ABSTRACT

REGAN, SHANNON MARIE. Factors Affecting Monoecious Hydrilla (*Hydrilla verticillata*) in Dynamic Systems (Under the direction of Dr. Robert J. Richardson).

Monoecious hydrilla (*Hydrilla verticillata* L.f. Royle) is an invasive submersed macrophyte, and is one of the most difficult weeds to control in the United States. It is steadily invading more northern latitudes and increasingly dynamic systems such as high biodiversity rivers, estuaries, and reservoirs with high water fluctuation. It behaves as a herbaceous perennial that senesces in the late fall to early winter. Regrowth is dependent upon sprouting of vegetative propagules called turions which are deposited in vast numbers upon or within the substrate each growing season. Sprouting of these propagules appears to be partly a function of temperature. Timing of sprouting and growth rates were evaluated for monoecious hydrilla axillary and subterranean turions across a water temperature gradient over a period of twelve days. Temperatures evaluated included: T1=41.0˚C, T2=34.9˚C, T3=29.3˚C, T4=24.0˚C, T5=17.6˚C, and T6=12.3˚C. Neither axillary nor subterranean turions sprouted in the hottest and coldest temperatures of 41.0 and 12.3˚C. Optimum growth for both propagules types occurred at 29.3˚C. Shoot lengths were significantly reduced for both turion types in 17.6˚C. Timing of sprouting was not significantly different between axillary and subterranean turions.

Salinity tolerance was also evaluated for both sprouted and unsprouted monoecious hydrilla subterranean turions. As hydrilla continues to invade rivers that empty into brackish waters, salinity will likely be a major factor limiting its expandable range. Salinities evaluated included a range of 0 to 24 ppt at exposure times of 2 to 8 weeks followed by a two-week recovery period. Shoot length, number of lateral branches, and fresh and dry weights were
determined. Sprouted subterranean turions tolerated 8 weeks in 6 ppt and 4 weeks in 9 ppt, following the recovery period. Sprouting occurred in salinities up to 12 ppt. Lateral branching increased in salinities of 3 to 6 ppt.

A final research area focused on managing hydrilla in dynamic systems. Current hydrilla management options are limited for flowing systems with high biodiversity, and have not been extensively studied. A two year pilot herbicide trial was implemented in the Eno River, located in the piedmont region of North Carolina, to investigate the efficacy of treating hydrilla in a lotic system with high biodiversity and threatened or rare species. Two consecutive years of treatment were conducted using a low rate of fluridone maintained over a window of 60-100 days. Treatment impacts to selected target and non-target aquatic species were evaluated. Efforts included quantitative sampling of hydrilla, *Somatogyrus virginicus* Walker (a rare, endemic snail), and *Podostemum ceratophyllum* Michx. (a native macrophyte and habitat of *S. virginicus*) at seven spatially separated sites along the Eno River. Biweekly vegetation monitoring and monthly snail sampling began two weeks before treatment in 2015, and continued through 2016. Hydrilla biomass, shoot length, and tuber density significantly decreased within the treated section of the river. *P. ceratophyllum* densities and lengths were not significantly different between sampling years. *S. virginicus* densities exhibited patterns consistent with annual reproductive cycles. Overall, fluridone effectively controlled hydrilla within the treated area with no apparent negative impacts to the monitored non-target species.
Factors Affecting Monoecious Hydrilla (*Hydrilla verticillata*) in Dynamic Systems

by

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DEDICATION

To my ridiculously supportive parents, siblings, and husband
BIOGRAPHY

Shannon Regan was born and raised in northwestern Pennsylvania. Her love of the water was evident from a young age and lead her to pursue a degree in Marine Science at Coastal Carolina University. Upon receiving her BS, she moved to Raleigh, NC where she was given the opportunity to continue her education in a Master’s program at NC State University where she began studying aquatic plant management and ecology. Outside of school she can be found walking in the woods with her dog or cooking elaborate meals.
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CHAPTER 1

Literature review

*Hydrilla verticillata* (L.f.) Royle is a submersed aquatic macrophyte in the Hydrocharitaceae family. It is distributed world-wide with populations found on every continent except Antarctica (Madeira et al. 1997; Dayan and Netherland 2005). Hydrilla was first introduced into the United States in the 1950s in Florida, and is now established in waters extending as far north as Maine (Maine DEP), and as far west as California and Washington (Figure 1) (Netherland 1997; USGS 2016).

There are two distinct biotypes of hydriolla established within the U.S.: a monoecious biotype with staminate and pistillate flowers located on the same plant, and a female dioecious biotype with only pistillate flowers present (Blackburn et al. 1969; Cook and Luond 1982). Dioecious hydriolla was first introduced to the U.S. in Florida and can be found throughout the southern and western states (Dayan and Netherland 2005; Haller 2014). Monoecious hydriolla was first documented in 1980 in Umstead Lake, NC and is typically suited to more temperate climates of the U.S. such as North Carolina northward (True-Meadows et al. 2016). Populations of monoecious hydriolla have also recently been confirmed in Guntersville Reservoir, Alabama and Lake J. Strom Thurmond, Georgia (Williams et al. 2017).
*H. verticillata* is a monocotyledonous, rooted species with highly branched shoots. Sessile, oblong, finely serrated leaves are 0.75 - 1.5 cm long and arranged in whorls of 3-8 along the length of the stem (Aulbach-Smith and Kozlowski 1996). It is similar in appearance to *Elodea canadensis* Michx. and *Egeria densa* Planch., but can be distinguished by its rougher texture. Hydrilla also has an average of 4+ leaves per whorl whereas *E. canadensis* leaves are arranged in whorls of three (Aulbach-Smith and Kozlowski 1996).

Hydrilla has spread across much of the United States due in part to its multiple modes of reproduction. It is capable of reproducing via fragments, stolons, rhizomes, seed (monoecious biotype only), and vegetative propagules (Cook and Luond 1982; Langeland and Smith 1984). These vegetative propagules called turions form in leaf or branch axils and at the terminal end of positively geotropic rhizomes (Yeo et al. 1984). Subterranean formed turions are commonly referred to as tubers throughout the literature, and will be referred to as such throughout the remainder of this text. Turions and tubers are formed in vast numbers each growing season. Monoecious hydrilla tuber densities in North Carolina lakes have been reported to be as high as 1,312 to 1,512 T/m² (Hodson et al. 1984; Harlan et al. 1985). A more recent study reported an even higher density of 3,077 T/m² (Nawrocki 2011). Undisturbed monoecious tubers can remain viable within the hydrosol in North Carolina for at least six years (Nawrocki 2016). Prolific tuber production leads to the establishment of a tuber bank within the sediment of infested waterbodies. This is essentially akin to a terrestrial seed bank, and is the mechanism that allows the regrowth of monoecious hydrilla in
temperate climates and is responsible for the rapid revegetation after ecological stress or control treatments (Netherland 1997).

Hydrilla has adaptations that enable it to withstand and even thrive in a variety of environments. It is able to grow in varying nutrient and pH levels as it is found in oligotrophic to eutrophic waters as well as highly acidic to alkaline environments (Cook and Luond 1982). Nawrocki et al. (2011) reported little difference in initial growth of monoecious hydrilla tubers sprouted in pH ranging from 4-10. Some level of salinity tolerance has also been reported for this notoriously freshwater species. However, there are discrepancies within the literature. Some report that hydrilla will have little productivity in 4 ppt, will not grow in 6 ppt, will die 10 ppt, and will not survive even 1 day of exposure to 15 ppt (Haller et al. 1974; Twilley and Barko 1990; Frazer et al. 2006). However, Steward and Van (1987) reported tolerances up to 13 ppt. The ability of hydrilla to tolerate brackish waters in its native range of south and southeast Asia has also been noted (Cook and Luond 1982). Hydrilla has lower light requirements for photosynthesis than many other submerged species. It can photosynthesize in < 1% sunlight (Langeland 1996). This lends to its competitive advantage in both time and space as it can begin photosynthesizing earlier in the morning and colonize greater depths than other aquatic species (Langeland 1996). In Crystal River, Florida, the plant was found growing 15 meters deep (Langeland 1996). The dioecious biotype also has special adaptations that allow it to sequester carbon more efficiently. These include utilization of the bicarbonate ion and the ability to switch between C3 and C4-like
acid metabolism (Van et al. 1976). It is unknown whether the monoecious biotype fits into this same photosynthetic category, but speculation suggests it does (Steward and Van 1987).

These physiological adaptations and competitive strategies facilitate rapid hydrilla spread within infested waterbodies. Its aggressive, opportunistic growth characteristics allow it to form dense monocultures that reach the water’s surface (Sutton 1986). These topped out mats of vegetation shade out native species, alter fish populations, and can incite rapid fluctuations in temperature, pH, and dissolved oxygen levels (Dayan and Netherland 2005). Hydrilla has been shown to outcompete other submerged species including *Elodea canadensis*, *Vallisneria americana* Michx., *Potamogeton crispus* L., and *Myriophyllum spicatum* L. (Haller and Sutton 1975; True-Meadows 2013). This superior competition and enhanced dominance is due to its ability to limit light penetration throughout the water column and due to the “presence of millions of meristematic tissues per ha” (Haller and Sutton 1975). Colle and Shireman (1980) determined that large monocultures of hydrilla negatively affect fish species such as redear (*Lepomis microlophus* Günther) and bluegill (*Lepomis macrochirus* Rafinesque). They also reported that largemouth bass (*Micropterus salmoides* Lacepède) of harvestable size were negatively affected by hydrilla cover exceeding 30%, but that the condition of small sized largemouth bass was not affected until coverage exceeded 50% (Colle and Shireman 1980). Colle et al. (1987) also found that redear and bluegill populations were negatively correlated with hydrilla coverage estimates. However, they reported that increased coverage did not affect harvestable populations of largemouth bass or black crappie (*Pomoxis nigromaculatus* Lesueur). Hydrilla has also been identified as the host of a
neurotoxin-producing cyanobacteria, *Aetokthonos hydrollicola* which is linked to a neurological disease (Avian Vacuolar Myelinopathy- AVM). This toxin leads to the formation of brain lesions that affect bald eagles (*Haliaeetus leucocephalus* Linnaeus), American coots (*Fulica americana* Gmelin), grass carp (*Ctenopharyngodon idella* Val.), and herbivorous turtles like *Chrysemys picta* Schneider (Mercurio et al. 2014; Wilde et al. 2014). In addition to causing ecological harm, hydrilla also causes major economic damage by reducing flow in drainage canals, clogging water intakes, interfering with recreational activities such as boating, fishing, and swimming, and reducing lakefront property values (Langeland 1996).

The aggressive growth, high propagule production, and ability of this hardy plant to tolerate a wide range of disturbances are all characteristics of an invasive species (Cook and Luond 1982; Kolar and Lodge 2001). Therefore, it is not surprising that hydrilla, deemed “the perfect aquatic weed”, is listed on the United States Federal Noxious Weed List, and costs millions of dollars to manage each year (Langeland 1996; USDA 2012).

Macrophyte growth in lotic systems is often limited by high water velocity, ever changing substrate, and variable water depths (Sousa et al. 2010). In Brazil, hydrilla has been shown to be able to colonize deeper areas within a river than a similar native species, *Egeria najas* Planch (Sousa et al. 2010). As such, hydrilla is better able to overcome these challenging environmental conditions as compared to egeria. As monoecious hydrilla continues to invade more lotic systems in the U.S. such as the Ohio River, the Eno River in the Piedmont
region of NC, and the Chowan River in Eastern NC, it may outcompete the native species in these lotic systems as well. However, the data to support this conclusion has yet to be reported in the published literature. Invasive species characteristically take hold in disturbed areas (Frazer et al. 2006). Lotic systems provide the dynamic environment necessary to create intermittently disturbed areas. For invasive species that can survive in lotic systems, the intermittent disturbance provides competition free zones for rapid colonization. This pattern was observed in Kings Bay, FL where native species were replaced by the invasive macrophytes *Myriophyllum spicatum* and hydrilla (Frazer et al. 2006). It also occurred in the Potomac River where native species were displaced in the 1930s (apparently due to storm damage, nutrient enrichment, and grazing) and hydrilla became dominant by the 1980s (Carter and Rybicki 1986). Lotic systems represent a relatively new and challenging area of hydrilla research.

Long term hydrilla management is difficult due to its perennation through prolific production of tubers and turions. Current management techniques include cultural control, biological control, mechanical removal, and chemical control (Haller 2014). However, most of these practices, particularly in North Carolina, have been studied in low biodiversity, lentic environments (Nawrocki 2011), and may not be practical or feasible to implement in lotic systems with high biodiversity.

Cultural control methods involve physically altering the environment to discourage weed growth. Water level drawdowns are an example of this type of control and have previously
been used for hydrilla management. This exposes the plants and sediment to the air, thus eliminating standing biomass and disrupting the life cycle of the plant. (Haller et al. 1976). This method has been successful in providing temporary control of dioecious hydrilla in Florida (Langeland 1996). Haller et al. (1976) proposed that two drawdowns would be effective in controlling hydrilla. The first would kill the existing biomass and aerate the soil to stimulate tuber germination. The second drawdown would kill the resulting biomass and should be initiated before additional tubers are formed. However, this method is not feasible in natural flowing systems where a mechanism to draw down water levels does not exist. Furthermore, it would prove fatal to the native flora and fauna of the system.

Biological control methods for hydrilla include stocking the waterbody with herbivorous grass carp, *Ctenopharyngodon idella* Val., or releasing the leaf-mining fly, *Hydrellia pakistanae* Deonier. Triploid (sterile) grass carp have been an effective tool in aquatic plant management since the 1970s. These fish can consume more than 100% of their body weight per day, and have been successful in significantly reducing, even eliminating populations of invasive macrophytes (Opuszynski 1972; Leslie et al. 1987). However, due to their selective generalist feeding habits, they will feed on more palatable vegetation first which may or may not be the invasive for which they were prescribed (Leslie et al. 1987). The majority of grass carp research to date has focused on the eradication of invasive plants (Dibble and Kovalenko 2009). Partial control of plant populations is rarely achieved (Bonar et al. 2002). It is for these reasons, coupled with the lack of research pertaining to the mobility of grass
carp in lotic systems, that we believe this biocontrol agent is not a viable option for hydrilla management in flowing, high biodiversity systems.

*Hydrellia pakistanae*, a fly whose larvae mine hydrilla leaves, is another biocontrol option (Buckingham et al. 1989). *H. pakistanae* is a host specific feeder that prefers hydrilla (Driesche et al. 2002). “The overwintering stage is unknown but larvae have been found on hydrilla throughout the entire winter” (Driesche et al. 2002). While this does not present a problem for management of dioecious hydrilla that overwinters, it may for monoecious hydrilla which acts a herbaceous perennial in North Carolina with senescence occurring in late fall/early winter (Harlan et al. 1985). Past releases of this fly in NC have not been successful due to its failure to overwinter (Nawrocki 2011). Therefore, *H. pakistanae* do not provide a means for monoecious hydrilla control in temperate climates.

Mechanical control methods involve physical removal of the plant via hand-pulling or a harvester that sheers off vegetation. As hydrilla spreads rapidly via fragmentation, mechanical removal may not be the best option for this species as fragments are difficult to contain particularly in flowing waters. It also does not address the issue of the tuber bank for only aboveground biomass is removed. Serafy et al. (1994) reported that mechanical harvesting had a “pruning effect” on hydrilla which resulted in even denser plant stands than at un-harvested sites. They also reported a 23% decline in fish numbers and biomass. Haller et al. (1980) also reported declines in fish populations and biomass due to mechanical harvesting techniques. Costs for this control option are estimated at over $2000/hectare.
Mechanical harvesting causes more harm than good in some situations as it may lead to the significant decline of fish populations and biomass, and may exacerbate the spread of hydrilla via fragmentation (Haller et al. 1980). It would also be nearly impossible to implement in a rocky river environment with large boulders scattered throughout.

Chemical control with the use of aquatic herbicides is an effective and popular means of hydrilla management. Currently, eight herbicides are registered for hydrilla control in the United States. These include bispyribac-sodium, copper, diquat, endothall, flumioxazin, fluridone, imazamox, and penoxsulam (Table 1). Herbicides used in hydrilla management are either fast or slow acting. Fast acting herbicides, such as copper, diquat, endothall, and flumioxazin, do not require long exposure times. Whereas, slow acting translocated herbicides, such as bispyribac, fluridone, imazamox, and penoxsulam, require days or weeks of contact with the target plant to provide control. Hydrilla may require multiple applications of fast acting herbicides in one growing season due to its rapid growth rates and asynchronous sprouting. Hydrilla tubers contain multiple growth buds within a single tuber. Therefore, sprouting may occur subsequently from the same tuber post application with fast acting herbicides that do not typically translocate to below ground tissue (Nawrocki 2011). Van and Conant (1988) reported that both biotypes of hydrilla could be controlled with applications of 1 mg/L of organic copper, 0.25 mg/L of diquat, or 0.5-1 mg/L of endothall. However, in a flowing system with high water exchange, a minimum of 2.0 mg/L diquat or 5.0 mg/L of endothall and a contact time of six hours would be required. Consequently, these higher required rates may be lethal to valuable non-target species as well (Van and Conant 1988).
Flumioxazin has been reported to reduce chlorophyll levels in dioecious hydrilla at concentrations of 100-1,600 µg/L (Mudge et al. 2012). However, efficacy is reduced in high pH or at low light levels. Penoxsulam has been shown to be effective on monoecious hydrilla at rates of 20 µg/L with a 90 day exposure time (Getsinger et al. 2011). The efficacy of imazamox on monoecious hydrilla has also been reported at rates of 200 µg/L (Getsinger et al. 2011). Limited research on bispyribac-sodium shows that it has strong activity on dioecious hydrilla at rates between 10-25 µg/L (Netherland 2011).

Of the slow acting herbicides, fluridone was the first herbicide to provide long-term hydrilla control and has been reported to inhibit tuber and turion production at rates of 5-50 µg/L (MacDonald et al. 1993). Netherland and Getsinger (1995) reported greater than 90% control with treatments of 5 µg/L maintained over a period of 105 days. Netherland et al. (1993) reported 88% control with 12 µg/L maintained for 90 days, and concluded that low rates of fluridone maintained over long periods would be effective in controlling hydrilla in lotic systems. While there is ample research pertaining to the efficacy of chemical control on hydrilla, much of the research has been focused on the dioecious biotype (True-Meadows et al. 2016). Even fewer studies address the efficacy of use in dynamic, flowing systems with high biodiversity and in the presence of rare, threatened, or endangered species (Getsinger et al. 2008).

As hydrilla continues to invade more dynamic, high biodiversity systems such as rivers, estuaries, and reservoirs with high water fluctuation as has been reported in North Carolina
(True-Meadows et al. 2016), there is an increasing urgency to address these gaps in the literature in the areas that pertain to the biology, ecology, and control of this species in such systems.
References

Aulbach-Smith CA, Kozlowski SJ de (1996) Aquatic and Wetland Plants of South Carolina, second. South Carolina Department of Natural Resources, Columbia, SC


Dayan FE, Netherland MD (2005) Hydrilla, the Perfect Aquatic Weed, Becomes More


Haller WT, Sutton DL (1975) Community Structure and Competition Between Hydrilla and


UF / IFAS Center for Aquatic and Invasive Plants. Details About the Aquatic Herbicides Used in Florida – Plant Management in Florida Waters.

https://plants.ifas.ufl.edu/manage/control-methods/chemical-control/details-about-the-aquatic-herbicides-used-in-florida/


Figure 1. Monoecious and dioecious hydrilla distribution in the United States (USGS 2016).
Table 1. Current EPA registered herbicides for aquatic plant management with activity on hydrilla (Nawrocki 2011, UF / IFAS Center for Aquatic and Invasive Plants)

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Mode of Action</th>
<th>Application Rates</th>
<th>Fast or Slow Acting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bispyribac-sodium</td>
<td>Acetolactate synthase inhibitor</td>
<td>5-100 µg/L</td>
<td>Slow</td>
</tr>
<tr>
<td>Copper</td>
<td>Cell wall disrupter</td>
<td>0.4-1 mg/L</td>
<td>Fast</td>
</tr>
<tr>
<td>Diquat</td>
<td>Photosystem I disrupter</td>
<td>0.25-0.37 mg/L</td>
<td>Fast</td>
</tr>
<tr>
<td>Endothall</td>
<td>Protein phosphatase inhibitor</td>
<td>2-5 mg/L</td>
<td>Fast</td>
</tr>
<tr>
<td>Flumioxazin</td>
<td>Protoporphyrinogen oxidase inhibitor</td>
<td>100-400 µg/L</td>
<td>Fast</td>
</tr>
<tr>
<td>Fluridone</td>
<td>Phytoene desaturase inhibitor</td>
<td>5-90 µg/L</td>
<td>Slow</td>
</tr>
<tr>
<td>Imazamox</td>
<td>Acetolactate synthase inhibitor</td>
<td>150-200 µg/L</td>
<td>Slow</td>
</tr>
<tr>
<td>Penoxsulam</td>
<td>Acetolactate synthase inhibitor</td>
<td>5-40 µg/L</td>
<td>Slow</td>
</tr>
<tr>
<td>Topramezone</td>
<td>4-HPPD enzyme inhibitor</td>
<td>30-50 µg/L</td>
<td>Slow</td>
</tr>
</tbody>
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CHAPTER 2
The Effect of Temperature on Monoecious Hydrilla Tubers and Turions

Abstract
Monoecious hydrilla (*Hydrilla verticillata*) is a problematic submersed weed across the United States and is steadily spreading into more northern latitudes. It behaves as a herbaceous perennial that senesces in the late fall to early winter. Regrowth is dependent upon sprouting of vegetative propagules called turions, which are deposited in vast numbers upon or within the substrate each growing season. Sprouting appears to be at least partly a function of temperature. Timing of sprouting and shoot elongation rates were evaluated for monoecious hydrilla axillary and subterranean turions across a water temperature gradient over a period of twelve days. Propagules were floated in glass jars and placed on a thermal gradient table in row with rows corresponding to six different temperatures. Temperatures evaluated included: T1=41.0°C, T2=34.9°C, T3=29.3°C, T4=24.0°C, T5=17.6°C, and T6=12.3°C. Neither axillary nor subterranean turions sprouted in the hottest and coldest temperatures of 41.0 and 12.3°C. Optimum shoot elongation for both propagules occurred at 29.3°C. Shoot lengths were significantly reduced for both turion types in 17.6°C. Timing of sprouting was not significantly different between axillary and subterranean turions. Results will be useful for predicting the timing of sprouting, and therefore will aid in determining the appropriate timing of herbicide treatments and survey efforts.

Introduction
Hydrilla (*Hydrilla verticillata* (L.f.) Royle) is a submersed macrophyte often called “the perfect aquatic weed” (Langeland 1996), and is one of the most expensive and difficult to
control aquatic weeds in the U.S. (Langeland 1996). Two genetically distinct biotypes exist in the United States; a dioecious biotype with only female flowers present, and a monoecious biotype with male and female flowers located on the same plant (Cook and Luond 1982). Monoecious hydrilla was first documented in the U.S. in 1980 in Umstead Lake, NC (True-Meadows et al. 2016), and has been steadily making its way across much of the Atlantic basin and parts of the Pacific and Interior basins of the continental U.S. (Madeira et al. 2000; True-Meadows et al. 2016). In contrast to the dioecious biotype, the monoecious strain appears to be better suited to temperate climates in the U.S., such as regions from North Carolina northward. It also acts as a herbaceous perennial (Harlan et al. 1985). Therefore, regrowth is dependent upon sprouting of subterranean and axillary turions (Harlan et al. 1985). Hydrilla produces large amounts of subterranean and axillary turions, and it is the sprouting of these vegetative propagules that help facilitate its persistence throughout the United States. Subterranean turions form at terminal rhizome nodes, and are widely referred to as tubers and will be referred to as such from here on. Axillary turions, often referred to as just turions, are formed in leaf or branch axils (Yeo et al. 1984). Turions become detached from the parent plant via development of an abscission zone. Whereas, tubers become detached when the parent rhizome senesces (Yeo et al. 1984). Once detached, tubers and turions “serve as a persistent meristem bank (analogous to a seed bank)” (Netherland, 1997), thereby hindering management.

In general, there is considerably less research pertaining to monoecious hydrilla as compared to dioecious hydrilla (Steward and Van 1987; True-Meadows et al. 2016), especially
regarding the sprouting of tubers and turions under varying temperatures. Steward and Vann (1987) reported that monoecious tubers sprout at lower temperatures than dioecious. However, turions were not considered in their studies. Carter et al. (1987) determined that a chilling period is required in order to induce sprouting in monoecious tubers, however temperature induced sprouting was not explored. As successful management of this plant is largely dependent upon understanding factors which influence the sprouting and growth of tubers and turions (Netherland 1997), there is an increasing need to investigate sprouting requirements for both tubers and turions. This study aims to determine differences in sprouting frequencies and growth rates for both tubers and turions across a water temperature gradient.

**Methods**

Lab studies were conducted at North Carolina State University in 2015 and 2016. Hydrilla propagules were collected from Shearon-Harris Reservoir located near New Hill, NC. Prior to the study, propagules were refrigerated for approximately 15 to 30 days. Hydrilla propagules were floated in glass jars (Pyrex 100 x 80 mm No. 3250) and placed on a thermal gradient table to determine differences in sprouting frequencies and shoot elongation rates at varying temperatures. Jars were filled with 1.5 cm of pea gravel with a filter paper placed on top followed by 320 ml of deionized water. Five tubers and five turions were placed in each jar. Watch glasses were placed on all jars to limit water loss through evaporation (Figure 2). Jars were arranged in rows on a thermal gradient table with rows corresponding to six different temperatures. Treatments were replicated five times with jars as replications. Temperature of each jar was determined every other day via an electronic thermometer.
Average temperature by row was as follows T1=41.0˚C, T2=34.9˚C, T3=29.3˚C, T4=24.0˚C, 
T5=17.6˚C, and T6=12.3˚C. Sprouting frequency and maximum shoot length were
determined every other day for 12 days. Shoot length was measured in millimeters via
electronic calipers. Two runs were completed with run 1 conducted in February 2015 and run
2 conducted in March 2016.

Shoot length data were analyzed using a linear mixed model in JMP Pro 12 (SAS Institute
Inc., Cary, NC). Prior to analysis, data were log transformed [Log (length+0.5)] to improve
normality. However, untransformed means are presented for clarity. Run, as a fixed effect,
was determined to be insignificant (p > F = 0.93; α=0.05), therefore, data were combined
across both runs. Sprouting frequency data were modeled using a logistic regression model
(PROC LOGISTIC in SAS) (SAS Institute Inc., Cary, NC) as number of sprouted
propagules/number available. Neither tubers nor turions sprouted in the highest or lowest
temperatures (T1=41.01˚C and T6=12.30˚C). Therefore, data for T1 and T6 are not
presented.

**Results & Discussion**

In this study, the attached tuber or turion served as the only nutrient source for the plant. Use
of deionized water free from the artificial addition of nutrients as the test solution eliminated
outside influences on sprouting rates and stem elongation. Both tubers and turions sprouted
more rapidly and had maximum shoot lengths at T3, with average shoot lengths of 42.0 and
33.9 mm, respectively (Figure 3). Shoot lengths were significantly reduced at T5 for both
propagule types (Figure 3). Turions sprouted faster than tubers, however final tuber shoot
lengths exceeded final turion shoot lengths at all temperatures. These results are similar to Mcfarland and Barko (1987) who reported optimum biomass production at 28 and 32˚C and severely reduced growth at 12 and 16˚C.

Earlier sprouting of turions is consistent with the findings of Spencer and Ksander (2001). However, sprouting frequency by type (tuber vs. turion) was not significant (p = 0.97) in our model. Temperature was a significant factor influencing both turion and tuber sprouting rates (p < 0.0001). Near complete sprouting was observed for both tubers and turions at T2 and T3 with average sprouting at 96-100% at T2 and 96% at T3 (Table 2). Steward and Van (1987) found similar results at 22 and 30˚C. After twelve days, 68-100% of tubers and turions had sprouted at our lowest temperature (T5=17.6˚C). This is a higher sprouting frequency than reported by Steward and Van (1987) who observed only 35-68% sprouting after three weeks of exposure to 15˚C. These differences could be due to our slightly higher temperature of 17.6˚C versus their temperature of 15˚C indicating that a mere two degrees could have serious impacts on sprouting rates in situ.

Previous research indicates that hydrilla has the capacity to become established in more northern latitudes than where it is currently found. These include Alaska and the sub-arctic ranges of Canada (Hartis 2013). These latitudes exhibit cooler water temperatures in the spring compared to spring water temperatures in monoecious hydrilla’s current range. Therefore, sprouting may be delayed in these regions. Elevation may also influence the timing of sprouting due to lower temperatures found at higher elevations (Meays 2000).
Temperature gradients witnessed on a smaller spatial scale should also be considered. Within a single stream, temperatures typically increase with distance from the source (Hawkins et al. 1997). Strong vertical thermal stratification has also been documented in lentic environments with temperature differences reaching 10 to 15˚C between surface and bottom waters in the peak of summer stratification (Imberger 1985).

Timing of sprouting or propagule emergence may also play a role in competitive plant interactions (Spencer and Ksander 2001). Hydrilla has been shown to outcompete other submersed species including *Elodea canadensis* Michx., *Vallisneria americana* Michx., *Potamogeton crispus* L., and Eurasian watermilfoil (*Myriophyllum spicatum* L.) (True-Meadows 2013). Eurasian watermilfoil is also a highly invasive aquatic weed that is a large problem in the northern U.S. and southern Canada. Sprouting followed by rapid growth occurs when water temperatures reach 15˚C (Smith and Barko 1990). Hydrilla’s previously documented competition with Eurasian watermilfoil coupled with its ability to sprout in similar temperatures further points to the potential for hydrilla to become a nuisance species in northern latitudes.

The results of this study have practical implications because management practices can be optimized by monitoring environmental conditions. Low temperatures that reduce sprouting or subsequent growth should also delay herbicide applications. Failure to monitor sediment temperatures or identify temperature gradients in a water body could lead to reduced control and/or wasted resources. Surveys should not be conducted until water temperatures reach
17°C for at least two weeks. This is especially important in lotic systems that often exhibit cooler temperatures in their headwaters. Our results should also be considered when implementing management programs across latitudes or elevations.
References


Figure 2. Picture of experimental setup with test jars placed on thermal gradient table in their respective temperature row.
Figure 3. Average shoot length of sprouted hydrilla tubers and turions over time.
Table 2. Hydrilla sprouting frequency means for tubers and turions over a period of 12 days at T2-T5 (34.9-17.6°C).

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>Sprouting frequency</th>
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<tbody>
<tr>
<td>℃</td>
<td>#</td>
<td>Tuber</td>
</tr>
<tr>
<td>34.9</td>
<td>2</td>
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</tr>
<tr>
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<td></td>
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</tr>
<tr>
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</tr>
<tr>
<td></td>
<td></td>
<td>Day 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day 12</td>
</tr>
<tr>
<td>29.3</td>
<td>3</td>
<td>Day 3</td>
</tr>
<tr>
<td></td>
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<td>Day 10</td>
</tr>
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<td></td>
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<tr>
<td>24.0</td>
<td>4</td>
<td>Day 3</td>
</tr>
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<td>Day 10</td>
</tr>
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CHAPTER 3

Salinity Tolerance of Monoecious Hydrilla: Survivability, Growth, and Sprouting

Abstract

Monoecious *Hydrilla verticillata* is one of the most difficult weeds to control in the United States. As hydrilla continues to invade increasingly dynamic systems, namely rivers that empty into brackish waters, salinity will likely be the greatest factor in determining its expandable range. Salinity tolerance was evaluated for both sprouted and unsprouted monoecious hydrilla tubers. Sprouted tubers were placed in an incubator held at 23°C with a photoperiod of 14 h light: 10 h dark. Unsprouted tuber trials were conducted in a greenhouse facility. Tested salinities included a range of 0 to 24 ppt at exposure times of 2 to 8 weeks followed by a two-week recovery period. Shoot length, number of lateral branches, and fresh and dry weights were determined. Sprouted tubers tolerated 8 weeks in 6 ppt and 4 weeks in 9 ppt, following the recovery period. Fresh weights of sprouted tubers increased from starting weights in salinities up to 9 ppt, however, all dry weights were reduced from the control. Unsprouted tubers sprouted in salinities up to 12 ppt, although shoot lengths and fresh weights were reduced from the control. Lateral branching increased in salinities of 3 to 6 ppt, relative to the control. Results indicate the potential for hydrilla to spread into brackish waters and withstand periodic salt intrusion events.

Introduction

*Hydrilla verticillata* (L.f.) Royle is a highly invasive submersed weed. It was first introduced to the United States in 1960 in Florida, and has made its way as far west as California (Harlan et al. 1985) and as far north as Maine (Maine DEP). There are two distinct biotypes
of hydrilla established within the U.S.: a monoecious and a female dioecious biotype (Blackburn et al. 1969; Cook and Luond 1982). The monoecious biotype is typically found in more temperate climates within the U.S. (Netherland 1997) such as North Carolina and northward. Whereas, the dioecious biotype is found in warmer climates of the U.S. (Netherland 1997).

Hydrilla is notoriously difficult and costly to control due in part to its prolific production of vegetative propagules (True-Meadows et al. 2016). These vegetative buds, called turions, are formed in leaf or branch axils and at the terminal end of positively geotropic rhizomes (Van et al. 1978). Rhizome formed turions, or subterranean turions, are widely referred to as tubers and will be referred to as such from here on. Tubers and turions detach from the parent plant either from an abscission zone for turions, or as the parent rhizome senesces for tubers (Yeo et al. 1984). Great numbers of tubers are formed in the sediment each growing season adding to the “tuber bank” which acts as a seed bank. This is the mechanism that allows monoecious hydrilla to overwinter in temperate climates, and aids in rapid revegetation after control treatments or ecological stress (Netherland 1997).

As monoecious hydrilla continues to invade increasingly dynamic and high biodiversity systems such as “flowing rivers, estuaries, and reservoirs with high water fluctuation” (True-Meadows et al. 2016), there is a need to determine how hydrilla will respond when exposed to varying environmental stressors that the aforementioned systems may present. Determining salinity tolerance is of particular importance since many of these more recent
sites of hydrilla invasion, such as the Chowan and Roanoke rivers in eastern NC, empty into brackish waters which imparts a salinity gradient that may determine hydrilla’s expandable range. Additionally, current climate change models predict that salinity variability will increase along the eastern mid-Atlantic coastal waters, and that salt water intrusion events lasting greater than a month will be more frequent (Najjar et al. 2010). Salinity tolerance of hydrilla has been previously reported, however, there are discrepancies within the literature. It was reported that dioecious hydrilla failed to grow after four weeks in 6 ppt (Haller et al. 1974), and monoecious hydrilla showed little productivity at 4 ppt (Twilley and Barko 1990). In contrast, Steward & Van (1984) reported a 13 ppt threshold for both monoecious and dioecious hydrilla. These studies did not address the effect of salinity on unsprouted tubers, nor did they evaluate the ability of the plant to recover after varying exposure times. Therefore, the objective of this study was to determine growth rate, biomass production, and survivability of both sprouted and unsprouted monoecious hydrilla tubers across a salinity gradient at multiple exposure times followed by a recovery period in fresh water.

Methods

Test propagules. Unsprouted monoecious hydrilla tubers were collected from Shearon-Harris Reservoir located near New Hill, NC with the exception of tubers used in run 2 of the greenhouse salinity tests. Tubers used in run 2 were collected from stock plants grown in outdoor mesocosms which originated from NC sources. Tubers intended for laboratory tests were sprouted in de-chlorinated tap water until average shoot length reached approximately 40 millimeters. Tubers intended for greenhouse tests were refrigerated for a minimum of one week.
Laboratory salinity tolerance test. Salinity tolerance tests on sprouted tubers were conducted in the Aquatic Toxicology Laboratory in the Department of Applied Ecology, North Carolina State University. Instant Ocean (Aquarium Systems, Mentor, OH) was mixed with reconstituted soft water to achieve our desired salinities of 0, 3, 6, 9, 12, 18, and 24 ppt. No fertilizer was added to the test solution in order to eliminate outside influences on stem elongation rates. Sprouted tubers were randomly selected and placed in Pyrex No. 3250 jars containing our test salinity solution with five tubers per jar. Watch glasses were placed on all jars to limit evaporation and changes in salinity. Initial shoot lengths were measured, and total fresh weight by jar was determined. Jars were placed in an incubator at 23°C with a photoperiod of 14 h light: 10 h dark using Phillips Natural Sunshine bulbs. Sprouted tubers were subjected to the various salinities for 2, 4, 6, or 8 weeks. Three replicates were included for each concentration (salinity) exposure time combination. After their respective exposure time, sprouted tubers were rinsed and placed in fresh reconstituted soft water for an additional two weeks. Shoot length was measured weekly and number of lateral branches was noted. Weekly measurements were collected on a subsample of the population (105 shoots; 3 jars per salinity). Ending fresh and dry weights were determined for each jar. Fifty percent water renewals occurred every week for every treatment. Water quality tests were performed at the start of the trial and during water renewals by testing a composite sample of each salinity. Parameters measured included temperature, salinity, dissolved oxygen, conductivity, pH, hardness, and alkalinity. Standard methods as described by Bringolf et al. (2005) were followed for all water chemistry analyses.
Greenhouse salinity tolerance tests. Salinity tolerance tests on unsprouted tubers were performed in the greenhouse facility at the Weed Control Lab at North Carolina State University. Instant Ocean was mixed with de-chlorinated tap water to achieve the same salinities as above. No nutrients were added to the test solution in order to eliminate outside influences on sprouting and stem elongation rates. Tubers were floated in Pyrex No. 3250 jars with five tubers per jar. Watch glasses were placed on jars to limit evaporation and changes in salinity. Beginning tuber fresh weight by jar was determined prior to treatment. Shoot length (mm), sprouting frequency, and number of lateral branches were determined weekly. Ending biomass was harvested and fresh and dry weights were recorded. Two rounds were completed with three replications.

Analysis. Data including shoot length, fresh and dry weights, and lateral branching were analyzed using a linear model in JMP Pro 12 (SAS Institute Inc., Cary, NC). Prior to analysis, lateral branching data were log transformed [Log (#branches+1)] to improve normality. However, untransformed means are presented for clarity. Tubers exposed to 18 and 24 ppt in our greenhouse trials did not sprout, therefore, data from these salinities are not presented. Data from both runs in the greenhouse trials were not able to be combined, therefore they are presented separately.

Results & Discussion

Laboratory salinity tolerance tests. Salinity was a significant factor affecting shoot length, number of lateral branches, and biomass production of sprouted hydrilla tubers.
**Water quality.** Average temperature was 22.7°C. Dissolved oxygen values ranged from 6.01-10.90 mg/L with an average value of 8.35 mg/L. Average pH was 8.21 with a range of 7.47-9.48. Conductivity, alkalinity, and hardness increased with increasing salinity (Table 3). Hardness values in salinities of 6 ppt and above exceeded 600 mg CaCO\(_3\)/L and were higher than could be measured with our equipment and methods.

**Shoot length.** Shoot length decreased with increasing salinity. After eight weeks of exposure, average shoot length was reduced between 32 to 88 % from the control at all salinities (Figure 4). Although extension rates were reduced, shoot length continued to increase in salinities of 6 ppt or lower after eight weeks of exposure (Figure 5). Shoots exposed to 9 ppt or higher did not exhibit any significant increase in shoot length regardless of exposure time.

**Shoot length following recovery period.** Shoots subjected to salinities up to 3 ppt showed little to no change in shoot length after the recovery period of two weeks in fresh water (Figure 6). Change in shoot length after recovery was not significant. Shoots exposed to 6 ppt were able to recover after eight weeks of exposure, and those subjected to 9 ppt recovered from four weeks of exposure. However, no increase in shoot length occurred in exposures greater than four weeks for 9 ppt salinity. Shoots subjected to salinities of 12 ppt and higher did not exhibit any ability to recover even after the shortest exposure time (Figure 6) (Recovery is indicated by a positive change in shoot length after exposure to fresh water).

**Number of lateral branches.** Salinity significantly affected the number of lateral branches (p < 0.0003) (Figure 7). Shoots exposed to salinities of 3 and 6 ppt had significantly more lateral branches than any other treatments with means of 9.07 and 7.33, respectively. Shoots
exposed to 0 and 9 ppt had the next highest levels of lateral branching with means of 6.25 and 4.01, respectively. All other treatments averaged less than two lateral branches per shoot.

**Biomass production.** Overall biomass increased in treatments of 0, 3, 6, and 9 ppt as indicated by a positive change in ending versus starting fresh weights (Figure 8). However, biomass decreased in treatments of 12 ppt and higher indicated by a negative change of fresh weights. Decreased biomass is due to the necrosis of a portion of the shoots. Dry weights of all treatments were significantly reduced from the control (p = 0.0029) (Figure 9). Weights were averaged over all exposure times including the two-week recovery period.

*Greenhouse salinity tolerance tests.* Salinity significantly affected stem extension rates, lateral branching, and biomass production of unsprouted monoecious tubers.

**Sprouting.**

*Run 1.* Sprouting was significantly affected by salinity (p < 0.0001). Tubers began sprouting within the first five days of exposure for salinities of 0-9 ppt (Figure 10). Average sprouting frequencies for these salinities reached 70-90%. Sprouting in 12 ppt did not occur until 4 weeks after exposure, and only an average of 30% of the tubers sprouted.

*Run 2.* Sprouting was significantly affected by salinity (p < 0.0001). Tubers exposed to 0 ppt began sprouting within the first five days (Figure 10), and average sprouting frequencies reached 90% over the course of the study. Sprouting in 3 and 6 ppt did not occur until 2 weeks after exposure with average sprouting frequencies reaching 6-13%. Tubers exposed to 9 ppt and above did not sprout.

**Shoot length.**
Run 1. Shoot length was inversely related to salinity. After eight weeks of exposure, all shoot lengths were significantly reduced from the control (p = 0.0105) with average ending shoot length for 0 ppt at 55.2 mm. However, shoots subjected to 3 ppt, albeit reduced, were not significantly different from the control up to the seven-week mark (Figure 11). Shoots exposed to 6 ppt and above were reduced from the control for the entire length of treatment (Figure 11).

Run 2. All shoot lengths were reduced from the control (p = 0.0096) with average ending shoot length for 0 ppt at 39.7 mm. Shoots in 3 ppt and 6 ppt were not significantly different from each other with ending average shoot lengths of 9.8 and 14.0 mm, respectively (Figure 12).

Number of lateral branches.

Run 1. Salinity affected the number of lateral branches (p < 0.0001). Shoots exposed to 3 ppt had the highest number of lateral branches with an average of 5.6 branches (Figure 13). Shoots from 0 and 6 ppt had the next highest level of lateral branching with means of 4.5 and 4.8, respectively. Salinities of 9 and 12 ppt further reduced lateral branching with means of 3.1 and 0.3, respectively (Figure 13).

Run 2. Lateral branching was reduced in run 2 as compared to run 1. Average ending number of lateral branches in the control was 2.5 and less than 1 in all other treatments (Figure 14).

Biomass production.

Run 1. Salinity significantly affected ending fresh (p = 0.0004) and dry weights (p = 0.0193). Fresh weights were reduced from the control in salinities of 6 ppt and above (Figure 15). Shoots in 0 and 3 ppt had the highest fresh weights at 3.19 and 2.5 grams, respectively.
(Figure 15). In contrast, dry weight did not reflect as much variability between salinities. Only shoots subjected to 12 ppt had dry weights that were significantly reduced from the control (Figure 16).

**Run 2.** Ending average fresh weights were reduced from the control at all salinities (p = 0.0025). Fresh weights from all other salinities were not significantly different from each other (Figure 17). No differences were detected in dry weights between the control and all other treatments (data not shown).

In this study, monoecious *Hydrilla verticillata* was negatively affected by long periods of exposure to high salinities. However, it appears to be tolerant to short periods of exposure to low salinities. Shoots from sprouted tubers were still growing at eight weeks of exposure to 6 ppt and were able to recover after four weeks at 9 ppt as evidenced by increased shoot length and fresh weight. These results differ from previous studies that report that hydrilla did not grow in 6.66 ppt, died in 10 ppt, and did not survive after 1 day of exposure to 15 ppt (Haller et al. 1974; Frazer et al. 2006). The difference in results could be due to variances in salinity tolerance between biotypes of monoecious and dioecious hydrilla. Both Haller et al. (1974) and Frazer et al. (2006) conducted their studies on the dioecious biotype (Haller et al. biotype assumed to be dioecious due to location of plant collection). However, Twilley and Barko (1990) reported little productivity of monoecious hydrilla above exposure to 4 ppt which is also lower than our reported range, and Steward and Van (1987) reported no discernible differences between biotypes. Studies that reported lower salinity tolerance than our study were conducted using shoot cuttings that were not attached to a tuber or turion (Haller et al. 1974; Frazer et al. 2006).
In contrast, Steward and Van (1987) used sprouted tubers in their salinity exposure tests. They reported tolerances up to 13 ppt (regardless of biotype) which more closely aligns with our results. Therefore, it seems likely that the notable discrepancies within the literature are more a factor of whether or not the plants were attached to the tuber or turion during the length of exposure rather than biotype.

Monoecious hydrilla may be able to survive in these low salinities by increased lateral branching at 3 ppt and 6 ppt, relative to the control. This, in turn, may increase the number of viable fragments which could lead to further spread and new infestations of this highly invasive plant should the fragments be transported to areas with favorable conditions.

Previous studies with dioecious hydrilla had contradictory results in which the control had the highest branching and no branches were added in 15 and 25 ppt (Frazer et al. 2006).

These results indicate that plants grown from sprouted monoecious hydrilla tubers show little difference in growth parameters as compared to the control in salinities up to 3 ppt, and were able to recover after four weeks in 9 ppt and at least eight weeks at 6 ppt. Minimal recovery was seen in the control and in 3 ppt. As aforementioned, this was likely due to variances between subsamples. Recovery conditions were not drastically different (or not different at all in the case of the control) from treatment conditions for these salinities. This resulted in little change. Recovery conditions were drastically different from treatment conditions in the higher salinities resulting in greater recovery. Therefore, the impact of the variance between subsamples in 0 and 3 ppt was exacerbated by these lower stem extension rates.
Unsprouted tubers appeared to be less tolerant to salinity with reduced shoot lengths and fresh weights occurring in salinities of 6 ppt and above in the greenhouse trials. Although dry weights at 6 ppt were not reduced. Sprouting occurred in salinities up to 12 ppt. This is higher than the previously reported maximum sprouting salinity of 9 ppt (Carter et al. 1987). Run 2 of our unsprouted tuber trial showed even greater sensitivity, and sprouting did not occur in salinities of 9 ppt and higher. However, this run of the trial was conducted toward the end of the year when day lengths were shorter. This and the difference in tuber origin may have contributed to reduced sprouting and stem extension rates in run 2.

Monoecious hydrilla produces tubers in vast numbers each growing season. In a laboratory study, a single monoecious tuber produced over 6,000 new tubers in just 16 weeks (Sutton et al. 1992). In hydrilla infested water bodies in North Carolina, tuber bank densities have been reported to be as great as 1,312 – 3,077 tubers/m² (Harlan et al. 1985; Nawrocki 2011). Recent research suggests that monoecious hydrilla tubers can remain viable within the hydosoil for at least six years (Nawrocki 2016). These structures allow hydrilla to withstand and recover from ecological stress (Netherland 1997). Our results suggest that once a tuber bank becomes established it may be able to withstand temporary salt intrusion of low salinities.

If current climate change models are correct in predicting more frequent, month-long salt water intrusion events along the east coast (Najjar et al. 2000; Najjar et al. 2010), hydrilla
may be able to survive when other submersed fresh water species may not. This lowered interspecific competition coupled with predicted increased spread through fragmentation could exacerbate the spread of hydrilla along the mid-Atlantic tidal waters.
References


Table 3. Average water quality conditions for laboratory hydrilla salinity testing.

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<th>DO mg/L</th>
<th>pH</th>
<th>Alkalinity mg CaCO₃/L</th>
<th>Hardness mg CaCO₃/L</th>
<th>Conductivity µS</th>
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Figure 4. Average ending shoot length of sprouted hydrilla tubers (including those that no longer had shoots attached) after eight weeks of exposure to varying salinities. Average starting shoot length was 40 mm. Shoot lengths were reduced from the control (0 ppt) at all salinities (p < 0.001). Error bars were constructed using 1 standard error from the mean.
Figure 5. Average change in hydrilla shoot length across multiple salinities and exposure times with error bars representing 1 standard error from the mean. Shoot length was significantly affected by exposure time ($p = 0.0481$) and salinity ($p < 0.0001$). Positive values indicate an increase in shoot length. Negative values indicate mortality of a portion of the shoots.
Figure 6. Mean change in hydrilla shoot length across multiple exposure times to salinities of 0-24 ppt followed by a two-week recovery period in fresh water. Shoot lengths were significantly affected by exposure time ($p = 0.0481$) and salinity ($p < 0.0001$). Positive values indicate an increase in shoot length. Negative values indicate further necrosis. Error bars represent 1 standard error from the mean.
Figure 7. Mean number of lateral branches by salinity averaged over all exposure times. Treatments of 3 and 6 ppt had the highest number of lateral branches (p < 0.0001). Means with different letters are significantly different. Significance was determined using log transformed means. Error bars represent 1 standard error from the mean using untransformed means.
Figure 8. Mean change in fresh weight of hydrilla averaged over all exposure times after a 2-week recovery period. Salinity significantly affected the change in fresh weight (p < 0.0001). Positive values indicate an increase in biomass, whereas, negative values indicate that a portion of the plants had died. Error bars represent 1 standard error from the mean.
Figure 9. Average hydrilla dry weight by salinity averaged over all exposure times. Error bars indicate 1 standard error from the mean. Dry weights were reduced from the control at all salinities ($p = 0.0029$).
Figure 10. Mean hydrilla sprouting rates from unsprouted tubers placed in varying salinities over eight weeks of exposure, separated by run 1 and run 2. Sprouting was significantly different by run (p < 0.0001) and salinity for run 1 (p < 0.0001) and run 2 (p < 0.0001). Error bars indicate one standard error from the mean.
Figure 11. Mean hydrilla shoot length from unsprouted tubers placed in varying salinities after eight weeks of exposure, run 1. Shoot lengths were reduced from the control (0 ppt) after 8 weeks at all salinities ($p = 0.0105$). Error bars indicate one standard error from the mean.
Figure 12. Mean hydilla shoot length from unsprouted tubers placed in varying salinities after eight weeks of exposure, run 2. Shoot lengths were reduced from the control (0 ppt) at all salinities ($p = 0.0096$). No sprouting occurred in 9 and 12 ppt. Error bars indicate one standard error from the mean.
Figure 13. Mean number of lateral branches from unsprouted tubers placed in varying salinities after eight weeks of exposure, run 1. Lateral branching was highest at 3 ppt and significantly reduced at 9 and 12 ppt (p < 0.0001). Means not connected by the same letter are significantly different. Significance was determined by using log transformed means. Error bars indicate one standard error from the mean using untransformed means.
Figure 14. Mean number of lateral branches from unsprouted tubers placed in varying salinities after eight weeks of exposure, run 2. Lateral branching was reduced at all salinities relative to the control ($p < 0.0001$). Means not connected by the same letter are significantly different. Significance was determined by using log transformed means. Error bars indicate one standard error from the mean using untransformed means.
Figure 15. Mean fresh weight from unspouted tubers placed in varying salinities after eight weeks of exposure, run 1. Salinity significantly affected fresh weight (p = 0.0004). Fresh weight of shoots exposed to 3 ppt did not differ from the control (0 ppt). Error bars indicate one standard error from the mean.
Figure 16. Mean hydrla dry weight from unsprouted tubers placed in varying salinities after eight weeks of exposure, run 1. Salinity significantly affected dry weight ($p = 0.0193$). Shoots in 12 ppt had reduced dry weight from all other salinities. Error bars indicate one standard error from the mean.
Figure 17. Mean hydrilla fresh weight from un sprouted tubers placed in varying salinities after eight weeks of exposure, run 2. Fresh weights were reduced from control at all salinities (p = 0.0025). Error bars indicate one standard error from the mean.
CHAPTER 4

Hydrilla in the Eno River: A Case Study for the Management of Hydrilla in Lotic, High Biodiversity Systems

Abstract

Flowing waters such as rivers and streams are important sources for drinking water, energy production, irrigation, and recreational activities. The increased dispersal of invasive species is one of the greatest threats to these systems and the species they contain. *Hydrilla verticillata*, a submersed macrophyte, is one of the worst aquatic invaders in the United States. Current hydrilla management options are limited for flowing systems with high biodiversity, and have not been extensively studied. A pilot project was implemented in the Eno River, located in the Piedmont region of North Carolina, to investigate the efficacy of treating hydriilla in a lotic system with high biodiversity and threatened or rare species. Two consecutive years of treatment were conducted using a low fluridone rate maintained over a window of 60-100 days. Treatment impacts to selected target and non-target aquatic species were evaluated. Efforts included quantitative sampling of *H. verticillata*, *Somatogyrus virginicus* (a rare, endemic snail), and *Podostemum ceratophyllum* (a native macrophyte and habitat of *S. virginicus*) at seven spatially separated sites along the Eno River. Biweekly vegetation monitoring and monthly snail sampling began two weeks before treatment in 2015, and continued through 2016. Hydrilla biomass, shoot length, and tuber density significantly decreased within the treated section of the river. *P. ceratophyllum* densities and lengths were not significantly different between sampling years. *S. virginicus* densities exhibited patterns with high annual variation which were consistent with reproductive cycles
of this species. Overall, fluridone effectively controlled hydrilla within the treated area with no apparent negative impacts to the studied non-target species.

**Introduction**

Although flowing waters only make up about 0.0001% of the water on earth, they are of enormous importance to humans (Wetzel 2001). They provide a source for drinking water, energy production, irrigation, navigation, and recreation (Friberg 2014). Flowing water, or lotic, systems are distinguishable from standing water, or lentic, systems by their unidirectional flow and water retention time. Water is constantly cycling through a flowing system. Therefore, downstream areas are affected by upstream waters, and water retention times are much shorter than in a lentic system such as a lake or pond (Wetzel 2001). The erosive action of flowing waters produces ever-changing channel morphology and substrate. Flow is variable as lotic systems often respond rapidly to precipitation. Physical, chemical, and biological characteristics are also highly variable within and across different systems. These aspects make lotic systems more difficult to generalize than standing waters (Wetzel 2001).

Lotic ecosystems are highly susceptible to anthropogenic influences, and are threatened world-wide through the construction of dams and impoundments, pollution, climate change, direct habitat destruction, and increased dispersal of invasive species (Van Wilgen et al. 2007; Friberg 2014). Of these, invasive species are one of the most significant threats to global biodiversity (Van Wilgen et al. 2007). While submersed aquatic plants provide many
benefits to lotic systems such as nutrient cycling, habitat availability, increased water clarity, and decreased erosion, these beneficial traits can become harmful with excessive growth; as is the case with invasive submersed species (Santos et al. 2011). Invasive aquatic plants have no native predators and have competitive growth advantages such as low light requirements and high propagule production. Therefore they often grow to nuisance levels, and out-compete native species for space and resources (Santos et al. 2011).

*Hydrilla verticillata* (L.f.) Royle is an extremely costly and difficult to control submersed invasive aquatic plant in the United States (Langeland 1996). Hydrilla’s aggressive growth habits allow it to form dense monocultures that displace native vegetation thereby causing detrimental effects such as clogged waterways, decreased dissolved oxygen, and altered fish populations (Langeland 1996). It has also been identified as the host of a neurotoxin-producing cyanobacteria, *Aetokthonos hydrllicola*. This toxin is linked to a neurological disease (Avian Vacuolar Myelinopathy- AVM) that affects bald eagles (*Haliaeetus leucocephalus* Linnaeus), American coots (*Fulica americana* Gmelin), grass carp (*Ctenopharyngodon idella* Val.), and painted turtles (*Chrysemys picta* Schneider) (Mercurio et al. 2014; Wilde et al. 2014).

Two biotypes of this plant exist in the United States: a monoecious and a dioecious strain (Blackburn et al. 1969; Cook and Luond 1982). The monoecious biotype, typically found in more temperate climates of the United States such as North Carolina northward, acts as a herbaceous perennial with senescence occurring in the late fall/early winter and regrowth
occurring in the spring/early summer (Harlan et al. 1985). Hydrilla reproduces rapidly through fragmentation, stolons, seed, and vegetative propagules called turions (Cook and Luond 1982; Langeland and Smith 1984). These propagules are formed in leaf or branch axils and in the sediment at the end of rhizomes (Yeo et al. 1984). Subterranean formed turions are often called tubers, and will be referred to as such from here on. Large numbers of tubers are produced in the sediment each growing season allowing monoecious hydrilla to overwinter in temperate climates and regrowth to occur after ecological stress or control treatments (Netherland 1997).

Several methods are commonly used to control hydrilla in the United States. These include mechanical harvesting, introduction of sterilized triploid grass carp, and herbicide applications (Langeland 1996; True-Meadows et al. 2016). There is a large amount of peer-reviewed research pertaining to the control of dioecious hydrilla. However, there is considerably less research focused on controlling monoecious hydrilla; particularly in flowing systems with high biodiversity and water fluctuation (Getsinger et al. 2008; True-Meadows et al. 2016). Mechanical harvesting may do more harm than good due to its destructive nature and tendency to increase fragmentation of hydrilla. This method has been reported to decrease fish biomass and sometimes exacerbate the growth of hydrilla (Haller et al. 1980; Serafy et al. 1994). Introducing grass carp to a lotic, high biodiversity system to control hydrilla is also not a feasible option as grass carp are “selective generalist” feeders and may, in turn, feed on desirable native species (Leslie et al. 1987). Additionally, more research is needed to determine if introduced fish will remain where stocked in a lotic system.
with varying flow. Aquatic herbicides have successfully been used to control hydrilla. Two of the most commonly applied herbicides for the control of hydrilla in lentic systems include fluridone and the dipotassium salt of endothall (Archambault and Cope 2016). However, there are still some unanswered questions pertaining to their use in flowing systems that have short water retention times and protected or threatened native species. Netherland et al. (1993) reported that low rates of fluridone maintained over long periods will effectively control hydrilla in lotic systems. While this study was an important first step, it focused on dioecious hydrilla in a laboratory setting. Netherland (2015) reported that low rates of fluridone (6-12 ppb) prevented sprouting monoecious hydrilla tubers from emerging, and even lower rates (1.5-3 ppb) reduced biomass by 84 to 96%. However, this study was also conducted in a greenhouse setting and did not address the potential effects on non-target species. The current study sought to address these gaps in the literature.

The impetus for this study was the lack of research pertaining to management of monoecious hydrilla in flowing systems in the presence of native, desirable, and threatened species. The study site for this pilot project was the Eno River, a high biodiversity river located in the Piedmont region of North Carolina in Durham and Orange counties. The Eno River is a municipal water source, and is also recreationally important for it flows through most of the Eno River State Park. It also has high rates of endemism, and is home to several threatened and endangered species (Archambault and Cope 2016). One of these said species is a rare aquatic snail, the Panhandle pebblesnail Somatogyrus virginicus Walker. S. virginicus is listed as a species of greatest conservation need and as a federal species of concern in the
North Carolina Wildlife Action Plan (NCWRC 2015). The only confirmed population in North Carolina is within the Eno River (Ratcliffe et al. 2016). It lives within the native macrophyte species _Podostemum ceratophyllum_ Michx., commonly referred to as riverweed or riffleweed. Riffleweed is an extremely important native plant species for it provides surface area for algal growth which benefits scrapers, and provides stable habitat for aquatic macroinvertebrates such as filter feeders (Hutchens et al. 2004). The invasion of alien aquatic plants in this system, particularly hydrilla, is a serious threat to these invaluable native plants and their respective inhabitants.

Hydrilla has been an ongoing problem within the Eno River since it was first documented in 2005. A two-year pilot project was implemented in 2015 for an evaluation utilizing a metered system to maintain low concentrations of fluridone (market name Sonar® Genesis Aquatic Herbicide) within the hydrilla infested areas of the Eno River from late spring through the summer in order to control hydrilla. Prior to this, a metered herbicide treatment within a lotic system had never been reported in North Carolina. The objective of this research was to evaluate the efficacy of treatment on monoecious hydrilla, and elucidate impacts to the non-target species, riffleweed and the panhandle pebblesnail.

**Methods**

*Vegetation.* Hydrilla and riffleweed were monitored pre, during, and post treatment to determine any potential effects on density, shoot length, and chlorophyll levels. Monitoring began in May 2015 and occurred biweekly through September 2015. Winter and spring sampling was conducted approximately bi-monthly from October 2015 through May 2016.
Biweekly sampling resumed in June through September 2016. Six sites were monitored; two controls located outside (upstream) of the treatment zone and four located within the treatment zone (Figure 18). Parameters measured included riffleweed density, shoot length, and percent bottom coverage, and hydrilla length and percent bottom coverage. Riffleweed density was determined by randomly selecting four rocks per site and counting the number of stems per 25 cm². Shoot length for each species was determined by measuring the four longest shoot lengths at each site. Percent bottom coverage of the stream bed was visually estimated at the same swath at each site. In 2015, samples of each species at each sampling date were collected for analysis of chlorophyll $a$ concentrations. Temperature (°C), pH, and dissolved oxygen (mg/L) were also recorded with the use of a handheld YSI meter. Tuber sampling was conducted prior to treatment in November 2014 and again in April 2016 after one year of treatment. Metal quadrats (50 cm x 50 cm) were used to determine area. Quadrats were randomly placed in areas that were known to have contained hydrilla in the previous growing season. Substrate from four quadrats per site was sampled to an approximate depth of 30 cm. Substrate was sieved and turions/tubers were counted. Sites sampled in 2014 included Pleasant Green, Cole Mill, and Guess Road. Sites sampled in 2016 included Dumont, Pleasant Green, Cole Mill, Guess Road (Figure 18), and an additional four untreated sites near Weaver Street Market in Hillsborough, NC where hydrilla growth was prolific in 2015.

*Panhandle pebblesnail.* Snails were monitored pre, during, and post treatment to identify any potential treatment effects on population numbers. Monthly monitoring began May 2015 and
continued through October 2015. Winter sampling occurred bi-monthly from December 2015 through April 2016. Monthly sampling resumed in May and continued through September 2016. Seven sites were monitored: two controls located outside of the treatment zone and five located within the treatment zone (Figure 19). Snail density was calculated by randomly selecting ten riffleweed-covered rocks at each site from optimal habitat and counting the number of snails on a per unit effort basis. This was achieved by rinsing the rock off into a clear plastic bucket and counting the number of snails for ten minutes per rock. Two rocks per site were recounted to determine detection probability. Optimal habitat here is defined as fast flowing portions of the stream, or riffles. Maximum height, length, width, and percent riffleweed coverage of the top surface of each rock was calculated to determine area.

River survey. Kayak surveys were conducted on the Eno River for two consecutive years in order to assess vegetation density and to determine stream morphology in the treated section of the channel. The first survey was conducted in May 2015, prior to treatment, and the second survey was conducted in May 2016 after one year of treatment. Approximately sixteen miles of the river were surveyed, and data was collected at roughly 0.25 km intervals. Survey data included presence/absence data of riffleweed, hydilla, and waterwillow (*Justicia americana* (L.) Vahl), a native shoreline emergent plant. Species were given a rating of 0-3 with 0 being absent and 3 being very dense. Both sides of the channel were given a rating, and ratings were averaged for each point in post-hoc analyses. Stream width and five depth readings across the channel were also recorded at every survey point to determine an average channel depth throughout the treatment area. Stream morphology was also classified at each
point as either a run, riffle, or pool. ArcGIS was used to create shapefiles and publication quality maps from the data.

**Analysis.** Neither hydrilla nor panhandle pebblesnails were found in the untreated control sites. Therefore, reference sites were removed from the hydrilla and snail analyses. Pre- and post-treatment sampling for both years corrected for this bias. Results from the vegetation and snail sampling data were analyzed using a linear model and kayak survey data were analyzed using an ordinal logistic model. All analyses were performed using JMP Pro 12 (SAS Institute Inc., Cary, NC). Snail density and riffleweed length data were log transformed \[\log (\text{snails/m}^2 + 0.5)\] and \[\log (\text{length} + 1)\] to improve normality. Untransformed means are presented for clarity.

**Results & Discussion**

**Vegetation.** Hydrilla shoot length and percent coverage were significantly different by year and sampling occasion \((p < 0.0001)\), but did not differ by sampling site. In 2015, shoot lengths and percent coverage increased from May into June, and then sharply dropped off roughly half-way through the treatment window (Figure 20). Recovery was seen after treatment ceased, and shoot lengths continued to increase into September (Figure 20). Plants then began to naturally senesce until no stems were found at any sites during our December sampling. Biomass, measured as percent coverage, did not return to pre-treatment levels once treatment ceased (Figure 21). In 2016, hydrilla shoots were an average of 13.7 cm long at our May sampling, and were not significantly different from shoot lengths found in May of 2015 (Figure 20). Treatment effects were seen much earlier in the treatment window in 2016.
Average shoot length declined from May into June, and hydrilla was not found at any sampling sites after the last sampling occasion in June. Percent hydrilla coverage in 2016 never reached greater than 1% (data not shown). Temperature, pH, and dissolved oxygen were not significant factors affecting shoot length or percent coverage.

Treatment in 2015 began in late May and continued through mid-July. Average fluridone concentration over the 2015 treatment period was 4.3 ppb (Heilman 2016). As this was a pilot-project slated to continue at least two years, the goal was not eradication of hydrilla. Therefore, the recovery seen in August 2015, after treatment ceased, was due to the sprouting of previously unsprouted tubers or recovery from existing biomass and was not unexpected. Netherland et al. (1993) also reported recovery of dioecious hydrilla after fluridone treatments lasting 60 days or less. Treatment in 2016 began in early May and continued through late August. Average fluridone concentration over the 2016 treatment period was 1.8 ppb (Heilman 2016). Since immature plants are more susceptible to fluridone (MacDonald et al. 1993), the earlier start date could explain why treatment effects (bleached tips, shorter shoot lengths) were seen earlier in 2016 compared to 2015. The longer treatment window in 2016 provided better control as evidenced by the absence of hydrilla post June sampling and the lack of regrowth after treatment ceased.

Riffleweed density and shoot length were not different by year, but there were differences by site and sampling occasion. Lowest densities were found at our control sites 70 W and Gold Park with means of 29.4 and 24.7 cm, respectively (Figure 22). Shortest shoot lengths were
found at Gold Park and Dumont with means of 8.5 and 8.8 cm, respectively (Figure 23). Shoot lengths at the second control site, 70 W, were not significantly different from all other treated sites (Figure 23). Seasonality was observed with riffleweed growth. Shoot lengths decreased during warm summer months with low flow, and increased during cooler months with higher water levels. Percent riffleweed coverage was also significantly different by site. Gold Park and Pleasant Green had the lowest averages of 47 and 50%, respectively (data not shown). Temperature, pH, and dissolved oxygen were not significant factors in predicting shoot length, density, or percent coverage.

Riffleweed appears to be tolerant to low levels of fluridone maintained over several months indicated by the lack of difference in density and shoot length between sampling years. Similar shoot lengths found at our control site 70 W compared to the treated sites also indicates that observed changes in shoot length were most likely environmentally mediated and not an effect of treatment. Riffleweed’s growth is highly correlated with water levels; shoots lengthen as water levels rise and flowering occurs when levels drop and plants are exposed to the air (Philbrick et al. 2015). This trend was observed in the Eno where water levels are lowest during the hot, often dry summer months (Figure 23).

Chlorophyll $a$ concentrations were not significantly different between riffleweed and hydrilla over the course of the 2015 sampling season. Concentrations differed by site ($p < 0.0001$). Both control sites, 70 W and Gold Park, had significantly higher concentrations of chlorophyll $a$ than all other sites (Figure 24). Fluridone “inhibits the biosynthesis of
carotenoid precursors” causing chlorophyll photodegradation (McCowen et al. 1979). Therefore, the lower chlorophyll levels observed in plants located within the treatment area indicate the herbicide efficacy. While riffleweed may have had reduced chlorophyll levels due to treatment, it was not as sensitive as hydrilla and did not exhibit any long term effects. This may be a factor of the aforementioned seasonal growth patterns of riffleweed versus hydrilla for fluridone is more effective on actively growing, immature plants with low carotenoid levels (MacDonald et al. 1993; Philbrick et al. 2015).

Average pre-treatment tuber densities in 2014 were approximately 390 tubers m$^{-2}$ (data not shown). In 2016, tuber densities in the same sites were only 1 tuber/m$^2$ with tubers only found on one site, Pleasant Green. Average density in the untreated sites in 2016 was 9.5 tubers m$^{-2}$.

Decreasing the tuber bank is one of the greatest challenges of long term hydrilla management. Tuber density decreases as we observed bode well for the success of long-term hydrilla control in lotic systems. The significant reduction of hydrilla presence within the treatment area coupled with low tuber densities may indicate that these dynamic flowing systems exhibit higher sprouting frequencies than what has been reported in lentic systems actively managed for hydrilla (Nawrocki et al. 2016). Factors such as increasing soil depth and anaerobic conditions have been reported to inhibit tuber sprouting (Miller et al. 1976; Van and Steward 1990). The rocky substrate and oxygen rich waters of the Eno River could lead to shallow tuber deposition in oxygenated sediments, thus having the opposite effect on
tuber quiescence. Tuber densities in the untreated areas in 2016 were also relatively low. Qualitative observations of hydrilla in this area indicated that biomass was reduced in 2016 as compared to 2015. This is presumably a factor of increased turbidity associated with high flow for the Eno experienced record flow rates during the 2016 treatment. This also supports the “rapid tuber bank depletion” theory for if the majority of tubers produced during the previous growing season sprout each year, and tuber production for 2016 was inhibited by environmental factors, then we would expect to see low tuber densities following a year of reduced propagule production.

*Panhandle pebblesnail.* Average panhandle pebblesnail densities were positively correlated with percent coverage of riffleweed (p < 0.0001). Average snail densities were higher in 2015 compared to 2016 (p < 0.0001), and significantly different by site (p < 0.001) and sampling month (p < 0.0001) (Figure 26). Highest densities were found at Few’s Ford and were lowest at Pleasant Green. Highest densities by sampling month were found in June, July, August, and September of 2015 and in September of 2016. Lowest densities were observed in May of 2015 followed by February, April, and May of 2016. Our sampling encompassed the full life cycle of the panhandle pebblesnail. At our first pre-treatment sampling in May 2015, the overwintering population was laying eggs (Figure 27). Densities swiftly increased into June as the juveniles began hatching, and then slowly declined as the species returned to its overwintering population. Egg laying and juvenile hatching occurred during the same time frame in 2016. Recounted values were positively correlated with original counts (correlation = 0.95) indicating a high detection probability.
Patterns observed reflect an r-strategist “boom and bust” type reproductive periodicity typical for this species (Figueiredo-Barrosa et al. 2006; Johnson et al. 2013). Timing of egg laying and hatching were similar to what has previously been reported for this species in the Eno River (Archambault and Cope 2016). Snail densities were positively correlated with riffleweed coverage, therefore, it is not surprising that lowest snail numbers were found at Pleasant Green. This site exhibited the lowest percent coverage of riffleweed throughout the two years of sampling. It is unclear why overall densities were lower in 2016. However, it is likely that higher than average flow rates in 2016 may have negatively impacted this species. While increased flow rates on their own may not pose a problem (due to the characteristically strong foot of the Somatogyrus genus (Archambault and Cope 2016), associated turbidity would. S. virginicus prefers clean water and has been notably absent in areas of light siltation (Archambault and Cope 2016). Furthermore, laboratory studies confirm that concentrations of fluridone found throughout the treatment were much lower than concentrations needed to cause any detrimental effects to the egg, juvenile, or adult life stages (Archambault and Cope 2016). Quantitative studies assessing panhandle pebblesnail populations within the Eno have not been previously conducted. Given the aforementioned reasons, we assume that a level of variation in population size from year to year is normal and may be a factor of increased siltation due to high turbidity.

*River survey.* Ninety-eight points within the treatment area were surveyed in 2015 and again in 2016. In 2015, hydrilla was present in 38% of points. Of these occurrences, 92% had an average rating of 0.5-1 and were considered sparse, while 8% were rated as moderate with
average density ratings of 1.5-2 (Figure 28). No dense stands (2.5-3 rating) were found at this time. Riffleweed was present in 29% of points in 2015 with 71% of occurrences rated as sparse, 25% as moderate, and 3% as dense (Figure 29). Waterwillow was present in 37% of points surveyed in 2015 with 58% classified as sparse, 31% as moderate, and 11% as dense (Figure 30). In 2016, hydrilla was only found in 6% of the points surveyed. All occurrences were rated as sparse (Figure 31). Riffleweed occurred in 26% of survey points. Of these, 44% had average density ratings of sparse, 36% as moderate, and 20% as dense (Figure 32). Waterwillow was found in 44% of points in 2016 with 67% rated as sparse, 21% as moderate, and 12% as dense (Figure 33). Densities and occurrences of riffleweed and waterwillow were not significantly different by year (p = 0.2085 & p = 0.3711, respectively). However, hydrilla densities were significantly reduced in 2016 (p < 0.0001). Neither riffleweed nor waterwillow presence were significant in predicting occurrence of hydrilla.

Average channel depth was approximately 2.6 ft, and average channel width was approximately 74 ft (Figure 34). Channel depth and width were averaged across survey years as differences were minimal. The majority of the Eno River channel morphology within the surveyed area can be classified as a run (~67% of points surveyed) (Figure 35).

This survey showed significant reduction in hydrilla presence/density with no notable differences in native plant populations after one year of treatment. This supports the results from our bi-weekly vegetation point sampling and further points to the success of this project.
Overall, this two-year pilot project provided excellent selective control of hydrilla within the treated area. Low rates of fluridone maintained for approximately 90 days were effective at controlling early stage growth of hydrilla located within and adjacent to populations of native and threatened species while limiting detrimental effects to said species. This protocol provides a framework on which to base future management plans that call for the complex management of hydrilla in high biodiversity, flowing systems.
References


Figure 18. Eno River vegetation sampling sites. 70 W and Gold Park are control sites located upstream of the treatment zone.
Figure 19. Eno River panhandle pebblesnail sampling sites. 70 W and Gold Park are control sites located upstream of the treatment zone.
Figure 20. Mean hydrilla shoot length by sampling month and day of the month in a standard twelve month calendar year. Lengths differed by year and sampling occasion (p < 0.0001). Shaded portions represent the period of treatment. Error bars indicate one standard error from the mean.
Figure 21. Average hydrilla percent coverage by month in 2015. The shaded portion represents the period of treatment. Error bars indicate one standard error from the mean.
Figure 22. Average riffleweed stem density by site pooled across 2015 and 2016. 70 W and Gold Park, the control sites, have lower densities in comparison to all other sites (p = 0.0031). Error bars indicate one standard error from the mean.
Figure 23. Average riffleweed shoot length by site separated by month within year. Gold Park and Dumont shoot lengths are shorter in comparison to all other sites (p < 0.0001). The shaded portion represents the period of treatment. Error bars indicate one standard error from the mean.
Figure 24. Average chlorophyll $a$ concentration of hydrilla and riffleweed over the course of the 2015 sampling season by site. The shaded portion represents the period of treatment. FW = fresh weight. Highest concentrations were found at our reference sites, 70W and Gold Park ($p < 0.0001$) Error bars represent one standard error from the mean.
Figure 25. Panhandle pebblesnail density over two years by site. Shaded portions represent the period of treatment. Densities were higher in 2015 compared to 2016 ($p < 0.0001$) and varied by site ($p < 0.001$) and sampling month ($p < 0.0001$). Error bars represent one standard error from the mean.
Figure 26. Panhandle pebblesnails laying eggs on the bottom of riffleweed covered rock.
Figure 27. Eno River hydrilla abundance during 2015 kayak survey. Density ratings correspond to a scale of 0-3, with 0 being absent, and 3 being dense. Frequency of occurrence is included in the legend. Ratings were averaged for left and right bank data.
Figure 28. Eno River rifflweed abundance during 2015 kayak survey. Density ratings correspond to a scale of 0-3, with 0 being absent, and 3 being dense. Frequency of occurrence is included in the legend. Ratings were averaged for left and right bank data.
Figure 29. Eno River waterwillow abundance during 2015 kayak survey. Density ratings correspond to a scale of 0-3, with 0 being absent, and 3 being dense. Frequency of occurrence is included in the legend. Ratings were averaged for left and right bank data.
Figure 30. Eno River hydrilla abundance during 2016 kayak survey. Density ratings correspond to a scale of 0-3, with 0 being absent, and 3 being dense. Frequency of occurrence is included in the legend. Ratings were averaged for left and right bank data.
Figure 31. Eno River riffleweed abundance during 2016 kayak survey. Density ratings correspond to a scale of 0-3, with 0 being absent, and 3 being dense. Frequency of occurrence is included in the legend. Ratings were averaged for left and right bank data.
**Figure 32.** Eno River waterwillow abundance during 2015 kayak survey. Density ratings correspond to a scale of 0-3, with 0 being absent, and 3 being dense. Frequency of occurrence is included in the legend. Ratings were averaged for left and right bank data.
Figure 33. Average channel depth for a portion of the Eno River. Five depth readings were taken across the channel at each survey point. Frequency of occurrence is included in the legend. Readings were averaged for each point over two years.
Figure 34. Eno River channel morphology classified as either a riffle, run, or pool. 67% of points were classified as a run. Frequency of occurrence is included in the legend.