

## Monoecious Hydrilla – A Review of the Literature

### Abstract

*Hydrilla verticillata* is a submersed aquatic weed that has become one of most expensive and difficult to manage in the United States. There are two biotypes of hydrilla found in the United States, a dioecious and a monoecious biotype. The monoecious biotype is typically found from North Carolina northward and is rapidly spreading. The body of research focusing on strictly the monoecious biotype is much smaller than that of the dioecious, as reflected in the literature. A search with BIOSIS yielded 1,246 articles with the topic 'hydrilla'; only 53 of those had the additional topic 'monoecious'. Many publications on hydrilla make no mention of biotype, therefore only an educated guess can be made based on study locations to decipher biotype. Provided is a comprehensive overview of published research on monoecious hydrilla.

### Species Overview

The submersed aquatic monocotyledonous angiosperm *Hydrilla verticillata* (L.f.) Royle is an aggressive, opportunistic, nuisance species in the Hydrocharitaceae family that has spread from its native Asia to every continent except Antarctica (Cook and Luond 1982, Pieterse 1981). Taxonomic classification for Hydrilla along with other genera of Hydrocharitaceae is shown below (USDA 2012), although the genus *Najas* is now placed within Hydrocharitaceae by some sources (Weakly 2012).

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Alismatidae
Order	Hydrocharitales
Family	Hydrocharitaceae
Genus	<i>Hydrilla</i>
Genus	<i>Blyxa</i>
Genus	<i>Egeria</i>
Genus	<i>Elodea</i>
Genus	<i>Enhalus</i>
Genus	<i>Halophila</i>
Genus	<i>Hydrocharis</i>
Genus	<i>Lagarosiphon</i>
Genus	<i>Limnobium</i>
Genus	<i>Nechamadra</i>
Genus	<i>Ottelia</i>
Genus	<i>Stratiotes</i>

Hydrilla is difficult to manage and causes significant economic and ecological damage across the US (Langeland 1996). Hydrilla may appear similar to other submersed aquatic plants (refer to taxonomic key in Figure 1), but what makes hydrilla unique are its vegetative reproductive structures and a well-developed root system for carbohydrate storage. These perennial structures allow hydrilla to rapidly resprout and revegetate after treatments or environmental stresses (Netherland, 1997; Owens & Madsen, 1998). Hydrilla causes severe problems in infested water bodies (Langeland 1996). One significant problem is the disruption of water flow in reservoirs or in drainage and irrigation canals. It negatively impacts recreational uses of a water body including boating, fishing, swimming, water skiing, etc. Hydrilla can also displace native plant life and shift balanced ecosystems to monocultures with altered fish populations. Hydrilla can also affect wildlife higher up the food chain. It has been documented to host an epiphytic cyanobacterium in the order Stigonematales. This cyanobacterium is believed to produce a neurotoxin that causes Avian Vacuolar Myelinopathy (AVM), a neurological disease that impacts waterfowl and their predators in the southeastern US, including bald eagles (Wilde et al. 2005, Williams et al. 2007).

In the US, both a female dioecious biotype (staminate and pistillate flowers on separate plants) and a monoecious biotype (staminate and pistillate flowers on the same plant) of hydrilla have become naturalized (Cook and Lüönd 1982). Optimal growth and survival for the dioecious type is found in warmer climates, while the monoecious form is better suited for more temperate climates with lower temperatures and shorter growing seasons (Ames et al. 1986; Van 1989; Madeira et al. 2000; Netherland 1997; Steward et al. 1987). Dioecious hydrilla typically thrives all year in the warm waters of the southern US, while monoecious hydrilla dies back completely in the winter and acts as a herbaceous perennial (Harlan et al. 1985).

Monoecious and dioecious biotypes of hydrilla found in the US have been shown to be genetically distinct (Verkleij et al. 1983; Ryan et al. 1991). A method of distinction between the two biotypes was conducted by Ryan and Holmberg (1994) and Ryan et al. (1995). They used random amplified polymorphic DNA (RAPD) and detected a marker that was only present in dioecious hydrilla, not the monoecious biotype. Madeira et al. (1997, 2000) continued this research examining samples of both US hydrilla biotypes from around the world to examine origins of introductions. In 2004, Madeira et al. published an improved tool for distinguishing between monoecious and dioecious hydrilla that could be done without the reference samples required for the original method.

## Introduction and Spread

Monoecious hydrilla may have been first documented within the US in Delaware, and then in Washington, DC and the Potomac River in 1980 (Haller 1982, Steward et al. 1984). Steward et al. (1984) suggested that it was possibly introduced during caging and transplanting experiments where hydrilla, mistakenly identified as *Elodea canadensis*, was transplanted from Kenilworth Gardens in Washington, DC. Lilypons Water Gardens, located in Adamstown, Maryland, may have been the source of the Kenilworth Gardens infestation, as they were a popular commercial supplier of aquatic ornamental plants in the area, and Haller (1982) reported seeing a hydrilla-like plant with tubers there during a 1980 visit. Monoecious hydrilla was first documented in North Carolina in 1980 after reports of dense, weedy vegetation in Umstead Lake in the mid to late 1970's (Figure 2). The Delaware and North Carolina invasions seem to be relatively synchronous and it is possible that early monoecious hydrilla spread in the US was facilitated by contaminated aquatic plant (esp. water lily) shipments from Lilypons (Madeira et al. 2000) or other sources. Local spread and dispersal of monoecious hydrilla is often attributed to boaters moving plant fragments unintentionally, and new infestations often occur near boat ramps. Intentional spread also occurs when individuals believe that hydrilla will benefit fish and waterfowl habitat (Langeland 1996). Non-human dispersal is possible as well, with waterfowl able to transport turions, fragments, and seeds (Coley 1997, Joyce et al. 1980, Langeland 1996, Langeland and Smith 1984, Miller 1998).

The monoecious biotype spread and became the dominant hydrilla biotype found in the Mid-Atlantic States (Kay 1992; Langeland 1996). In 2000, Madeira et al. showed that monoecious hydrilla was found in drainages of the Atlantic Basin from central Georgia up to Pennsylvania and Connecticut. It was not found in Gulf Basin drainages, but was sporadically located in the Pacific basin, in California and Washington (Madeira et al. 2000). Monoecious hydrilla is also found in the Interior Basin in Maryland and North Carolina. Langeland (1996) suggested that monoecious hydrilla could spread as far north as southern Canada, based on its range in Europe. Les et al. (1997) compiled the northernmost hydrilla distributions worldwide and Balciunas and Chen (1993) provided a comparison of January temperatures in North America to those in Asia where hydrilla has been documented. Based on reported worldwide hydrilla distribution and climate patterns, there are vast areas in North America at risk of invasion by hydrilla (Figure 3).

US monoecious hydrilla populations have been linked to a population found in Seoul, Korea (Medeira et al. 1997). Monoecious and dioecious hydrilla are both triploid, and the diploid parents of monoecious hydrilla have not been determined. However, Benoit (2011) suggests that monoecious hydrilla is a hybrid of Indian and Indonesian lineages and that dioecious hydrilla tested from India, Nepal, and the United States should be considered a distinct species. Benoit (2011) also postulated that Japan/Korean/European hydrilla and Indonesian/Malaysian hydrilla should each be considered distinct species as well, postulating a total of 3 species within *Hydrilla*.

### **Anatomy/Life Cycle**

Hydrilla grows as a rooted and submersed plant. Detached hydrilla stems can also survive on their own or in mats and may grow roots to attach to sediment. Hydrilla produces many stolons and rhizomes, as well as turions in the leaf axils which detach upon maturity. Axillary turions are small compact buds that are green in color. Subterranean turions are produced on the terminal end of rhizomes. Subterranean turions are larger and vary more in color, from white to yellow, or gray to red depending on the sediment. They are often referred to as tubers, although tubers and turions are distinct structures. Hydrilla leaves are whorled and serrated. The morphology of hydrilla has been described in detail by Cook & Lüönd (1982) and Yeo et al. (1984).

Pesacreta (1990) examined carbohydrate allocation in monoecious hydrilla and found that the majority of starch accumulation occurred in plant shoots when exposed to short photoperiods. Starch levels in tubers were found to decrease mostly in the first two weeks after sprouting (Pesacreta 1990). Pesacreta (1990) also found that monoecious hydrilla displayed enhanced fragmentation after 8 weeks of high temperatures (32° C).

Harlan et al. (1985) described the phenology of monoecious hydrilla in North Carolina. A comparison of monoecious hydrilla phenology in North Carolina to the phenology of dioecious hydrilla in Florida is shown in Figure 4. Tubers began sprouting in late March and continued through August, when the hydrosol was 11 to 13° C. The sprouting of axillary turions usually occurs prior to the sprouting of tubers in monoecious hydrilla (Spencer and Ksander 2001). Spencer and Ksander (2001) found that half of axillary turions sprout by mid-June, and half of tubers by mid-July. After subterranean and axillary turions sprout, rapid growth occurs laterally. On the Chickahominy River, the most abundant hydrilla biomass was found in October (Shields et al. 2012). Turion production occurs during long-day

photoperiods in the summer. Tubers were formed from June through October (Harlan et al. 1985). Axillary turions formed from October through December (Harlan et al. 1985). Following a final burst of subterranean turion production induced by short days, monoecious hydrilla biomass declines and then breaks away from the substrate and mats loaded with axillary turions can shift locations (Steward and Van 1987). Harlan et al. (1985) found the majority of sprouting axillary turions in depths less than 0.5 m as a result of these mats floating to the shore. Monoecious hydrilla behaves as a herbaceous perennial in North Carolina and farther north, as plant stands and fragments do not over-winter (Harlan et al. 1985; Owens and Smart 2007; Sutton et al. 1992). Maximum growth is seen in the summer, in winter there is no shoot growth and plants die back, and regrowth is dependent on turions sprouting in the spring (Harlan et al. 1985; Sutton et al. 1992). Monoecious hydrilla does, however, show some perennial characteristics in studies done in southern Florida, with growth all year long, but somewhat limited in winter months (Steward 1993; Sutton et al. 1992). Maki and Galatowitsch (2008) studied monoecious hydrilla axillary turion overwintering capacity, and found that 67 and 42% survived cold treatments (4°C) of 63 and 105 days, respectively.



Monoecious hydrilla subterranean turions/tubers



Monoecious hydrilla axillary turions

Initial identification of hydrilla can often be difficult due to its highly polymorphic tendencies, and it is often misidentified as the morphologically similar *Egeria* spp. and *Elodea* spp. (Rybicki et al. 2013). Distinguishing between biotypes furthers the confusion, as growing conditions can have a significant effect on the appearance of the plant, and there is no way to visually definitively distinguish between biotypes without flowers (Figure 1). There are, however, some characteristics that can lead to a reasonable conclusion as to which biotype is being examined. For example, the monoecious plant is much less robust than the dioecious with leaf width of 1 to 3 mm and 2.25 to 3.5mm, respectively (Benoit 2011). Monoecious hydrilla produces more tubers than does dioecious hydrilla; however the

tubers are smaller and weigh less (Sutton et al. 1992; Van 1989.) A comparison of hydrilla tuber weights and densities is shown in Tables 1 and 2. Sprouting monoecious tubers send shoots out laterally, rather than vertically towards the surface as the dioecious tubers do (Van 1989). And, staminate flowers are present with monoecious, but not dioecious hydrilla.

Monoecious hydrilla tubers have a very high germination rate in laboratory trials, often greater than 90% (Harlan et al. 1985, Van and Steward 1990, personal experience). However, while monoecious hydrilla tubers readily germinate when removed from sediment, when left undisturbed in situ the germination rate is much lower (Van and Steward 1990). Carter et al. (1987) found that monoecious hydrilla tubers require a chilling period prior to sprouting which may prevent sprouting the same year of formation. Monoecious hydrilla tubers have been shown to remain in undisturbed soil for more than 4 years after production (Van and Steward 1990), and six year tubers have still been viable in North Carolina (unpublished data). There appears to be an environmental factor imposed dormancy which prevents depletion of tuber populations. Axillary turions will germinate within one year or not at all (Van and Steward 1990). Nawrocki et al. (2011) also found that monoecious hydrilla tubers have multiple axillary buds preformed within dormant tubers that can produce secondary shoots, even after terminal shoot removal.

Spencer and Anderson (1986) found in a greenhouse study that 38% of monoecious hydrilla grown from tubers and exposed to a 10 hour photo period produced tubers by 28 days, and 100% produced tubers by 56 days. They also observed that tuber production was the priority for the plants, over production of new root or shoot tissue (Spencer and Anderson 1986). Van (1989) found that monoecious hydrilla produced tubers under both a 10 and 16 hour (h) photoperiod, but production was much higher for the 10 h photoperiod. Tubers were produced in Van's study (1989) after 4 weeks of exposure to this photoperiod. Spencer et al. (1994) had similar results; monoecious hydrilla produced more tubers with an 11 h photoperiod than with a 15 h photoperiod. Spencer et al. (1994) also examined the carbon and nitrogen allocation of monoecious hydrilla and found that 43 times more carbon was allocated to new tubers than nitrogen.

Monoecious hydrilla produces tubers at a lower temperature than does dioecious (Steward and Van 1987). This, along with its annual growth habit, shows why monoecious hydrilla is adapted for northern areas, which are colder and have short growing seasons. However, McFarland and Barko (1999) found

that monoecious hydrilla may be more adapted to warmer temperatures than previously reported. In their study, subterranean turion production occurred at unexpectedly high temperatures (35 °C), although in reduced amounts (McFarland and Barko 1999). This lends to the theory that monoecious hydrilla could possibly spread and thrive farther south than typically thought. Nawrocki et al (2011) also found similarities in sprouting of both biotypes under temperature and light manipulation.

Hydrilla turions vary greatly in abundance, size, and weight. Axillary turions are on average half the size of tubers (Van and Steward 1990). Spencer et al. (1987) found that the mean fresh weight for monoecious tubers ranged from 117 to 202 mg and 36 to 77 mg for axillary turions. Nawrocki (2011) reported that monoecious subterranean turion weight varied from 30 to 320 mg in North Carolina lakes. Harlan et al. (1985) showed that the majority of monoecious hydrilla tubers in North Carolina lakes were found at soil depths from 0 to 8 cm, but that soil depths of 8 to 12 cm could hold up to 50% of the total turions. Tubers were infrequently found deeper than 12 cm (Harlan et al. 1985). Monoecious hydrilla tubers have been found in densities of over 3000 tubers per m<sup>2</sup> in North Carolina (Nawrocki 2011). Nawrocki et al. (2011) examined monoecious hydrilla tuber sprouting dynamics at varying pH levels (4-10), and found few differences in initial growth. Monoecious hydrilla can tolerate salinities up to 13 ppt (Steward and Van 1987); however, Twilley and Barko (1990) reported little salinity tolerance in monoecious hydrilla. Shields et al. (2012) reported that hydrilla was limited to the upper estuary of the Chickahominy River where salinity stays less than 3 ppt. Carter et al. (1987) reported that monoecious hydrilla vegetative growth was reduced when exposed to salinities of 7 and 9 ppt, and that there is a negative correlation between salinity and tuber germination. Greater than 92% of tubers in fresh water sprouted, 4 to 20% of tubers exposed to 5 to 9 ppt salinity sprouted, while no tubers sprouted with salinities greater than 9 ppt (Carter et al. 1987). Nawrocki et al. (2011) exposed tubers to salinity for two weeks. Salinity of 12 ppt had little to no effect on tuber sprouting, while tubers exposed to 24 ppt did not sprout under constant salinity exposure but sprouted when moved into a solution of deionized water (Nawrocki et al. 2011).

While there have been no reports of seed production from the dioecious biotype of hydrilla (Steward 1993), viable seed production has been reported in monoecious hydrilla (Conant et al. 1984; Langeland and Smith 1984; Langeland 1996; Lal and Gopal 1993). A monoecious population in New Delhi, India produced seed profusely during late winter (Lal and Gopal 1993). The seeds showed light sensitivity and germinated within a week at 23 to 28°C, and readily germinated when exposed to light after being

stored wet or dry in darkness for up to one year (Lal and Gopal 1993). Sexual reproduction of monoecious hydrilla would result in variations that would allow adaptations to a wider range of environments than are already exploited by this plant, by aiding in dispersal and overwintering. Lal and Gopal (1993) suggest that hydrilla seed production may offer a long-term strategy for plant survival after long dry periods, such as in regions with a monsoon climate.

Steward (1993) found that 71% of test crosses between dioecious and monoecious hydrilla resulted in the production of seed. Of the seeds from these successful crosses, 90% were viable and the majority of seedlings survived (Steward 1993). Lake Gaston, on the North Carolina-Virginia border, is the first location where both biotypes of hydrilla were found in one body of water (Ryan et al. 1995). Steward (1993) hypothesized that if hydrilla starts sexually reproducing, there could be serious detrimental effects. Genetic variability would increase, and individuals could develop with adaptations for thriving in a greater number of environments. This could lead to greater difficulty in managing and controlling hydrilla.

Monoecious hydrilla is an extremely tolerant and competitive plant. It can establish and then displace native plants. Monoecious hydrilla can persist alone and competitively with *Elodea canadensis* (Michx.) in flowing systems like streams and waterways in New Zealand (Hofstra et al. 2010). Spencer and Ksander (2000) showed the strong competitive ability of monoecious hydrilla mixed with American pondweed, and Meadows and Richardson (2012) found that monoecious hydrilla out-competed four other submersed aquatic plants (Eurasian watermilfoil (*Myriophyllum spicatum* L.; invasive), curly leaf pondweed [*Potamogeton crispus* L.; (invasive), *Elodea canadensis* Michx. (native), and *Vallisneria americana* Michx. (native)] in a mesocosm trial. Chadwell and Engelhardt (2007) reported that monoecious hydrilla establishment was inhibited by previous colonies of *Vallisneria americana* (Michx.) in greenhouse trials, but not in field trials, while Steward (1991b) showed that monoecious hydrilla biomass was on average 45% higher when grown with *Vallisneria americana* in mesocosms. Meadows and Richardson (2012) and Hofstra et al. (1999) found hydrilla growth in outdoor tanks to be greater when planted close to the same time as competitor species than if the competitor was given more time to establish before monoecious hydrilla introduction.

Steward (1991a) found that monoecious hydrilla in the Potomac River would most likely not grow at less than 5% of incident solar PAR, and therefore would be restricted to the limnetic zone. Estes et al. (2011)

conducted a survey after the discovery of monoecious hydrilla in Cumberland County, Tennessee, the first infestation found in a rocky, rugged mountain stream system. They found hydrilla mainly in pool and run habitats (Estes et al. 2011). Hydrilla can take advantage of disturbances and rapidly colonize these areas. On the Chickahominy River a high salinity, low water clarity disturbance event occurred in 2001 to 2002 which led to bare sediment (Shields et al. 2012). Monoecious hydrilla took advantage of the lack of competition and became the dominant species, however smaller amounts of non-invasive SAV also became established in these areas (Shields et al. 2012). In North Carolina, monoecious hydrilla is now invading highly disturbed systems like flowing rivers, estuaries, and reservoirs with high water fluctuation.

### **Control/Management**

Hydrilla is a difficult weed to eradicate, and research on management of the monoecious biotype is more limited than dioecious. Monoecious hydrilla active management should begin several weeks after tubers first sprout, or as soon as possible after discovery of new infestations. However, new infestations of monoecious hydrilla are often not detected until much later in the season, when shoot growth reaches the surface of the water body, which increases the level of difficulty to achieve control. Treatments to control monoecious hydrilla are generally the same as for dioecious hydrilla, with chemical control and grass carp being the most historically used methods. Mechanical control is usually not recommended for hydrilla management due to the fragmentation that occurs, the cost, and other negative impacts. Serafy et al. (1994) found that hydrilla biomass was greater 21 days after harvesting than at an undisturbed site, and mechanical harvesting had short term negative effects on fish populations. Haller et al. (1980) estimated that mechanical harvesting of dioecious hydrilla caused a loss of 32% of fish numbers and 18% of fish biomass in Orange Lake, FL.

Chemical herbicide treatments are a popular and effective method for managing hydrilla infestations. Current herbicides registered for hydrilla control in the US include bispyribac-sodium, copper, diquat, endothall, flumioxazin, fluridone, imazamox, and penoxsulam, although the majority of research conducted for product registration was done on dioecious hydrilla, and not the monoecious biotype. Treatments of diquat and endothall (both dipotassium and monoamine salts) produced similar results on both US biotypes (Steward and Van 1986, 1987, Van et al. 1987). Diquat and endothall are often more successful when applied early to mid-June, when monoecious hydrilla biomass is more manageable (Langeland and Pesacreta 1986). A second application can be applied in mid-August if

regrowth occurs (Langeland and Pesacreta 1986). Van et al. (1987) reported that a concentration of 0.25 mg/l diquat for 2 days was lethal to both monoecious and dioecious hydrilla. Hodson (1984) showed that endothall was effective on monoecious hydrilla, but appropriate exposure times must be met. Langeland and Pesacreta (1986) also reported that granular endothall was more effective in areas with more water exchange, while Poovey and Getsinger (2010) found that endothall applied at 2 mg ai l<sup>-1</sup> with 72 hour exposure times reduced monoecious hydrilla biomass grown from shoot fragments. Monoecious hydrilla sprouted tubers needed an increased rate (4 mg ai l<sup>-1</sup>) or longer exposure times (96 hours) to achieve the same result (Poovey and Getsinger 2010). Bensulfuron methyl, when applied in rates of 0.05-0.2 mg/L to monoecious hydrilla, causes biomass reduction for only two months before regrowth occurs (Van and Vandiver 1992). However, it has been shown to inhibit subterranean turion production in monoecious hydrilla (Van and Vandiver 1992). Fluridone will control monoecious hydrilla (Langeland and Pesacreta 1986) and has been documented to cause tuber numbers to decrease over time (Nawrocki 2011). However after years of repetitive Fluridone treatments within Florida, fluridone-resistant dioecious hydrilla has been documented (Michel et al. 2004). The exclusive annual use of fluridone (or any single herbicide mode of action) is not recommended; it is important to alternate herbicide modes of action or other management techniques in any long-term weed management plan.

Grass carp [*Ctenopharyngodon idella* (Val.)] are often recommended and used for hydrilla management, as stocking a body of water with these fish can cause a reduction or elimination of aquatic plants. These fish have been called 'selective generalists' as they feed on aquatic plant species in order of decreasing palatability; hydrilla and pondweeds (*Potamogeton* spp.) are the most common species reported to be consumed by grass carp (Dibble and Kovalenko 2009). A major concern with using grass carp as a vegetation control method is the possible negative impacts on aquatic communities, both direct and indirect, and more research is needed in this area. One major benefit of using grass carp is that it is a multi-year solution. Regulations for grass carp stocking differ on a state by state basis. In many states a permit from a state resource management agency is needed for stocking grass carp. The majority of published research on grass carp and hydrilla has been done with the dioecious biotype, however, Hodson et al (1984) found a stocking rate of 50 or more grass carp per hectare effectively controlled monoecious hydrilla when stocked in winter or spring before significant hydrilla growth. In North Carolina, recommendations on stocking grass carp are 15 fish per acre in small ponds, and 15-20 fish per vegetated acre in larger water bodies (Buhler 2011). Combining two methods of control is often recommended and can be beneficial. Stocking a water body with grass carp is often used in tandem

with herbicide treatments. However, timing is critical, as treated hydrilla plants have been found to be less desirable for grass carp in greenhouse trials (Kracko and Noble 1993).

The hydrilla leaf-mining fly (*Hydrellia pakistanae* Deonier and *H. balciunasi* Bock), has been suggested as a biological control agent for monoecious hydrilla, as it has had success on dioecious hydrilla (Doyle et al. 2002, 2007; Owens et al. 2006, 2008). However, Dray and Center (1996) suggest that *H. pakistanae* would be useful for control of monoecious hydrilla only where it can grow as a perennial, and Grodowitz et al. (2010) and Harms and Grodowitz (2011) show that monoecious hydrilla is not a suitable host, as the plant provides no over-wintering habitat for the fly. The search for new biological control agents should be conducted in temperate climates to find agents with compatible overwintering strategies to monoecious hydrilla.

Prolific tuber production is one of the main challenges to monoecious hydrilla management. Nawrocki (2011) predicted that with consistent yearly herbicide treatments, it would take 7-10 years to reduce an initial monoecious hydrilla tuber bank 99.5%. When one year of treatment was omitted, tuber densities rebounded to 74% of the original amount (Nawrocki 2011). Spencer and Ksander (1999) found that exposure to acetic acid inhibited sprouting of tubers by 80 to 100%, and suggested this method may be useful in tandem with drawdown treatments. Hodson et al. (1984) found that drawdown treatments were ineffective on monoecious hydrilla in North Carolina due to the location of the tubers in the soil profile; many tubers were found in the clay substrate layer under an organic detrital layer, and drawdowns of a few months were not successful in completely drying this clay layer. Poovey and Kay (1998) further examined summer drawdowns as a control measure for monoecious hydrilla and found that a drawdown of only one week on sandy soil completely killed hydrilla and allowed for no tuber production, while on silt loam a two week or more drawdown period was needed to greatly reduce tuber numbers and suppress regrowth. Unfortunately the timing of summer drawdowns is not feasible for many water bodies. Another method proposed for control of monoecious hydrilla and other aquatic invasives is the altering of organic matter contents of sediments (Gunnison and Barko 1989). However, this method has had mixed results, and Spencer et al. (1992) found it unsuccessful for monoecious hydrilla biomass reduction.

While hydrilla is one of the most often studied submersed aquatic plants, the majority of research has been conducted with the dioecious biotype and additional research is greatly needed on the

monoecious biotype. Development of strategies to increase tuber sprouting within a narrow time frame is an area of great importance, as this would allow management efforts to be more effective. In situ documentation of the relevance of monoecious hydrilla seed production is also needed. This includes quantity of seed produced, viability of seed, potential for seed dispersal, and ability of seed to establish new plants. Additionally, research to increase the effectiveness of management techniques, while limiting damage to non-target organisms is also needed. As monoecious hydrilla spreads into glacial lakes, a different species spectrum will be present from that in Florida lakes and management techniques will need specificity for glacial lake plant diversity. This would include best management practices for herbicide use, but also other management options and proper integration of multiple techniques. In order to achieve these goals, the cooperation between industry, academia, regulatory agencies, and land managers is vital.

#### Literature Cited

- Benoit, L.K. "Cryptic Speciation, Genetic Diversity and Herbicide Resistance in the Invasive Aquatic Plant *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae)" (January 1, 2011). *Dissertations Collection for University of Connecticut*. Paper AAI3492149.  
<http://digitalcommons.uconn.edu/dissertations/AAI3492149>
- Blackburn, R.D., L.W. Weldon, R.R. Yeo, and T.M. Taylor. 1969. Identification and distribution of certain similar-appearing submersed aquatic weeds in Florida. *Hyacinth Control J.* 8(1): 17-22.
- Bowmer, K.H., S.W.L. Jacobs, and G.R. Sainty. 1995. Identification, Biology, and Management of *Elodea canadensis*, Hydrocharitaceae. *Journal of Aquatic Plant Management.* 33: 13-19.
- Buhler, W. 2011. Aquatic Weed Control. *In 2012 North Carolina Agricultural Chemicals Manual*. College of Agriculture and Life Sciences, Raleigh, NC. Available online:  
<http://ipm.ncsu.edu/agchem/7-toc.pdf>.
- Carter, V., N. B. Rybicki and C. L. Schulman. 1987. Effect of salinity and temperature on germination of monoecious hydrilla propagules. *Journal of Aquatic Plant Management.* 25: 54-57.
- Chadwell, T. B. and K. A. Engelhardt. 2008. Effects of pre-existing submersed vegetation and propagule pressure on the invasion success of *Hydrilla verticillata*. *Journal of Applied Ecology.* 45(2): 515-523.
- Cook, C. D. K. and R. Lüönd. 1982. A revision of the genus *Hydrilla* (Hydrocharitaceae). *Aquatic Bot.* 13: 485-504.
- Coley, C. 1997. Hydrilla tubers recovered from waterfowl Lake Mattamuskeet, North Carolina. *Aquatic*

- Plant News. 54:2.
- Conant, Jr., R.D., T.K. Van, and K.K. Steward. 1984. Monoecious hydrilla produces viable seeds in the United States. *Aquatics*, 6 (3):10.
- Crow, Garrett E. and B. Hellquist. 2000. *Aquatic and Wetland Plants of Northeastern North America Volume Two Angiosperms: Monocotyledons*. The University of Wisconsin Press, Madison.
- Dibble, E.D. and K. Kovalenko. 2009. Ecological impact of grass carp: a review of the available data. *J. Aquat. Plant Manage.* 47:1-15.
- Doyle, R. D., M. J. Grodowitz, R. M. Smart, and C. S. Owens. 2002. Impact of herbivory of *Hydrellia pakistanae* (Diptera: Ephydriidae) on growth and photosynthetic potential of *Hydrilla verticillata*. *Biol. Control*. 24: 221-229.
- Doyle, R. D., M. J. Grodowitz, R. M. Smart, and C. S. Owens. 2007. Separate and interactive effects of competition and herbivory on the growth, expansion, and tuber formation of *Hydrilla verticillata*. *Biol. Control* . 41:327-338.
- Dray, F. A., Jr., and T. D. Center. 1996. Reproduction and development of the biocontrol agent *Hydrellia pakistanae* (Diptera: Ephydriidae) on monoecious hydrilla. *Biol. Cont.* 7:275-280.
- Estes, L.D., C. Fleming, A.D. Fowler, and N. Parker. 2011. The distribution, abundance, and habitat colonization of the invasive submersed macrophyte, *Hydrilla verticillata*, in a high-gradient riverine system. Proc. 72<sup>nd</sup> Annual Association of Southern Biologists (ABS) Meeting, 13-16 April, Huntsville, AL; Oral presentation.
- Godfrey, Robert K., and Jean W. Wooten. 1979. *Aquatic and Wetland Plants of Southeastern United States: Monocotyledons*. The University of Georgia Press, Athens.
- Grodowitz, M., J. Nachtrieb, N. Harms, and J. Freedman. 2010. Suitability of using introduced *Hydrellia* spp. for management of monoecious *Hydrilla verticillata* (L.f.) Royle. APCRP Technical Notes Collection, ERDC/TN APCRP-BC-17. Vicksburg, MS: U.S. Army Engineer Research and Development Center.
- Haller, W. T. 1982. Hydrilla goes to Washington. *Aquatics* 4(4):6-7.
- Haller, W.T., J.V. Shiremana, and D.F. Duranta. 1980. Fish harvest resulting from mechanical control of hydrilla. *Transactions of the American Fisheries Society*. 109(5): 517-520.
- Haller, W.T., D. L. Sutton and W. C. Barlowe. 1974. Effects of salinity on growth of several aquatic macrophytes. *Ecology*. 55(4): 891-894.
- Harlan, S. M., G. J. Davis, and G. J. Pesacreta. 1985. Hydrilla in three North Carolina lakes. *J. Aquat. Plant Manage.* 23:68-71.

- Harms, N.E. and M.J. Grodowitz. 2011. Overwintering biology of *Hyrella pakistanae*, a biological control agent of hydrilla. *J. Aquat. Plant Manage.* 49:114-117.
- Hodson, R.G. G.J. Davis, and K.A. Langeland. 1984. Hydrilla management in North Carolina. *Water Resources Research Inst. Of Univ. of N.C. Rep. No. 217.*
- Hofstra, D.E., P.D. Champion, and J.S. Clayton. 2010. Predicting invasive success of *Hydrilla verticillata* (L.f) Royle in flowing water. *Hydrobiologia.* 656:213-219.
- Hofstra, D.E., J. Clayton, J.D. Green, and M. Auger. 1999. Competitive performance of *Hydrilla verticillata* in New Zealand. *Aquatic Botany.* 63(3):305-324.
- Joyce, J.C., W.T. Haller, and D. Colle. 1980. Investigation of the presence and survivability of of hydrilla propagules in waterfowl. *Aquatics* 2(3):10-11.
- Kracko, K.L. and R.M. Noble. 1993. Herbicide inhibition of grass carp feeding on hydrilla. *J. Aquat. Plant Manage.* 31:273-275.
- Langeland, K.A. and G.J. Pesacreta. 1986. Management program for hydrilla (a monoecious strain) in North Carolina. *Water Resources Research Inst. Of Univ. of N.C. Rep. No. 225.*
- Langeland, K.A. 1996. *Hydrilla verticillata* (L.F.) Royle (Hydrocharitaceae), "The Perfect Aquatic Weed". *Castanea.* 61(3): 293-304.
- Langeland, K.A. and C.B. Smith. 1984. Hydrilla produces viable seed in North Carolina Lakes. *Aquatics.* 6(4):20-21.
- Lal, C. and B. Gopal. 1993. Production and germination of seeds in *Hydrilla verticillata*. *Aquat. Bot.,* 45: 257-261.
- Les, D.H., L.J. Mehrhoff, M.A. Cleland, and J.D. Gable. 1997. *Hydrilla verticillata* (Hydrocharitaceae) in Connecticut. *J. Aquat. Plant Manage.* 35:10-14.
- Madeira, P.T., T.K. Van, K.K. Steward, and R.J. Schnell. 1997. Random amplified polymorphic DNA analysis of the phonetic relationships among world-wide accessions of *Hydrilla verticillata*. *Aquatic Biology.* 59:217-236.
- Madeira, P.T., C.C. Jacono, and T.K. Van. 2000. Monitoring hydrilla using two RAPD procedures and the nonindigenous aquatic species database. *J. Aquat. Plant Manage.* 38:33-40.
- Madeira, P.T., T.K. Van, and T.D. Center. 2004. An improved molecular tool for distinguishing monoecious and dioecious hydrilla. *J. Aquat. Plant Manage.* 42:28-32
- Maki, K.C. and S.M. Galatowitsch. 2008. Cold tolerance of two biotypes of hydrilla and northern watermilfoil. *J. Aquat. Plant Manage.* 46:42-50.
- McFarland, D.G. and J.W. Barko. 1987. Effects of temperature and sediment type on growth and

- morphology of monoecious and dioecious hydrilla. *J. Freshwater Ecol.* 4(2):245-252.
- McFarland, D.G. and Barko, J.W. 1999. High-temperature effects on growth and propagule formation in hydrilla biotypes. *J. Aquat. Plant Manage.* 37:17-25.
- Michel, A., B. E. Scheffler, R. S. Arias, S. O. Duke, M. D. Netherland and F.E. Dayan. 2004. Somatic mutation-mediated evaluation of herbicide resistance in the invasive plant hydrilla. *Mol. Ecol.* 13:3229-3237.
- Miller, R.W. 1998. The First State's experiences controlling the northern monoecious form of hydrilla. *Aquatics* 10(2):16-23.
- Meadows, S.T. and R.J. Richardson. 2012. Competition of monoecious hydrilla with other submersed macrophytes. Proc. 52<sup>nd</sup> Annual Meeting Aquatic Plant Management Society (APMS), July 22-25, Salt Lake City, UT. Abstract pp.
- Nawrocki, J.J. 2011. Environmental and Physiological Factors Affecting Submersed Aquatic Weed Management (Masters Thesis). North Carolina State University. Available online: <http://repository.lib.ncsu.edu/ir/handle/1840.16/7126>
- Nawrocki, J.J., S.T. Hoyle, J. Scott, and R.J. Richardson. 2011. Proc. 51<sup>st</sup> Annual Meeting Aquatic Plant Management Society (APMS), 24-27 July, Baltimore, MD. Abstract pp. 37-38.
- Netherland, M. D. 1997. Turion Ecology of Hydrilla. *J. Aquat. Plant Manage.* 35: 1-10.
- Owens, C. S., M. J. Grodowitz, R. M. Smart, N. E. Harms, and J. M. Nachtrieb. 2006. Viability of hydrilla fragments exposed to different levels of insect herbivory. *J. Aquat. Plant Manage.* 44:145.
- Owens, C. S., M. J. Grodowitz, and R. M. Smart. 2008. Impact of insect herbivory on the establishment of *Hydrilla verticillata* (L.f) Royle fragments. *J. Aquat. Plant Manage.* 46: 199-202.
- Owens, C. S. & J. D. Madsen, 1998. Phenological studies of carbohydrate allocation in *Hydrilla*. *Journal of Aquatic Plant Management* 36: 40-44.
- Owens, C.S. and R.M. Smart. 2007. Sexual preference and alternative life cycles in hydrilla: monoecious and dioecious. Proc. 47<sup>th</sup> Annual Meeting Aquatic Plant Management Society (APMS), July 15-18, Nashville, TN. Abstract p. 47.
- Pesacreta, George J. 1990. "Pilot Study: Carbohydrate Allocation in *Hydrilla* Biotypes." Miscellaneous Paper A-90-2. US Army Engineer Waterways Experiment Station. Vicksburg. MS.
- Poovey, A.G. and K.D. Getsinger. 2010. Comparative response of monoecious and dioecious hydrilla to endothall. *J. Aquat. Plant Manage.* 48:15-20.
- Poovey, A.G. and S.H. Kay. 1998. The potential of a summer drawdown to manage monoecious hydrilla.

- J. Aquat. Plant Manage. 36:127-130.
- Pieterse, A. H. 1981. *Hydrilla verticillata* - a review. Tropical Agriculture. 7: 9-34.
- Ryan, F.J., C.R. Coley, and S.H. Kay. 1995. Coexistence of monoecious and dioecious hydrilla in Lake Gaston, North Carolina and Virginia. J. Aquat. Plant Manage. 33:8-12.
- Ryan, F. J., J. S. Thullen, and D. L. Holmberg. 1991. Non-genetic origin of isoenzymic variability in subterranean turions of monoecious and dioecious hydrilla. J. Aquat. Plant Manage. 29: 3-6.
- Rybicki, N.B., J.D. Kirshtein, and M.A. Voytek. 2013. Improved insight about the distribution of *Hydrilla*, *Egeria*, and *Elodea* (Hydrocharitacea): application of molecular techniques for identification. J. Aquat. Plant Manage. *In Press*.
- Serafy, J.E., R.M. Harrell, and L.M. Hurley. 1994. Mechanical removal of hydrilla in the Potomac River, Maryland: Local impacts on vegetation and associated fishes. Journal of Freshwater Ecology. 9(2): 135-143.
- Shields, E.C., K.A. Moore, and D.B. Parrish. 2012. Influences of salinity and light availability on abundance and distribution of tidal freshwater and oligohaline submersed aquatic vegetation. Estuaries and Coasts. 35:515-526.
- Spencer, D.F. and L.W.J. Anderson. 1986. Photoperiod responses in monoecious and dioecious hydrilla *verticillata*. Weed Sci. 34(4):551-557.
- Spencer, D.F., L.W.J. Anderson, M.D. Ames, and F.J. Ryan. 1987. Variation in *Hydrilla verticillata* (L.f.) Royle propagule weight. J. Aquat. Plant Manage. 25:11-14.
- Spencer, D., L. Anderson, G. Ksander, S. Klaine, and F. Bailey. 1994. Vegetative propagule production and allocation of carbon and nitrogen by monoecious hydrilla *verticillata* (L.f.) Royle grown at two photoperiods. Aquat. Bot. 48(2):121-132.
- Spencer, D.F. and G.G. Ksander. 1999. Influence of dilute acetic acid treatments on survival of monoecious hydrilla tubers in the Oregon House Canal, California J. Aquat. Plant Manage. 37:67-71.
- Spencer, D.F. and G.G. Ksander. 2000. Interactions between American pondweed and monoecious hydrilla grown in mixtures. J. Aquat. Plant Manage. 38:5-13.
- Spencer, D.F. and G.G. Ksander. 2001. Field evaluation of degree-day based equations for predicting sprouting of hydrilla (*Hydrilla verticillata*) turions and tubers. Journal of Freshwater Ecology. 16:479-486.
- Spencer, D.F., G.G. Ksander, and S.R. Bissell. 1992. Growth of monoecious hydrilla on different soils amended with peat or barley straw. J. Aquat. Plant Manage. 30:9-15.

- Steward, K.K. 1991a. Light requirements for growth of monoecious hydrilla from the Potomac River. Florida Sci. 54(3/4):204-214.
- Steward, K.K. 1991b. Competitive interaction between monoecious hydrilla and American eelgrass on sediments of varying fertility. Florida Sci. 54(3/4):135-147.
- Steward, K.K. 1993. Seed production in monoecious and dioecious populations of hydrilla. Aquatic Botany. 46:169-183.
- Steward, K.K. and T.K. Van. 1987. Comparative studies of monoecious and dioecious hydrilla (*Hydrilla verticillata*) biotypes. Weed Science. 35: 204-210.
- Steward, K.K., T.K. Van, V. Carter, and A.H. Pieterse. 1984. Hydrilla invades Washington, DC and the Potomac. American Journal of Botany. 71(1):162-163
- Sutton, D.L., T.K. Van, and K.M. Portier. 1992. Growth of dioecious and monoecious hydrilla from single tubers. J. Aquat. Plant Manage. 30:15-20.
- Twilley, R.R. and J.W. Barko. 1990. The growth of submersed macrophytes under experimental salinity and light conditions. Estuaries. 13(3):311-321.
- Van, T.K. 1989. Differential responses to photoperiods in monoecious and dioecious *Hydrilla verticillata*. Weed Science. 37:552-556.
- Van, T.K. and K.K. Steward. 1990. Longevity of monoecious hydrilla propagules. J. Aquat. Plant Manage. 28:74-76.
- Van, T.K., Steward, K.K., and Conant, R.D., Jr. 1987. Responses of monoecious and dioecious hydrilla (*Hydrilla verticillata*) to various concentrations and exposures of diquat. Weed Science. 35(2):247-52.
- Van, T.K. and V.V. Vandiver Jr. 1992. Response of monoecious and dioecious hydrilla to bensulfuron methyl. Journal of Aquatic Plant Management. 30:41-44.
- Verkleij, J.A.C, A.H. Pieterse, G.J.T. Horneman, and M. Torenbeek. 1983. A comparative study of the morphology and isoenzyme patterns of *Hydrilla verticillata* (L.f.) Royle. Aquat. Bot. 17:43-59.
- Weakley, A. S. 2012. Flora of the Southern and Mid-Atlantic States. The University of North Carolina Herbarium. Available online: <http://www.herbarium.unc.edu/flora.htm>.
- Wilde, S.B., T.M. Murphy, C.P. Hope, S.K. Habrun, J. Kempton, A. Birrenkott, F. Wiley, W.W. Bowerman, and A.J. Lewitus. 2005. Avian vacuolar myelinopathy linked to exotic aquatic plants and a novel cyanobacterial species. Environ. Toxicol. 20:348–353.
- Williams, S.K., J. Kempton, S.B. Wilde, and A. Lewitus. 2007. A novel epiphytic cyanobacterium associated with reservoirs affected by avian vacuolar myelinopathy. Harmful Algae. 6:343–353.

Yeo, R. R., R. H. Falk and J. R. Thurston. 1984. The morphology of Hydrilla (*Hydrilla verticillata* (L.f.) Royle). *Journal of Aquatic Plant Management* 22: 1–17.

USDA, NRCS. 2013. The PLANTS Database (<http://plants.usda.gov>, 23 December 2012). National Plant Data Team, Greensboro, NC 27401-4901 USA.

Aquatic herbs with leaves attached at multiple stem nodes. Leaves less than 4cm long and opposite or occurring in whorls of 2-8.

1 Leaves opposite or occurring in 4 or fewer per whorl.

2 Leaves slightly narrowed or straight-sided to base, sessile; perianth present...**Elodea**

2 Leaves broadened and sheathing at base, narrowing towards apex via “shoulders”; perianth absent....**Najas**

1 Leaves in whorls of 3 to 8, with 4-5 leaves per whorl common.

3 Leaves 2 to 3 cm long or longer serrulate margins and rarely the midrib beneath; young leaves not noticeably scabrous (not rough to touch); leaf whorls generally spaced regularly on all stems; petals white and 9 to 12 mm long .....**Egeria**

3 Leaves mostly 0.75 to 1.5 cm long, serrate margins and toothed along the midrib beneath; young leaves scabrous; leaf whorls generally crowded at stem terminal, not crowded on mature stems; petals translucent and 2 to 5 mm long...**Hydrilla**

### **Elodea**

1 Most leaves 1.75 mm wide or greater.... **2**

1 Most leaves less than 1.75 mm wide ....**4**

2 Leaves often overlapping with regular rows near stem apex and lying along stem, may appear oblong or ovate. ***E. canadensis***

2 Leaves not usually overlapping near stem apex and may appear irregular and spreading, mostly elliptic to linear or lanceolate....**3**

3 Leaves often present in whorls of 4, lanceolate to elliptic, not usually parallel-sided, apex usually obtuse to widely acute.... ***E. potamogeton***

3 Leaves often present in pairs, no whorls of four; mostly linear and parallel sided with acute apex. ***E. bifoliata***

4 Most leaves folded along midrib, slightly recurved, margins undulate, usually not greater than 10 mm long.... ***E. nuttallii***

4 Most leaves flat, margins straight and spreading, some greater than 10 mm long.... ***E. callitrichoides***

### **Hydrilla**

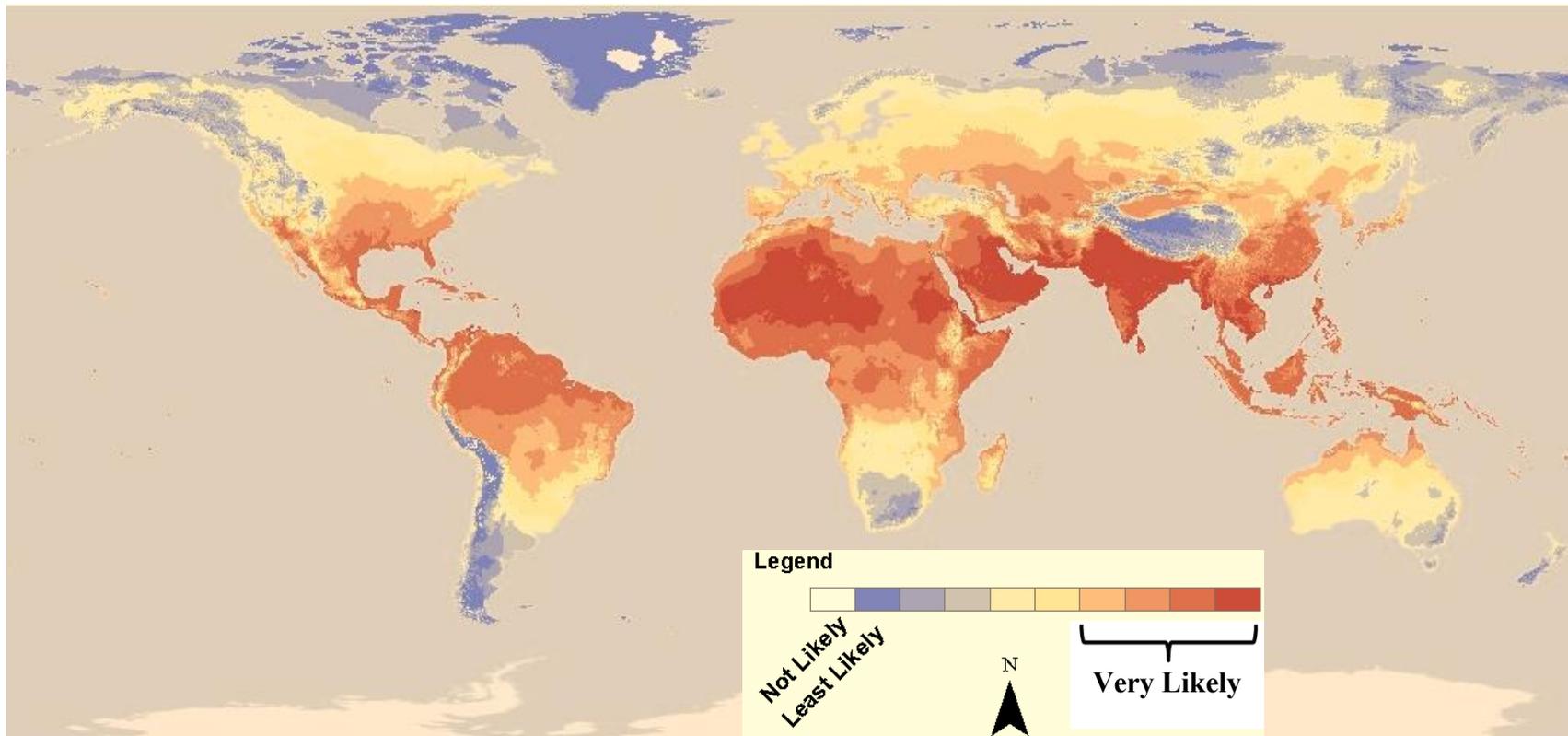
Leaf width 2.25 to 3.5mm; staminate flowers not present...***dioecious***

Leaf width 1.0 to 3.0 mm; staminate flowers sessile, at anthesis deciduous from plant...***monoecious***

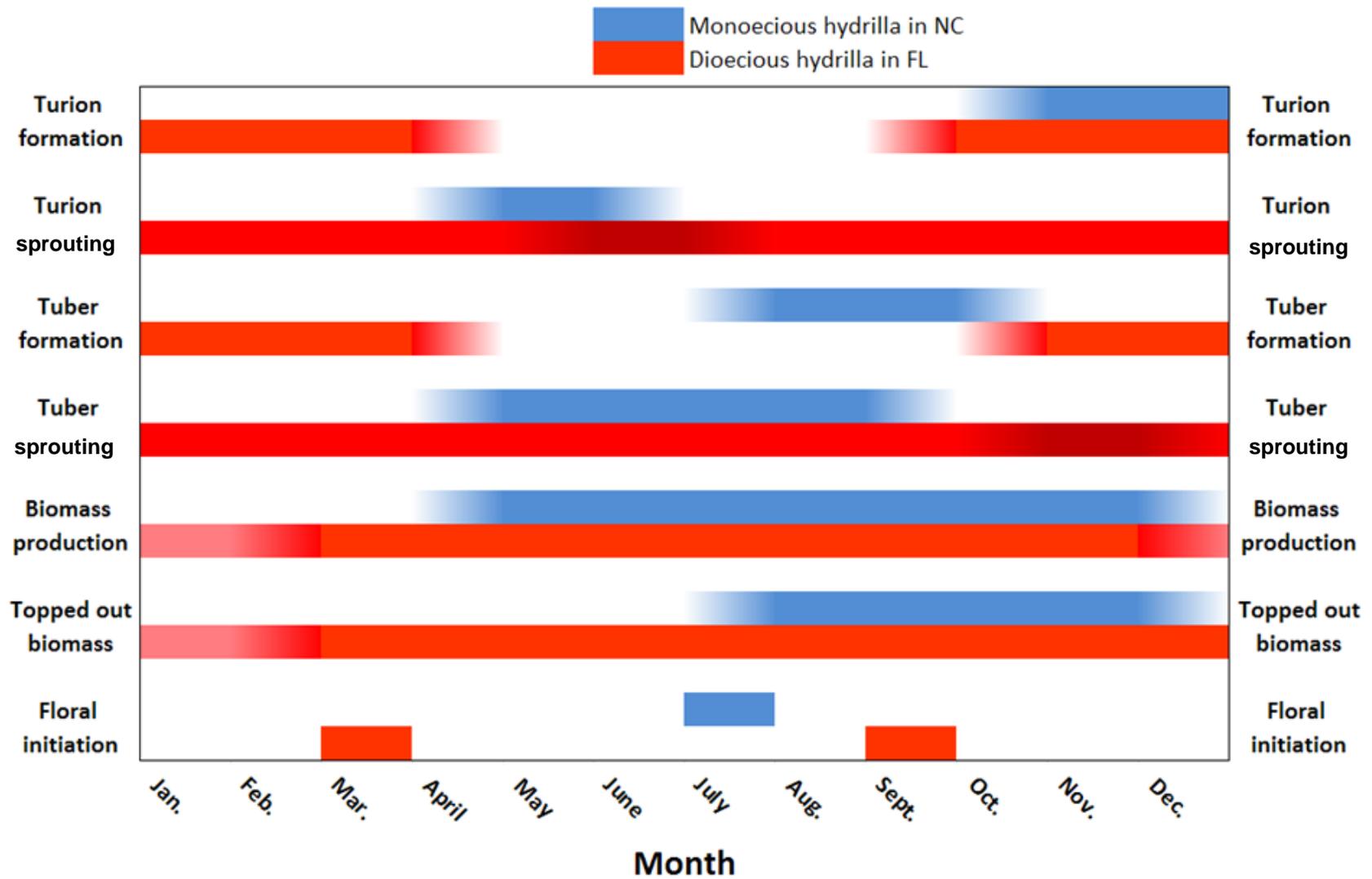
**Figure 1.** Taxonomic key for Hydrocharitaceae genera *Elodea*, *Najas*, *Egeria*, and *Hydrilla*. *Hydrilla* keyed to biotype and *Elodea* keyed to species (Godfrey and Wooten 1979; Weakley 2012).



**Figure 2.** Herbarium specimen of monoecious hydrilla from Umstead Lake, Wake County, North Carolina, dated Dec. 9, 1980.



**Figure 3.** NCSU generated model of likely climates for supporting hydrilla growth June-August. Model based on current reported geographic distribution of hydrilla and average minimum temperatures from 1950-2000).



**Figure 4.** A comparison of monoecious hydrilla phenology in North Carolina to dioecious hydrilla phenology in Florida. Modified from Harlan et al. (1985).

**Table 1.** Reported tuber weight for dioecious and monoecious hydrilla.

Biotype	Tuber weight (mg)	Situation	Citation
Dioecious	63-91	Mesocosm	Sutton et al. (1992)
	160-386	Mesocosm	Spencer et al. (1987)
	42-44	Mesocosm	Van (1989)
	188-290	Field, lake	Miller et al. (1976)
Monoecious	42-53	Mesocosm	Sutton et al. (1992)
	117-202	Mesocosm	Spencer et al. (1987)
	33-34	Mesocosm	Van (1989)
	30-320	Field, lake	Nawrocki (2011)

**Table 2.** Reported tuber densities for dioecious and monoecious hydrilla.

Biotype	Tuber density	Situation	Citation
Dioecious	2,153	Mesocosm, 12 mo.	Steward and Van (1987)
	2,293	Mesocosm, 7 mo.	Steward (1980)
	257	Field, ponds	Haller and Sutton (1975)
	293-605	Field, lake	Miller et al. (1976)
	62-900	Field, ponds	Steward (1980)
Monoecious	2,099-9,053	Mesocosm, 16-28 mo	Steward and Van (1987)
	910-2,985	Mesocosm, 2 mo	Poovey and Kay (1998)
	189-1,312	Field, 3 lakes	Harlan et al. (1985)
	101-1,705*	Field, 2 lakes	Nawrocki (2011)