ABSTRACT

FOLEY, KARA JEAN. Ecology and Management of dioecious *Hydrilla verticillata* in a Virginia Piedmont Reservoir. (Under the direction of Dr. Robert J. Richardson and Dr. Ramon G. Leon).

Philpott Reservoir, a United States Army Corps of Engineers-managed impoundment located in the Virginia Piedmont, is relatively unstudied. Philpott Reservoir serves as a source of hydroelectric power, facilitates flood control for downstream communities along the Smith River, is a destination for outdoor recreation opportunities, and functions as a major freshwater ecosystem. This system provides habitat for many key species, potentially including the Roanoke Logperch (*Percina rex*), an endangered freshwater fish. At this time, the most northeastern US population of dioecious hydrilla (*Hydrilla verticillata* (L.f.) Royle), a Federally-listed noxious aquatic weed, exists in Philpott Reservoir. We investigated the ecology and management of this uncharacteristic population of dioecious hydrilla in a reservoir system. Our results can be applied to future monitoring and management efforts and demonstrate the adaptable behavior of this invasive species in the United States.

To gain a better understanding of the biological, chemical, and physical factors that drive the distribution of hydrilla within Philpott Reservoir, a shoreline point intercept survey of submersed aquatic vegetation (SAV) was conducted annually for three consecutive years and the presence of hydrilla was compared to documented environmental factors within the reservoir. Multivariate analysis revealed that the dioecious hydrilla population at Philpott Reservoir is driven by light availability and sediment hardness and shares a similar niche with *Egeria densa* (Planch), another invasive aquatic plant species in the state of Virginia. When compared to the other SAV species in Philpott Reservoir, hydrilla exhibited a more generalist response to tested physical and chemical environmental conditions.
Next, the temperature and climate-induced phenology patterns of this Virginia dioecious hydrilla population was studied in small-scale laboratory and mesocosm experiments. The water temperatures at Philpott Reservoir are generally colder than those of other waterbodies with documented populations of dioecious hydrilla in the United States. Consequently, the understood benchmark water temperatures for successful dioecious hydrilla growth are either rarely reached or significantly delayed within the constraints of the growing season at this location. The sprouting success of vegetative propagules (tubers) from the Virginia dioecious hydrilla population was compared to that of a Florida population of dioecious hydrilla along a water temperature gradient. Sprouted stems were then planted in outdoor mesocosm facilities that experience dissimilar climates and their development throughout a growing season was compared. It was determined that tubers from Philpott Reservoir’s population of dioecious hydrilla sprouted in warmer water temperatures and can produce tubers earlier in the fall when compared to the tested Florida population. These results suggest that the population of dioecious hydrilla at Philpott Reservoir may have developed methods to best guarantee successful longevity at the northern extreme of its introduced range in the United States by condensing the timing of its major growth and reproductive phenological events to avoid potentially-detrimental cold environmental conditions.

Lastly, three chemical control strategies for management of the dioecious hydrilla population at Philpott Reservoir were tested in a field-based trial. Treatment efficacy was determined using both physical biomass samples and hydroacoustically-acquired biovolume estimates and the results were compared. By two months after treatment, combinations of copper/diquat and endothall/diquat effectively reduced dioecious hydrilla biomass in the treated plots.
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Ecology and Management of dioecious *Hydrilla verticillata* in a Virginia Piedmont Reservoir

by

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DEDICATION

To mom and dad;
Thank you for always encouraging me to follow my dreams
(especially this one).
BIOGRAPHY

Kara grew up in Amherst, New Hampshire and was inspired to pursue a career that allowed her to study natural resources after taking a high school conservation biology course taught by Ms. Melissa Chapman. She went on to study environmental science at the University of New Hampshire and earned her Bachelor’s degree in 2016. For her senior capstone project, Kara worked in the Center for Freshwater Biology and studied the movement of cyanotoxins through aquatic food webs with Dr. James Haney. Post-graduation, Kara was fortunate to intern at the New Hampshire Department of Environmental Services under Ms. Amy Smagula. Through this internship, Kara learned about the importance of aquatic plant management and was inspired to continue her studies in the field.

Kara began her pursuit of a Master’s degree at North Carolina State University after connecting with a former intern for Ms. Smagula, Dr. Erika Haug. In 2017, Kara moved down to Raleigh, North Carolina and started her program at North Carolina State University in Crop Science and Fisheries, Wildlife and Conservation Biology under the direction of Dr. Rob Richardson. Kara looks forward to continued work in the field of aquatic plant management and working towards conserving and protecting our natural resources. In her free time, Kara loves to bake, enjoy the outdoors, and spend time with her family and friends.
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CHAPTER 1: Literature Review

Overview

Aquatic plant management is a key factor for the sustainability of reservoirs in the United States. Reservoir systems are engineered into landscapes and are closely tied to complex ecological processes and key societal needs. Reservoirs are geologically young, have short water retention capacity, experience high shoreline disturbance events, and support low autochthonous inputs of nutrients (Thornton 1980). Due to these factors, native aquatic macrophyte species that require constant and undisturbed environmental conditions often have difficulty establishing and persisting in reservoirs. Reservoir systems can be particularly vulnerable to invasions of non-native aquatic species because of their close connection with other water resources and the dampened effects of interspecific competition due to the minimal presence of native seed banks (Smart and Dick 1999; Havel et al. 2005).

Hydrilla (*Hydrilla verticillata* (L.f.) Royle) is a Federally-listed noxious aquatic weed species in the United States. Three biotypes (strains) of hydrilla have been introduced to the United States from their native range in Asia. These biotypes are differentiated by their distinct reproductive structures and include a monoecious, dioecious, and a novel, undescribed strain (Tippery et al. 2020). Hydrilla is well adapted to a variety of aquatic habitats and can be an aggressive, persistent invader when left unmanaged (Langeland 1996). Because of its vigorous growth and reproductive strategies, hydrilla can withstand the unpredictable environmental conditions that reservoirs provide (Gettys et al. 2014; Thomaz et al. 2015).

This research is centered on Philpott Reservoir, a United States Army Corps of Engineers (USACE) impoundment in the Piedmont ecoregion of Virginia. Philpott Reservoir experiences high shoreline disturbance, is exceptionally deep, and lacks native submersed aquatic vegetation.
In 2015, personnel from the USACE Wilmington District documented the presence of hydrilla at multiple points along the shoreline (M. Hosey, personal communication). In 2017, it was confirmed via molecular methods that the population of hydrilla was of the dioecious biotype (R. Thum, personal communication), making it one of the most northern documented occurrences of dioecious hydrilla in an Atlantic seaboard state.

**Aquatic Plant Management in Piedmont Reservoirs**

*The Piedmont Ecoregion*

The southern Piedmont is an ecoregion in the eastern United States that spans over 1.5 million square kilometers from Alabama to Virginia (USDA 1981). As a transitional area between the Appalachian Mountains and the Coastal Plain, the topography of the region is relatively flat with elevations ranging from just over 300 m above sea level on its western edge to 100 m above sea level on its eastern edge (Hack 1982). The climate in the southern Piedmont is mild with average temperatures between 12 and 18°C and mean annual precipitation totals of approximately 120 cm (USDA 1981). According to a 1981 report, major land uses in the region include forests (60%), human development (15%), grasslands (11%), or croplands (9%) (USDA 1981). The southern Piedmont was never glaciated (Jefferson and McGee 2012), therefore, very few naturally formed lakes exist within the region. As a result, all of the major standing water bodies within the Piedmont are human-engineered reservoirs.

*The Significance of Reservoirs*

Reservoir systems are common features throughout the global landscape. Some of the oldest reservoirs were constructed over 6,000 years ago, such as the one formed by the Kosheish Dam along the Nile River in 4900 BC (Jorgensen et al. 2005; Milanovic 2012). Today, an
estimated 16.7 million reservoirs exist worldwide (Lehner et al. 2011). Estimates of the total global surface area that they occupy range from 260,000 km$^2$ to as much as 1,500,000 km$^2$ (St. Louis et al. 2000; Messager et al. 2016). Gleick (2000) reported that the total global storage capacity of reservoirs is over 8,000 km$^3$ or about 10% of the total terrestrial freshwater storage.

Reservoirs provide cultural, supporting, regulating, and provisioning ecosystem services. Some common uses include flood control, agricultural irrigation, navigation, fish production/aquaculture, recreation, energy production, industrial assistance, and conservation (Kennedy and Gaugush 1988; Mallin et al. 1994; Straskraba 2005; Tundisi and Tundisi 2012; Nawrocki 2016; Youth et al. 2016). Worldwide, many communities rely on reservoir systems to support growing populations and depend on having a high-functioning resource with long term sustainability (Avakyan 1967; Baron et al. 2002). Because reservoirs are alterations of the natural landscape, they are closely related to local and global ecosystem health and need to be properly managed to ensure the wellbeing of the environment and society (Suen and Eheart 2006; Jager and Smith 2008; Ho and Goethals 2019).

The majority of reservoir projects in the United States have been completed in areas where natural lakes do not occur, which is mainly in unglaciated mid-latitude regions, and coincide with areas where water shortage is a high concern (Thornton 1980; Kennedy and Walker 1990; Jorgensen et al. 2005). Reservoir construction in the United States is considerably slower than it has been in the past, which is likely due to the loss of suitable area, the increased efficiency of other power generation options in replacement of hydropower, and a shift in the political views of some in “big dam” ethics (Benson 1982; Rosenberg et al. 2000; Billington et al. 2013).
Currently, the human population density within the southern Piedmont is undergoing a period of rapid expansion and landscape development is putting significant pressure on available freshwater resources (Lenat and Crawford 1994; Walters et al. 2005; Freeman and Marcinek 2006). Conroy et al. (2003) reported that nearly all of the major river systems in the southern Piedmont are influenced by flow manipulation via impoundment systems. Major Piedmont reservoirs include John H. Kerr Reservoir (VA), J. Strom Thurmond Reservoir (GA/SC), Lake Hartwell (GA), Lake Murray (SC) and Lake Lanier (GA) (Ruddy and Hitt 1990).

Reservoir Limnology

To manage reservoir systems well, it is important to understand the inherent biological, physical, and chemical processes that define how they function. Despite filling a similar niche within the landscape by sustaining aquatic ecosystems and hydrological processes, reservoirs and glacial lakes are different and should be treated as separate types of systems (Thornton 1980). In contrast to glacial lakes, reservoirs are relatively young and have been engineered into the landscape as opposed to evolving naturally within the landscape (Straskraba 2005). Reservoirs often experience more dramatic, short-term changes when compared to glacial lakes that most often undergo gradual successional change. Events that impact reservoir stability can be human-induced, such as manipulation of water levels, or can occur due to natural circumstances such as heavy wind or rain events (Tundisi and Tundisi 2012). Consequently, reservoirs tend to experience higher shoreline disturbance through frequent water level fluctuations and have more unstable water quality conditions due to short water residence times and minimal autochthonous sources of nutrients when compared to glacial lakes (Geraldes and Boavida 2005). These factors contribute to the potential success of submersed aquatic vegetation (SAV) in reservoir systems.
and ultimately affect all levels of the aquatic food web, reservoir water quality, and associated hydrological processes (Madsen et al. 2001).

Aquatic Plant Management in Reservoirs

Native aquatic macrophytes play an essential role in aquatic ecosystems by providing food and habitat to higher trophic levels, participating in the primary production of dissolved oxygen, stabilizing sediments and shorelines through root formation, and consuming excess nutrients in the water column (Madsen et al. 2001; Pokorny and Kvet 2004; Cronin et al. 2006; Xu et al. 2020). Like many other biological organisms, aquatic macrophytes are highly dependent on their surrounding environment for successful growth and reproduction. Generally, aquatic plant species favor habitats that have minimal disturbance (Furey et al. 2004). Because of this, many reservoir systems do not provide ideal habitat for aquatic macrophytes (Pelteir and Welch 1970; Wetzel 1990; Bates and Smith 1994; Havel et al. 2005; Mjelde et al. 2013). However, some species of aquatic plants have been recognized as ecosystem engineers as they can drive local environmental factors towards more suitable conditions for their growth (Koch 2001; Emery-Butcher et al. 2020).

In the United States, many freshwater resources are threatened by the introduction of non-native aquatic plant species (Hussner et al. 2017). Due to having low natural drivers of population suppression, non-native species populations are not balanced by ecosystem processes and can have undesirable impacts on the environment. Non-native aquatic plant species can be further classified as invasive or nuisance species if they have negative impacts on the economy, environment, or human health in their introduced range (Lodge et al. 2006). Invasive species tend to have weedy characteristics that enhance their ability to tolerate environmental
disturbance (Baker 1974; Wilcox and Meeker 1991; Barrat-Segretain and Cellot 2007; Schooler et al. 2010; Sousa 2011). For example, Poovey and Kay (1998) found that short term water level drawdowns did not suppress invasive aquatic plant growth as would be expected and, instead, potentially stimulated an increase in propagule production when compared to plants that were not exposed to water level manipulation.

According to the USGS Nonindigenous Aquatic Species (NAS) Database, 30 of the 95 (31%) major reservoir systems in the Piedmont ecoregion have been impacted by documented infestations of hydrilla (*Hydrilla verticillata* (L.f.) Royle), a non-native, invasive aquatic macrophyte (USGS 2020a; Figure 1). If left unmanaged, nuisance aquatic plant species such as hydrilla threaten the societal and ecological functions of Piedmont reservoir systems because of their aggressive growth and reproductive capabilities (Thomaz et al. 2009). Reservoirs are especially vulnerable to aquatic plant invasions as their infrastructure can be compromised by dense vegetative growth that clogs intake pipes and generators and limit their ability to perform essential functions such as power generation and the manipulation of water levels (Kirk and Henderson 2006; Getsinger et al. 2014).

**Hydrilla verticillata**

*Overview*

The genus *Hydrilla* is a monotypic taxonomic group containing a single species – *H. verticillata*. Hydrilla is an obligate submerged aquatic macrophyte belonging to the Hydrocharitaceae family. Some of its closest phylogenetic relatives include the genera of *Vallisneria*, *Nechamandra*, and *Maidenia* (Chen et al. 2012). Hydrilla is considered native to Asia but has now expanded its range to all continents except for Antarctica (Cook and Luond
In most regions where it has been introduced, hydrilla tends to exhibit the characteristics of an aggressive, weedy species. This is not the case, however, in select locations within central and eastern Europe where there is concern that hydrilla may go extinct (Efremov et al. 2018). To manage its spread, the United States has listed hydrilla as a Federal Noxious Weed and its import, export, or movement within the country is prohibited under the Plant Protection Act of 2000.

*Introduction and History in the United States*

Like many other plant species, multiple biotypes (strains) of hydrilla exist worldwide. In the case of hydrilla, biotypes are genetically isolated by sexual structures. The dioecious strain is characterized by the production of unisexual flowers on separate plants. A monoecious strain also exists in which unisexual flowers are produced on the same plant. Additionally, a morphologically-distinct strain of hydrilla was recently discovered in Connecticut within the Connecticut River system (Tippery et al. 2020). The sexual reproductive behaviors of this population are not yet known.

The first introduction of hydrilla in the United States was recorded in Florida in the 1950s after a release of aquarium pants occurred in the local canal systems (Williams 1980). While the original reports of this population did not differentiate between biotypes, it is now well understood that this original introduction was of the dioecious biotype and it likely originated from India (Madeira et al. 1991). Today, dioecious hydrilla has been recorded in 18 states within the United States (USGS 2020a; Figure 2). In general, most of the states where dioecious hydrilla has been found occur in southern latitudes.
Later in 1980, the first introduction of the monoecious biotype was documented somewhat simultaneously in multiple Mid-Atlantic states including Delaware, Washington D.C., and North Carolina (True-Meadows et al. 2016). It is speculated that these introductions were the result of misidentified aquarium plants that were transplanted from the same source (True-Meadows et al. 2016). Monoecious hydrilla has now spread into 25 states in the United States (USGS 2020a; Figure 2).

In 2016, an uncharacteristic population of hydrilla was reported in Connecticut within the Connecticut River system (Tippery et al. 2020). A molecular investigation by Tippery et al. (2020) revealed that the new strain of hydrilla is genetically unique from all previously-sequenced strains of hydrilla. It is understood that this novel population of hydrilla is most closely related to a native Eurasian strain but is not an identical match. It is hypothesized that this strain may have hybridized in cultivation and, as a result, it may be nearly impossible to trace its exact origin. At this time, no other populations of this novel biotype exist in the United States and further information on its ecological behavior is unknown.

Ecology

The rapid spread of hydrilla throughout the United States may be attributed to its broad habitat requirements and multiple reproductive pathways. Dioecious hydrilla can successfully reproduce by subterranean turions (tubers), axillary turions (turions), stolons, and stem fragmentation (Netherand 1997; Madsen and Smith 1999). Monoecious hydrilla is also able to reproduce by viable seed (Lal and Gopal 1993; Steward 1993). Of the reproductive methods, stem fragmentation is important for long-distance dispersal and tuber production ensures the longevity of populations throughout multiple growing seasons. Studies have described that a
single monoecious hydrilla plant can produce over 4,500 tubers within a single growing season and those tubers can remain viable in sediments for at least 6 years (Sutton et al. 1992; Nawrocki et al. 2016). Vectors of hydrilla movement over land include shipment/aquarium trade, boat traffic from infested to uninfested waters, flood events and, potentially, waterfowl migration (Johnstone et al. 1985; Langeland 1996; Kay and Hoyle 2001; Baniszewski et al. 2016).

Hydrilla can thrive in both lentic and lotic systems and has been documented in water depths of 15 m but is most commonly found no deeper than 3 m (Langeland 1996; Hofstra et al. 2010; Efremov et al. 2018). Hydrilla can grow under both high and low light conditions and can even successfully develop in total darkness (Haug et al. 2019). This explains why hydrilla can occupy deep, turbid, or densely vegetated habitats.

Research has demonstrated that water temperature significantly impacts hydrilla growth. Monoecious hydrilla tubers have exhibited a sprouting response to temperature from 11 to 15°C and dioecious hydrilla tubers have been noted to sprout in warmer water temperatures of at least 18 to 35°C (Haller et al. 1975; Harlan et al. 1985; Steward and Van 1987; Rybicki and Carter 2002). After sprouting, it has been observed that both monoecious and dioecious hydrilla produce peak biomass at water temperatures between 28 and 32°C (McFarland and Barko 1987).

Both monoecious and dioecious hydrilla can withstand water chemistries with low salinity (9 - 12 ppt), allowing it to colonize estuarine waterways and waterbodies that are subjected to saltwater intrusion (Carter et al. 1987; Steward and Van 1987; Regan 2017). Multiple studies have confirmed that both biotypes can also survive under varying levels of pH (4.0 – 10.5) with the optimum of pH 7 – 8 (Efremov et al. 2018; Song et al. 2018). Additionally, hydrilla is one of just a few aquatic plant species that is capable of both C₃ and C₄ photosynthetic pathways through a facultative response which gives individuals a competitive advantage in
environments where dissolved carbon dioxide concentrations are low (Bowes et al. 2002). Hydrilla is most productive in nutrient-rich systems, with nitrogen concentrations having a significant impact on growth (Van et al. 1999; Bellinger and Davis 2018).

Monoecious and dioecious hydrilla exhibit contrasting growth habits and phenological dynamics. Where dioecious hydrilla has been documented in the United States, it tends to have a perennial growth pattern in which its biomass does not completely die back in the winter. Conversely, monoecious hydrilla tends to have an annual growth pattern. These observations agree with those of all other herbs – as a general rule most dioecious plants are perennials and monoecious plants are annuals (Bawa 1980). In North Carolina, the growth of monoecious hydrilla from axillary turions begins in the spring after sediment temperatures pass the 11°C threshold (March – May), tubers continue to sprout throughout the summer months (April – August), and peak biomass has been documented in mid-Fall just before winter dieback occurs (Harlan et al. 1985; Meadows 2013). Monoecious hydrilla tuber formation is initiated by the onset of short daylength and occurs from June through October in North Carolina (Harlan et al. 1985; Netherland 1997). In its southern range in the United States, the development of dioecious hydrilla from overwintering stems and root crowns has been documented from May through September and tuber formation tends to begin in October and lasts throughout the winter (Netherland 1997; Madsen and Owens 1998). Phenological information is important to consider when planning management strategies for hydrilla and treatment timings should be prescribed appropriately for each respective biotype.
Identification

Hydrilla is a rooted, obligate submersed aquatic plant species and is highly polymorphic depending on environmental conditions (Verkleij et al. 1983). Leaves are lanceolate with serrated margins and arranged in whorls of 3 – 8 (most commonly 5) in both monoecious and dioecious biotypes (Cook and Luond 1982). The leaves of dioecious hydrilla are often longer, wider, and have more pronounced midrib spines on their underside than those of monoecious hydrilla. Stems are thin, brittle and often support adventitious roots. Monoecious hydrilla often has more stem branching and stoloniferous growth before reaching the water surface when compared to dioecious hydrilla which does not tend to branch until stems are high in the water column. Both monoecious and dioecious hydrilla exhibit rapid stem elongation (up to nearly 200 inches per day) and form dense canopy mats that often shade out other species beneath them in the water column (Yeo et al. 1984; Glomski and Netherland 2012). In comparison to the morphology of monoecious and dioecious hydrilla, the novel Connecticut River strain of hydrilla has been observed to have more leaves per whorl and longer internode distances and has been noted to be more robust and darker in color (Tippery et al. 2020).

Both monoecious and dioecious hydrilla form flowers. They are small, inconspicuous, and infrequently present and therefore should not be relied on for identification purposes. Only female-flowering dioecious plants have been documented in the United States. When in bloom, female flowers are solitary and have three small white sepals (usually less than 3 mm long) and three clear radial petals (Cook and Luond 1982). The male flowers of monoecious hydrilla are morphologically similar to female flowers however they are often smaller and sometimes brown or red in color (Langeland 1996). Female flowers arise from stem apices and float on the surface of the water. At maturity, male flowers are released from a spathe and freely float for a short
period of time before they disperse pollen into the air (Yeo et al 1984; Haynes 1988). Pollination occurs at the water’s surface and viable seeds are produced (Lal and Gopal 1993).

Both biotypes also produce hibernacula in the form of subterranean turions (tubers) and auxiliary turions (turions). Tubers vary in shape and color and are mainly composed of starch (Haug 2018). They develop on rhizomes and can be found buried in sediments up to 30 cm deep. When mature, they detach from the rhizome and are completely self-sufficient. Axillary turions are smaller than tubers are most often green in color, cone-shaped, and are spinier than tubers (Langeland 1996). Research has shown that dioecious hydrilla produces fewer but larger tubers than monoecious hydrilla when exposed to identical environmental conditions (Steward and Van 1987).

The morphology and growth habit of hydrilla is similar to other submersed aquatic plant species, especially that of *Egeria densa* (Planch) and *Elodea spp.* (Blackburn et al. 1969; Les et al. 1997). *E. densa* can be distinguished from hydrilla by its smooth midrib and short internode distance. Occasionally, *E. densa* also exhibits “double nodes” in which two leaf whorls are essentially superimposed on top of one another, establishing a region that is important for stem branching and vegetative growth (Getsinger and Dillon 1984). Hydrilla has not been observed to produce such a region along its stem. A key distinguishing factor between hydrilla and *Elodea spp.* is the leaf arrangement. Leaves of *Elodea spp.* are consistently arranged in whorls of 3 and do not have serrated margins. In instances where these plants are not easily identifiable through visual methods, molecular analysis can more accurately discern differences between species (Madeira et al. 2004; Rybicki et al. 2013).

In addition to molecular tests, recent research has begun to assess the applicability of analysis of environmental DNA (eDNA) to accurately identify and quantify the presence of aquatic plant
species. With the use of eDNA, it is possible to gain information on organism presence, distribution, and even population size without needing to physically handle specimens as all organisms leave traceable genetic information simply by existing within the environment. The ability to capture this genetic information and synthesize it into valuable information is an emerging tool that has proven to be useful in many scientific fields outside of aquatic weed science such as herpetology, ichthyology, mammalogy, and entomology where small and/or inconspicuous study subjects make it difficult to get an accurate representation of organism distribution in nature (Bohmann et al. 2014; Thomsen and Willerslev 2015). The eDNA method can be a viable option for the detection of some aquatic plant species including hydrilla, *Egeria densa* and *Elodea canadensis* (Matsuhashi et al. 2016; Angles d’Auriac et al. 2019).

**Impacts**

Hydrilla invasions have impacts on a multi-dimensional scale. Freshwater resource stakeholders are often concerned about the societal and economic expenses that can be incurred due to hydrilla growth at nuisance levels. Activities such as boating, swimming, and fishing may be impeded by hydrilla because of issues associated with access, safety, and decreased sportfish populations (Colle et al. 1987). Additionally, it has been shown that the property values of lakeside residences have been reduced by the abundant presence of exotic species (Horsch and Lewis 2009). In reservoirs that generate hydroelectric power, floating mats of hydrilla can clog generators and water intake pipes and lead to a significant loss in power generation (and its associated monetary earnings) as evidenced by such events at Tom Miller Dam on Lake Austin (TX) in 2003 and the St. Stephen hydroelectric facility on Lake Marion (SC) in 1991 (Kirk and Henderson 2006; Getsinger et al. 2014). Similarly, reservoirs that are used for agricultural
irrigation purposes can have reduced efficiency when infested with densely-growing aquatic vegetation, impacting yield capacity and impeding operation of the reservoir infrastructure (Manz and Westhoff 1987).

In the United States, a significant amount of funding has been allocated to hydrilla-related control and prevention projects. In Florida alone, nearly $750 million of state and local government funding has been dedicated to hydrilla management in the last 30 years (approx. $25 million per year) (Madsen and Wersal 2012). Economic models have confirmed that these efforts are cost-effective as there is a net-benefit of almost $60 million per year for aquatic invasive species control in Florida (Adams and Lee 2007). Unfortunately, despite these efforts and investments, hydrilla has continued to spread, possibly due to political and cultural obstacles, and is now invading 70% of the freshwater resources in the state (Makowski and Finkl 2019). It is conceivable that the spread of this troublesome aquatic plant will eventually subside in the United States with increased research coupled with continuous and intensified control efforts.

From an ecological standpoint, uncontrolled hydrilla growth can impact the environment in many ways. In a study of the Tennessee-Tombigbee Waterway in Alabama, Miranda and Hodges (2000) found that morning samples of dissolved oxygen concentrations in areas of dense plant growth (plant species were reported to be a mix of hydrilla, *Ludwigia spp*, *Myriophyllum spicatum* and *Ceratophyllum demersum*) were, on average, nearly 5 mg l\(^{-1}\) lower than those in areas of open water. When dense hydrilla beds are formed, the surrounding aquatic environment is often not able to support the respiration demands of the biomass which leads to shifts in water chemistry. These impacts influence ecosystem dynamics and can have negative consequences such as increased fish kills and changes in the structure of local aquatic communities at multiple levels throughout the food web (Schmitz and Osborne 1984; Kirk and Henderson 2006). This
phenomenon was observed in Lake Marion (SC) in 1991 after dense hydrilla biomass led to the largest documented fish kill in South Carolina's history (Kirk and Henderson 2006).

Studies have shown that hydrilla can outcompete many native species including *Vallisneria americana* and *Elodea canadensis*, as well as other invasive species such as *Egeria densa*, *Potamogeton crispus* and *Myriophyllum spicatum* (Van et al. 1999; Mony et al. 2007; Meadows 2013; Silveira et al. 2018). This competition from aggressive invasive species ultimately decreases the biodiversity of submersed aquatic vegetation and reduces habitat availability for aquatic organisms that require specific ecological niches for survival (Lishawa et al. 2019; Ceschin et al. 2020).

Recently, hydrilla has been identified as a host for a novel epiphytic cyanobacterial species, *Aetokthonos hydrillicola*, belonging to the order Nostocales (Wilde et al. 2005; Wilde et al. 2014). It is hypothesized that *A. hydrillicola* is toxigenic and its associated toxins are related to the development of a neurological condition called Vacuolar Myelinopathy (VM) in organisms at the top of the food chain – most notably bald eagles (Wilde et al. 2013). VM is characterized by the growth of microscopic lesions in the central nervous system of susceptible organisms and causes ataxia, loss of reflex, abnormal swimming patterns, and compromised flight (Larsen et al. 2002). These physical symptoms cause a decline in fitness which ultimately assists in the continued transfer of *A. hydrillicola* toxins throughout the food web.

Many Piedmont reservoirs with infestations of hydrilla have tested positive for *A. hydrillicola*. These reservoirs include J. Strom Thurmond (GA/SC), Lake Juliette (GA), Woodlake Reservoir (NC), Lake Murray (SC), Smith Reservoir (GA), and Lake Horton (GA) (Wilde et al. 2014). The appropriate management of hydrilla in these systems and the prevention
of its future spread into related reservoirs will help mitigate the impact that A. *hydrillicola* has on aquatic ecosystems in the southeastern United States.

*Management*

Today, many techniques for hydrilla management are employed in the United States. These methods are of biological, mechanical, chemical, physical, or cultural means (Gettys et al. 2014). Biological control employs organisms that are naturally adapted to interact with the target species and suppress their growth through ecological pathways. Methods that involve the manipulation of the environment to create unsuitable growing conditions for the target species are considered to be physical control methods. Mechanical control methods are options that involve machinery that physically removes biomass out of a system. Chemical control methods utilize herbicide technology to target undesirable species. Lastly, cultural control methods are those that are related to public education and perception change and the application of long-term management plans that increase prevention strategies for hydrilla introduction and spread. Cultural control methods can be a powerful and important tool for hydrilla management practices because its spread is largely attributed to anthropogenic activities (Johnstone et al. 2001; Kay and Hoyle 2005).

Of the biological control options to manage hydrilla, the most widely deployed in the United States is the triploid grass carp (*Ctenopharyngodon idella*). Grass carp are considered to be a species of herbivorous fish. However, they will also consume other small fish species, insects, worms, and zooplankton in the absence of macrophyte options (Cudmore and Mandrak 2004). Grass carp prefer to consume soft-bodied submersed macrophytes including hydrilla, *Potamogeton, Elodea, and Egeria* (Pine and Anderson 1991). Caution needs to be exercised
when considering grass carp as a control method because they are non-selective and mobile. As such, grass carp can negatively impact native species of aquatic vegetation and higher tropic level interactions within the food web (Pipalova 2006). In appropriate situations, grass carp are cost-effective, efficient, and provide good long-term control of nuisance weeds. Young grass carp can consume hydrilla at rates of over 100% of their body weight and can survive for over 10 years (Chilton and Muoneke 1992).

While other biological control options have been evaluated for hydrilla, an effective option has not yet been determined that can withstand the environmental conditions presented in the United States. For example, the weevil Bagous affinis feeds on hydrilla but requires long-term drawdown periods to complete its life cycle and has not been able to establish a self-sustained population in the United States (Purcell et al. 2019). The tunnel-boring weevil Hydrellia pakistanae has demonstrated high affinity to hydrilla in laboratory experiments and has even been able to establish populations in some regions of the United States since its initial introduction in 1987 (Wheeler and Center 2001). Unfortunately, due to its high sensitivity to cold winters and heavy rain events, H. pakistanae has not been able to establish large enough population densities to effectively reduce impact extensive hydrilla infestations (Wheeler and Center 2001). Current research is evaluating other biological control options in the Hydrellia clade that have native ranges that overlap with that of hydrilla (McCulloch et al. 2019).

Mechanical control options such as hand-pulling and biomass harvesting by cutter boats and removal harvesters offer unsatisfactory control for hydrilla at high population densities. Unless target populations are localized to small areas, hand-pulling is not a cost-effective method and is often time-prohibitive. While other mechanical control options such as the use of weed harvesting boats could be more efficient than hand-pulling, they often cannot effectively manage
hydrilla’s underground rhizomes. Therefore, these methods are only able to suppress a population of hydrilla for a short period of time as its exponential regrowth pattern can result in the production of almost 200 inches of stem tissue per day (Glomski and Netherland 2012). Additionally, many mechanical harvesting tools often foster the spread rather than suppress the growth of hydrilla due to stem fragmentation (Umetsu et al. 2012).

Because of hydrilla’s capacity to occupy waterbodies under a wide range of environmental conditions, physical control methods are difficult to effectively utilize. For example, Haug et al. (2019) determined that hydrilla can survive in the absence of light for at least 10 weeks which eliminates control options that rely on shading such as benthic barriers and pond dyes. Another method for aquatic plant control is the use of manipulated water level drawdowns to target hydrilla growth. However, drawdowns are ineffective at suppressing hydrilla growth because tuber viability remains high even when left in non-inundated, damp sediments for long periods (Doyle and Smart 2001). Other options for physical control can involve water quality manipulation such as increasing dissolved oxygen concentrations through aeration (Cooley et al. 1980) but may ultimately have many undesirable non-target impacts.

Chemical control methods for hydrilla have been widely studied and employed. Like all other herbicides, aquatic herbicides can be classified as having either contact or systemic modes of action. When considering all of the control options for hydrilla, some of the systemic herbicides can be the most selective choice (with the exception to hand-pulling) and will therefore minimize impacts on non-target macrophyte species. There are currently 17 registered active ingredients that have been approved by the US Environmental Protection Agency for use in aquatic systems (UF IFAS 2020). Of these, less than half are effective for hydrilla
management and include fluridone, copper, diquat, endothall, and florpyrauxifen-benzyl (Richardson 2008; Netherland and Richardson 2016; Hussner et al. 2017; Haug 2018).

Recently, resistance to fluridone through a somatic point mutation has been discovered in some dioecious hydrilla populations in Florida (Dayan and Netherland 2005; Netherland and Jones 2015). This finding is especially worrisome because of the large-scale, global use of fluridone in the United States and the limited alternative options for systemic chemical control (Richardson 2008). Best management practices indicate that further resistance issues can be mitigated by avoiding repeated applications of products with similar modes of actions at low dose rates and through diversification of management approaches (Norsworthy et al. 2012).

Cultural management techniques are important tools for aquatic plant management. These include the development of prevention strategies such as early detection rapid response protocols, education and outreach materials, and legislation related to the transport of invasive species. Additionally, having long-term management plans in place for infested waterbodies will aid in the reduction of populations and mitigate further spread to vulnerable waterbodies. Lastly, the continued research on aquatic invasive species ecology and management will provide the information needed to develop effective resources that can be used by lake managers and other public water users in their efforts to conserve and protect water resources globally. In a study that assessed the success of outreach efforts to Illinois boaters, those who recognized prevention slogans and were able to recognize species of concern were also those that were most likely to demonstrate behaviors that discouraged the spread of invasive species (Cole et al. 2016).

Every management situation presents unique challenges; therefore it is important to be prepared with multiple options for control. Many management plans incorporate integrated pest management techniques in which multiple methods are implemented simultaneously to achieve
maximum control of target species with minimal damage to non-target species. For example, it is common to utilize biological control options, such as grass carp, in conjunction with a chemical-based management plan to ensure that excess growth that is not captured by herbicide treatments is addressed by the biological control agent.

Effective management is highly influenced by species ecology and biology. As such, the timing of control efforts must be in sync with the most appropriate phenological stage. Ideally, hydrilla biomass should be targeted after tuber sprouting events but before new tuber development is initiated to best control aboveground biomass before it is able to add propagules to the sediment seed bank. With applications of systemic herbicides, timing should be prioritized based on the period of the most active growth to allow for the highest absorption of the active ingredient. Because monoecious and dioecious hydrilla has different phenological patterns and respond differently to environmental cues, biotype-specific management protocols need to be employed.

**Philpott Reservoir**

Philpott Reservoir is a 1,165 hectare (2,880 acre) impoundment located in the Piedmont region of southcentral Virginia (36.811592, -80.058381; Figure 3). The USACE maintains the reservoir for hydroelectric power generation and flood control (USACE 2020). In addition to its primary ecosystem services, Philpott Reservoir provides important wildlife habitat as well as a variety of recreational opportunities for public water users.

The USACE completed the construction of the Philpott Dam on the Smith River in 1953, an event that officially marked the incorporation of the reservoir into the landscape (Hartwig 1998). At full pool, Philpott Reservoir’s shoreline length measures approximately 100 miles (161
km), its average depth is 60.7 feet (18.3 m) and its maximum depth reaches 191 feet (58.3 m) (Hartwig 1998). Based on the last 15 years of record, the water surface elevation of the reservoir has averaged 971.92 feet (296.2 m) with minimum and maximum recorded surface elevations measuring 965.55 ft (294.3 m) and 974.98 ft (297.1 m), respectively (USGS 2020b). Because the USACE owns much of the land surrounding Philpott Reservoir, most of its shoreline has remained undeveloped except for some boat access areas, public parks and campgrounds, and hiking trails.

Many other minor tributary streams contribute to flow into Philpott Reservoir and include Rennet Bag Creek, Ryan’s Branch, Beards Creek, Nicholas Creek, Goblintown Creek/Fairystone Lake, and Bowens Creek (Figure 3). Philpott Reservoir is the first in a series of impoundments within the Roanoke River basin and is followed by John H. Kerr Reservoir, Lake Gaston, and Roanoke Rapids Reservoir before connecting with the Albemarle Sound in eastern North Carolina (Figure 3). Smith Mountain Lake and Leesville Reservoir are also significant impoundments within the Roanoke River basin and regulate flow along the Roanoke River in central Virginia before flowing into John H. Kerr Reservoir.

The Virginia Department of Environmental Quality (VA DEQ) classifies Philpott Reservoir as a cool water fishery based on a metric that is dependent on year-round striped bass, hybrid striped bass, and/or walleye populations (Gilinsky 2009); however, warm water, cool water, and coldwater fish species have all been documented in the reservoir (Hartwig 1998). Extensive research efforts have been focused on the population size and regional habitat suitability for the Roanoke Logperch (*Percina rex*), an endangered fish species that has been documented upstream and downstream of Philpott Reservoir in the Smith River, but not yet in the reservoir itself (Roberts and Angermeier 2012).
Approximately 15 hectares (37 acres) of dioecious hydrilla was documented growing densely along the shoreline of Philpott Reservoir in 2017 (T. Harris, personal communication). This population of dioecious hydrilla is located in the northern extreme of its introduced range in the United States. It’s continued expansion throughout the reservoir threatens the integrity of the Philpott Dam and the fishery within the system, which together account for over $1.2 million in economic value per year (Hartwig 1998). Additionally, due to Philpott Reservoir’s position at the start of the chain of reservoirs within the Roanoke River watershed, this population has the potential to spread downstream into other highly valuable systems, including John H. Kerr reservoir and Lake Gaston, and to other nearby waterbodies via boat traffic. It is imperative that the ecological aspects of this population of hydrilla are well understood in order to most effectively manage its spread to ultimately reduce its potential impact to the environment.

**Research Objectives**

The purpose of this research was to gain a better understanding of the ecology and management options for the uncharacteristic population of dioecious hydrilla in Philpott Reservoir. Specifically, this work addressed three research questions: 1) How is dioecious hydrilla spatially distributed throughout Philpott Reservoir and what are some physical environmental factors that drive its establishment and success? 2) Does a northern population of dioicous hydrilla exhibit different phenological patterns when compared to a southern population of dioecious hydrilla? 3) What are the best management practices for dioecious hydrilla control in Philpott Reservoir?

To address these questions, the hydrilla from Philpott Reservoir was studied on multiple levels which ranged from an isolated laboratory experiment, a small-scale outdoor mesocosm
study, and multiple years of field-based efforts. The goal of this work was to best understand the factors that drive the distribution and success of a northern population of dioecious hydrilla and, in turn, determine the most efficacious management strategy for the long-term sustainability of Philpott Reservoir.
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Figure 1-1: Large reservoirs (normal capacity > 5,000 acre-feet) in the Piedmont ecoregion in the United States. Reservoirs where hydrilla has been documented in the USGS Nonindigenous Aquatic Species database are denoted by ‘●’. Philpott Reservoir is denoted by ‘★’. All other reservoirs are represented by ‘○’.
Figure 1-2: Distribution of documented monoecious and dioecious hydrilla populations in the contiguous United States based on average year of introduction to HUC-8 watersheds. Design updated from: U.S. Geological Survey, 2016, Hydrilla verticillata. Nonindigenous Aquatic Species Program, Gainesville, FL. https://nas.er.usgs.gov/XIMAGESERVERX/2016/20161027161554.jpg

Data sources: USGS NAS Database (Accessed Jan 2020); USGS WBD Dataset (Accessed Jan 2020)
**Figure 1-3:** Spatial location of Philpott Reservoir (Patrick/Henry/Franklin Counties, VA) in the United States.
CHAPTER 2: Littoral physical, chemical, and biological characteristics determining submersed aquatic vegetation distribution in freshwater reservoirs

Abstract

Hydrilla (*Hydrilla verticillata* (L.f) Royle) is classified as a Federal noxious aquatic macrophyte in the United States. Philpott Reservoir (VA) supports a dominating population of dioecious hydrilla despite providing unideal conditions for submersed plant growth due to standard reservoir operations which include a high incidence of shoreline disturbance, minimal littoral area, and physically dense sediment composition. Three years of point-intercept surveys for submersed aquatic vegetation (SAV) presence, water quality data, and physical basin characteristics were evaluated using multivariate analyses to determine the factors that may be the most influential to SAV composition and distribution and hydrilla dominance in the reservoir. Analyses utilized ordination, classification, and regression techniques through the application of principal components analyses (PCA), linear discriminant analysis (LDA), and multiple logistic regression (MLR). MLR produced three significant predictors of hydrilla: *Egeria* presence (*p* = 0.0321), secchi depth (*p*<0.0001), and sediment composition (*p* = 0.0092). PCA revealed that these predictors are closely related to other environmental factors including the concentration of total nitrogen in the water column, water pH, and water depth. In an attempt to group species by the collected environmental variables in a constrained space, an LDA model correctly classified 64.4% of points by species presence. These multivariate models suggest that, despite the fairly generalist needs of hydrilla in aquatic ecosystems, the distribution of hydrilla in Philpott Reservoir may be largely driven by the underlying physical properties that influence higher chemical and ecological processes.
Introduction

Aquatic ecosystems offer dynamic habitat for organisms due to the interconnectedness and variation of their abiotic and biotic components. Submersed aquatic vegetation (SAV) interacts with temporal, spatial, chemical, physical, and biological factors in freshwater environments (Lacoul and Freedman 2006). Like all organisms, the success of SAV establishment and distribution within aquatic systems is facilitated by the collective conditions of their surrounding environment. Thus, the presence or absence of aquatic macrophytes can be indicators of overall ecosystem health and trophic state (Tarkowska-Kukuryk and Mieczan 2016). Major abiotic factors that influence the distribution of SAV in freshwater systems include light, temperature, water nutrient content, sediment characteristics, water movement, and frequency of disturbance events (Bornette and Pijulon 2011). Bakker et al. (2013) adds that biotic factors such as propagule availability, herbivory, and competition should also be considered to best understand aquatic macrophyte distribution. Anthropogenic activities can alter these biotic and abiotic factors through the introduction of SAV (invasive or native) to aquatic systems, fisheries management, nutrient pollution, and the manipulation of impounded hydrological systems.

While it is clear that environment affects SAV success, it is also important to note that the presence or absence of SAV can regulate species occurrence within aquatic food webs. For example, by providing habitat for certain zooplankton and epiphytic algal species, native SAV participates in a positive feedback loop that facilitates the enhancement of water clarity and stabilizes nutrient concentrations, which ultimately improves conditions for their self-sustainment (Burkholder and Wetzel 1990; Dodson et al. 2005). The protected zooplankton communities in SAV beds regulate phytoplankton populations through grazing (Timms and
Moss 1984), and the presence of epiphytic algal species aids in the uptake of excess nutrients in the water column. Also, SAV provides food and habitat for organisms of higher trophic levels, stabilizes shorelines through root formation and absorbing energy impacts from waves, and utilizes photosynthesis pathways to contribute to the production of dissolved oxygen and the uptake of dissolved nutrients and carbon dioxide (Boyd 1971; Carpenter and Lodge 1986; Ferreira et al. 2018).

Non-native, invasive SAV, however, can have negative impacts within aquatic food webs. Often, non-native SAV is not naturally limited and can outcompete native species, alter water quality, and reduce biodiversity (Hussner et al. 2017). *Hydrilla verticillata* (L.f.) Royle (“hydrilla”), an obligate aquatic angiosperm belonging to the Hydrocharitaceae family, is a non-native species that has been classified as a noxious weed in the United States (The Plant Protection Act – Title 7 Chapter 104; USDA-NRCS 2020). Hydrilla has been described as “the perfect aquatic weed” with “disastrous” effects in the United States (Verkleij et al. 1983; Langeland 1996). Similar to other plant species with weedy characteristics, hydrilla has a quick growth rate, reproduces via multiple pathways, persists under broad environmental conditions, and can produce high densities of resilient offspring (Sousa et al. 2011; True-Meadows et al. 2016). When left unmanaged, hydrilla is capable of outcompeting native aquatic plant species, reducing habitat availability for fish and other aquatic organisms, altering water quality, clogging intake grates used for hydropower generation, and reducing flow rates in irrigation and drainage canals (Schultz and Dibble 2012).

Hydrilla was first introduced to the United States in the 1950s (Williams 1980) and has since become widespread. The distribution of hydrilla throughout the country is generally attributed to its vegetative reproductive capabilities, however, separate introductions have
occurred that have aided geographic spread. The first introduction of hydilla in the United States was recorded in Florida and was of a female-flowering dioecious strain (Joyce 1992). Later, in the 1980s, a monoecious strain was introduced to Delaware and nearby areas including the Potomac River (VA) (Steward et al. 1984; True-Meadows et al. 2016). At around the same time, an established monoecious hydilla population was also documented in the Piedmont region of North Carolina (Harlan et al. 1985). Most recently, a novel strain of hydilla has been documented in Connecticut (Tippery et al. 2020). To date, most populations of dioecious hydilla are spread across the southern United States and most monoecious hydilla populations have been observed from North Carolina northward. However, unusually-located populations of both biotypes have been documented outside of their “normal” respective ranges in the country (Madeira et al. 2007; Alix and Scribailo 2009).

Entities concerned with the distribution of native and non-native species of aquatic vegetation, such as state and local agencies, consultants, and citizen groups, have developed monitoring programs to aid in the documentation of aquatic plant communities throughout time. Such programs can range in intensity and may require significant amounts of time and resources to accomplish. Nevertheless, these efforts are prioritized because they provide valuable information about the health of aquatic ecosystems which ultimately aids in the prescription of appropriate management practices when necessary.

The SAV community of Philpott Reservoir, a 1,165 hectare (2,880 acre) impoundment in southcentral Virginia, is relatively unstudied. The physical characteristics of Philpott Reservoir are similar to those of other reservoirs as it is geologically young (formed approximately 70 years ago), experiences high shoreline disturbance due to water level fluctuation, and has a sparse native SAV community (Thornton et al. 1990). These characteristics favor the
establishment and success of invasive plant species, especially hydrilla, that thrive in disturbed conditions and are habitat generalists that can grow under a broad range of environmental factors (Langeland 1996). The presence of hydrilla was documented in Philpott Reservoir in 2014 by the United States Army Corps of Engineers (USACE) (M. Hosey, personal communication). In 2017, the hydrilla population was confirmed to be of the dioecious biotype after genetic analysis (R. Thum, personal communication), making it the northernmost-known find of the dioecious strain of hydrilla in an Atlantic state.

To analyze dynamics of SAV in Philpott Reservoir, a geographic information system (GIS) was used to interpret discrete data points and adapt them into a general model based on spatial proximity. GIS is a powerful tool that has been widely utilized for investigations in aquatic plant community dynamics for nearly 50 years. In 1975, the USACE Aquatic Plant Control Research Program outlined their activities related to the remote sensing of aquatic plants (Long 1979). This was some of the earliest work that considered the use of GIS for aquatic plant identification, mapping, and management. Today, GIS has been applied to many facets of the aquatic plant management field including detecting, mapping, modeling, and land conservation (Boylen et al. 2006; Valley 2016; Rodriguez-Merino et al. 2020; Song and Park 2020).

In conjunction with GIS, multivariate statistical models were used to evaluate the relationships between the presence or absence of SAV and some of the physical and chemical characteristics of Philpott Reservoir. Multivariate analyses are those that consider the simultaneous relationships of many measured properties of the same individual and are commonly used in ecological studies to capture patterns and structure within complex environmental settings (Gauch 1982). We tested the applicability of an ordination technique (principal components analysis - PCA), a classification technique (linear discriminant analysis -
LDA) and a regression technique (multiple logistic regression - MLR). Ordination analyses are those that describe data and summarize trends by reducing dimensionality and comparing the variation between variates (Kenkel et al. 2002). Classification analyses attempt to group continuous observational variables into categorical responses (Greenacre and Primicerio 2013). And, the most widely applied method, regression, uses combinations of explanatory variables to predict a continuous, applicable response (Greenacre and Primicerio 2013). The goal of this study was to apply our collected data to these statistical models and determine how SAV has changed in Philpott Reservoir over time and identify the significant environmental factors that may be influencing hydrilla presence or absence within the system.

**Methods**

**Study Site**

Philpott Reservoir (36.811592, -80.058381) is located at the conjunction of Henry, Patrick, and Franklin Counties in southcentral Virginia. It was formed after the completion of the Philpott Dam along the Smith River in 1953 and is maintained by USACE for flood control, hydropower generation, and recreation. At full pool, Philpott Reservoir spans 1,1165 hectare (2,880 acres) with approximately 100 miles of shoreline. Philpott Reservoir is the first impoundment in a series of several within the Roanoke Rapids watershed and eventually drains to the Albemarle Sound in eastern North Carolina.

In 2014, USACE personnel conducted the first documented SAV survey at Philpott Reservoir. At this time, hydrilla, *Egeria densa* (Planch), *Najas guadalupensis* (Sprengel) Magnus, *Najas minor* (All.), and the macroalga *Chara spp*, were documented at randomly selected points throughout the littoral zone (M. Hosey, personal communication). The survey
was repeated by USACE in 2015 and 2016, incorporating additional survey points where greater resolution was deemed necessary. North Carolina State University researchers began work in 2017 at Philpott Reservoir and initiated an in-depth, systematic survey methodology for annual monitoring efforts. Species presence and absence patterns within the reservoir generally agreed with the previous records of USACE efforts.

Data Acquisition:

SAV presence within the littoral zone of Philpott Reservoir was consistently documented for three consecutive years (2017 – 2019) via a boat-based point-intercept survey. The survey was completed in August or September of each year when plant biomass was sufficiently established to document the maximum number of species present. Survey points were randomly positioned in approximately 500 m intervals along the shoreline of Philpott Reservoir prior to survey initiation in ArcMap 10.61. The spatial distribution of survey points remained unchanged throughout all years of survey. For on-board navigational purposes, the survey point shapefile was converted to .gpx and uploaded to a Lowrance HDS-7 commercial-grade echosounder2. At each point, a double-sided throw rake was tossed towards the shoreline and collected vegetation was identified and recorded. Points where species were present were assigned a value of 1 for each unique species and those with species absent were assigned a value of 0. The number of points surveyed each year ranged from 235 to 253 depending on reservoir water level and the associated accessibility to shallow areas.

In conjunction with the point intercept survey, hydroacoustic data points were passively captured along the approximate 3 m contour around the full reservoir shoreline from a boat-mounted transducer and an associated Lowrance HDS 7 commercial grade ecosounding device2.
All collected hydroacoustic data were instantaneously logged to a microSD card in .sl2 format in 1 hr (approximately 1 GB) segments and uploaded to a cloud-based post-processing service (ciBioBase\textsuperscript{3}). In the ciBioBase online platform, the collected hydroacoustic data were validated and the water depth and sediment composition were geostatistically interpolated through an automated algorithm. The ciBioBase internal algorithm assigned the sediment composition a value from 0 (soft) to 0.5 (hard) depending on second echo amplitudes generated by the sonar signal sent through the hydroacoustic technology. The trip segments were merged by year and data offsets were applied to standardize for water surface elevation.

Discrete measurements of water quality collected by the Virginia Department of Environmental Quality (VA DEQ) at six long-term monitoring stations within Philpott Reservoir were accessed with permission through the US EPA’s Water Quality Portal (Read et al. 2017; NWQMC 2020). The data were filtered by year and all samples recorded before 2015 were removed. Parameters measured with sufficient data for analysis included secchi depth ("secchi") (ft), pH, and total nitrogen ("nitrogen") (mg/L). A two-way factorial ANOVA determined that there was no significant interaction between station location and year for each monitored parameter (p = 0.4929 (secchi); p = 0.2504 (nitrogen); p = 0.4604 (pH)), so the measured values were pooled over year at each monitoring station for subsequent analysis.

Water surface elevation data was obtained from a USGS monitoring situated at Philpott Dam (Gage ID = USGS 02071900; USGS 2020). The National Water Information System’s web interface was queried for daily records of elevation beginning in August 2016. Those records were averaged by year from August to August to determine the status of water level between survey times. Means were subjected to an ANOVA and Tukey HSD test in JMP Pro (v. 14.3.0;
Cary, NC; SAS Institute, Inc.) to determine if there was a difference in water level between surveys and for mean separation, respectively.

Data Processing:

Because active management of hydrilla occurred between years of data collection, it was unsuitable to compound vegetation survey results into a robust continuous variable such as probability of occurrence. Instead, SAV presence at each surveyed point was averaged over year by species and all points with a value > 0 were assigned value of 1 such that all points with at least one occurrence a species over the three survey years was considered as suitable habitat for that species. The resultant binary SAV presence data was joined with their respective georeferenced survey points.

Water quality data were separately interpolated within the waterbody's surface using the "Spline with Barriers" (Spatial Analyst) tool. The spline interpolation technique is a deterministic method that uses minimum curvature to predict a surface while preserving trends (Arun 2013). It was important to choose a technique that allowed for the incorporation of a barrier shapefile to prohibit the interpolation of waterbody characteristics over land features.

Water depth and sediment composition data were exported from ciBioBase in grid format, entered as XY data in ArcMap, transformed to the NAD coordinate system, and subjected to interpolation using the “Spline with Barriers” (Spatial Analyst) (Valley 2016). Shoreline slope and aspect was determined through manipulation of the rasterized depth data with the appropriate “Slope” and “Aspect” tools in the Spatial Analyst toolbox.

The values of the rasterized data layers (pH, secchi, nitrogen, depth, composition, aspect, and slope) were amended to the SAV points with joined binary species presence data through a
bilinear interpolation accomplished by the “Extract Multi Values to Points” (Spatial Analyst) tool. Values were exported in tabular format as a matrix for statistical analysis.

**Statistical Model Analysis:**

All statistical analysis was accomplished in JMP 14.3.0\textsuperscript{4}. The mean, standard error, and minimum and maximum values were determined for all continuous environmental variables at all sampled points (n = 253). A correlation matrix was generated on the environmental variables using the row-wise method and Spearman’s ρ to determine significant pairwise relationships.

Next, an MLR was performed to identify the predictor variables that best explain the presence or absence of hydrilla in Philpott Reservoir. Due to the low presence of naiads at sampled points (n = 4), they were not included in the analysis. The model of best fit was determined by following a backward, forward, and mixed stepwise selection procedure and the R\textsuperscript{2}, AICc, and BIC values of each were compared. For all tests, the stopping rule was regulated by a p-value threshold such that a p-value of 0.25 or less was needed to enter the model and a p-value of less than 0.05 was needed to stay in the model. Ultimately, a backwards selection method was chosen (R\textsuperscript{2} = 0.25, AICc = 233.5, BIC = 247.5). The coefficients for each significant variable were applied to a predictive model for hydrilla presence in this specific system. The overall significance of the model was tested using the \( \chi^2 \) test on the Wald statistic (\( \alpha = 0.05 \)).

Next, an unsupervised PCA analysis on correlations was performed to better understand the relationships that the explanatory environmental variables had with each other. Before analysis, the data were centered by subtracting the mean from each value and scaled by dividing by their standard deviation to account for differences in units. All continuous environmental
variables were added to the model and the species were input as supplementary variables. The results of the model are presented numerically in a loading matrix and visually in biplot format.

Lastly, an LDA with common covariances was prepared in an attempt to categorize macrophyte composition in Philpott Reservoir by the collected environmental predictor variables in a confined space. Again, all continuous environmental variables were added to the model as covariates and the species present at each point were considered as categorical data. A canonical plot was produced to visualize the relationship that the species have with each of the environmental variables and score summaries informed the strength of the model. The accuracy of the LDA species classifications was assessed in a classification count table. The resultant matrix compares the model-predicted species at each study point to the actual species recorded in the field. The percent of misclassified points can determine the overall model strength.

Results

Four genera of SAV (Hydrilla, Egeria, Chara, and Najas) were consistently documented in Philpott Reservoir between 2014 – 2019 (Table 1). Eleocharis spp. was also documented in 2018 and 2019. Hydrilla was the dominant species present during all surveyed years (Table 1; Figure 1). The presence of hydriilla in Philpott reservoir peaked in 2017 (33% occurrence) and then dropped in 2019 (16.6% occurrence), likely in response to the active control efforts that began in 2018. Native species to Philpott Reservoir occurred frequently in early years of survey, became minimally present during peak hydriilla occurrence in 2017, and have gradually become more common at survey points in recent years (Table 1). When averaged over the past three years of survey, native species were documented co-occurring with hydriilla at 20% of the points where hydriilla was occurred. Despite surveying fewer points, USACE data generally agreed with
the NCSU data in species present and their relative abundance within the waterbody. However, misidentification of morphologically-similar genera (such as hydrilla and Egeria) may have occurred before 2016 and thereby skewed the data (M. Hosey, personal communication; Table 1).

According to daily measurements of water level by the USGS, there was a significant difference in mean annual water levels between 2017 – 2019. Of the three years, water levels were the lowest in 2018 (mean = 971.8 ft (296.2 m)) and were highest in 2019 (mean = 974.6 ft (297.1 m)) (Figure 2; USGS 2020).

Biological, chemical, and physical parameters exhibited high variability throughout the 253 surveyed points (Table 2). When averaged over the three years of the survey, hydrilla was most likely to be occupying a survey point (22.5%), and Egeria, Chara, and Najas had relatively low occurrence rates (3.7%, 1.7%, and 0.6%, respectively). Based on the gridded interpolation of water quality values at sampled points, average secchi depth = 3.1 feet (0.94 m), the average concentration of total nitrogen = 0.2 mg/L and average pH = 7.0. In terms of reservoir topography, the mean aspect of surveyed points = 184.2, signifying that southern slope direction was most common along the shoreline. The mean sediment composition was of medium hardness based on its average value of 0.34. The sediment slope at survey points averaged 17°, indicating steep shoreline topography. Average water depth at survey points was 14.9 feet.

Many of the environmental variables exhibited significant pairwise relationships (Table 3). Secchi depth was strongly correlated to 4 of the 6 other environmental variables including nitrogen ($\rho = -0.74$), pH ($\rho = 0.61$), slope ($\rho = 0.19$) and depth ($\rho = 0.26$). All of the physical variables, except for basin aspect, were also correlated in pairwise relationships (composition and slope ($\rho = -0.23$), composition and depth ($\rho = -0.88$), and depth and slope ($\rho = 0.21$)).
A significant logistic regression model was produced (p < 0.0001) that accounted for 25% of the variability in hydrilla occurrence (Table 4). Significant factors for predicting hydrilla occurrence included every type of factor (biological, chemical, and physical). These included *Egeria* presence (p < 0.0321), secchi depth (p < 0.0001), and sediment composition (p = 0.0092). The MLR equation is as follows:

$$\log \left( \frac{p}{1 - p} \right) = -12.00 + 1.18x_1 + 2.63x_2 + 9.85x_3$$

Where:

- $p$ = Probability of hydrilla occurrence
- $x_1 = Egeria$ occurrence
- $x_2 = $ Secchi depth (ft)
- $x_3 = $ Sediment composition

The subsequent ordination in PCA found that the first and second principal components axes accounted for 34.1% and 25.5% of the variation in the data, respectively, or 59.6% when combined (Table 5). Both PC1 and PC2 had eigenvalues greater than 1 and were the main axes for analysis. The eigenvalues of PC3 and PC4 were also relatively high at 0.99 and 0.90 respectively, suggesting that they may also be worth interpreting. PC1 demonstrated a strong positive relationship between secchi depth, pH, and water depth and their inverse relationship with nitrogen concentration. PC2 revealed that pH was also positively correlated with sediment composition and water depth. Aspect and slope became important variables in PC3 and PC4, respectively. When combined with the supplementary species variables in the biplot, both PC1 and PC2 variables appear to be positively correlated with the presence of hydrilla and *Egeria* (Figure 3). In some cases, PCA can be used as a tool to reduce the size of datasets by identifying the most prominent variables that explain the most variance in the data. However, in the case of this study, essentially all variables tested were recognized as important, unique factors.
LDA confirmed that hydilla and *Egeria* share similar niches in Philpott Reservoir due to the close spatial proximity of the plotted 95% confidence ellipses (Figure 4). Again, secchi depth and sediment composition appear to be significant drivers of their presence. The presence of *Chara* trends strongly with sediment composition. It should be noted that the 95% confidence ellipse for hydilla was plotted around centroid of the LDA biplot. This suggests that hydilla exhibits a more generalist behavior in Philpott Reservoir when compared to *Egeria* and *Chara* whose 95% confidence ellipses were plotted more closely to specific environmental variables. The success of LDA was moderate, with 37 (35.6%) of points misclassified (Table 6). Of the misclassified points, 22 *Egeria* were incorrectly classified as hydilla, 3 hydilla were incorrectly classified as *Egeria*, 9 *Chara* were incorrectly classified as hydilla and 3 hydilla were incorrectly classified as *Chara*. *Egeria* was never misclassified as *Chara* and vice versa.

**Discussion**

Likely due to management efforts through chemical control, the occurrence of hydilla and *Egeria* in Philpott Reservoir have both declined since peaking in 2017. It is apparent that *Chara* and *Najas* were highly abundant in the early years of survey efforts before the expansion of non-native species throughout the reservoir. These findings may be misleading due to differences in surveyors, sampling methodology, and sample size between early and late survey years (Table 1). Nevertheless, when only considering data collected in more recent years after the development of a standard survey protocol, non-native species have remained maintained dominance within the SAV community (Table 1; Figure 1).

Our multivariate models suggest that hydilla in Philpott Reservoir is likely to occur with other species of SAV where light attenuation into the water column is high and sediments are
relatively hard. This combination of physical, biological, and chemical water quality factors was an anticipated result of this work and demonstrates the complexity of aquatic environments. These results agree with other studies such as that of Sousa et al. (2011) who found that a mix of physical and chemical factors (depth, slope, light, and phosphorous concentration) had a strong influence on aquatic plant distribution in a Brazilian lake. Our findings demonstrate that, in Philpott Reservoir, hydrilla fits the mold of being the “perfect aquatic weed” as it appears to be driven by essentially only basic aquatic plant needs – the availability of some sunlight and a reliable place to root.

Many studies have examined hydrilla’s independent relationships with *Egeria* presence, water column secchi depth, and sediment composition. Generally, it is understood that hydrilla is unlikely to cohabit with other SAV due to its strong competitive abilities (Mony 2007; Wang 2008; Silveira et al. 2018). Alternatively, some studies cite that invasive SAV species could have synergistic relationships with other SAV and their developmental success is supported by one another. Such a relationship was documented in the Potomac River where hydrilla was documented co-occurring with other SAV in areas where light attenuation through the water column was high, which potentially could be attributed to the decrease of turbidity after SAV establishment stabilized sediments (Rybicki and Landwehr 2007). Other studies confirm that light availability positively influences hydrilla development (Madsen and Owens 2000; Bianchini et al. 2010; Dan-Ting et al. 2011), but, under ideal conditions, hydrilla is still able to grow in only 5% incident sunlight and can develop in total darkness (Steward 1991; Haug et al. 2019). Additionally, previous research has concluded that sediment composition does influence hydrilla biomass production, but interestingly may not significantly change tuber production (Netherland 1997).
When combined, the results from MLR, PCA, and LDA represent multiple levels of understanding of the structure and processes that drive macrophyte community assemblages in Philpott Reservoir. At the base of our analysis, MLR defined the strongest predictors of hydrilla presence that can be applied to estimations of potential hydrilla distribution at untested points or in the future. Next, PCA allowed us to gain a better understanding of how those predictors are related to other environmental phenomena in the reservoir and what drives their magnitude at any time. For instance, secchi depth, a significant indicator of hydrilla presence, was highly correlated with the combined influence of nitrogen concentrations, water pH and water depth in PC1. One interpretation of this relationship could be that pH is positively correlated with nitrogen uptake which decreases light attenuation in the water column due to biological consumption and ultimately reduces water transparency and the related secchi depth value. Concurrently, water depth may drive the base of the relationship as biological consumption also relies on light availability and, in general, light attenuation is lower in deeper regions when compared to shallow areas.

It is important to remember that the data incorporated into this model are based on an interpolation process and therefore this is not a discrete account of the processes occurring at Philpott Reservoir. The efforts that would need to be met to obtain exact values for water quality, sediment composition, and basin topography at all sampling points would require a significant amount of time and resources and were not feasible to obtain for this small-scale study. The six long-term water quality monitoring stations are distributed throughout the reservoir such that a general pattern could be obtained. Generally, these patterns match those of other reservoirs with similar morphology. For example, secchi depth was lowest in the upstream riverine portions of the reservoir and high at the reservoir’s outlet near the dam. Nitrogen concentrations spatially
trended closely with secchi depth, as expected. With this, LDA confirmed the ability of our data to classify species diversity and was nearly 65% accurate. To reduce the number of misidentified points, future studies could increase sample size or expand variable diversity.

An untested factor that may have influenced SAV compositional patterns over time is shoreline disturbance due to water level fluctuation. Physical shoreline disturbance due to water level fluctuation is a common characteristic of reservoir systems. Shoreline disturbance is a well-understood antagonistic effect on SAV growth due to the manipulation of water availability, the transformation of local water chemistry, and its influence on light availability. In Philpott Reservoir, water levels are driven by downstream flood control needs, requirements for the generation of hydroelectric power, and upstream water inputs. The significant difference in water levels between survey years may be a confounding factor in the analysis of hydrilla distribution in the reservoir over time.

Alternatively, the high frequency of shoreline disturbance at Philpott Reservoir may also be encouraging the growth of the elodeid species that make up its SAV community. Elodeid species are those that support short leaves along an elongated, submersed stem and include hydrilla, *Egeria*, *Chara* and *Najas*, the documented SAV in Philpott Reservoir (Figures 5 – 8). The intermediate disturbance hypothesis states that ecosystems with a very high or very low frequency of disturbance events often have a low diversity of SAV as most species are unable to either (a) compete with the most competitive species that dominate in low disturbed environments, or, (b) withstand high environmental stress in the face of frequent disturbance events (Sousa 1984). Bornett and Puijalon (2011) suggested that small caulescent or rosette species such as *Elodea spp.* and hydrilla are those that are most likely to dominate in highly disturbed systems due to their flexibility and ability to withstand both high and fairly low water
levels. Our observations at Philpott Reservoir support this phenomenon as all of the species present express similar morphological features that are also shared by those species that are able to dominate in highly disturbed environments (Figures 5 – 8).

Subsequent research should attempt to validate these models in the field. This could be done by surveying points that were not utilized for the statistical analysis and determining their species presence in relation to other physical parameters. Attempts could also be made to validate this model in other reservoir systems where dioecious hydrilla is a dominant species. Future models should also consider more environmental variables such as propagule distribution, water fetch and flow patterns, and sediment nutrient concentrations and could incorporate multiple survey time points to capture seasonality changes within the reservoir.

Our models were successful at characterizing the environmental factors that influence the distribution of hydrilla in Philpott Reservoir and can be applied to future research and management efforts. They can be further strengthened through the incorporation of more data and field validation. These models should be viewed as another “tool in the toolbox” and should not replace other strategies for the long-term monitoring and management of hydrilla. As such, continuing the application of field-based monitoring efforts will be important for defining the further distribution of hydrilla in Philpott Reservoir. Future SAV surveys can be focused in regions of the waterbody that our models have highlighted to be more vulnerable to invasion such as those regions with high secchi depths and hard sediments. Revegetation projects may also help establish an introduction of native species to replace hydrilla and *Egeria* populations and may be able to facilitate similar relationships with other native species. Current revegetation projects at Philpott Reservoir are attempting to introduce *Vallisneria americana* and *Justicia*
*americana* to the system and could be a key factor in the transition from non-native invasive species dominance to native SAV dominance with continued management.

** SOURCES OF MATERIALS **

1. ArcMap 10.7.1, Environmental Systems Research Institute (ESRI), 380 New York Street, Redlands, CA 92373.

2. Lowrance HDS-7 Gen3, Navico Inc., 4500 South 129th East Avenue Suite 200, Tulsa, OK 74134.


4. JMP Pro 14.2.0, SAS Institute Inc., 101 SAS Campus Drive, Cary, NC, 27513
REFERENCES


Williams MC. 1980. Purposefully introduced plants that have become noxious or poisonous weeds. Weed Science. 28: 300 – 305.
Figure 2-1: Results from 3 consecutive years of SAV point-intercept survey at Philpott Reservoir.
Table 2-1: % occurrence of genera present at Philpott Reservoir over 6 years of survey.

<table>
<thead>
<tr>
<th>Genera</th>
<th>Survey Year</th>
<th>2014$^a$</th>
<th>2015$^a$</th>
<th>2016$^a$</th>
<th>2017$^b$</th>
<th>2018$^b$</th>
<th>2019$^b$</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrilla</td>
<td></td>
<td>23.8%</td>
<td>19.6%</td>
<td>17.9%</td>
<td>33.1%</td>
<td>18.4%</td>
<td>16.6%</td>
<td>21.2 ± 2.1%</td>
</tr>
<tr>
<td>Egeria</td>
<td></td>
<td>4.8%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>6.7%</td>
<td>4.0%</td>
<td>1.6%</td>
<td>2.5 ± 0.9%</td>
</tr>
<tr>
<td>Najas</td>
<td></td>
<td>11.9%</td>
<td>17.4%</td>
<td>6.0%</td>
<td>0.4%</td>
<td>0.4%</td>
<td>0.8%</td>
<td>6.2 ± 2.3%</td>
</tr>
<tr>
<td>Chara</td>
<td></td>
<td>16.7%</td>
<td>4.3%</td>
<td>3.0%</td>
<td>1.3%</td>
<td>2.4%</td>
<td>2.0%</td>
<td>5.0 ± 1.9%</td>
</tr>
<tr>
<td>Eleocharis</td>
<td></td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.4%</td>
<td>0.4%</td>
<td>0.4 ± 0%</td>
</tr>
</tbody>
</table>

No. points sampled | 42 | 46 | 67 | 239 | 249 | 253 | 146 ± 33

$^a$ USACE Survey Data  
$^b$ NCSU Survey Data
Figure 2-2: Surface elevation data of Philpott Reservoir water levels averaged over 1 year before survey. Data obtained from USGS National Water Information System (USGS 2020). Means separated by Tukey HSD ($\alpha = 0.05$).
Table 2-2: Overview of the studied biological, chemical, and physical parameters at Philpott Reservoir.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean</th>
<th>SE</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biological</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Najas</em></td>
<td>253</td>
<td>0.6%</td>
<td>0.2%</td>
<td>0.0%</td>
<td>33.2%</td>
</tr>
<tr>
<td><em>Chara</em></td>
<td>253</td>
<td>1.7%</td>
<td>0.4%</td>
<td>0.0%</td>
<td>42.2%</td>
</tr>
<tr>
<td><em>Egeria</em></td>
<td>253</td>
<td>3.7%</td>
<td>0.8%</td>
<td>0.0%</td>
<td>94.4%</td>
</tr>
<tr>
<td><em>Hydrilla</em></td>
<td>253</td>
<td>22.5%</td>
<td>1.8%</td>
<td>0.0%</td>
<td>100.2%</td>
</tr>
<tr>
<td><strong>Chemical</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secchi Depth (ft)</td>
<td>253</td>
<td>3.129</td>
<td>0.039</td>
<td>1.257</td>
<td>3.747</td>
</tr>
<tr>
<td>Nitrogen (mg/L)</td>
<td>253</td>
<td>0.235</td>
<td>0.003</td>
<td>0.151</td>
<td>0.411</td>
</tr>
<tr>
<td>pH</td>
<td>253</td>
<td>7.019</td>
<td>0.014</td>
<td>6.484</td>
<td>7.294</td>
</tr>
<tr>
<td><strong>Physical</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspect (°)</td>
<td>253</td>
<td>184.213</td>
<td>4.022</td>
<td>-1.000</td>
<td>321.193</td>
</tr>
<tr>
<td>Composition</td>
<td>253</td>
<td>0.345</td>
<td>0.003</td>
<td>0.257</td>
<td>0.457</td>
</tr>
<tr>
<td>Slope (°)</td>
<td>253</td>
<td>17.273</td>
<td>0.739</td>
<td>0.000</td>
<td>51.789</td>
</tr>
<tr>
<td>Depth (ft)</td>
<td>253</td>
<td>14.900</td>
<td>0.389</td>
<td>2.500</td>
<td>34.315</td>
</tr>
</tbody>
</table>
Table 2-3: Pairwise correlations of environmental variables estimated by row-wise method. Values in top half of matrix represent prob > |ρ|. Significant values are bolded (α = 0.05).

Spearman’s ρ Correlation Coefficients, N = 253

<table>
<thead>
<tr>
<th></th>
<th>Secchi</th>
<th>Nitrogen</th>
<th>pH</th>
<th>Aspect</th>
<th>Composition</th>
<th>Slope</th>
<th>Depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secchi</td>
<td>--</td>
<td>&lt;.0001</td>
<td></td>
<td>0.1083</td>
<td>0.1091</td>
<td>0.0017</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>-0.7385</td>
<td>--</td>
<td></td>
<td>0.3395</td>
<td>0.8790</td>
<td>0.1397</td>
<td>0.0602</td>
</tr>
<tr>
<td>pH</td>
<td>0.6154</td>
<td>-0.4078</td>
<td>--</td>
<td>0.3675</td>
<td>0.6587</td>
<td>0.4370</td>
<td>0.2188</td>
</tr>
<tr>
<td>Aspect</td>
<td>0.0921</td>
<td>-0.0429</td>
<td>0.0773</td>
<td>--</td>
<td>0.7665</td>
<td>0.7252</td>
<td>0.5035</td>
</tr>
<tr>
<td>Composition</td>
<td>-0.1508</td>
<td>0.0685</td>
<td>0.0331</td>
<td>0.0199</td>
<td>--</td>
<td><strong>0.0005</strong></td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Slope</td>
<td>0.1887</td>
<td>-0.0631</td>
<td>-0.0542</td>
<td>0.0002</td>
<td>-0.2306</td>
<td>--</td>
<td><strong>0.0002</strong></td>
</tr>
<tr>
<td>Depth</td>
<td>0.2569</td>
<td>-0.1647</td>
<td>0.0834</td>
<td>0.0569</td>
<td>-0.88260</td>
<td>0.2061</td>
<td>--</td>
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</tbody>
</table>
Table 2-4: Results from multivariate logistic regression.

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<tr>
<th>Variable</th>
<th>Parameter Estimate</th>
<th>Standard Error</th>
<th>ChiSquare</th>
<th>Prob &gt; ChiSq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-12.00</td>
<td>2.30</td>
<td>27.32</td>
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</tr>
<tr>
<td>Egeria [1]</td>
<td>1.18</td>
<td>0.550</td>
<td>4.59</td>
<td>0.0321</td>
</tr>
<tr>
<td>Secchi</td>
<td>2.63</td>
<td>0.478</td>
<td>30.22</td>
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</tr>
<tr>
<td>Composition</td>
<td>9.85</td>
<td>3.78</td>
<td>6.79</td>
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<table>
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<th>DF</th>
<th>ChiSquare</th>
<th>Prob&gt;ChiSq</th>
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<tr>
<td>Difference</td>
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<td>Full</td>
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<tr>
<td>Reduced</td>
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</table>

R² 0.25 AICc 233.5

Observations 253 BIC 247.5
Table 2-5: Results of PCA ordination. Loadings > |0.5| are bolded.

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<tr>
<td></td>
<td>1</td>
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<tr>
<td>% of Variance</td>
<td></td>
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<tr>
<td></td>
<td>34.1%</td>
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<tr>
<td>secchi</td>
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<td>nitrogen</td>
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<td>pH</td>
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<tr>
<td>aspect</td>
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<tr>
<td>composition</td>
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<tr>
<td>slope</td>
<td>0.30065</td>
</tr>
<tr>
<td>depth</td>
<td>0.61484</td>
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Figure 2-3: PCA Biplot on PC1 and PC2 with categorical supplementary species variables plotted.
Figure 2-4: LDA Canonical Plot on points with species present with biplot rays and 95% means confidence ellipses plotted.
Table 2-6: Score summaries of LDA on points with species present.

<table>
<thead>
<tr>
<th>Actual</th>
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<tbody>
<tr>
<td></td>
<td>Chara</td>
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<td>Hydrilla</td>
</tr>
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<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Egeria</td>
<td>0</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Hydrilla</td>
<td>9</td>
<td>22</td>
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</tr>
</tbody>
</table>

- \( N = 104 \)
- # Missclassified: 37
- % Classified: 64.4%
- Entropy \( R^2 \): -0.1804
Figure 2-5: Line drawing of *Hydrilla verticillata* (IFAS 1990). Accessed from: http://plants.ifas.ufl.edu/plant-directory/Hydrilla-verticillata/
Figure 2-6: Line drawing of *Egeria densa* (IFAS 1990). Accessed from: http://plants.ifas.ufl.edu/plant-directory/egeria-densa/
**Figure 2-7:** Line drawing of *Chara spp.* (IFAS 1990). Accessed from: [http://plants.ifas.ufl.edu/plant-directory/chara-species/](http://plants.ifas.ufl.edu/plant-directory/chara-species/)
Figure 2-8: Line drawing of *Najas guadalupensis* (IFAS 1990). Accessed from: http://plants.ifas.ufl.edu/plant-directory/najas-guadalupensis/
CHAPTER 3: A comparison of the growth and reproductive success of northern and southern populations of dioecious *Hydrilla verticillata* (L.f.) Royle in the United States.

Abstract

Hydrilla (*Hydrilla verticillata* (L.f.) Royle) is an invasive aquatic plant that has negatively impacted the physical, biological, and chemical aspects of freshwater ecosystem dynamics in the United States. Populations of the dioecious biotype of hydrilla are most commonly documented in southern states including: Florida, Georgia, Alabama, South Carolina, Louisiana, and Texas. Recently, a population of dioecious hydrilla was documented in Philpott Reservoir (Bassett, VA) at the northern end of its reported, introduced geographic range. This study compared the tuber sprouting dynamics of this Virginia (VA) population of dioecious hydrilla with those of a Florida (FL) population along a temperature gradient. By the end of the 2-week study, sprouted tubers produced by the FL population had significantly longer stem lengths at cooler water temperatures (25°C) than sprouted tubers produced by the VA population of dioecious hydrilla (*p* < 0.0002). Next, differences in the phenological patterns of the same VA and FL dioecious hydrilla populations were compared in a replicated outdoor mesocosm trial in two dissimilar climates. Results demonstrated that the FL and VA dioecious hydrilla plants followed comparable developmental patterns throughout the growing season at both mesocosm facilities, however, VA hydrilla produced tubers earlier in the growing season compared to FL hydrilla under cool climatic conditions. This research suggests that there may be differences in sprouting and growth dynamics between FL and VA populations of dioecious hydrilla which could have implications on its future spread throughout the United States.
Introduction

Philpott Reservoir is located along the Smith River near Martinsville, VA and is a part of the Roanoke River basin in the Piedmont ecoregion of the United States (USACE 2018). The 1,165 hectare (2,880 acre) impoundment is owned and maintained by the United States Army Corps of Engineers and provides flood control and hydroelectric power generation (VDGIF 2016). A submersed aquatic vegetation survey by North Carolina State University researchers identified approximately 15 hectares (37 acres) of dioecious *Hydrilla verticillata* (L.f.) Royle (“hydrilla”) in Philpott Reservoir in August 2017. Other species present at the time of the survey included *Egeria densa* (Planch), *Najas guadalupensis* (Spreng) Magnus, and *Chara* spp. Subsequent surveys of subterranean turions (tuber) densities in selected coves revealed tuber bank densities that ranged from 12.3 to 65 tubers m\(^{-2}\) in between June 2018 and December 2019 (T. Harris, personal communication). This tuber bank is relatively sparse compared to the density of other documented tuber banks which have reportedly ranged from 1 – 1,000 tubers m\(^{-2}\) (Best and Boyd 1996).

To date, three recognized strains of hydrilla have been documented in the United States: a monoecious, dioecious, and a novel genetically distinct, but phenologically uncharacterized strain (Tippery et al. 2020). The female-flowering dioecious hydrilla strain was introduced to the United States from its native range in India in the 1950s (Langeland 1996). Since then, it has rapidly dispersed throughout the southeast and south-central region of the country (Harms et al. 2014). Due to having limited natural competition from plants and herbivores in its introduced range in the region as well as aggressive growth and reproductive behaviors, hydrilla has become a problematic aquatic weed in the United States. It is currently classified as 1 of 112 plants listed as a Federal Noxious Weed under the Plant Protection Act and is recognized on 17 state noxious
weed lists in the United States (The Plant Protection Act – Title 7 Chapter 104; USDA-NRCS 2020).

Philpott Reservoir currently supports the northernmost established dioecious hydrilla population on the east coast of the United States. However, it is not the first dioecious hydrilla population that has been recorded in an area that experiences a relatively cool climate. In 1997, dioecious hydrilla was identified in a small pond in Mystic, Connecticut. This population has since been eradicated (Les et al. 1997). Additionally, there are known populations of dioecious hydrilla within a warm geothermal area of Boise, Idaho and multiple locations in Kentucky (Rybicki et al. 2013; USFWS 2017; USGS 2017). In 1995, dioecious hydrilla was also found in another Virginian lake, Lake Gaston, coexisting with monoecious hydrilla (Ryan et al. 1995). According to the available reports, none of these instances of dioecious hydrilla populations in cool climates were of the same infestation level of the population in Philpott Reservoir, however, they do provide insight into the adaptable nature of the "perfect aquatic weed" (Langeland 1996).

Infestations of both monoecious and dioecious biotypes of hydrilla have similar negative ecological and economic consequences in the United States. Morphologically, however, monoecious and dioecious hydrilla are quite different. Compared to monoecious hydrilla, dioecious hydrilla tends to have longer leaves, shorter internodes and exhibits less branching near the hydrosoil (Ryan et al. 1995). The novel, uncharacterized strain of hydrilla can be distinguished from the monoecious and dioecious biotypes by its wider leaves in whorls of 6 – 8 (Tippery et al. 2020). In southern climates, dioecious hydrilla has been observed to have perennial growth patterns and sustain aboveground biomass during winter months (Owens et al. 2012). Conversely, the aboveground biomass of the monoecious biotype tends to die off in the
winter and regrows in the spring from hibernacula (overwintering tubers and turions) when water temperatures return to optimal levels (Netherland 1997).

Water resource managers have utilized various methods for dioecious hydrilla control that incorporate physical, biological, and chemical-based options and often formulate their management decisions on the phenology of hydrilla for the most appropriate treatments. The phenology of hydrilla must be well understood for such control measures to achieve maximum effectiveness and to mitigate the further spread of this problematic species throughout the US and other regions of the world.

Previous studies have demonstrated that some phenological patterns of monoecious and dioecious hydrilla tubers tend to be dependent on water temperature. Haller et al. (1975) found that the optimal water temperature for dioecious hydrilla tuber sprouting ranged from 15 to 35°C. Steward and Van (1987) studied hydrilla sprouting rates and found that monoecious hydrilla sprouts more successfully in 15°C water than dioecious hydrilla does. The results of these studies agree with the geographic distribution of hydrilla biotypes in the United States as dioecious plants tend to be found in warmer climates when compared to monoecious plants. McFarland and Barko (1987) determined that the thermal optimum for biomass production in both monoecious and dioecious hydrilla is between 28 and 32°C. Based on the last 10 years of record, the mean water temperature at Philpott Lake has been 9.3°C with a minimum of 4.3°C and a maximum of 17.6°C (USGS 2020). Based on the aforementioned previously completed studies, dioecious hydrilla should not be successful in Philpott Reservoir and its phenological patterns may follow different environmental cues in order to survive. The goal of this research was to examine the current understanding of the constrictions of climate on dioecious hydrilla,
relate that information to Philpott Reservoir, and to uncover potential implications for future management of this problematic species in the northeastern United States.

It was hypothesized that the hydrilla population at Philpott Reservoir is able to survive in the northern end of its extreme range due to an evolved ability to sprout and reproduce in cooler water temperatures when compared to a population adapted to a more southern, warmer climate. To address this hypothesis, the environmental response of a cool-water Virginia (VA) population dioecious hydrilla population were directly compared to those of a warm-water Florida (FL) dioecious hydrilla population in two related studies. The first (hereby referred to as the “Controlled Conditions Trial”), analyzed the tuber sprouting success of each population along a water temperature gradient in a laboratory setting. The second (hereby referred to as the “Outdoor Mesocosm Trial”), focused on the post-sprouting phenology of the two populations when grown in dissimilar climates.

Methods

Controlled Conditions Trial

Dioecious hydrilla tubers were collected from Philpott Reservoir (Patrick County, VA) and from the UF/IFAS Center for Aquatic and Invasive Plants research ponds (Alachua County, FL) in March 2018 and were sprouted under greenhouse conditions at North Carolina State University (Wake County, NC). Sprouted stems were planted in an outdoor mesocosm facility in Raleigh, NC at North Carolina State University Research Farm #2 (35.810278, -78.721714). Each sprouted tuber was planted individually in topsoil amended with Osmocote™ and topped with a thin layer of sand in a plastic Classic 300s six-inch nursery pot. The pots were placed in a 1040 L mesocosm filled with pond water suitable for submersed plant growth (Henry 2017). The
VA and FL populations were planted in adjacent mesocosms so that they were subjected to identical growing conditions while remaining separate to avoid contamination from stem fragments of the opposite population. In December 2018, the tubers produced by both populations were harvested and immediately refrigerated for 72 hours. The first-generation descendants from the original hydrilla populations were studied in the subsequent research trials to avoid interactions from maternal effects from each populations' respective home environment and to highlight the genetic component of the phenotypes (Mousseau and Fox 1998).

A temperature gradient was produced in a laboratory setting by the flow of hot (50°C) and cold (5°C) water through opposite ends of a stainless steel table. With this setup, a randomized complete block design was achieved as the temperature treatments remained constant along a vertical line but were variable along its horizontal length. Atop the table, 28 50mL beakers were arranged in 7 columns and 4 rows such that 7 temperature treatments were replicated 4 times. Each beaker was filled with 1.5 cm of pea gravel and 100 mL DI water. Six 125 mL test tubes filled with 100 mL DI water and 0.5 mL water conditioner were also placed within each beaker. The table's water flow was initiated one week before the start of the study to ensure that water temperatures were constant during the study period. Once equilibrated, water temperature treatments within test tubes averaged 9.35 ± 0.03°C, 14.93 ± 0.03°C, 19.70 ± 0.2°C, 24.52 ± 0.02°C, 28.55 ± 0.02°C, 32.60 ± 0.03°C and 35.74 ± 0.03°C throughout the duration of the study.

The fresh weight of all studied tubers was recorded before exposure to the temperature treatments. Three tubers from the VA population and three tubers from the FL population were randomly assigned to each beaker and individually placed within a labeled test tube. Tubers were kept in separate experimental mesocosms (test tubes) to most effectively monitor every
experimental unit throughout the course of the study and to avoid potential interaction by allelopathic effects from sprouted tubers on non-sprouted tubers. Allelopathy has not been reported in hydrilla growth studies, however, it has been documented in numerous other aquatic plant species (Jones 1995). Test tubes were capped with aluminum foil to prevent water evaporation. The water within each test tube was aerated daily with compressed air for 30 seconds. The water temperature in each test tube was recorded every 24 hours with a Fisher Scientific Traceable™ digital thermometer. Stem lengths of each sprouted tuber were recorded for 14 consecutive days and were measured with electronic calipers.

The study was completed in December 2018 and was repeated in January 2019. Data were analyzed in JMP Pro 14.2.0. No temperature by run interaction was detected for either stem length of tuber sprouting (p = 0.6753 and p = 0.0806, respectively. Therefore the data from both runs were combined for subsequent analysis. Final stem lengths were compared using a one-way ANOVA and means were separated with a Tukey HSD test (p ≤ 0.05). Additionally, the tuber data were regressed using JMP software and graphed in SigmaPlot 14.0³. The best fit lines for the regression analysis of tuber response to temperature were fitted to Gaussian three-parameter curves along the general equation:

\[ f = a \exp \left[ -0.5 \left( \frac{x - x_0}{b} \right)^2 \right] \]

Where:

- \( a \) = peak value
- \( x_0 \) = critical temperature at peak value
- \( b \) = growth rate
- \( x \) = water temperature (°C)
- \( f \) = Hydrilla response
Outdoor Mesocosm Trial

An additional 100 first-generation tubers from both FL and VA dioecious hydrilla populations were collected from established plants that had been subjected to identical growing conditions in Raleigh, NC at North Carolina State University Research Farm #2 (35.810278, -78.721714). Post-harvest, the tubers were refrigerated for 72 hours and then exposed to ideal sprouting conditions in a greenhouse setting for approximately 2 weeks. When tubers showed signs of sufficiently sprouted stems, they were planted in small pots and allowed to develop for approximately 4 weeks. Then, 45 6-inch apical stems were gathered from each population and were replanted at the North Carolina State University Research Farm #2 in Raleigh, NC (35.810278, -78.721714) and another 45 from each population were planted at the North Carolina Department of Agriculture & Consumer Service’s Upper Mountain Research Station in Laurel Springs, NC (36.39956, -81.30965). Laurel Springs, NC is located in North Carolina’s Blue Ridge Mountains [Elevation = 2,800 ft (853 m)] and has a significantly cooler climate when compared to Raleigh, NC [Elevation = 315 ft (96 m)] (Table 1). The nearly identical mesocosm facilities in Raleigh, NC and Laurel Springs, NC allow for direct comparison of hydrilla behavior in response to climate. At each location, individual stems were planted in 6-inch round pots in soil amended with Osmocote™ and topped with a thin layer of sand. Six 1040 L totes were filled with pond water and 15 pots were positioned within each tote such that 3 totes contained 15 pots each of each respective FL and VA population of dioecious hydrilla.

Destructive harvests occurred monthly from May 2019 to December 2019 at both outdoor mesocosm locations. At the time of each harvest, water quality parameters (dissolved oxygen, pH, and temperature) of each mesocosm were measured with a YSI™ handheld water quality probe⁴. One plant in each triplicate tote was harvested and the longest stem length, number of
stems, average leaves/whorl, average leaf length, number of tubers, number of turions and above- and below-sediment biomass dry weight of each plant was recorded.

In December 2019, five of the remaining plants in each triplicate tote at both research stations were randomly selected and destructively harvested. The tubers were collected and returned to the laboratory for further analysis. Tuber fresh weight was recorded and viability was determined by subjecting the tubers to ideal sprouting conditions in a greenhouse setting. After 14 days, the stem lengths of the sprouted tubers were measured. The tubers that did not exhibit signs of sprouting by this time were considered to be not viable/dormant.

Results were analyzed in JMP Pro 14.2.0\(^2\) to determine differences in recorded continuous variables between FL and VA dioecious hydrilla in the two tested dissimilar climates. Environmental data were analyzed via a one-way ANOVA test. Dioecious hydrilla phenology over time in (tuber production, longest stem length, and dry weight) were analyzed and compared by study site (Raleigh vs Laurel Springs) by non-linear regression in SigmaPlot 14.0\(^3\) and fit to Gaussian three parameter curves (see general equation above).

**Results**

*Controlled Conditions Trial*

Florida tubers were significantly heavier than the tested Virginia tubers (0.31 ± 0.17 g (FL) 0.23 ± 0.06 g (VA), p < 0.0001)(Figure 1). The water temperature treatments along the temperature gradient table were significantly different based on treatment row, but, within the replicate columns, both FL and VA tubers were exposed to identical treatments (Table 2).

Sprouted stem length of FL tubers was influenced by both water temperature (p = 0.0039) and starting weight (p < 0.001) but no significant interaction between water temperature and
starting weight was detected ($p = 0.0732$). The sprouted stem lengths of VA tubers were also significantly associated with water temperature ($p < 0.001; \alpha = 0.05$) and starting weight ($p = 0.0085$) and there was a significant interaction between water temperature and starting weight ($p = 0.0213$). When directly compared at the tested temperature treatments, the FL and VA tubers did not differ in sprouting dynamics at any treatment except for the $25^\circ\text{C}$ treatment where FL tubers had longer stem lengths and a higher percentage of total tubers sprouted when compared to the VA tubers (Table 3).

The regression analysis indicated that the peak sprouted stem lengths for FL tubers occurred at $25.6^\circ\text{C}$ and were 10.3 cm ($p < 0.0001$). For VA tubers, peak sprouted stem lengths occurred at $29.9^\circ\text{C}$ and were 4.3 cm ($p < 0.0001$) (Figure 2; Equation 1). Both VA and FL tubers exhibited similar peak of the percentage of tubers sprouted at $30.7^\circ\text{C}$ ($p < 0.0001$) and $29.3^\circ\text{C}$ ($p < 0.0001$), respectively. However, for both parameters, FL tubers began to show a response to temperature at cooler temperatures when compared to the VA tubers (FL growth rate = 7.7 ($p < 0.0001$); VA growth rate = 3.0 ($p < 0.0001$) (Figure 3; Equation 2). All regression equations exhibited a strong fit to the data (Equations 1 and 2).

**Outdoor Mesocosm Trial**

Air temperatures throughout the duration of the outdoor mesocosm study were averaged $11.5 \pm 0.42^\circ\text{C}$ in Laurel Springs and $16.84 \pm 0.45^\circ\text{C}$ in Raleigh and ranged from -11.00 to 23.28$^\circ\text{C}$ and -4.89 to 30.72$^\circ\text{C}$, respectively (Table 1). Throughout the duration of the study, air temperatures in Raleigh were significantly warmer than those in Laurel Springs ($p < 0.0001$). Concurrently, mean water temperatures were significantly colder in Laurel Springs ($15.1 \pm 0.1^\circ\text{C}$) than in Raleigh ($19.8 \pm 0.02^\circ\text{C}$) ($p = 0.014$; Figure 4) throughout the duration of
the study. On average, water temperatures at Laurel Springs were warmer than those at Philpott Reservoir from June to August but cooler than those at Philpott Reservoir from September to December, probably because of its location at high elevation in the Blue Ridge Mountains (Figure 4).

In Laurel Springs, maximum tuber production occurred in November for VA dioecious hydrilla plants (6.1 MAP; $p < 0.0001$). The FL dioecious hydriilla never reached a significant peak of tuber production in Laurel Springs ($p = 0.99$). In Raleigh, neither VA nor FL dioecious hydrilla exhibited peak tuber production ($p = 0.97$ (FL); $p = 0.97$ (VA)) (Figure 5). Averaged over the study period, more tubers were produced in Laurel Springs compared to Raleigh over the course of the growing season ($p = 0.0053$), however, there was no difference in the total amount of tubers produced the differing populations of dioecious hydrilla at either research site ($p = 0.9537$).

In Laurel Springs, the longest stem length of FL and VA hydrilla populations peaked in August (4.6 MAP; $p < 0.0001$) and September (5.0 MAP; $p < 0.0001$). In Raleigh, the longest stem lengths were at a maximum in August for both VA and FL populations (4.2 MAP; $p < 0.0001$ (VA) and 4.6 MAP; $p < 0.0001$ (FL))(Figure 5). There was no difference in the total mean sprouted stem lengths between research site locations ($p = 0.7409$) or between hydrilla populations ($p = 0.0562$) averaged over the study period.

Biomass production of both FL and VA hydrilla Laurel Springs peaked in September (5.5 MAP; $p < 0.0001$ and 5.7 MAP; $p < 0.0001$, respectively). In Raleigh, the total biomass peaked in September for VA hydrilla (5.5 MAP; $p < 0.0001$) and in November for FL hydrilla (6.2 MAP; $p < 0.0001$) (Figure 5). There was no difference in the total mean sprouted stem
lengths between research site locations ($p = 0.7908$) or between hydrilla populations ($p = 0.6698$) averaged over the study period.

At the time of the final harvest, the FL dioecious hydrilla plants grown in Laurel Springs produced more tubers than the FL dioecious hydrilla plants in Raleigh (Figure 6a). Of those tubers produced by the FL dioecious hydrilla plants, more viable tubers were produced in Laurel Springs ($85 \pm 10 \%$) than in Raleigh ($20 \pm 20\%$) (Figure 6b), despite having no significant difference in mean fresh weight (Figure 6d). There was no significant difference in the number of tubers produced, the viability, or the fresh weight of the tubers produced by VA plants in Raleigh and Laurel Springs (Figures 6a, 6b, 6d). However, sprouted tubers produced by VA dioecious hydrilla in Laurel Springs were significantly longer for tubers produced in Laurel Springs ($13.5 \pm 4 \text{ mm}$) than those produced in Raleigh ($4.4 \pm 1 \text{ mm}$) (Figure 6c).

**Discussion**

Results from the Controlled Conditions Trial did not support the hypothesis that tubers from VA dioecious hydrilla populations would sprout at cooler temperatures compared to tubers from FL dioecious hydrilla populations. Instead, we found the opposite, in which FL tubers were able to sprout at cooler temperatures when compared to VA tubers. One possible explanation for these results could be that the population of dioecious hydrilla in VA may have adapted to cold water environments by delaying germination until water temperatures increase. If this is the case, previously understood control techniques used for dioecious hydrilla may need to be reevaluated for later-sprouting plant stems in these more northern populations.

In reference to published literature, the tested tuber sprouting behavior from FL dioecious hydrilla populations appears to generally agree with previous findings. Peak FL tuber sprouting
occurred at 29.3°C (Figure 3). Haller et al. (1975) reported peak tuber germination at water temperatures between 18 to 33°C and McFarland and Barko (1987) found that peak biomass production for both monoecious and dioecious biotypes occurs between 28 and 32°C. With the findings of differences in tuber sprouting behaviors between geographically distinct strains of hydrilla, our study has demonstrated hydrilla’s flexible capacity for sprouting and growth under a wide range of conditions.

It is important to consider that the fresh weights of the dioecious hydrilla tubers from the VA population were lower than the fresh weights of the FL population at the start of the Controlled Conditions Trial (Figure 2). Because initial tuber size is directly related to shoot length (Bowes et al. 1979), this is likely a confounding factor and should be addressed in future studies. However, it should be noted that a tuber’s response to temperature may not be dependent on its size so our findings between populations are likely still comparable.

Results from the Outdoor Mesocosm Trial demonstrate that FL and VA dioecious hydrilla populations exhibited similar phenological patterns in both cool and warm environments except for the timing of tuber production in a cool climate. The VA dioecious hydrilla rapidly produced many tubers in early winter at Laurel Springs research site (Figure 5). This is especially interesting to contrast to the FL population in Laurel Springs as it did not exhibit a rapid response of tuber production at any point throughout the study (Figure 5). This may have occurred because the VA hydrilla population has acclimated to produce tubers relatively early before water temperatures drop to the point that tuber production is not possible. In this way, a new generation of vegetative propagules will have been contributed to sustain the population into future growing seasons. The response of the FL tubers is consistent with the literature that demonstrates that tuber production normally occurs in the winter months from October through
April (Harlan et al. 1985). Because this study was terminated before a full year of growth could be observed, this section of growth was unable to be captured.

When combined, our findings from the controlled conditions trial and the outdoor mesocosm trial complement each other nicely and paint a clear picture as to how this uncharacteristic population of dioecious hydrilla is existing in Philpott Reservoir. Contrary to our hypotheses, we found that the VA hydrilla is not growing and developing in-than-usual colder conditions, but has instead learned how to avoid such unfavorable conditions by condensing its life cycle down into a shorter active window and ultimately reducing its risk of mortality due to seasonal water temperature fluctuations.

Henry (2017) conducted a similar study on monoecious hydrilla phenology at the Laurel Springs and Raleigh research sites. This work revealed that monoecious hydrilla produced more tubers in Raleigh than in Laurel Springs. It was also demonstrated that monoecious hydrilla tubers produced in Raleigh, though more numerous, were smaller in size compared to those produced in Laurel Springs. This indicates that monoecious hydrilla responds to cool climatic conditions and environmental stress by investing more resources to increase the fitness of future generations. In our study, dioecious hydrilla from FL populations produced more tubers in Laurel Springs and there was no significant difference in the size of collected tubers from each study site (Figure 6). Although the results of these two studies don't directly agree, they suggest that monoecious and dioecious hydrilla responds to climate cues differently, and they also both reveal some of the adaptive characteristics of hydrilla to environmental variation along latitudinal gradients in various climatic regimes.

Although water temperatures at the Raleigh, NC and Laurel Springs, NC research sites were different throughout the course of the study, neither replicated the observed water
temperatures of Philpott Reservoir (Figure 4). Small mesocosm size likely allowed for more substantial water temperature fluctuations throughout the growing season that are not normally experienced in large reservoir systems, such as Philpott Reservoir. Because of this, it is difficult to directly compare the phenology of the studied plants with that of the plants growing in Philpott Reservoir. Nevertheless, this research still provides important insight into the potential adaptive ability of dioecious hydrilla in response to environmental pressure.

The first reported introduction of dioecious hydrilla in the United States occurred in Florida in the 1950s (Hoyer et al. 2005). It rapidly spread into nearly all of the watersheds in the state of Florida and spread northward and westward into watersheds in Georgia, Louisiana, and Alabama. More recently, it has been documented in eastern Texas and South Carolina. It is important to note that, despite the significant amount of time and resources that water resource managers have dedicated to counteracting the growth hydrilla and mitigating its spread throughout the United States, it is still expanding its range into new environments. As it is exposed to these new ecosystems, it is subjected to new environmental pressures and could be slowly adapting to these new systems in the same way that it may be adapting to life in Virginia. It is important to take note of behavioral and phenological differences in such dioecious hydrilla populations to most successfully manage it as we move forward.

Although dioecious hydrilla is unable to reproduce sexually in the US due to the lack of male plant populations, selection events that lead to adaptation to environmental conditions in dioecious plants have still been documented. Examples of potential processes for such events could involve non-genetic inheritance and somatic mutations spread through vegetative propagation (Arias et al. 2005; Day and Bonduriansky 2011).
Non-genetic inheritance has not been widely discussed in the scientific community, especially when compared to the well-accepted theory of genetic or Mendelian inheritance. Non-genetic inheritance has been defined as, “any effect on offspring phenotype brought about by the transmission of factors other than DNA sequences from parents or more remote ancestors” (Bonduriansky and Day 2009, p. 106). Pathways of non-genetic inheritance can involve hormones, microbes, RNA, chromatin modifications, and prions (misfolded proteins) (Toth 2015). Adaptations that arise from events that involve non-genetic inheritance are usually not permanently ingrained into generational succession because they are not caused by changes in the DNA sequence and instead may only be expressed for a few generations depending on environmental conditions (Toth 2015). Non-genetic inheritance is thought to be more prevalent in plants than in animals because plants are believed to have a higher incidence of phenotypic plasticity and, by definition, only plastic traits can be manipulated through non-genetic pathways (Bonduriansky and Day 2009). Furthermore, invasive plant populations, such as hydrilla in the US, tend to have more phenotypic plasticity when compared to native plant populations (Richards et al. 2006; Henry 2017). The results of this study suggest that non-genetic inheritance could be impacting the growth and success of dioecious hydrilla in Virginia but a more detailed comparison of their genetic differences would need to be completed to support this hypothesis. Perhaps delayed tuber sprouting and rapid tuber production are “learned” traits that this population has sustained over time in the face of harsh environmental pressure.

Also, dioecious hydrilla has already exhibited gene manipulation through the development of resistance to fluridone, a major aquatic herbicide that has been used in hydrilla management for nearly 40 years (Hoyer et al. 2005). In this case, resistance occurred through a somatic cell mutation at fluridone’s target site on the *pds* gene (Arias et al. 2005). Somatic cell
mutations differ from germ cell mutations as they are unrelated to sexual reproduction processes. However, in contrast to animals, somatic cell mutations in plants can be passed on to evolutionary descendants, which further complicates the issue of the presence of herbicide resistant hydrilla populations in Florida (Simberloff and Leppanen 2018). In any case, it is also feasible that a somatic cell mutation could be a factor related to the fitness of the dioecious hydrilla population at Philpott Reservoir which could be revealed through genetic testing.

In conclusion, the results of this research indicate that there is a difference in the sprouting and growth dynamics of FL and VA dioecious hydrilla populations. The next steps of this research should involve truthing these findings with observational and quantitative data from the field. If the growth and reproductive dynamics of the dioecious hydrilla population within Philpott Reservoir behave in the same way that was revealed through these laboratory and mesocosm trials, then management efforts must appropriately reflect the phenology of this population of hydrilla to maximize efficacy. For example, herbicide treatments should be applied in the spring when water temperatures reach at least 25°C to best target newly sprouted tubers from the tuber bank. Additionally, all treatments should be completed before October to target plants before maximum tuber production occurs.

**SOURCES OF MATERIALS**

1Fisher Scientific Traceable Digital Thermometer, Thermo Fisher Scientific, 168 Third Avenue, Waltham, MA, 02451

2JMP Pro 14.2., SAS Institute Inc., 101 SAS Campus Drive, Cary, NC, 27513

3SigmaPlot 14.0, Systat Software Inc., 2107 North First Street, Suite 360, San Jose, CA, 95131

4YSI 556 MPS Multiprobe System, YSI Inc./Xylem Inc., 1700/1725 Brannum Lane, Yellow Springs, OH, 45387
REFERENCES


Jones HL. 1995. Allelopathic ability of various aquatic plants to inhibit the growth of *Hydrilla verticillata* (L.f.) Royle and *Myriophyllum spicatum* L. Technical Report A-95-1, U.S. Army Corps of Engineer Waterways Experiment Station, Vicksburg, MS.


Table 3-1: Mean, minimum, and maximum air temperatures (°C) recorded in Laurel Springs, NC (Upper Mountain Research Station) and Raleigh, NC (Reedy Creek Field Laboratory) throughout the duration of the mesocosm study. Means separated by Student’s t test (α = 0.05). Data from the North Carolina State Climate Office (http://climate.ncsu.edu/)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SE</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laurel Springs, NC</td>
<td>11.50 ± 0.42</td>
<td>a</td>
<td>-11.00</td>
</tr>
<tr>
<td>Raleigh, NC</td>
<td>16.84 ± 0.45</td>
<td>b</td>
<td>-4.89</td>
</tr>
</tbody>
</table>
**Figure 3-1:** Starting weight of studied dioecious hydrilla tubers. Means were separated by Student’s t-test ($\alpha = 0.05$).
**Table 3-2:** Observed water temperatures (°C ± SE) in test tubes throughout the extent of study. Means separated by Tukey HSD test ($\alpha = 0.05$).

<table>
<thead>
<tr>
<th>Column on Table</th>
<th>State</th>
<th>Average Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>FL</td>
<td>35.73 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>35.75 ± 0.04</td>
</tr>
<tr>
<td>B</td>
<td>FL</td>
<td>32.63 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>32.57 ± 0.04</td>
</tr>
<tr>
<td>C</td>
<td>FL</td>
<td>28.56 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>28.54 ± 0.03</td>
</tr>
<tr>
<td>D</td>
<td>FL</td>
<td>24.53 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>24.50 ± 0.03</td>
</tr>
<tr>
<td>E</td>
<td>FL</td>
<td>19.67 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>19.71 ± 0.03</td>
</tr>
<tr>
<td>F</td>
<td>FL</td>
<td>14.94 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>14.92 ± 0.04</td>
</tr>
<tr>
<td>G</td>
<td>FL</td>
<td>9.26 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>9.44 ± 0.04</td>
</tr>
</tbody>
</table>
Table 3-3: Percentage of sprouted tubers and their associated mean stem lengths on Day 14 of the study. Means separated by Tukey HSD test ($\alpha = 0.05$).

<table>
<thead>
<tr>
<th>Average Temperature (C)</th>
<th>State</th>
<th>Sprouted Stem Length (cm)</th>
<th>% Sprouted</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>FL</td>
<td>0.0 ± 0.0 d</td>
<td>0 ± 0.0%</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>0.0 ± 0.0 d</td>
<td>0 ± 0.0%</td>
</tr>
<tr>
<td>15</td>
<td>FL</td>
<td>0.2 ± 0.2 d</td>
<td>4.8 ± 4.8%</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>0.0 ± 0.0 d</td>
<td>0 ± 0.0%</td>
</tr>
<tr>
<td>20</td>
<td>FL</td>
<td>3.0 ± 1.0 bcd</td>
<td>42.9 ± 14.0%</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>0.0 ± 0.0 d</td>
<td>0 ± 0.0%</td>
</tr>
<tr>
<td>25</td>
<td>FL</td>
<td>11.5 ± 2.5 a</td>
<td>61.9 ± 11.3%</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>0.9 ± 0.5 d</td>
<td>14.3 ± 9.9%</td>
</tr>
<tr>
<td>29</td>
<td>FL</td>
<td>6.7 ± 1.7 ab</td>
<td>61.9 ± 8.7%</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>4.0 ± 1.1 bcd</td>
<td>66.7 ± 7.3%</td>
</tr>
<tr>
<td>33</td>
<td>FL</td>
<td>5.9 ± 1.3 bc</td>
<td>76.2 ± 9.5%</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>3.6 ± 1.0 bcd</td>
<td>61.9 ± 15.3%</td>
</tr>
<tr>
<td>36</td>
<td>FL</td>
<td>1.6 ± 0.4 cd</td>
<td>42.9 ± 15.8%</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>0.5 ± 0.3 d</td>
<td>14.3 ± 9.9%</td>
</tr>
</tbody>
</table>
Figure 3-2: Stem lengths of sprouted tubers by day 14 of the study. Bars indicate ± SE. The dotted line is the best-fitted curve for tested Florida dioecious hydrilla tubers ($r^2 = 0.87$). The solid line is the best-fitted curve for tested Virginia dioecious hydrilla tubers ($r^2 = 0.99$). See Equation 1 for additional curve information.
Equation 3-1: Sprouted stem lengths of a) FL and b) VA dioecious hydriella tubers in response to temperature 14 days after study initiation ($y = $ stem length (cm); $x = $ water temperature ($^\circ$C)).

a. FL: $y = 10.3 \exp[-0.5 \left(\frac{x-25.7}{5.1}\right)^2]$; $r^2 = 0.87$

b. VA: $y = 5.0 \exp[-0.5 \left(\frac{x-29.9}{2.9}\right)^2]$; $r^2 = 0.99$
Figure 3-3: Percent of spouted tubers by day 14 of the study. Bars indicate ± SE. The dotted line is the best-fitted curve for tested Florida dioecious hydrilla tubers ($r^2 = 0.94$). The solid line is the best-fitted curve for tested Virginia dioecious hydrilla tubers ($r^2 = 0.99$). See Equation 2 for additional curve information.
**Equation 3-2:** Percent of a) VL and b) VA dioecious hydrilla tubers sprouted by day 14 of study in response to temperature ($y = \%$ sprouted; $x = $ water temperature (°C)).

a. FL: $y = 71.6 \exp[-0.5 \left(\frac{x-29.3}{7.7}\right)^2]; \ r^2 = 0.94$

b. VA: $y = 80.8 \exp[-0.5 \left(\frac{x-30.7}{3.0}\right)^2]; \ r^2 = 0.99$
Figure 3-4: Collected water temperature data (°C) from the Raleigh, NC and Laurel Springs, NC research stations as well as mean water temperatures representing those at Philpott Reservoir (Bassett, VA). Data for Philpott Reservoir was obtained from the USGS National Water Information System (https://waterdata.usgs.gov/). Bars represent ± SE.
Figure 3-5: Results from outdoor mesocosm trial. Number of tubers produced, longest stem length (cm), and harvested total dry weight (g) by FL and VA dioecious hydrilla in Laurel Springs, NC and Raleigh, NC over time. Bars indicate ± SE. The dotted line is the best-fitted curve for tested FL dioecious hydrilla. The solid line is the best-fitted curve for tested VA dioecious hydrilla. R² from non-linear regression analysis included in respective plots.
Figure 3-6: (a) Mean number of tubers, (b) mean percent of viable tubers, (c) mean stem length of sprouted tubers, and (d) mean fresh weight of tubers produced by Florida and Virginia populations of dioecious hydrilla 8 months after planting in Raleigh, NC and in Laurel Springs, NC. Viability and sprouted stem length of tubers were determined after 14 days of exposure to ideal sprouting conditions in a greenhouse setting. Error bars represent SE. Letters denote significant differences according to a Student's t-test.
CHAPTER 4: Assessing management options for dioecious *Hydrilla verticillata* in Philpott Reservoir (VA)

Abstract

*Hydrilla* (*Hydrilla verticillata* (L.f) Royle) is a troublesome non-native aquatic weed in the United States. After its introduction to the United States, hydrilla rapidly dispersed to many freshwater resources, and its excessive growth has disrupted native ecosystems and impacted local economies. Recently, dioecious hydrilla was identified in Philpott Reservoir, a 1,165 hectare (2,880 acre) United States Army Corps of Engineers impoundment located in Virginia’s southcentral Piedmont geographic region. To determine an appropriate management strategy for this population of hydrilla, the efficacy of fluridone, copper/diquat and endothall/diquat on dioecious hydrilla were assessed in repeated field trials at Philpott Reservoir. Study plots were monitored at 1 and 2 months after treatment and compared to pre-treatment conditions using both physical biomass samples and biovolume estimates acquired via hydroacoustic technology. When directly correlated, the collected biomass and estimated biovolume of hydrilla in treatment plots exhibited a positive non-linear trend of moderate strength (Spearman $\rho = 0.49; p = < 0.001$). By 2 months after treatment, dioecious hydrilla biomass was reduced from pre-treatment levels by 70% in the fluridone treatment plot, 100% in the endothall/diquat plot, and 100% in the copper/diquat treatment plot. The biovolume estimates measured similar patterns in treatment efficacy but were complicated by the presence of other submersed aquatic vegetation due to its non-selective nature and shallow water depths of some treatment plots. This research suggests that, despite a moderately high degree of agreement between biovolume and biomass estimates, monitoring methods can influence the perceived effect of treatment efforts in aquatic environments. This work will also inform future management decisions that are associated with the population of dioecious hydrilla at Philpott Reservoir.
Introduction

Philpott Reservoir is operated and managed by the United States Army Corps of Engineers (USACE) and contains the northern-most population of dioecious *Hydrilla verticillata* (L.f.) Royle (“hydrilla”) on the eastern coast of the United States (US). Hydrilla is a federally listed noxious weed in the US and is recognized as an invasive species with an invasive rank of “high” in the state of Virginia (Heffernan et al. 2014; USDA-NRCS 2020;). This troublesome submersed aquatic macrophyte is understood to have significant negative ecological and economic impacts where it invades if left unmanaged (Langeland 1996). In reservoir systems, some of these impacts include decreased water quality and biodiversity, impeded recreational opportunities, and interference with power generation and dam maintenance (Clayton 1996; Clayton and Champion 2006; Getsinger et al. 2014). To avoid the outcomes of the aforementioned negative impacts, methods for managing this invasive species should be prioritized (Hussner et al. 2017).

Chemical control options were selected as the most suitable management option for the hydrilla population at Philpott Reservoir. Biological controls, such as triploid grass carp (*Ctenopharyngodon idella*) are widely utilized for hydrilla management in Piedmont reservoirs; especially those that do not sustain native populations of submersed aquatic vegetation, such as Philpott Reservoir (Kirk et al. 2014). However, active spawning populations of the endangered Roanoke Logperch (*Percina rex*) have been documented both upstream and downstream from Philpott Reservoir within the Smith River (Roberts and Angermeir 2012). In an effort to avoid interference with the sensitive population dynamics of this native fish species, grass carp were not considered as an option for hydrilla control at Philpott Reservoir.
Chemical control methods for dioecious hydrilla have been widely studied. There are currently 17 registered active ingredients for aquatic plant control in the US (UF IFAS 2020). Of those, 8 are considered to be efficacious for dioecious hydrilla control (Hussner et al. 2017; Netherland and Richardson 2016). Chemical methods can achieve results more rapidly and with higher species selectivity when compared to other control options such as grass carp and mechanical harvesting. Since so many herbicide active ingredients show activity on hydrilla, it was important to test some of the available options.

Endothall is commonly used for both monoecious and dioecious hydrilla control in the US (Netherland et al. 1991; Poovey and Getsinger 2010). Although it classified as a contact herbicide, and impacts treated vegetation in a rapid manner, it has also demonstrated systemic activity (Ortiz et al. 2019). Vegetation is effectively controlled by endothall through interference with cell membranes and cellular respiration and division processes, however, its mode of action is not fully understood and is listed as 'unknown' in the WSSA classifications (USEPA 2005; Tresch et al. 2011). Netherland et al. (2001) determined that endothall requires slightly longer exposure times and higher rates when targeting hydrilla compared to other aquatic plant species. Specifically, they determined that treatments of 2.0 mg/L with 48-hour exposure time or 3.0 mg/L with 24-hour exposure time effectively reduced hydrilla biomass by >85% in growth chamber and mesocosm trials. Endothall has an additive relationship with diquat when applied together for hydrilla control (Chiconela and Haller 2013).

Diquat is a contact herbicide and is classified as a photosystem I inhibitor (WSSA Group 22) and destroys cells by harvesting light energy to form hydroxyl radicals that target plant cell membranes (Selden 2015). Diquat is considered to be a non-selective contact herbicide and plant symptomology is often visualized rapidly (within hours) after application, especially when
environmental conditions are sunny and warm. Diquat is relatively short-lived in the environment by rapidly and irreversibly binding to suspended sediments in the water column. This quality, in turn, makes diquat making it a poor choice for waterbodies with high turbidity. In addition to endothall, diquat is also often used in combination with copper products for hydrilla control because of their synergistic relationship (Sutton et al. 1972; Chiconela and Haller 2013).

Copper, a naturally occurring element, is essential to all life at low concentrations. When exposed to excessive quantities of copper, however, aquatic plants are often negatively impacted. Copper was registered for aquatic use in the 1950s making it the first regulated chemical control option for aquatic vegetation (Netherland 2014). When applied to aquatic environments, copper is fast-acting and selectivity is rate dependent. Because it is fast-acting, it requires a relatively short contact time (minutes to hours) when compared to other active ingredients. Copper is long-lasting in the environment due to its elemental structure. As such, repeated applications of copper in waterbodies can lead to a buildup of copper in some sediments and potentially lead to an increase in sediment toxicity (Rader et al. 2019). However, studies of waterbodies where copper-based products have been utilized in multi-year management plans have demonstrated that copper concentrations are not long-lasting in natural systems and where copper concentrations have built up, they are not biologically available to aquatic organisms (Iwinski et al. 2016).

Fluridone is a systemic herbicide classified as a phytoene desaturase (PDS) inhibitor (WSSA Group 12) that interferes with the biosynthesis of essential pigments that hydrilla uses to carry out photosynthesis (Van and Steward 1986). Due to this mechanism of action, the typical visual symptomology of plants post-treatment includes bleached (white) leaf tips. Because of its
slow activity, it requires a longer exposure time (weeks to months) to effectively control hydrilla when compared endothall, diquat, or copper (Van and Steward 1986).

Plant biomass is a commonly used metric for post-treatment analysis for field studies of submersed aquatic vegetation (SAV) management (Johnson and Newman 2011; Nichols 1984). Plant biomass can be influenced by many factors including light availability, plant phenology, water and sediment chemistry, water temperature, natural disturbance (wave energy, water level fluctuation, etc.), and anthropogenic disturbance (management-based, recreational, etc.) (Madsen 1993). Multiple methods for biomass sampling of SAV have been used by researchers in their field trials. Biomass samples are often collected using destructive methods such as dredging, coring, and raking. To assess changes in community dynamics over time, biomass sampling is often conducted at precisely-defined points within a study plot and revisited at predetermined time intervals. Incorporating biomass sampling into field-based research is often beneficial for water research managers because it allows for the ability to directly monitor species specific plant growth dynamics over time. However, the time intensive nature of this sampling method can severely limit the number of data points collected per unit area, thereby potentially reducing the accuracy of estimates in a large-scale study site as compared to other methods.

Boat-based hydroacoustic technology has been developed for submersed aquatic vegetation monitoring and is now widely used among researchers. Hydroacoustic methods are an intermediary option for monitoring between the spectrum of direct, physical collection methods and indirect estimation based upon remote sensing and aerial imagery analysis. Data collected by hydroacoustic means are acquired through a boat-mounted transducer that sends acoustic signals at specific frequencies down through the water column (Sabol et al. 1998). Those signals are associated with a spatial coordinate point and can be interpreted as either
bottom hydrosoil or SAV, depending on their strength upon returning to the transducer (Sabol et al. 1998). After post-processing, collected hydroacoustic data can provide pertinent information on multiple site characteristics such as depth, sediment composition, and the biovolume (a metric derived from dividing plant height by water depth) of SAV. With this tool, large-scale systems can be monitored relatively quickly and with high resolution. However, these biovolume estimates incorporate all vegetation as one estimate and lack species specific classification that other monitoring methods provide.

Philpott Reservoir is a 1,165 hectare (2,880 acre) impoundment with just over 100 miles (161 km) of shoreline located along the Smith River in Virginia’s southcentral piedmont region (36° 48’ 41.73”N, 80° 03’ 30.17”W; elev. 297 m.). This reservoir is situated within the second-largest watershed in the state of Virginia, the Roanoke River watershed, which covers a total of 6,274 mi² (16,249.6 km²). The US Army Corps of Engineers (USACE) has maintained Philpott Reservoir for flood control and hydroelectric power generation since the completion of its dam in 1953. In 1995, the recreational value of the Smith River and Philpott Reservoir fisheries and combined with value of the generated hydropower from the Philpott Dam was estimated to be over $1,200,000 (Hartwig 1998). In addition to its significant economic value, the reservoir itself plays an important ecological role for fish and wildlife enhancement within the Roanoke River watershed.

Philpott Reservoir is deep, has hard sediment composition, and does not support an expansive littoral zone due to steep sloping shorelines. Additionally, the reservoir experiences frequent water level fluctuations resulting in high shoreline disturbance that has led to considerable erosion. Water quality at Philpott Reservoir is typically rated as “good” with high dissolved oxygen levels, neutral pH, and secchi depths of over 2.5 meters (NWQMC 2020).
Despite having a less than ideal physical habitat, a healthy population of dioecious hydrilla exists within Philpott Reservoir. Likely due to environmental factors, the hydrilla has a particularly patchy distribution and is mainly concentrated at the 3 m depth contour along the shoreline and in isolated shallow coves. Other SAV present in Philpott Reservoir include egeria (Egeria densa (Planch)), chara (Chara spp.), and naiad (Najas guadalupensis (Spreng) Magnus), however, hydrilla is most certainly the dominant species of aquatic vegetation. Current revegetation projects at the reservoir are also actively supporting the introduction of native squarestem spikerush (Eleocharis quadrangulata (Michx.) Roem. & Schult.), American water willow (Justicia americana (L.) Vahl), and vallisneria (Vallisneria americana (Michx)). This project is the first account of active hydrilla management within Philpott Reservoir.

The goal of this study was to: 1) determine the efficacy of selected chemical control options for the unique population of Virginian dioecious hydrilla, and, 2) compare post-treatment biomass and biovolume sampling efforts at Philpott Reservoir to determine a long-term monitoring protocol that best captures the dynamics of hydrilla management over time. Because this population of dioecious hydrilla has remained unmanaged in Philpott Reservoir, it is important to determine the most efficacious methods for management and monitoring of the population over time through small-scale trials before moving to reservoir-wide efforts. The results of this research will inform future best management practices for the dioecious hydrilla population in Philpott Reservoir.

Methods

Herbicide Treatments:

Three herbicide treatments [150 ppb fluridone (Sonar™ SRP); 1 ppm copper (Current™)/2 gal SA⁻¹ diquat (Tribune™); 1.5 ppm endothall/0.3 ppm diquat (Aquastrike™ (pre-
mixed)) were selected to target the dioecious hydrilla population in Philpott Reservoir. All treatments were replicated in either time or space and were randomly assigned to a pre-determined study plot along the shoreline of Philpott Reservoir (Table 1). Treatment with fluridone and copper/diquat were applied to the same plot in two separate years after sufficient regrowth from the established tuber bank occurred. Treatment with endothall/diquat was applied to two separate plots within the same year. All plots were spatially isolated in protected shoreline areas to prevent cross contamination, and with minimal water exchange and supported comparable levels of hydrilla pre-treatment (Figure 1; Table 2). Hydrilla was the dominant species in all plots, however chara and egeria were also present in low densities. An untreated reference plot was also established for treatment efficacy comparison through time.

Granular fluridone granules were applied via a boat-based pellet-spreader and was divided into 3 monthly applications of 50 ppb to maintain concentrations over the growing season. Liquid copper/diquat and endothall/diquat applications were applied through subsurface injection via on-board drop hoses combined with targeted spot treatment via handheld sprayer gun for near-shoreline, difficult to access regions of the treatment polygons. All treatments were administered in July 2018 and repeated in July 2019 with the exception of the endothall/diquat combination which was tested only in 2019 at two locations.

Vegetation biomass samples were collected from each study plot at 3 – 4 geo-referenced points following methods described by Johnson and Newman (2011) at pre-treatment, 1 month after treatment (MAT), and 2 MAT time points. Because of the patchy distribution of hydrilla within treatment zones pre-treatment, sample points were determined based on hydrilla presence at the pre-treatment sampling time point and georeferenced for future assessment. At each point, a vertical ¼ meter sampling rake was lowered straight down into the water column, rotated 360°,
and slowly pulled back up to the surface. Collected plants were hand-separated by species, rinsed, dried to a constant mass, and weighed.

Concurrently, at each sampling period, hydroacoustic data was recorded throughout each treatment plot following methods inspired by Howell and Richardson (2019). A Lowrance HDS-7 Gen3\(^1\) echosounder coupled with a down-scanning boat-mounted transducer logged sonar signals to a micro-SD memory card. During data collection, boat speed was maintained at approximately 8 km hr\(^{-1}\) (5 mph) in areas within the treatment plot with water depths greater than 0.76 m. The collected data were uploaded to a cloud-based post-processing platform (ciBioBase\(^2\)) and vegetation biovolume within the water column was determined based on an internal algorithm. Plant biovolume represents the ratio of the vegetated area within the water column over the total water depth at that same point along a traveled track \([\text{biovolume} = (\text{plant height} / \text{water depth}) \times 100]\); Valley 2005]. During post-processing, the collected data were condensed into a point-grid by averaging 5 – 30 individual sonar signals to a GPS-referenced point via a ciBioBase proprietary algorithm (Navico 2019). During this time, the data were also amended such that points where water depths were too shallow for proper analysis of plant biovolume were excluded (Navico 2019).

**Data Processing**

In the ciBioBase web interface, data offsets were applied to all sonar tracks so that water depths were normalized for full-pool conditions (296.9 meters above sea level). Vegetation data were exported as a CSV in grid format and imported as XY data into ArcMap 10.7.1\(^3\) in the WGS 1983 geographic coordinate system. In ArcMap, all shapefiles were projected to NAD 1983 and transformed into a raster surface with the 'Spline with Barriers' tool in the Spatial
Analyst toolbox (Inputs: Z value field = 'biovolume'; Input Barrier features = reservoir polyline; Output cell size = 1.5; Smoothing factor = 0). Mean biovolume data was extracted with the 'Zonal Statistics as Table' tool (Spatial Analyst) from 2.5 m² quadrats centered on the biomass sampling points of each respective treatment plot. Quadrats were utilized to calculate mean biovolume to account for spatial discrepancies between the GPS-referenced biomass point and the actual point sampled in the field.

All biovolume estimates were linked to biomass samples that were taken on the same day except for one sampling time point in the endothall/diquat treatment plot at 2 MAT. The biovolume data acquired at this timepoint was insufficient for analysis and therefore biovolume data acquired 1 week before the biomass samples were utilized. Additionally, insufficient biovolume data was collected during pre-treatment sampling at the untreated reference plot, therefore those data points are not included.

**Statistical Analysis**

The extracted biovolume data was coupled with the associated biomass data for each sampled point and were imported into JMP Pro 14.2.04 software for statistical analysis. Before analysis, the biovolume data points were tested for an accurate representation of hydrilla presence with a paired t-test between the raw biovolume values and biovolume values corrected for % hydrilla biomass (corrected biovolume = total biovolume * (hydrilla dry weight/total dry weight)) collected at each sample point. The analysis resulted in a significant difference between raw and corrected biovolume values (t = 2.22; p = 0.0290). Subsequent analysis was performed on corrected biovolume to best describe the change in hydrilla presence over time despite having
negative implications for the applicability of biovolume data as a standalone parameter for pre- and post-treatment monitoring.

A bivariate orthogonal regression with equal variances was performed on standardized paired biovolume and biomass values (Figure 2). Unlike simple linear regression, orthogonal regression predicts the relationship between two variables that contain error and is commonly applied to studies that are interested in measuring the same outcome with two different methods (Carroll and Ruppert 1994). Variable standardization followed the equation \([ (x - \bar{x})/\sigma ]\). To test the strength of their nonlinear relationship, a Spearman’s Rank test was also performed between standardized biomass and biovolume values. Insignificant plot by treatment interactions for biomass \((p = 0.7039)\) and biovolume \((p = 0.1764)\) indicated that the data collected in repeated treatments (either by space or time) could be pooled for herbicide efficacy analysis. Control of hydrilla by treatment type was determined by comparing pre- and post-treatment values of biovolume and biomass within each sampled plot with one-way ANOVA. Means were separated by the nonparametric Wilxcon method (Table 2).

**Results**

Biovolume (BV) and biomass (BM) exhibited a significant linear orthogonal relationship with a relatively low level of correlation \((r^2 = 0.2929; p < 0.0062;\) Figure 2). These results indicate that there is a fairly weak positive trend in the relationship between hydrilla biovolume and biomass. A Spearman’s Rank analysis between biomass and biovolume estimates revealed a slightly stronger association \((Spearman \rho = 0.4933; p < 0.0001)\), indicating that the two variables likely follow a nonlinear trend.
Copper/diquat and endothall/diquat treatments effectively reduced dioecious hydrilla in Philpott Reservoir. Both biovolume and biomass data captured changes in vegetation presence over time (Table 2). According to both measurements, hydrilla growth in the untreated reference plot remained constant over the course of study.

In the copper/diquat treatment plot, hydrilla biomass was reduced from 3.7 ± 0.6 g at pre-treatment levels to 0.3 ± 0.2 g by 1 MAT (-92%). Biovolume was also reduced 1 MAT from 17 ± 5% to 3 ± 1% (-82%). At 2 MAT, hydrilla in the copper/diquat treatment plot remained at a reduced level compared to pre-treatment conditions of 0 ± 0 g biomass (-100%) at 3 ± 1% biovolume (-82%). Biomass and biovolume estimates generally agree in terms of treatment efficacy at this treatment site.

Hydrilla biomass exhibited a similar response to endothall/diquat treatment. A decrease in biomass from 5.0 ± 1.3 g at pre-treatment to 0.8 ± 0.5 g 1 MAT (-84%) and 0 ± 0 g 2 MAT (-100%) was observed. Biovolume data did not reflect comparable results in the endothall/diquat plot at 1 MAT as estimates increased from 20 ± 7% pre-treatment to 22 ± 7% 1 MAT (+10%). However, at 2 MAT, biovolume was significantly reduced to 3 ± 1% (-85%).

In the fluridone treatment plots, neither biovolume nor biomass decreased from pre-treatment levels by 2 MAT. Hydrilla biomass decreased from 2.7 ± 1.1 g pre-treatment to 0.8 ± 0.6 g 2 MAT (-70%). It should be noted that the fluridone treatment plot had significantly shallower sample points (mean depth = 1.0 ± 0.3 ft) when compared to the rest of the plots, which may be a confounding factor for both biomass and biovolume estimates. Below a depth of 0.76 m, hydroacoustic data may exhibit false-positive readings (Howell and Richardson 2019).
Discussion

To date, multiple studies have applied hydroacoustic data to the study of SAV population density and distribution (Maceina and Shireman 1980; Maceina et al. 1984; Duarte 1986; Thomas et al. 1989; Sabol et al. 2009; Valley et al. 2015; Howell and Richardson 2019). Findings from this study generally agree with aspects of previously published literature. Howell and Richardson (2019) correlated biovolume estimates and sampled biomass of SAV in two Piedmont reservoirs and found a strong positive trend in their nonlinear correlation with a Spearman $\rho = 0.79$. The stronger correlation realized by Howell and Richardson (2019) in comparison to the present study could be attributed to their more diverse dataset with more samples and better representation of samples at varying water depths. Further, Torso et al. (2020) sampled biomass and biovolume of SAV in multiple temperate waterbodies and determined Spearman $\rho$ values that ranged from 0.49 – 0.75 and cited that differences in correlative strength reflected waterbody-specific characteristics. Both Torso et al. (2020) and Howell and Richardson (2019) recognized that the non-linear relationship between biomass and biovolume could be explained by the capability of SAV biomass to occupy greater than 100% of the water column through the formation of surface mats and branching.

Control of dioecious hydrilla with copper/diquat, fluridone, and endothall/diquat was successful at Philpott Reservoir, however, pre- and post-treatment monitoring protocols can influence the perceived efficacy. At Philpott Reservoir, mixed SAV composition in some treatment zones warranted the use of at least one sampling method that allowed for speciation within samples. Time and effort could probably have been spared by only implementing hydroacoustic-based analyses (Sabol et al. 2009) but a trade-off of reduced detection for small-scale change over time would need to be made.
All studied plots supported comparable levels of hydrilla biomass and biovolume prior to treatment. The fluridone treatment plot also supported a fairly dense egeria population when compared to the other sites. Although studies have suggested that egeria is the weaker of the two competitors in a mesocosm analysis (Mony et al. 2007), the response of hydrilla to interspecific competition may interact with its response to chemical control. Fluridone is understood to effectively control egeria (Netherland 2004; Caudill et al. 2019), however, minimal research has questioned the effects of biological competition on herbicide efficacy in aquatic environments.

It should be noted that the physical characteristics of Philpott Reservoir may have caused complications with the observed herbicide efficacy in all treatment plots. For example, frequent water level fluctuation due to the maintenance of the Philpott Dam causes shoreline disturbance that directly impacts SAV growth due to dewatering and water quality variability. By 2 MAT, water levels were down 2.6 feet and 3.15 feet from the full pool in Year 1 and Year 2 of treatment, respectively (USGS 2020). However, while these disturbances could have directly influenced the results of this study, it could be assumed that all studied plots experienced similar effects of the disturbance and that the hydrilla would have responded similarly in all situations.

A separate study has been monitoring hydrilla tuber bank dynamics at Philpott Reservoir since 2017. Densities have ranged from a minimum of 20.2 ± 3.1 tubers m⁻² (Winter 2019) to a maximum of 30.9 ± 6.7 tubers m⁻² (Spring 2019) (T. Harris, personal communication). These densities are relatively low when compared to studies of other water bodies with dioecious hydrilla infestations (Netherland 1997), however, their sustained presence in the hydrosol indicates that continued management needs to be completed at Philpott Reservoir to most effectively control the dioecious hydrilla population. Due to the successful control achieved with this management program, future management efforts can continue to rely on copper/diquat and
endothall/diquat combinations at Philpott Reservoir, however, other options should also be considered as there. Changes in application rates and timing as well as a rotation in the products used year after year on each site should occur in an effort to mitigate the risk of resistance development and to continue to achieve successful control over this highly invasive aquatic weed in a dynamic environmental system.

**SOURCES OF MATERIALS**

1. Lowrance HDS-7 Gen3, Navico Inc., 4500 South 129th East Avenue Suite 200, Tulsa, OK 74134.


3. ArcMap 10.7.1, Environmental Systems Research Institute (ESRI), 380 New York Street, Redlands, CA 92373

4. JMP Pro 14.2.0, SAS Institute Inc., 101 SAS Campus Drive, Cary, NC, 27513
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Hartwig JJ. 1998. Recreational use, social and economic characteristics of the Smith River and Philpott Reservoir fisheries, Virginia. Master’s Thesis. Virginia Polytechnic Institute and State University; Blacksburg, VA.


Netherland MD, GW Reed, WD Getsinger. 1991. Endothall concentration and exposure time relationships for the control of Eurasian watermilfoil and hydrilla. Miscellaneous Paper A-91-4. US Army Engineer Waterways Experiment Station, Vicksburg, MS.


Van TK and Steward KK. 1986. The use of controlled-release fluridone fibers for the control of Hydrilla (Hydrilla verticillata). Weed Science. 34:70-7
Figure 4-1: Spatial distribution of treatment plot at Philpott Reservoir.
Table 4-1: Herbicide application rates and treatment schemes targeting dioecious hydilla in Philpott Reservoir, VA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plot</th>
<th>Surface Hectares</th>
<th>Mean Depth (m)</th>
<th>( n ) Sample Points</th>
<th>Years Sampled</th>
<th>Species Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated Reference</td>
<td>1</td>
<td>0.4</td>
<td>4.6</td>
<td>4</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><strong>Fluridone</strong>, 150 ppb <em>(50 ppb applied monthly for 3 consecutive months)</em></td>
<td>2</td>
<td>1.0</td>
<td>2.0</td>
<td>3</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><strong>Copper</strong>, 1 ppm + <strong>Diquat</strong>, 2 gal SA(^1) (Tank Mix)</td>
<td>3</td>
<td>1.2</td>
<td>3.0</td>
<td>4</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><strong>Endothall</strong>, 1.5 ppm + <strong>Diquat</strong>, 0.3 ppm (Pre-Mix)</td>
<td>4</td>
<td>1.5</td>
<td>3.0</td>
<td>4</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.0</td>
<td>3.0</td>
<td>4</td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>

*DHV = dioecious *Hydrilla verticillata* (L.f.) Royle; ED = *Egeria densa* (Planch); CH = *Chara spp.*
Figure 4-2: Bivariate fit of standardized hydrilla dry weight (g) (DW) and standardized vegetation biovolume corrected for proportional hydrilla biomass (%) (BV) at sampled points with equal variance orthogonal regression line plotted ($r^2 = 0.2929; p = <0.0062$).
Table 4-2: Monitoring results at pre- and post-treatment sampling timepoints. Biovolume values are corrected based on % hydrilla biomass present. Letters indicate significantly different records within columns based on nonparametric comparisons using the Wilcoxon method ($\alpha = 0.05$).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Depth at Sample Points (m)</th>
<th>Timing$^a$</th>
<th>Biovolume ($\pm$ SE)</th>
<th>Biomass (g) ($\pm$ SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Untreated Reference</strong></td>
<td>3.2 + 0.5 $^a$</td>
<td>Pre-Treatment</td>
<td>23 + 6% $^a$</td>
<td>4.1 + 2.7 $^a$</td>
</tr>
<tr>
<td></td>
<td>1 MAT</td>
<td></td>
<td>26 + 6% $^a$</td>
<td>4.5 + 1.8 $^a$</td>
</tr>
<tr>
<td></td>
<td>2 MAT</td>
<td></td>
<td>16 + 5% $^a$</td>
<td>8.9 + 2.8 $^a$</td>
</tr>
<tr>
<td><strong>Copper/Diquat</strong></td>
<td>3.0 + 0.5 $^a$</td>
<td>Pre-Treatment</td>
<td>17 + 5% $^a$</td>
<td>3.7 + 0.6 $^a$</td>
</tr>
<tr>
<td></td>
<td>1 MAT</td>
<td></td>
<td>3 + 1% $^c$</td>
<td>0.3 + 0.2 $^b$</td>
</tr>
<tr>
<td></td>
<td>2 MAT</td>
<td></td>
<td>3 + 1% $^c$</td>
<td>0.0 + 0.0 $^b$</td>
</tr>
<tr>
<td><strong>Endothall/Diquat</strong></td>
<td>2.2 + 0.4 $^b$</td>
<td>Pre-Treatment</td>
<td>20 + 7% $^a$</td>
<td>5.0 + 1.3 $^a$</td>
</tr>
<tr>
<td></td>
<td>1 MAT</td>
<td></td>
<td>22 + 7% $^a$</td>
<td>0.8 + 0.5 $^b$</td>
</tr>
<tr>
<td></td>
<td>2 MAT</td>
<td></td>
<td>3 + 1% $^c$</td>
<td>0.0 + 0.0 $^b$</td>
</tr>
<tr>
<td><strong>Fluridone</strong></td>
<td>1.0 + 0.3 $^c$</td>
<td>Pre-Treatment</td>
<td>12 + 7% $^{abc}$</td>
<td>2.7 + 1.1 $^{ab}$</td>
</tr>
<tr>
<td></td>
<td>1 MAT</td>
<td></td>
<td>13 + 6% $^{abc}$</td>
<td>5.2 + 4.1 $^{ab}$</td>
</tr>
<tr>
<td></td>
<td>2 MAT</td>
<td></td>
<td>19 + 8% $^{abc}$</td>
<td>0.8 + 0.6 $^b$</td>
</tr>
</tbody>
</table>

$^a$Treatments occurred on 10 Jul 2018 and 30 July 2019. Pre-treatment sampling occurred 24 hours prior to herbicide application.