered in monoculture (Table 4). However, this was not an unexpected encounter; as previous research has suggested areas established with *H. verticillata* often reduce populations of *Potamogeton* spp. (Spenser and Ksander 2000; Meadows and Richardson 2012).

Frequency of species co-occurrence was greatest in 2015 (Table 4); although average community richness was highest in 2012 (1999: $\mu=1.48 \pm 0.66$; 2012: $\mu=1.87 \pm 0.80$; 2014: $\mu=1.60 \pm 0.81$; and 2015: $\mu=1.60 \pm 0.81$). Nevertheless, maximum species richness occurred in 2015 (community of five plant taxa), though just appearing once (Figure 3). In general, exotic SAV co-occurred with other exotic species more frequently than with native species which parallels the findings of Santos et al. (2011) that an increase in exotic species richness decreases the richness of native SAV communities. With exception of survey year 2014, *H. verticillata* most consistently co-occurred with *M. spicatum* (24-40% of total occurrence) (Table 4), whereas during the 2014 survey, *H. verticillata* more frequently co-occurred with *C. caroliniana* (21% of total occurrence) (Table 4). Native species co-occurrence was greatest among communities inhabited with *C. caroliniana* and *C. demersum* (1-8% of total occurrence), though both these native species co-occurred with exotic SAV with greater regularity (Table 4). With exclusion to *H. verticillata*, both native and exotic submersed species co-occurred more often than in monoculture.

We assessed within and between seasonal dominance of each submersed species to identify the pairwise influence on occurrence and co-occurrence (Table 5). Across survey years, *H. verticillata* was the dominant SAV species ($\geq 61\%$) with *M. spicatum* as the
subsequent dominate species (≥ 25%), with exception of 2014 when *C. caroliniana* was determined the second most dominant (39%). *C. caroliniana* was the dominant (15-39%) native species after 1999 with *Potamogeton* spp. found least dominant of all species identified (Table 5). Across study years, dominance was highest in 2015, with the least seasonal dominance detected in 2012 (Table 5). Dominance records parallel patterns of seasonal occurrence and co-occurrences findings (Table 4), suggesting that species which dominate lakewide SAV detections are more likely to co-occur at greater frequency than non-dominant species (eg. co-occurrence with *N. minor* or *Chara* spp.).

**Water column zonation**

We also analyzed the influence of species richness against average depth profiles (Figure 3). Average species occurrence and co-occurrence among depth gradients was significantly different (P < 0.05) between survey years according to Kruskal-Wallis non-parametric testing (1999: µ=1.45 ± 1.24; 2012: µ=1.50 ± 1.30; 2014: µ=1.66 ± 1.28; and 2015: µ=1.33 ± 1.05). However, monocultures of native species occurred at greater depths than exotic SAV with exception of *M. spicatum*, which was the deepest inhabited macrophyte on average during the 1999 and 2015 surveys (Figure 3). Apart from 2014, *M. spicatum* occurred at greater depths than the dominant species, *H. verticillata*, which in general occupied shallower depths than all other species when in monoculture. Previous lakewide surveys also indicate *M. spicatum* occurrence frequency to increase with depth (Lillie 1996; Wagner and Falter 2002). Further, *H. verticillata* is archived to most commonly colonize shallow littoral regions with the densest stands initially, rather than zones of greater
depth to optimize competitiveness with native species (Smart et al. 1994; Langeland 1996). Apart from *C. caroliniana*, SAV found in a community richness of four developed in depths greater than or equal to SAV established in a multispecies richness of two or three (Figure 3). For both native and exotic species, a species richness of two or three resulted in higher variability in depth occurrence among all four surveys. Consequently, variability among community zonation and frequency of colonization, is dependent upon the species composition within the community, rather than the number of species comprising the community (Chambers and Kalff 1985). Comparable outcomes were attained when we pooled all four datasets by average depth occurrence in monoculture (*Potamogeton* spp. not found in monoculture): [C. caroliniana > C. dermersum > M. spicatum > Chara spp. > H. verticillata > N. minor; deeper to shallower respectively].

**SAV spatial coverage**

Kernel-based spatial interpolations were higher in 2015 among lakewide macrophyte distributions (2015 total SAV: 760.02 ha\(^{-1}\)) and littoral percent area covered (PAC) (2015 total SAV: 71%) than in 1999 when compared to the maximum littoral occupancy threshold of 1067.61 ha\(^{-1}\) (100% theoretical occupancy) (Table 6). *H. verticillata* had the greatest distribution and expanse (PAC: 60-68%, 1999 and 2015 respectively) (Table 6; Figure 6). *M. spicatum*, the subsequent leading coverage species, comprised around 50% less of the littoral zone than *H. verticillata*; with a patchy spatial distribution (Figures 4 and 5). When in monoculture, *H. verticillata* and *M. spicatum* persisted within spatial locations at a higher rate of coverage (2-10 times greater) than compared to native species (Table 7; Figures 4 and
5). *Chara* spp. was not found to persist among the same spatial locations over time (Table 7), although proximity within the lake remained consistent (Figures 4 and 5). *C. caroliniana* co-occurred spatially at similar rates to *M. spicatum* in 2015 ($\leq 2$ ha$^{-1}$ area difference), although the greatest co-occurrence overlap was among communities containing *H. verticillata* or *M. spicatum* (Table 7). A reduction in overlap was observed among compositions of *M. spicatum* and *C. demersum* (Table 7). However, the highest overlap of native communities occurred between *C. caroliniana* and *C. demersum* in 2015. Excluding *Potamogeton* spp., communities exclusively composed with exotic species covered greater expanses than native species communities ($\sim 45$ ha$^{-1}$ difference). Conversely, communities composed of both native and exotic species had greater coverage than exotic communities in 2015 than in 1999 ($\sim 4$ ha$^{-1}$ larger). Temporally, spatial coverage among native species was greatest when co-occurring with exotic species (Table 7).

Density estimations indicate native and exotic SAV distributions vary greatly over space and time (Figures 4 and 5). Temporal cover alterations were primarily observed among *M. spicatum* and *N. minor*, as *M. spicatum* shifted littoral density to the center and northwestern portion of the lake, while *N. minor* distributed widely throughout the waterbody. Although not present in 1999, the highest density of *C. caroliniana* was found at locations not heavily occupied with *H. verticillata* or *M. spicatum* (Figure 6), a pattern not seen among other native species documented in 2015 (Figures 4 and 5). Verification of this inverse relationship was observed with *Chara* spp., as the greatest density of *Chara* spp. persisted in the northwestern portion of the waterbody which was highly invaded with exotic
macrophytes throughout this study (Figures 4 and 5). Overall, macrophyte density was greatest along shoreline coves, rather than within the center of the waterbody where littoral regions receive the greatest fetch and exposure from high variation in water levels (Madsen et al. 1996).

**Whole-lake response to native introduction and exotic persistence**

Deviations in lakewide patterns among native and exotic SAV directly correlate with the introduction of *C. caroliniana*. Notable shifts include: 1) *C. demersum* no longer the dominate native species, 2) decrease in co-occurrence among assemblages of *C. demersum* and *M. spicatum*, 3) *N. minor* decline in richness until 2015, 4) reduction of *Chara* spp. abundance, and 5) with exclusion to 2014, *H. verticillata* progressively increasing in spatial coverage. No significant difference was detected with *Potamogeton* spp. occurrence since 1999.

In general, *H. verticillata* and *M. spicatum* persist as the most dominant species within RRL over time. However, since earliest detection, *C. caroliniana* has remained the most frequently detected native species in monoculture, and co-occurred with greater incidence than other natives (Table 4). Furthermore, the expansion and competiveness of *C. caroliniana* may be associated to the temporal reduction in *M. spicatum* monoculture occurrence and dominance, as *C. caroliniana* had higher co-occurrence with *H. verticillata* in 2014 (Table 2). However, we recognize that a reduced sampling size in 2014 may contribute to some of the anomalies among dominance and co-occurrence values between *H. verticillata*, *M. spicatum* and *C. caroliniana*. Spatially however, *C. caroliniana* remains a
noteworthy competitor with exotic SAV, particularly, *M. spicatum* (Tables 6 and 7). *C. caroliniana* occurrence and spatial co-inhabitation with *M. spicatum* is positively attributed to an increase in depth zonation (Figure 3). Therefore, an increase among community stands of *C. caroliniana* and *M. spicatum* might highly correlate to the reduction of co-occurrence among *C. demersum* and *M. spicatum*, and an increase of *C. demersum* to deeper regions when in monoculture (Figure 3). Furthermore, since *C. demersum* is an un-rooted macrophyte, high-biomass yielding stands of *C. caroliniana*, *H. verticillata*, and *M. spicatum* likely displace monocultures of *C. demersum* to greater depths (Figure 3), although abundance of *C. demersum* has remained rather constant regardless of spatial location.

Though not considered an invasive species to NC, Schooler et al. (2009) reports *C. caroliniana* often displaces less vigorous SAV, which could explain the decline of *Chara* spp. and *N. minor* abundance since 1999. Although, *N. minor* spatial displacement is likely indirectly attributed to an increase of *C. caroliniana* monocultures, an increase in co-occurrence among *C. demersum* and *M. spicatum* is a likely culprit. Nevertheless, *N. minor* has begun to rebound in areas absent of *C. caroliniana* (Figure 5). However, scarcity of *Potamogeton* spp. is probably not correlated with the introduction of *C. caroliniana* since detection has remained low from the initial 1999 survey. Spencer and Rejmánek (1989), suggest that areas occupied with *H. verticillata* probably outcompete *Potamogeton* spp. once overwintering, benthic tuber-banks are established. Therefore, *Potamogeton* spp. has most likely remained in competition since *H. verticillata* was first introduced sometime during the 1980’s. We hypothesize, based on the lack of *H. verticillata* management at RRL, that *H.
*verticillata* is increasing lakewide tuber-banks which characterizes the continual expanse and co-inhabitance of *H. verticillata* within the waterbody.

**CONCLUSIONS**

Submersed macrophyte communities are highly dynamic structures which do not remain stable within the confines of one or two ecological parameters (Madsen et al. 1991). Though many environmental factors (eg. turbidity, fetch, water temperature, etc.) can indicate the success or failure of SAV populations, the introduction of competitive species greatly alters former macrophyte assemblages (Lehmann et al. 1997; Capers et al. 2007; Wilson et al. 2007). We analyzed four point-intercept surveys from 1999-2015 to address submersed plant population dynamics over time at RRL. In general, exotic SAV had the greatest incidence of detection, and the highest observed frequency of co-occurrence. Typically, exotic SAV co-occurred with other exotic species more frequently than with native species. However, spatial coverage among native species was greatest when co-occurring with exotic species. With exclusion to *H. verticillata*, both native and exotic submersed species co-occurred more often than in monoculture. Additionally, we discovered a native species, *C. caroliniana* was introduced at some point between the 1999 and 2012 survey. *C. caroliniana*, which is now the most prolific native species, has notably transformed past community richness values within the waterbody. Nevertheless, we established that an increase in exotic species richness decreased the richness of native SAV communities. Moreover, we analyzed bathymetric correlations of species presence in
monocultures to find that with exception of *M. spicatum*, native species occurred at greater depths than exotic SAV. Overall however, macrophyte density was greatest along shoreline coves, rather than within the center of the waterbody where littoral regions receive the greatest environmental disturbance.

Though we lack quantitative, historical plant population observations, we suggest future interactions and competition among spatial ranges will be determined by the aggressiveness of *C. caroliniana* and the persistence of *H. verticillata* and *M. spicatum*. As there is excessive change in water levels at RRL, bathymetric alterations will greatly contribute to species spatial occurrence and richness. Furthermore, species which can overwinter without significant loss in biomass will most likely remain dominant; specifically, *M. spicatum* and *C. caroliniana*. While *H. verticillata* senesces each season, the plant physiological properties which define it as the “perfect aquatic weed”, ensure its success as the dominant species in the future (Langeland 1996). Lacking a current management regime, we project littoral occupancy will remain around 70-100% at RRL for years to come.

**MANAGEMENT RECOMMENDATIONS**

The breadth of submersed vegetation occupancy is substantial within the littoral zone at RRL. Although most of the lake is dominated with exotic SAV, we believe these community assemblages provide sufficient diversity and edge habitat for cover, provisions, and spawn opportunities for aquatic organisms. Furthermore, the plentiful abundance of SAV enhance the lake ecologically, as RRL is much less turbid than most piedmont NC reservoirs.
Recreational opportunities during peak seasonal SAV growth (July-August) will likely remain hindered in areas fully saturated with submersed vegetation cover, specifically, areas dominated with *H. verticillata* and *M. spicatum*.

There are several potential management strategies for reviving the littoral zone at RRL. As a preventative measure, homeowners should be encouraged to remove all yard waste and lawn clippings to stabilize nutrient loads around docks and boat slips, to reduce nutrient rich debris leaching into the waterbody. Implementing a revegetation program with desirable native species (eg. *Vallisneria americana* Michx.) in a pilot project, as done upstream at both Lake Gaston and Kerr Lake reservoirs, may also prove beneficial. Likewise, incorporating triploid grass carp (*Ctenopharyngodon idella*), similar to Lake Gaston, may reduce the abundance of *H. verticillata* temporally. Drawdown is not a suitable option due to the fluvial prominence and arrangement of the waterbody. However, continuation of herbicide “spot” treatments may provide a suitable option for homeowner settings or near boat launches. Conversely, herbicide treatments are likely insufficient for a lakewide, holistic approach due to economic investment and littoral diversity of RRL. Nevertheless, water resource managers should ensure all herbicide treatments align with local guidelines and permitting requirements (Dominion 2015). Since management efforts rely on recurrently assessing the empirical attributes of submersed vegetation, we suggest conducting biennial evaluations to monitor trends in SAV abundance and species distribution.
SOURCES OF MATERIALS

1 Lowrance HDS-7 Gen2 chartplotter, Navico Inc., 4500 South 129th East Avenue, Suite 200, Tulsa, OK 74134.

2 ciBioBase 5.2 cloud-based software, Contour Innovations, LLC, 1229 Tyler Street NE, Suite 120, Minneapolis, MN 55413.

3 ArcGIS 10.2.2, Environmental Systems Research Institute, 380 New York Street, Redlands, CA 92373.
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Figure 1. Geographic location of Roanoke Rapids Lake along the Roanoke River chain.
Figure 2. Depth profile of Roanoke Rapids Lake created using pooled hydroacoustic transects from 2012 and 2015 survey years.
Figure 3. Community richness interactions among depth averages determined through species occurrence or co-occurrence observations (1999: $\mu=1.45 \pm 1.24$; 2012: $\mu=1.50 \pm 1.30$; 2014: $\mu=1.66 \pm 1.28$; and 2015: $\mu=1.33 \pm 1.05$). A species richness of one represents when submersed vegetation occurred in monoculture.
Figure 4. Species-wise kernel density interpolation maps depicting the distribution and expanse of submersed vegetation within Roanoke Rapids Lake based upon point-intercept sampling regimes and bathymetric parameters. Note, *Potamogeton* spp. and *C. caroliniana* were not detected during the 1999 whole-lake survey. Darker regions signify a greater likelihood of species incidence, while lighter regions denote less likely incidence.
Figure 5. Species-wise kernel density interpolation maps depicting the distribution and expanse of submersed vegetation within Roanoke Rapids Lake based upon bathymetric parameters and point-intercept sampling regimes during the 2015 whole-lake survey. Darker regions signify a greater likelihood of species incidence, while lighter regions denote less likely incidence.
Figure 6. Kernel density interpolation maps depicting the distribution and expanse of *H. verticillata* (red) and *C. caroliniana* (green) within Roanoke Rapids Lake based on spatial regions of interest among years 1999 and 2015. Note, *C. caroliniana* was not present in 1999 and thus, not shown. Darker regions signify a greater likelihood of species incidence, while lighter regions denote less likely incidence.
Table 1. Moran's ($I$) spatial autocorrelation report for submersed vegetation identified among survey years$^a$.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of points</th>
<th>$I$</th>
<th>z-score</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>812</td>
<td>0.21</td>
<td>6.41</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td><strong>362</strong></td>
<td><strong>0.04</strong></td>
<td><strong>1.14</strong></td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>2012</td>
<td>263</td>
<td>0.13</td>
<td>2.67</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2015</td>
<td>123</td>
<td>0.1</td>
<td>1.52</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><strong>370</strong></td>
<td>-0.03</td>
<td>-0.35</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td><strong>362</strong></td>
<td><strong>0.05</strong></td>
<td><strong>1.43</strong></td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

$^a$Years and values in bold denote spatial autocorrelation elements used for kernel density estimations.
Table 2. Outline of the seven submersed vegetation species identified at Roanoke Rapids Lake between years 1999-2015\textsuperscript{a,b}.

<table>
<thead>
<tr>
<th>Species(^c)</th>
<th>Common name</th>
<th>Status(^d)</th>
<th>1999 Detections (%)</th>
<th>2012 Detections (%)</th>
<th>2014 Detections (%)</th>
<th>2015 Detections (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hydrilla verticillata</em> (L.f.) Royal</td>
<td>Water thyme or Hydrilla</td>
<td>Exotic</td>
<td>494 (61%)</td>
<td>170 (65%)</td>
<td>74 (60%)</td>
<td>268 (72%)</td>
</tr>
<tr>
<td><em>Myriophyllum spicatum</em> L.</td>
<td>Eurasian watermilfoil</td>
<td>Exotic</td>
<td>150 (18%)</td>
<td>85 (32%)</td>
<td>44 (36%)</td>
<td>70 (19%)</td>
</tr>
<tr>
<td><em>Najas minor</em> All.</td>
<td>Brittle naiad</td>
<td>Exotic</td>
<td>68 (8%)</td>
<td>0 (0%)</td>
<td>2 (2%)</td>
<td>34 (9%)</td>
</tr>
<tr>
<td><em>Potamogeton spp.</em></td>
<td>Pondweed's</td>
<td>Native/Exotic</td>
<td>0 (0%)</td>
<td>1 (0%)</td>
<td>0 (0%)</td>
<td>5 (1%)</td>
</tr>
<tr>
<td><em>Cabomba caroliniana</em> A. Gray</td>
<td>Carolina fanwort</td>
<td>Native</td>
<td>0 (0%)</td>
<td>61 (23%)</td>
<td>47 (38%)</td>
<td>39 (11%)</td>
</tr>
<tr>
<td><em>Ceratophyllum demersum</em> L.</td>
<td>Coontail</td>
<td>Native</td>
<td>73 (9%)</td>
<td>36 (14%)</td>
<td>27 (22%)</td>
<td>33 (9%)</td>
</tr>
<tr>
<td><em>Chara spp.</em></td>
<td>Musk grass</td>
<td>Native</td>
<td>54 (7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>7 (2%)</td>
</tr>
<tr>
<td><strong>Total SAV</strong></td>
<td></td>
<td></td>
<td>568 (70%)</td>
<td>189 (72%)</td>
<td>121 (98%)</td>
<td>284 (77%)</td>
</tr>
</tbody>
</table>

\(^a\)Percent occurrence in parenthesis beside observed detections is devised by points visited.
\(^b\)Number of spatial locations visited: 1999: 812 points; 2012: 263 points; 2014: 123 points; and 2015: 370 points.
\(^c\)Species include vascular and macroalgal species (ie. *Chara*).
\(^d\)*Potamogeton spp.* status is species dependent.
Table 3. Maximum limiting depth of each submersed species identified at Roanoke Rapids Lake between years 1999-2015a.

<table>
<thead>
<tr>
<th>Species</th>
<th>Limiting Depth (m(^{-1}))</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. verticillata</td>
<td>1 - 6</td>
<td>Langeland 1996; Unpublished data NCSU</td>
</tr>
<tr>
<td>M. spicatum</td>
<td>1 - 4</td>
<td>Nichols and Shaw 1986</td>
</tr>
<tr>
<td>N. minor</td>
<td>1 - 5</td>
<td>Unpublished data NCSU</td>
</tr>
<tr>
<td>Potamogeton spp.</td>
<td>1 - 5</td>
<td>Unpublished data NCSU</td>
</tr>
<tr>
<td>C. caroliniana</td>
<td>1 - 4</td>
<td>Schooler et al. 2009</td>
</tr>
<tr>
<td>C. demersum</td>
<td>1 - 3</td>
<td>Engel 1985</td>
</tr>
<tr>
<td>Chara spp.</td>
<td>&gt; 6</td>
<td>Steinman et al. 1997</td>
</tr>
</tbody>
</table>

aLimiting depth determined from reported maximum depth occurrence in U.S. waterbodies with average secchi readings 3±1 m\(^{-1}\).
bUnpublished data by North Carolina State University from previous surveys.
cChara spp. is reported to occur in depths greater than angiosperms.
Table 4. Submersed vegetation occurrence and co-occurrence matrix per point location among years 1999-2015<sup>a,b,c</sup>.

<table>
<thead>
<tr>
<th></th>
<th>1999</th>
<th>2012</th>
<th>2014</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. verticillata</td>
<td>256 (44%)</td>
<td>116 (20%)</td>
<td>60 (11%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>M. spicatum</td>
<td>24 (4%)</td>
<td>0 (0%)</td>
<td>16 (3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>N. minor</td>
<td>5 (1%)</td>
<td>0 (0%)</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Potamogeton spp.</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>C. caroliniana</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>C. demersum</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Bold values denote when species occurred in monoculture.

<sup>b</sup> Parentheses signify percent occurrence or co-occurrence compared to total observed SAV during survey.

<sup>c</sup> Note, co-occurrence does not account for species richness among discrete point locations.
Table 5. Lake-wide dominance of each submersed species identified by point-intercept methods at Roanoke Rapids Lake between years 1999-2015\(^a,b,c\).

<table>
<thead>
<tr>
<th>Species</th>
<th>1999 Dominance</th>
<th>2012 Dominance</th>
<th>2014 Dominance</th>
<th>2015 Dominance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. verticillata</em></td>
<td>87%</td>
<td>90%</td>
<td>61%</td>
<td>94%</td>
</tr>
<tr>
<td><em>M. spicatum</em></td>
<td>26%</td>
<td>45%</td>
<td>36%</td>
<td>25%</td>
</tr>
<tr>
<td><em>N. minor</em></td>
<td><strong>12%</strong></td>
<td>0%</td>
<td>2%</td>
<td><strong>12%</strong></td>
</tr>
<tr>
<td><em>Potamogeton spp.</em></td>
<td>0%</td>
<td>1%</td>
<td>0%</td>
<td>2%</td>
</tr>
<tr>
<td><em>C. caroliniana</em></td>
<td>0%</td>
<td>32%</td>
<td><strong>39%</strong></td>
<td>14%</td>
</tr>
<tr>
<td><em>C. demersum</em></td>
<td>13%</td>
<td>19%</td>
<td><strong>22%</strong></td>
<td>12%</td>
</tr>
<tr>
<td><em>Chara spp.</em></td>
<td><strong>10%</strong></td>
<td>0%</td>
<td>0%</td>
<td>2%</td>
</tr>
</tbody>
</table>

\(^a\) Dominance percentage determined through species-wise occurrence compared to total species observed by year.
\(^b\) Total SAV occurrence per survey: 1999: 568 counts; 2012: 189 counts; 2014: 121 counts; and 2015: 284 counts.
\(^c\) Bold values denote year with greatest species dominance.
Table 6. Spatial statistics summary of locations occupied with submersed vegetation at Roanoke Rapids Lake in years 1999 and 2015\textsuperscript{a,b,c,d}.

<table>
<thead>
<tr>
<th>Species</th>
<th>1999 Survey</th>
<th>2015 Survey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area (ha\textsuperscript{-1})</td>
<td>Littoral PAC</td>
</tr>
<tr>
<td>H. verticillata</td>
<td>639.74</td>
<td>(60%)</td>
</tr>
<tr>
<td>M. spicatum</td>
<td>316.38</td>
<td>(30%)</td>
</tr>
<tr>
<td>N. minor</td>
<td>214.97</td>
<td>(20%)</td>
</tr>
<tr>
<td>Potamogeton spp.</td>
<td>0.00</td>
<td>(0%)</td>
</tr>
<tr>
<td>C. caroliniana</td>
<td>0.00</td>
<td>(0%)</td>
</tr>
<tr>
<td>C. demersum</td>
<td>183.10</td>
<td>(17%)</td>
</tr>
<tr>
<td>Chara spp.</td>
<td>107.15</td>
<td>(10%)</td>
</tr>
<tr>
<td>Total SAV</td>
<td>736.27</td>
<td>(69%)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Area derived from kernel density models of SAV occurrence.
\textsuperscript{b}Percent area covered (PAC) determined by devising interpolated species area by the littoral profile area (1067.61 ha\textsuperscript{-1}).
\textsuperscript{c}Bold values denote year with greatest SAV spatial coverage.
\textsuperscript{d}Note, calculated areas do not account for species richness among lakewide SAV distributions.
Table 7. Submersed vegetation spatial (ha⁻¹) co-occurrence matrix comparing temporal persistence and within season community overlap among years 1999 and 2015\(^a,b,c,d,e\).

<table>
<thead>
<tr>
<th></th>
<th>H. verticillata(^E)</th>
<th>M. spicatum(^E)</th>
<th>N. minor(^E)</th>
<th>Potamogeton spp.(^E/N)</th>
<th>C. caroliniana(^N)</th>
<th>C. demersum(^N)</th>
<th>Chara spp.(^N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. verticillata(^E)</td>
<td>69.67</td>
<td>29.14 (+7.48)</td>
<td>15.00 (-1.87)</td>
<td>2.29 (na)</td>
<td>16.46 (na)</td>
<td>12.73 (+2.44)</td>
<td>3.37 (-5.68)</td>
</tr>
<tr>
<td>M. spicatum(^E)</td>
<td>21.66</td>
<td>12.99</td>
<td>9.86 (+4.37)</td>
<td>0.00 (na)</td>
<td>9.12 (na)</td>
<td>4.41 (-7.26)</td>
<td>2.66 (+1.25)</td>
</tr>
<tr>
<td>N. minor(^E)</td>
<td>16.87</td>
<td>5.49</td>
<td>6.08</td>
<td>0.88 (na)</td>
<td>3.99 (na)</td>
<td>2.54 (-0.17)</td>
<td>2.60 (-0.61)</td>
</tr>
<tr>
<td>Potamogeton spp.(^E/N)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.91 (na)</td>
<td>1.15 (na)</td>
<td>0.15 (na)</td>
</tr>
<tr>
<td>C. caroliniana(^N)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.83 (na)</td>
</tr>
<tr>
<td>C. demersum(^N)</td>
<td>10.29</td>
<td>11.67</td>
<td>2.71</td>
<td>-</td>
<td>-</td>
<td>6.34</td>
<td>0.89 (+0.61)</td>
</tr>
<tr>
<td>Chara spp.(^N)</td>
<td>9.05</td>
<td>1.41</td>
<td>3.21</td>
<td>-</td>
<td>-</td>
<td>0.28</td>
<td>0.00</td>
</tr>
</tbody>
</table>

\(^a\)Capitalized superscript letters beside each species define macrophyte status (E: exotic; N: native).
\(^b\)Area determined using kernel density interpolation methods and raster algebra.
\(^c\)Bold values in unshaded area represent intraspecific spatial persistence among years 1999 and 2015.
\(^d\)Within season spatial co-occurrence denoted in light grey shaded area (1999) and dark grey shaded area (2015).
\(^e\)Parenthesis beside 2015 values signify temporal change (ha⁻¹) among co-occurrences as either increase (+) or decrease (-) in coverage; (na) values represent species interactions which did not previously co-occur.
CHAPTER 5

Evaluation of Potential Control Measures for Established *Arundo donax*
Global demands for reduced greenhouse gas emissions have prompted research of alternative energy sources from second-generation biofuel crops. *Arundo donax* (giant reed), a high biomass yielding cane species within the *Poaceae* family, continues to gain recognition for lignocellulosic biofuel production. However, the physiological properties which make giant reed a prime candidate for biofuel production may also promote invasiveness. Nevertheless, bio-energy advocates support planting giant reed in regions east of the Mississippi River. Our objective was to evaluate techniques for eliminating established giant reed populations. In spring 2013, a greenhouse trial was conducted to identify appropriate herbicide chemistries for field evaluation which included various rates of glyphosate (4.48-8.96 kg a.e. ha\(^{-1}\)), imazapyr (0.56-1.12 kg a.e. ha\(^{-1}\)), metsulfuron-methyl (0.17 kg a.i. ha\(^{-1}\)), and combinations of each. Except metsulfuron-methyl, all herbicide treatments provided excellent control. In summer 2014 and 2015, a range of foliar applications using glyphosate (2.60-9.95 kg a.e. ha\(^{-1}\)), imazapyr (1.12-2.24 kg a.e. ha\(^{-1}\)), and combinations thereof, occurred among two duplicate field trials in eastern North Carolina. Apart from the lowest rate of glyphosate (2.60 kg ha\(^{-1}\)), all herbicide applications controlled giant reed completely 6 and 9 months after treatment, with no green shoots observed 12 months after treatment. Based upon results, well-established giant reed may be readily controlled with glyphosate to eliminate escapes or allow crop rotation.
INTRODUCTION

Over the last several decades, global demands for reduced carbon (CO$_2$) and other greenhouse gas emissions due to fossil fuel consumption, have prompted research of alternative energy sources from biofuel crops (Ragauskas et al. 2006; Hastings et al. 2008; Naik et al. 2010; Xu et al. 2015). In the United States, both federal and state level incentives (eg. The United States Energy Policy Act of 1992 and 2005; The Food, Conservation, and Energy Act of 2008, which is inclusive in the Energy and Independence Act of 2007; fuel tax exceptions and credits) have promoted alternative bio-ethanol production to reduce the annual ecologic hindrance and economic dependence accompanying petroleum usage (Parcell and Westhoff 2005; Birur et al. 2008; Yacobucci 2012). Existing requests from environmental groups have shifted bio-energy research from first-generation commodities, such as corn (Zea mays), to second-generation biofuels; encouraging carbon neutral energy consumption options which endorse alternative energy, while also meeting the requirements of food sustainability (Kheshgi et al. 2000; Mohr and Raman 2013; Impagliazzo et al. 2016). High yielding, second-generation lignocellulosic biomass crops, offer a cost-efficient feedstock for conversion of carbohydrate structures (cellulose and hemi-cellulose) and organic polymers (lignin) to bio-ethanol, through innovative ensilage refining processes such as anaerobic pyrolysis and catalytic conversion (Alonso et al. 2010; Naik et al. 2010; Xu et al. 2015; Zucaro et al. 2016).

Giant reed (Arundo donax L.), a robust cane species within the Poaceae family, continues to gain recognition among researchers examining second-generation raw materials
(Birur et al. 2008; Mantineo et al. 2009; Baldini et al. 2016; Corno et al. 2016). Classified as a perennial grass species which prefers riparian zones (Wijte et al. 2005; Tarin et al. 2013; Corno et al. 2014), giant reed closely resembles the phenotypic and morphologic growth patterns of *Phragmites* spp. and *Bambusa* spp.; having an erect growth stature of 7-10 m$^{-1}$ (Coffman et al. 2010; Lambert et al. 2010; Saltonstall et al. 2010). Though tall, dense stands of giant reed have sufficient vigor to resist lodging from excessive rainfall and wind events (Nassi o Di Nasso et al. 2010; Hicks 2015). The clump-forming giant reed (Lambert et al. 2010), follows a C$_3$ photosynthetic pathway; although it is more comparable to the prolific growth patterns of C$_4$ plants (Rossa et al. 1998). Once established, annual yield potential has been documented to be $>20$ t/ha$^{-1}$ of standing biomass with minimal energy inputs, soil fertility amendments, and low irrigation allocation (Perdue 1958; Angelini et al. 2009; Impagliazzo et al. 2016). Therefore, bio-energy proponents have concurrently supported the plantings of giant reed to reduce anthropogenic nutrient loading (Quinn et al. 2007; Zucaro et al. 2016), reduce soil erosion potential (Pilu et al. 2012; Fagnano et al. 2015), add value to marginal parcels with unfavorable agronomic conditions (Nassi o Di Nasso et al. 2013), and provide increased soil carbon sequestration for environmental and economic credits (Williams et al. 2008).

For thousands of years, giant reed has been cultivated for its vast utilitarian prospective. Modern usages have documented provisions for paper pulp (Shatalov and Pereira 2006, Ge et al. 2016), erosion control and fodder (Hardion et al. 2014), construction material (Lambert et al. 2010; Karahancer et al. 2016), and reeds for musical instruments.
(Perdue 1958). Given these practical characteristics, anthropologists and plant geneticists suggest human activity most likely contributed to the spread of giant reed from its origin in either Asia (Polunin and Huxley 1987; Angelini et al. 2009) or the Mediterranean (Tarin et al. 2013; Hardion et al. 2014); to eventually California, where it was planted for erosion control in the early 1800’s (Mariani et al. 2010).

Since initial introduction into the United States, giant reed has expanded its range longitudinally from California to Maryland (Bell 1997); and most recently, Delaware (USDA 2017). Giant reed reproduction in North American occurs asexually through clonal fragmentation of stems (apical internodes), and rhizomatous structures; as inflorescences are unable to produce viable seed (Wijte et al. 2005; Santín-Montanyá et al. 2013). Rouge colonies of giant reed, from cultivated areas, have exhibited invasive properties in aquatic habitats within the Southwestern United States (Rieger and Kreager 1989; Tarin et al. 2013), and along the Rio Grande from Texas into Mexico (Everitt et al. 2005); with recent estimates of unwarranted giant reed stands comprising ~6,000 ha¹ along the river (Yang et al. 2011). In areas experiencing invasion, giant reed monocultures have been recognized for displacing native flora (Koop et al. 2012), altering hydrologic morphology (Bell 1997), and increasing flood (Spencer et al. 2013) and wildfire potential (Coffman et al. 2010). Therefore, officials have declared giant reed a state noxious weed in California, Nevada, and Texas (Koop et al. 2012). Nevertheless, bio-energy advocates have supported the plantings of giant reed in regions east of the Mississippi River basin.
The ability of giant reed to proliferate under various environmental scenarios has raised concern among many invasive species ecologists. The inherent breeding proficiency and dispersion strategies of giant reed, resulting from hydrologic and human activities, has spawned numerous investigations of best management practices (Barney 2012); including: field and equipment sanitation guidelines (Iverson et al. 2011; USDA 2014), biological control measures (Dudley and Lambert 2007; Goolsby and Moran 2009), mechanical and cultural tactics (Lowrey and Watson 2004), and numerous herbicide programs. Of these control methods, those including herbicides have been most effective (Bell 1997; Bell 2011; Spencer et al. 2012; Spencer 2014; Santín-Montanyá et al. 2013). Glyphosate \((N\text{-}(\text{phosphonomethyl}) \text{glycine})\) has become a standard for giant reed control over the last two decades, with imazapyr \([2-(4\text{-methyl-5-oxo-4-propan-2-yl-1H-imidazol-2-yl}) \text{pyridine-3-carboxylic acid}]\) also utilized (Bell 2011). Though herbicide rates and timing have been reported, there remains a gap in the literature for the effectiveness of these herbicides on well-established agricultural plantings (ie. shoots > 6 m\(^{-1}\)) which have not been mechanically harvested or previously mowed in Southeastern United States biofuel production areas. Several investigators have suggested applying glyphosate (1.5-5% solution), imazapyr (0.6-1.6% solution), or a combination rate thereof (0.28 kg a.e./ha\(^{-1}\) imazapyr + 1.1 kg a.e./ha\(^{-1}\) glyphosate) for efficient control (Bell 2011; Spencer et al. 2012; Corno et al. 2014).

While phenological components encourage giant reed as a candidate for lignocellulosic bio-ethanol production in North Carolina, there remains some uncertainty to the invasiveness of giant reed. The objective of this study was to identify herbicides that may
be used to control established giant reed as part of crop rotations or control escapes from agricultural plantings.

**MATERIALS AND METHODS**

**Greenhouse Experiment**

Following a complete randomized block design with five replications, individual giant reed stem cuttings were planted into separate 30.5 cm$^2$ pots containing a soilless, commercial potting media (Fafard® 2 Mix). At time of transplant, Osmocote® slow-release fertilizer$^2$ (14-14-14) was applied at a rate of 15 g per pot. Plantings were kept saturated using overhead mist irrigation at 0.635 cm$^{-1}$ of water, twice daily until treatment. Supplementary artificial light was used to simulate field photoperiod environments of 12-h per day$^{-1}$. Day/night temperature was maintained at (35/21 °C). Herbicide treatments (Table 1) were applied 17 April 2013 when established plantings reached an average height of 1.37 m$^{-1}$. Treatments were applied using a pressurized CO$_2$ backpack sprayer at 187 L ha$^{-1}$ at 172 kPa with a with dual nozzle boom arrangement comprised of Teejet TP8003VS nozzles$^3$ with 48.3 cm$^{-1}$ spacing, applied 50.8 cm$^{-1}$ above the plant canopy. Visual ratings of percent control (efficacy appraisals of chlorosis and necrosis) from 0% (no injury) to 100% (complete desiccation) were estimated eight and twelve weeks after initial treatment to assess plant growth regulation by each treatment. Biomass was harvested at time of each visual rating, where plant samples were allowed to dry at 60 °C for 48 h prior to recording biomass dry weights (g dry wt per treatment).
Field Experiment

Trials were conducted at the NCSU Williamsdale Biofuel Field Laboratory (BFL; Duplin Co.; 34°45’39.4"N, 78°06’01.3"W) to investigate giant reed control from designated herbicides and rates identified during the greenhouse experiment. Trials occurred within pre-established, two-year-old giant reed fields which were originally established in 2012. Fields were mowed in fall 2013 to form N=72 (30.5 m\(^{-1}\) x 6.1 m\(^{-1}\) 186.5 m\(^2\) plots. Herbicide rates and suggested adjuvants followed label recommendations for field usage. Following a complete randomized block design with three replications, initial treatments occurred 17 October 2014 when plants averaged 5.4 m\(^{-1}\) in height and were applied using an electric pump sprayer, calibrated to 187.1 L ha\(^{-1}\) at 275.79 kPa, and implemented with an extension boom which applied treatments 91.4 cm\(^{-1}\) above the plant canopy using a high volume Hypro XT080 nozzle\(^4\) to achieve canopy coverage. The trial was repeated on 16 June 2015 when giant reed reached an average height of 2.7 m\(^{-1}\) among plots not previously treated. Visible ratings of plant injury were conducted six and nine months after initial treatment where percent control (efficacy appraisals of chlorosis and necrosis) scaled from 0% (no injury) to 100% (complete desiccation). A twelve-month evaluation of average green shoots per plot (shoots per m\(^2\)) took place using a 2 m\(^{-1}\) x 0.5 m\(^{-1}\) pole to gauge the number of remaining shoots which intersected the pole. This process was replicated three times per plot to estimate temporal treatment performance. No significant treatment by trial interactions were observed; therefore, data from both trials were pooled for average stem count analysis.
Data Analysis

All data were statistically evaluated using analysis of variance (ANOVA) and compared with Fisher’s protected LSD mean separation approach (P < .05) with RStudio 3.1.3 (Wickham 2011; de Mendiburu 2015; R Core Team 2015). Factors used for assessment of treatment-by-treatment effects included: location, trial year, herbicide formulation and rate, replication, and assessment intervals. Significance values were used to quantify the association among herbicide treatments for percent control or remaining giant reed green shoots. ANOVA normality and homogeneity assumptions were checked using Shapiro-Wilks test, Q-Q diagnostic plots, and Bartlett’s k test. Results from the greenhouse and field experiments are reported separately.

RESULTS AND DISCUSSION

Greenhouse Experiment

Giant reed control at 8 WAT was greatest with glyphosate (4.48-8.96 kg a.e. ha⁻¹) treatments (Table 1). Applications of glyphosate (4.48 kg ha⁻¹) in combination with imazapyr (1.12 kg a.e./ha⁻¹), were not significantly different than the highest rate of imazapyr (1.12 kg ha⁻¹) or metsulfuron-methyl (0.17 kg a.i./ha⁻¹) in combination with imazapyr (0.56 kg a.e./ha⁻¹). Similarly, imazapyr at the lowest rate (0.56 kg ha⁻¹) was not significantly different than metsulfuron-methyl in combination with imazapyr 8 WAT. The lowest rate of metsulfuron-methyl displayed no greater control than the untreated control 8 WAT (Table 1). Except for
metsulfuron-methyl, all herbicide treatments controlled giant reed completely (100% control) 12 WAT (Table 1). Similar to the current study, Hicks (2013) reported low injury among metsulfuron-methyl treatments in greenhouse trials.

At the 8 WAT evaluation, giant reed biomass was lowest among treatments of glyphosate (8.96 kg ha\(^{-1}\)), and imazapyr (1.12 kg ha\(^{-1}\)) in combination with glyphosate (8.96 kg ha\(^{-1}\)) (Table 1). At the 12 WAT evaluation, the only biomass collected was from plantings treated with metsulfuron-methyl or plantings among the untreated control (Table 1). Giant reed biomass was not collected 12 WAT among single formulations of imazapyr, glyphosate, or combinations within (Table 1). Metsulfuron-methyl was not included in the field trial due to low control observed in the greenhouse.

**Field Experiment**

At 6 MAT, all treatments except for the lowest rate of glyphosate (2.60 kg a.e. ha\(^{-1}\)) provided complete control (100%) (Table 2). Similar results occurred at the 9 MAT assessment (Table 2). No shoots were found among plots treated with the highest rate of glyphosate (9.95 kg a.e. ha\(^{-1}\)) or imazapyr (1.12-2.24 kg a.e. ha\(^{-1}\)) 12 MAT. Apart from the combination treatment of mid-rate imazapyr (1.68 kg ha\(^{-1}\)) with high-rate glyphosate (9.95 kg ha\(^{-1}\)), no shoots were found among combinations of imazapyr and glyphosate (Table 2). Though insignificant, differences among combination treatments are attributed to incomplete coverage during application. Residual soil activity was expected to reduce the number of
giant reed shoots from plots including imazapyr treatments (Bell 2011; Minogue and Wright 2016).

**MANAGEMENT RECOMMENDATIONS**

Systemic herbicides are proven as an effective method for control of giant reed (Spencer et al. 2008; Bell 2011; Spencer et al. 2012; Santín-Montanyá et al. 2013; Corno et al. 2014). Based on our results, excellent control of giant reed may be achieved using mid to high rates of glyphosate (6.64-9.95 kg a.e. ha⁻¹), low rates of imazapyr (1.12 kg a.e. ha⁻¹), or the lowest combination rate of imazapyr (1.12 kg a.e. ha⁻¹) and glyphosate (2.60 kg a.e. ha⁻¹).

If mowing a field, ensure plants are of sufficient structure prior to treatment, since treatments should be applied when leaf surface and plant height (ie. ~1.8-3.0 m⁻¹) are adequate for promoting translocation to the rhizome (Bell 2011). Furthermore, timing of treatment is most effective during active growth (late summer and fall season) (DOW 2017). Among dense stands, use a high-volume, over-the-top application to obtain maximum coverage. If applicable, the use of aerial applications is recommended when traditional spray equipment is unable to obtain proper coverage (Jones and Lehman 1987; Bell 1997; USDA 2014). If unwarranted plants persist at low numbers, conduct a follow up spot-spray application and spray-to-wet. If using imazapyr, ensure herbicide rates do not exceed the label recommendation which could cause carry-over injury to the forthcoming crop (Ulbrich et al. 2005). An appropriate rotational crop would include one with glyphosate resistance technology. Among areas near riparian and other aquatic habitats (eg. irrigation
canal or pond), the appropriate formulation of glyphosate and imazapyr would be ones labeled for aquatic use.

No significant benefit was observed when imazapyr was used in combination with glyphosate. Therefore, treatments incorporating glyphosate alone will lower the overall cost of treatment and provide a shorter soil residual in scenarios where injury to native vegetation is of concern (Bell 2011). However, if residual soil activity is desired, such as providing a containment zone or border, then a combination treatment of imazapyr with glyphosate should provide adequate results.

SOURCES OF MATERIALS

1 Fafard 2 Mix potting media, Conrad Fafard, Inc., P.O. Box 790, Agawam, MA 01001
2 Osmocote® slow-release fertilizer, The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43040
3 Teejet TP8003VS nozzle, Teejet Technologies Illinois, LLC, 1801 Business Park Drive, Springfield, IL 62703
4 Hypro XT080 nozzle, Pentair Plc. Hypro, Stationroad, Lonstanton, Cambridge, UK CB24 3DS
LITERATURE CITED


Minogue, P. and Wright, S. 2016. Biology, Control and Invasive Potential of Giant Reed (Arundo donax L.) in Florida. FOR328: A series of the School of Forest Resources and Conservation, UF/IFAS Extension. 2-6.


United States Department of Agriculture. 2017. Plants Database.


Table 1. Control of giant reed with selected herbicide treatments in the greenhouse.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>kg ha$^{-1}$</th>
<th>Percent Control (%)$^c$</th>
<th>Biomass (g$^{-1}$)</th>
<th>Weeks After Treatment (WAT)$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>imazapyr</td>
<td>0.56</td>
<td>23 c</td>
<td>100 a</td>
<td>42 bcd</td>
</tr>
<tr>
<td>imazapyr</td>
<td>1.12</td>
<td>33 b</td>
<td>100 a</td>
<td>54 bc</td>
</tr>
<tr>
<td>glyphosate</td>
<td>4.48</td>
<td>100 a</td>
<td>100 a</td>
<td>41 bcd</td>
</tr>
<tr>
<td>glyphosate</td>
<td>8.96</td>
<td>100 a</td>
<td>100 a</td>
<td>31 d</td>
</tr>
<tr>
<td>imazapyr + glyphosate</td>
<td>0.56 + 4.48</td>
<td>100 a</td>
<td>100 a</td>
<td>36 cd</td>
</tr>
<tr>
<td>imazapyr + glyphosate</td>
<td>1.12 + 8.96</td>
<td>100 a</td>
<td>100 a</td>
<td>30 d</td>
</tr>
<tr>
<td>imazapyr + glyphosate</td>
<td>1.12 + 4.48</td>
<td>100 a</td>
<td>100 a</td>
<td>39 bed</td>
</tr>
<tr>
<td>imazapyr + glyphosate (follow-by)</td>
<td>1.12 + 4.48</td>
<td>100 b</td>
<td>100 a</td>
<td>50 bc</td>
</tr>
<tr>
<td>imazapyr + glyphosate</td>
<td>0.56 + 8.96</td>
<td>100 a</td>
<td>100 a</td>
<td>40 bed</td>
</tr>
<tr>
<td>metsulfuron-methyl</td>
<td>0.17 a.i.</td>
<td>3 d</td>
<td>0 b</td>
<td>78 a</td>
</tr>
<tr>
<td>metsulfuron-methyl + imazapyr</td>
<td>0.17 a.i. + 0.56</td>
<td>25 bc</td>
<td>100 a</td>
<td>55 b</td>
</tr>
<tr>
<td>untreated control</td>
<td>--</td>
<td>2 d</td>
<td>0 b</td>
<td>85 a</td>
</tr>
</tbody>
</table>

$^a$ All treatments included a nonionic surfactant (NIS) at 0.25% v/v.

$^b$ Follow-by treatment incorporated imazapyr application at same period as other treatments with glyphosate applied one week later.

$^c$ Control estimated on a 0 (no control) to 100 (complete control) ranking.

$^d$ Means within the same column followed by the same letter do not significantly differ (P < .05).
Table 2. Visual ratings of giant reed control following selected herbicide treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>kg ha(^{-1})</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>imazapyr</td>
<td>1.12</td>
<td>100 a</td>
<td>100 a</td>
<td>0 b</td>
</tr>
<tr>
<td>imazapyr</td>
<td>1.68</td>
<td>100 a</td>
<td>100 a</td>
<td>0 b</td>
</tr>
<tr>
<td>imazapyr</td>
<td>2.24</td>
<td>100 a</td>
<td>100 a</td>
<td>0 b</td>
</tr>
<tr>
<td>glyphosate</td>
<td>2.60</td>
<td>90 b</td>
<td>93 b</td>
<td>4 ab</td>
</tr>
<tr>
<td>glyphosate</td>
<td>6.64</td>
<td>100 a</td>
<td>100 a</td>
<td>2 b</td>
</tr>
<tr>
<td>glyphosate</td>
<td>9.95</td>
<td>100 a</td>
<td>100 a</td>
<td>0 b</td>
</tr>
<tr>
<td>imazapyr + glyphosate</td>
<td>1.12 + 2.60</td>
<td>100 a</td>
<td>100 a</td>
<td>0 b</td>
</tr>
<tr>
<td>imazapyr + glyphosate</td>
<td>1.12 + 6.64</td>
<td>100 a</td>
<td>100 a</td>
<td>0 b</td>
</tr>
<tr>
<td>imazapyr + glyphosate</td>
<td>1.12 + 9.95</td>
<td>100 a</td>
<td>100 a</td>
<td>0 b</td>
</tr>
<tr>
<td>imazapyr + glyphosate</td>
<td>1.68 + 9.95</td>
<td>100 a</td>
<td>100 a</td>
<td>1 b</td>
</tr>
<tr>
<td>imazapyr + glyphosate</td>
<td>2.24 + 9.95</td>
<td>100 a</td>
<td>100 a</td>
<td>0 b</td>
</tr>
<tr>
<td>untreated control</td>
<td>--</td>
<td>0 c</td>
<td>0 c</td>
<td>8 a</td>
</tr>
</tbody>
</table>

\(^{a}\) All treatments included a nonionic surfactant (NIS) at 0.25% v/v.

\(^{b}\) Control estimated on a 0 (no control) to 100 (complete control) ranking.

\(^{c}\) Means within the same column followed by the same letter do not significantly differ (P < .05).