ABSTRACT

Title of Thesis:	TURION SIZE ADVANTAGE IN THE RESTORATION OF *VALLISNERIA AMERICANA*: THE IMPORTANCE OF GENETIC IDENTITY AND DIVERSTY.
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The restoration and rehabilitation of damaged ecosystems has become a worldwide endeavor utilizing vast resources and ecological knowledge to build functioning and resilient ecosystems. Biodiversity restoration increases the likelihood that present species are well-adapted to the environment or can complement each other in resource use. Genetic diversity in populations may increase establishment rate, resistance to invasion, and resilience in a changing world. In parallel field and greenhouse experiments, I established colonies of the submersed aquatic macrophyte *Vallisneria americana*. Colony survival and performance was affected by environmental conditions in the field and genotypic diversity in the greenhouse. In the presence of nonnative *Hydrilla verticillata*, *V. americana* height was reduced; however, biomass increased, suggesting resource partitioning in response to competition. These results suggest that genotypic identity and diversity are important in early establishment of plant populations and calls attention to designing restorations that incorporate genetic information about source populations.

TURION SIZE ADVANTAGE IN THE RESTORATION OF *VALLISNERIA AMERICANA:* THE IMPROTANCE OF GENETIC IDENTITY AND DIVERSITY

by

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2019

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Acknowledgements

First and foremost, my gratitude goes to my adviser, Katia Engelhardt, for her invaluable advice and feedback, and for being a personal and professional inspiration. I am also grateful to Maile Neel for her input on genetic information, as well as for genotyping my field samples. I am grateful to both Katia and Maile for their long-term work in the genetic diversity of *Vallisneria americana*, which laid the foundation for this study. Thanks to Stuart Findlay for help in selecting field sites, and for providing an indispensable second set of hands during the planting process. David Fischer, Carrie Perkins, and Kristin Ratliff assisted in various field, lab, and greenhouse capacities. The Hudson River Foundation provided funding for the field experiment. The faculty, staff, and students at the University of Maryland Center for Environmental Science Appalachian Laboratory, including Paul Gugger, Andrew Elmore, and Regina Trott, provided technical and personal support.

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Chapter 1: Background and Hypotheses

Introduction

The magnitude and prevalence of anthropogenic damage to natural ecosystems has encouraged the development of restoration ecology as a separate field of scientific inquiry (Cairns and Heckman 1996; Davis and Slobodkin 2004; Hobbs and Norton 1996). Although it is a relatively young field, restoration ecology utilizes principles from population, community, ecosystem, and landscape ecology to facilitate the repair of damaged ecosystems (Palmer, Ambrose, and Poff 1997; Cairns and Heckman 1996; Davis and Slobodkin 2004). The goal of most restoration ecology is returning disturbed or degraded ecosystems to their natural state and functions (or as close to that state as possible, given the persistence of human disturbance); however, these ecosystems still have function despite their damaged state (Palmer, Ambrose, and Poff 1997; Cairns and Heckman 1996; Davis and Slobodkin 2004). Submersed aquatic vegetation (SAV) beds in lakes, rivers, and estuaries are ecosystems at a heightened risk of damage from anthropogenic and natural stressors, motivating the study of understanding their resilience and approaches to facilitating their recovery.

SAV has many important ecological functions. These include the sequestration of nutrients from the water column, provision of habitat and food for animal species, wave attenuation, and sediment anchoring (Biernacki and Lovett-Doust 1997; Chadwell and Engelhardt 2008; Owens *et al.* 2008; McFarland and

Shafer 2008). Species native to the eastern United States, such as *Vallisneria americana,* are credited with cleaning and maintaining water quality in freshwater and oligohaline systems (Biernacki and Lovett-Doust 1997; Owens *et al.* 2008; McFarland and Shafer 2008). The introduction of nonindigenous species, such as *Hydrilla verticillata,* can displace desirable native species and negatively impact water flow, although the invader can fill a similar ecological niche to the species it displaced (Langeland 1996; True-Meadows *et al.* 2016).

Human activity is primarily responsible for widespread declines of SAV populations over the last century (Moore, Shields, and Jarvis 2010; Boustany 2003; Cho and May 2006; McFarland and Shafer 2008; Rybicki and Landwehr 2007). Elevated watershed inputs of nutrients and sediments, caused by urbanization and agriculture practices, increase freshwater and estuarine turbidity, thereby decreasing light availability for SAV and greatly impeding growth (Moore, Shields, and Jarivs 2010; Boustany 2003; Cho and May 2006; Batiuk *et al.* 2000; Kemp *et al.* 2004; Carter *et al.* 1994). SAV-dominated ecosystems are more vulnerable to natural disturbances when they are already stressed from eutrophication. For example, in 2011, precipitation from Hurricane Irene disturbed and degraded communities of SAV growing in the Hudson River Estuary (Hamberg *et al.* 2017).

Restoration efforts focus on the transplantation of young plants to sites deemed favorable based on their history and environmental characteristics (Moore, Shields, and Jarvis 2010; Boustany 2003; Cho and May 2006). However, these

restoration projects have seen limited success because seedlings are particularly vulnerable to low light and high-energy hydrology (Moore, Shields, and Jarvis 2010; Boustany 2003; Cho and May 2006). Alternative transplanting techniques—such as planting seeds, or allowing plants to establish in mats prior to placement at the restoration site—have been tested with varying success, again largely dependent on the quality of the planting site (Moore, Shields, and Jarvis 2010; Boustany 2003). Therefore, careful site selection is the most important part of any SAV restoration plan: light and substrate must be adequate for growth, and current velocity and wave action must be relatively low to allow seedlings to establish (Moore, Shields, and Jarvis 2010; Cho and May 2006).

Here I argue that genetic identity and diversity can also play a role in restoring ecosystems because environmental conditions vary in time and space. Planting different genotypes, which have different responses to the environment, can increase the chance that a restored population establishes effectively and is resilient to change. Ecological processes that maintain biodiversity have been a focus of scientific study since the inception of ecology as a scientific discipline, so there is much to learn from the community ecology literature. Entire books have been written on the importance of biodiversity (*i.e.* Kinzig *et al.* 2001; Naeem *et al.* 2009) with the general conclusion that when functional diversity is high in a community (that is, when organisms differ in their use of the environment), more species can be supported by the ecosystem. By extension, when communities are functionally more diverse, they may use resources more efficiently and are therefore more likely to support higher

productivity. This concept is commonly referred to in the community ecology literature as a niche differentiation effect (Kylafis and Loreau 2011), resulting in "complementarity" (Loreau and Hector 2001) in resource use, which has consequences in ecosystem functioning and resilience. Likewise, genotypes may differ in their phenotypic expression and use of resources (Kawecki and Ebert 2004; Bischoff *et al.* 2009; Engelhardt *et al.* 2014; Evans *et al.* 2017), so that populations with higher genotypic richness are more likely to support higher productivity (Evans *et al.* 2017). My experiments focus on this idea, using the submersed aquatic macrophyte *Vallisneria americana* as a study species.

Vallisneria americana

One common SAV species in freshwater and oligohaline estuaries is *Vallisneria americana*, a species that is widely used in estuarine restoration efforts in the eastern United States because of its ease of propagation and high tolerance to low light levels (Moore, Shields, and Jarvis 2010). *V. americana* is a perennial dioecious macrophyte with long, tape-like leaves (up to 2 m) and a deep root system native to the eastern seaboard of the United States and inland to South Dakota and parts of Canada (McFarland and Shafer 2008; Owens *et al.* 2008; Wigand *et al.* 2001). Population declines have been reported in the United States since the 1960s, and restoration work is in progress (McFarland and Shafer 2008; Moore, Shields, and Jarvis 2010; Boustany 2003; Cho and May 2006).

V. americana utilizes both sexual and vegetative reproduction (McFarland and Shafer 2008; Owens *et al.* 2008; Biernacki and Lovett-Doust 1997). The female (pistillate) flower lies on the surface of water at the end of a long stem; pollination occurs after male (staminate) flowers are released from a capsule at the base of the male plant (McFarland and Shafer 2008). Sexual reproduction in SAV species is difficult because the process is easily interrupted at any stage by hydrological disturbance (McFarland and Shafer 2008). Therefore, the primary form of reproduction in *V. americana* is horizontal clonal spread through stolons (McFarland and Shafer 2008; Owens *et al.* 2008; Biernacki and Lovett-Doust 1997). An individual plant can have between twenty and forty shoots, called ramets (McFarland and Shafer 2008; Biernacki and Lovett-Doust 1997). In addition to horizontal spread, *V. americana* produces vegetative propagules, known as turions, which allow the species to senesce in the soil over the winter (Owens *et al.* 2008). Because the turions reside in the soil, it is the sexually produced seeds that are primarily responsible for long-range dispersal of the species (McFarland and Shafer 2008).

Because of their dominant clonal growth habit, it might be expected that SAV species have low genetic diversity; however, it has been shown that many species do, in fact, have high genetic diversity (Evans *et al.* 2017; Lloyd *et al.* 2011). Because SAV species utilize both sexual and asexual (clonal) reproductive habits, high genetic diversity is maintained within and among populations (Evans *et al.* 2017). Previous studies have shown that *V. americana* populations can be high in genetic diversity (Lloyd *et al.* 2011; Lloyd *et al.* 2012), and that genotypes respond differently to

environmental conditions (Engelhardt *et al.* 2014; Evans *et al.* 2017). I sought to use this prior knowledge in a restoration context to predict that populations that support more genotypes have a greater chance of survival and clonal reproduction.

Hydrilla verticillata

Competing with *V. americana* in many aquatic systems in the southeastern United States is *Hydrilla verticillata*, another aquatic macrophyte with similar growth characteristics. *H. verticillata* is invasive and thought to originate in southeast Asia (Owens *et al.* 2008; Langeland 1996). *H. verticillata* was first found in the United States in 1960 in the state of Florida (Steward and Van 1987; Langeland 1996) and appeared in the Potomac River in Virginia in the 1980s (Steward *et al.* 1984; True-Meadows *et al.* 2016; Rybicki and Landwehr 2007; Rybicki and Carter 2002). Since then, it has continued to spread through the United States, and analysis of its worldwide range suggests that it could reach southern Canada (Langeland 1996; True-Meadows *et al.* 2016). The presence of *H. verticillata* in water bodies often causes severe problems. The species' thick growth disrupts water flow, and it displaces native plant species, thereby shifting an ecosystem toward monoculture and altering the entire ecosystem structure (Langeland 1996; True-Meadows *et al.* 2016). These effects can negatively impact recreational use of water bodies as well as their natural ecological functioning (True-Meadows *et al.* 2016).

In addition to vegetative propagules, *Hydrilla verticillata* spreads and disperses via fragmentation; the fragments are viable vegetative offspring and can

float long distances before establishing (Baniszewski *et al.* 2016; True-Meadows *et al.* 2016; Steward and Van 1987; Langeland 1996; Chadwell and Engelhardt 2008; Owens *et al.* 2008). The mobility and resilience of these fragments make them effective and aggressive perpetrators of *H. verticillata* colonization (Baniszewksi *et al.* 2016; True-Meadows *et al.* 2016). The species is known for its rapid stem elongation and canopy-forming habit, which allow it to out-compete other species for available light (Langeland 1996; Steward and Van 1987; True-Meadows *et al.* 2016). *H. verticillata* has shown a higher tolerance for low light, oligotrophic, and eutrophic conditions than other species of SAV, and its turions have been known to persist in the sediment for several years before sprouting (Steward and Van 1987; Langeland 1996; True-Meadows *et al.* 2016).

Because of its growth habits and ecological tolerance, *Hydrilla* is an excellent invader and has been classified as "the perfect aquatic weed" (Langeland 1996). The species is difficult to manage because of its prolific turion production, easy fragmentation, and fragment spread and persistence (True-Meadows *et al.* 2016; Owens *et al.* 2008). However, genetic diversity in a competitor, such as *V. americana*, may limit *H. verticillata*'s success because genetic diversity might allow *V. americana* to resist invasion through higher resource utilization and increased productivity (Langeland 1996). Thus, I predicted that *V. americana* populations with higher diversity had a lower chance of being invaded by *H. verticillata* during establishment.

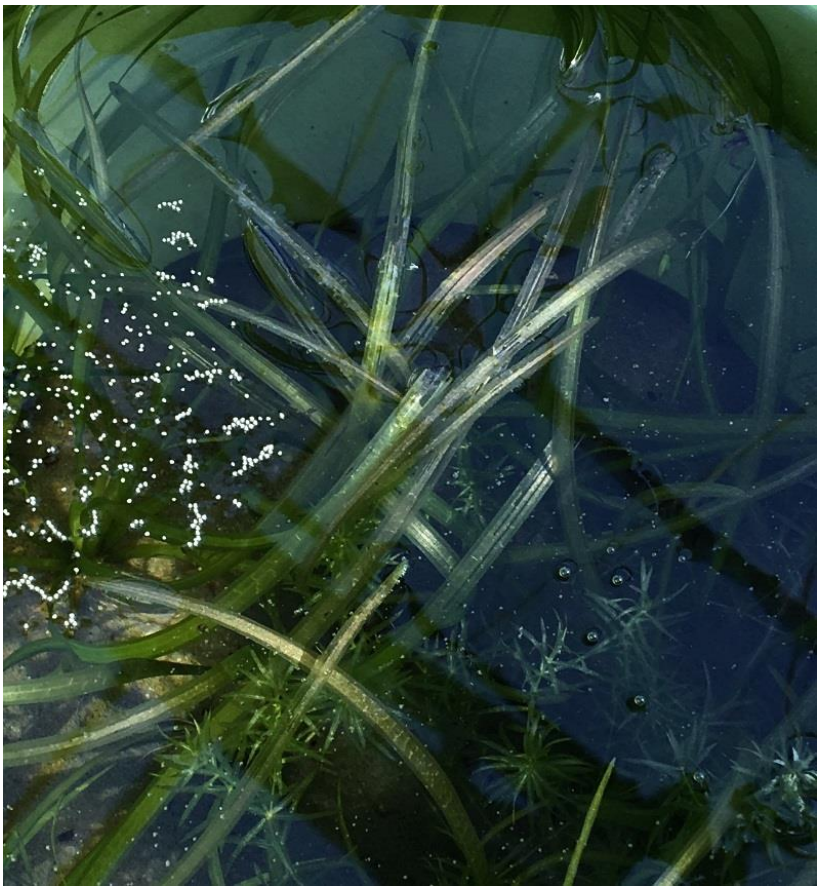


Figure 1.1. *V. americana* and *H. verticillata* growing together in the greenhouse at the University of Maryland Center for Environmental Science Appalachian Laboratory. Photo credit: A. Carew.

Abiotic drivers of SAV growth

Submersed aquatic macrophytes are subject to a variety of limiting conditions, both biotic and abiotic, such as salinity (Freedman and Lacoul 2006; Shields *et al.* 2012), light availability (Batiuk *et al.* 2000; Kemp *et al.* 2004; Freedman and Lacoul 2006; Moore and Wetzel 2000; Moore, Wetzel, and Orth 1997; Carter *et al.* 1994), hydrology (Koch 2001; Freedman and Lacoul 2006), and invasive species (Santos *et al.* 2011; Shea and Chesson 2002; Chadwell and Engelhardt 2008; Simberloff *et al.* 2013; Rybicki and Carter 2002; Rybicki and Landwehr 2007). The independent and combined influences of these factors can restrict the productivity and spread of submersed aquatic vegetation, which reduces its ability to provide necessary ecosystem services (Batiuk *et al.* 2000).

Species of submersed aquatic vegetation have an upper salinity tolerance (Freedman and Lacoul 2006; Shields *et al.* 2012). Exceptionally high salinity levels have been associated with declines in aquatic vegetation (Shields *et al.* 2012). Different species have differing levels of salinity tolerance; *Vallisneria americana* is a species that is less tolerant of high salinity (Freedman and Lacoul 2006), and *H. vertillata* is even less tolerant (Shields *et al.* 2012). The duration and intensity of salinity changes determines the magnitude of the effect on aquatic species (Freedman and Lacoul 2006; Shields *et al.* 2012).

Light availability is perhaps the best-studied of the environmental traits that drive SAV survival and performance (Batiuk *et al.* 2000; Kemp *et al.* 2004; Freedman and Lacoul 2006; Moore and Wetzel 2000; Moore, Wetzel, and Orth 1997; Madsen *et al.* 2001). Light availability is controlled by many interacting variables, both biotic and abiotic. The biotic, chemical, and physical composition of the water column—suspended sediments, phytoplankton, and dissolved nutrients and organic matter—influence light attenuation through the water column and thereby the depth at which plants can still obtain sufficient light (Moore and Wetzel 2000; Batiuk *et al.* 2000). The amount of light reaching the leaves of plants is further impacted by the presence of epiphytes (algae and bacteria) that grow on leaf surface (Moore and Wetzel 2000; Batiuk *et al.* 2000; Kemp *et al.* 2004). Eutrophication promotes this growth (Moore and Wetzel 2000). Periods of increased turbidity, caused by storm events, can further impede seedling growth or kill full-grown plants, depending on the duration and timing of events (Moore, Wetzel, and Orth 1997; Madsen *et al.* 2001). Increased nutrient and sediment loading in major estuaries therefore has contributed to the decline of aquatic vegetation (Batiuk *et al.* 2000; Madsen *et al.* 2001; Moore, Shields, and Jarvis 2010).

Hydrology can also influence aquatic vegetation growth (Koch 2001; Freedman and Lacoul 2006; Madsen *et al.* 2001). Excessive wave action or current velocity can uproot aquatic plants; seedlings and young plants are particularly vulnerable (Koch 2001; Madsen *et al.* 2001). Hydrology can also impact nutrient availability. Too much water flow can remove desirable sediments and nutrients

before they can be taken up by plants (Madsen *et al.* 2001). Low flow velocity leads to fewer dissolved nutrients in the water column because the long residence time allows for complete nutrient uptake by the macrophyte community (Koch 2001; Madsen *et al.* 2001). Wave action has similar effects to water current velocity: too much wave action scours a site of nutrients and sediments and can uproot existing plants, which magnifies the eroding effects of water movement (Koch 2001; Freedman and Lacoul 2006; Madsen *et al.* 2001). SAV communities reduce flow velocity but increase sedimentation rates, which in turn increases light availability and makes the site more suitable for SAV. However, in the absence of SAV, flow velocity is higher and sedimentation rates are lower, and SAV establishment and growth is inhibited in a habitat that is light limited (Madsen *et al.* 2001).

Effects of invasive species on SAV growth

The invasion of biotic communities by nonnative species has long been a topic of study and concern (Levine *et al.* 2004; Kennedy *et al.* 2002; Hooper *et al.* 2005). The concept of biotic resistance, introduced by Charles Elton (1958), suggests that communities with greater species richness tend to resist invasion because the existing plant community uses resources more fully (Kennedy *et al.* 2002; Levine *et al.* 2004), leaving less for potential invaders to exploit (Hooper *et al.* 2005). Genetic diversity may have the same effect if populations that support a greater number of functionally different genotypes are more productive and use up space and resources more fully. My thesis focuses on this idea, using the invasive submersed aquatic macrophyte *H. verticillata* as the species that invades native *V. americana* beds.

A species originating from a different geographic location than its current location can be classified as non-native (Santos *et al.* 2011). To be characterized as "invasive," a non-native species must exhibit harmful impacts on the structure and function of the ecosystem it occupies (Simberloff *et al.* 2013). Invasive species often out-compete native species through efficient resource use, fast growth, high fecundity, or resistance to local predators (Santos *et al.* 2011; Shea and Chesson 2002). An ecosystem can become susceptible to invasion if it has abundant resources, or if those resources are not adequately used by present species (Shea and Chesson 2002; Chadwell and Engelhardt 2008). Conversely, the preemption of habitat resources by native species can reduce the likelihood and severity of invasions (Owens *et al.* 2008).

Effects of Genetic Diversity on SAV growth

The level of species diversity in an ecosystem affects the function of that ecosystem. The functional characteristics and abundance of species dictate ecosystem properties, as do interactions between species (Hooper *et al.* 2005). A great deal of research illustrates the importance of species-level diversity on ecosystem structure and function (Hooper *et al.* 2005), but somewhat less research has been done on the effects of intraspecific (*i.e.*, genetic) diversity. The effects and importance of genetic diversity within a population are similar to the effects and importance of species diversity within a community (Hughes *et al.* 2008; Evans *et al.* 2017). A population's

productivity is increased by genetic diversity, as is its ability to resist and recover from disturbance (Hughes *et al.* 2008; Evans *et al.* 2017).

A higher amount of genetic diversity, often measured by the number of extant genotypes, increases population productivity (Ellers *et al.* 2011; Vellend *et al.* 2010; Crutsinger *et al.* 2006; Bischoff *et al.* 2009; Kettenring *et al.* 2014; Hughes and Stachowicz 2011; Kotowska *et al.* 2010; Evans *et al.* 2017). This increase in productivity is brought about by positive intraspecific interactions, such as resource partitioning, rather than competition or exclusion (Hughes and Stachowicz 2011; Evans *et al.* 2017). Likewise, the presence of greater genetic diversity in a population increases ecosystem functioning, such as the provision of food and habitat for animal species (Reusch *et al.* 2005; Evans *et al.* 2017; Kettenring *et al.* 2014; Reynolds *et al.* 2012). These effects are enhanced by the degree of difference between genotypes (Ellers *et al.* 2011; Bischoff *et al.* 2009) and local environmental conditions that genotypes respond to (Engelhardt *et al.* 2014; Ellers *et al.* 2011; Kawecki and Ebert 2004; Bischoff *et al.* 2009; Evans *et al.* 2017).

Studies conducted specifically on SAV have shown that genotypic diversity in SAV enhances productivity and resilience (Evans *et al.* 2017; Hughes and Stachowicz 2004, 2011). For example, Evans *et al.* (2017) tested the effects of shading on populations of the seagrass *Posidonia australis* that varied in their genetic diversity and found that populations with low diversity were particularly vulnerable to shading effects. Hughes and Stachowicz (2004, 2011) tested the effects of genetic diversity in

Zostera marina (eelgrass) on the species' ability to recover from disturbance. They found that *Z. marina* populations with higher genetic diversity recovered more fully from disturbances, in the form of grazing by geese and clipping intended to mimic this natural process. Although recovery was not necessarily accelerated by genetic diversity, populations with higher diversity showed higher biomass and shoot density by the end of the one- and two-year experiments (Hughes and Stachowicz 2004, 2011).

The effects of genetic diversity have implications in restoration. Some research suggests that increased biodiversity (at the community level as well as the population level) increases ecosystem resistance to disturbance as well as ability to recover, or resilience. The line between these two terms can become blurry, but the clear implication is that increased genetic diversity facilitates rapid, effective ecosystem recovery of both structure and function (Reusch *et al.* 2005; Hughes and Stachowicz 2004; Bischoff *et al.* 2009; Kettenring *et al.* 2014; Hughes and Stachowicz 2011; Reynolds *et al.* 2012; Evans *et al.* 2017).

Study goals and hypotheses

This study used several genotypes of *V. americana* from the Hudson River to test the biological hypothesis that genetic identity and diversity influences establishment and performance. I tested this in a field experiment by planting turions from a variety of genotypes at different field sites. Each planting, or "founder colony," consisted of eight turions in a cotton mesh bag which was weighed down

with gravel and anchored in the sediment with stakes. Furthermore, I hypothesized that increased genotypic diversity in *V. americana* founder colonies would increase the colonies' resistance to invasion. This was tested in a greenhouse experiment by planting *H. verticillata* in the same space as *V. americana* founder colonies.

Additionally, the selection of three field planting sites in the Hudson River Estuary, combined with a parallel common garden experiment in the greenhouse, tested the importance of site selection in SAV restoration projects. My planting technique anchored small colonies of clonally produced turions in the riverbed, which is different than previous techniques involving seedlings or seeds (Moore, Shields, and Jarvis 2010). The results of this study will inform ecological theory as well as SAV restoration goals and strategies.

Chapter 2: Turion size advantage in the restoration of *Vallisneria americana:* the importance of genetic identity and diversity

ABSTRACT

The restoration and rehabilitation of damaged ecosystems has become a worldwide endeavor that utilizes vast resources and ecological knowledge to build functioning and resilient ecosystems. Biodiversity restoration, a critical step in this process, increases the likelihood that present species are well-adapted to the environment or can complement each other in resource use through resource partitioning. At the population level, genetic diversity may increase establishment rate, increase resistance to invasion, and enhance resilience in a changing world. In parallel field and greenhouse experiments, I established founder colonies of the submersed aquatic macrophyte *Vallisneria americana* to test the effects of genotypic identity and diversity on colony establishment and invasibility. Environmental conditions in the field affected survival and performance of colonies. Turion size differed among genotypes and source populations and, combined with genetic diversity in the greenhouse, affected plant height, suggesting that the genetics of founder colonies influence plant establishment. This size advantage may have longer-term consequences if effective establishment influences the success of future generations. In the presence of the invader *Hydrilla verticillata*, *V. americana* height

was substantially reduced; however, biomass increased owing to a higher root-to-shoot ratio, suggesting resource partitioning in response to competition. These results suggest that genotypic identity and diversity are important in early establishment of plant populations and calls attention to designing restorations that incorporate genetic information about source populations.

INTRODUCTION

Restoration ecology is becoming increasingly prominent as a scientific field as expanding human populations put pressure on natural systems. Aquatic systems near coasts are especially imperiled and in need of conservation and restoration owing to eutrophication, climate change, and nonnative species invasions (Sala *et al.* 2000). Thus, understanding factors that contribute to the effective restoration of aquatic ecosystem functions are crucially needed. Here, I focus on the restoration of submersed aquatic macrophytes in tidal rivers, which affect ecosystem functioning locally, and provide important functions and services to downstream reaches.

Submersed aquatic vegetation (SAV) attenuates waves, captures sediments, immobilzes nutrients, and provides food web support (Biernacki and Lovett-Doust 1997; Benson *et al.* 2008; Rybicki and Landwehr 2007). Although SAV improves water quality (Batiuk *et al.* 2000; Biernacki and Lovett-Doust 1997), plant survival and productivity are lessened by excess nutrients and sediments in the water column, which reduce light availability (Moore *et al.* 2010; Batiuk *et al.* 2000; Rybicki and

Landwehr 2007; Carter *et al.* 1994). In the twentieth century, increased nutrient loading in many aquatic systems led to widespread declines in SAV (Batiuk *et al.* 2000). Recovery of SAV is impeded by large storm events that flood rivers with nutrients and sediment (Hamberg *et al.* 2017) as well as by continued chronic light limitation (Lefcheck *et al.* 2018). Furthermore, invasive species of SAV, such as *Hydrilla verticillata*, often out-compete native species by preempting available nutrients or blocking available light (Chadwell and Engelhardt 2008; Shea and Chesson 2002; Van *et al.* 1998). Given these environmental stresses and the negative consequences that SAV loss has on ecosystems, understanding processes that allow SAV-dominated systems to function as naturally as possible given continuing human disturbance is an important scientific inquiry with applications in restoration ecology and wetland management.

One prominent hypothesis in the quest for understanding processes underlying functioning and resilient ecosystems is that diversity at population and community levels plays a role in resource use efficiency, productivity, and response to and recovery from disturbances (Kylafis and Loreau 2011, Loreau and Hector 2001). At the community level, higher species richness can result in resource partitioning or increases the likelihood that a high-performing species is present (Crustinger *et al.* 2006; Tilman *et al.* 2001). Likewise, within species, genotypes can differ in their phenotypic expression and resource use (Kawecki and Ebert 2004; Bischoff *et al.* 2009; Engelhardt *et al.* 2014) such that populations with higher genotypic richness perform at a higher level in the absence or presence of disturbance (Hughes *et al.*

2008). The effects of increased diversity, at both the community and species level, have implications in restoration: higher biodiversity facilitates ecosystem recovery following a disturbance (Hughes and Stachowicz 2011; Kettenring *et al.* 2014) and faster establishment of populations if active intervention is necessary. My experiments focused on this idea, using the submersed aquatic macrophyte *Vallisneria americana* as my study species.

Vallisneria americana is a common SAV species in freshwater and oligohaline estuaries. *V. americana* is a perennial dioecious macrophyte with long, tape-like leaves and a deep root system compared to other SAV species (Wigand *et al.* 2001). It is widely used in estuarine restoration efforts because of its ease of propagation and high tolerance to low light levels (McFarland and Shafer 2008; Biernacki and Lovett-Doust 1997). In addition to sexual reproduction, *V. americana* can also expand clonally through the production of horizontal stolons, and turions that are produced when plants senesce for the winter (Biernacki and Lovett-Doust 1997; McFarland and Shafer 2008). Previous studies have shown that *V. americana* populations are highly variable in genetic diversity (Lloyd *et al.* 2011; Lloyd *et al.* 2012), and that genotypes respond differently to environmental conditions (Engelhardt *et al.* 2014).

Existing in the same habitat and therefore in direct competition with *V. americana* throughout eastern North America is the invasive aquatic weed *Hydrilla verticillata* (Langeland 1996; True-Meadows *et al.* 2016; Rybicki and Landwehr

2007; Rybicki and Carter 2002; Owens *et al.* 2008). The species is known for its rapid and aggressive growth, by which it out-competes other species for available light (Langeland 1996; Van *et al.* 1998). In addition to spreading via horizontal stolons and overwintering turions, *H. verticillata* disperses via fragmentation; pieces of stem that break off the main plant can root elsewhere after drifting (Owens *et al.* 2008). Furthermore, *H. verticillata* is tolerant of poor light conditions and eutrophication (Langeland 1996; True-Meadows *et al.* 2016). The similarity between the growth habits of *H. verticillata* and *V. americana*, as well as their overlapping North American ranges and their tendency to coexist in certain environments (Rybicki and Carter 2002), make these species useful to study the effects of native genotypic diversity on the invasibility of restored ecosystems.

I examined the effects of *Vallisneria americana* genotypic identity and diversity on submersed aquatic plant bed establishment and invasibility. I tested the general biological hypothesis that genetic diversity increases the chance that some genotypes are better adapted to local conditions and are therefore more productive, leading to faster establishment. If this hypothesis is true, survival and growth of colonies is higher and invasibility is lower, two important factors in the restoration of native plant populations. I planted *V. americana* genotypes in monocultures and in genotypic combinations using field and greenhouse experiments, and observed their performance relative to each other and relative to a competitor species, *H. verticillata.* I expected that *V. americana* genotypes and genotypic diversity levels would differ in survival and overall plant growth. Alternatively, I expected that field sites differing in

environmental conditions would drive colony survival, growth, and biomass production. In the greenhouse experiment, I expected that increased genotypic diversity would enhance establishment and increase colony resistance to invasion, decreasing the growth success of *Hydrilla verticillata* planted in the same space.

METHODS

For the study, I chose genotypes from a repository 187 *Vallisneria americana* genotypes that were sampled from three salinity reaches within the Hudson River—non-tidal freshwater (0 salinity; 14 genotypes), tidal freshwater (0 – 0.5 salinity; 71 genotypes), upper oligohaline (0.6 – 2.5 salinity; 55 genotypes), and lower oligohaline (2.5 – 5 salinity; 47 genotypes)—and cloned since 2015 (132 genotypes) in the University of Maryland Center for Environmental Science Appalachian Lab greenhouse and since 2011 (55 genotypes) in the University of Maryland College Park greenhouse. Some of the 2011 genotypes originated from populations that, as of Summer 2016, had not recovered from the 2011 storms that affected the Hudson River. Specific selection criteria for each experiment are described below.

Field experiment

Three field sites were selected in the tidal Hudson River Estuary (Figure 2.1) for experimental plantings in 2017. Sites were ca. 75 km apart to test hypotheses under different environmental conditions along the river gradient. Publicly available data from the Hudson River Environmental Conditions Observing System (HRECOS) showed that temperature and pH were relatively similar across planting sites, but

dissolved oxygen, turbidity, and salinity varied more widely (Table 2.1). As expected, salinity was highest at the most downstream location (Iona Island; Figure 2.1) and corresponds to an upper oligohaline salinity regime with occasional periods of higher salinity (lower oligohaline). Esopus Meadows (upper oligohaline) was less saline and equivalent to salinity at the farthest upstream site, Stuyvesant (tidal freshwater). Dissolved oxygen was lowest at Iona Island and highest at Esopus Meadows, whereas turbidity was the opposite (Table 2.1). All sites historically supported *V. americana* beds, but minimal growth had been documented since the 2011 storm season.

Twenty-four genotypes were selected for use in the experiment. Eight genotypes were planted at each site, consisting of 2 genotypes from each of 4 collection sites (referred to as populations). From among genotypes with sufficient numbers of turions for experimentation, populations used in the field experiment were selected by geographic proximity and genetic similarity (Neel *pers. comm.)* to the planting sites (Figure 2.1). This proximity and genetic similarity minimizes risks of potentially contaminating the natural genetic structure observed in the Hudson River.

Each of the three planting sites received 42 founder colonies (experimental units) that were planted with eight turions each (Figure 2.2). Prior to planting, the turions were measured individually (length) and collectively (total colony weight). The founder colonies were each placed in a cotton mesh bag with gravel (to weigh the bags down) and tied together at 1 m intervals on a 6 m string for efficient deployment in the field. This process created 6 transects of 7 founder colonies each (Figure 2.2,

2.3a). Twelve of the colonies were enclosed by small, cone-shaped cages of plastic mesh (Figure 2.2, 2.3b) to exclude herbivores and the rest remained as controls. Each genotype was planted in monoculture three times (8 genotypes x 3 replicates = 24 colonies), with 4 genotypes being planted in another set of 3 replicates for the herbivory exclosure (4 genotypes x 3 replicates = 12 colonies). All monocultures were planted with 8 turions of the target genotype. I planted 6 replicate polycultures containing one turion from each of the 8 genotypes (Figure 2.2). In all, 42 founder colonies were planted per site. Location of monocultures and polycultures were randomized along transects; herbivory exclosures were planted only at transect ends, so that they could be anchored in the riverbed with PVC pipes (Figure 2.3a).

In the field, the transect strings were spaced approximately 2 m apart and placed parallel to each other. The first, fourth, and seventh founder colonies on each transect were marked with a PVC pipe anchored in the riverbed; all other colonies were marked with pin flags (Figure 2.3a). All founder colonies were planted over the span of three days in mid-June 2017.

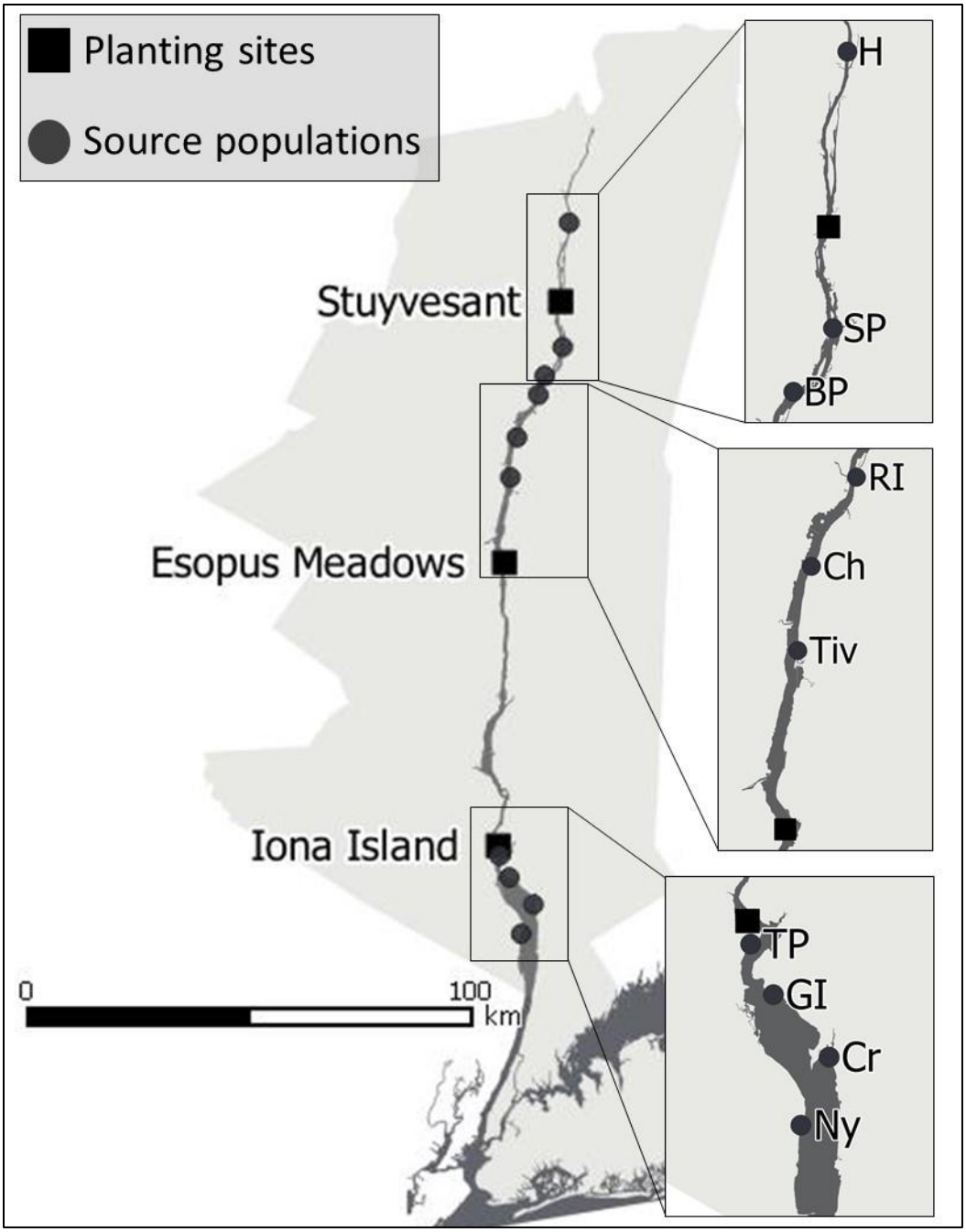


Figure 2.1. Locations of the three field study sites and genotype source populations in the Hudson River Estuary. Only the tidal portion of the river is pictured, which terminates at the Hudson River Lock & Dam in Troy, New York. Selection of source populations for each field site was based on genetic similarity, turion availability, and geographic proximity to the sites. The source population names, from north to south, are: Nutten Hooke (H), Stockport (SP), Brandow Point (BP), Roger's Island (RI), Cheviot (Ch), Tivoli (Tiv), Turning Point (TP), Georges Island (GI), Croton (Cr), and Nyack (Ny).

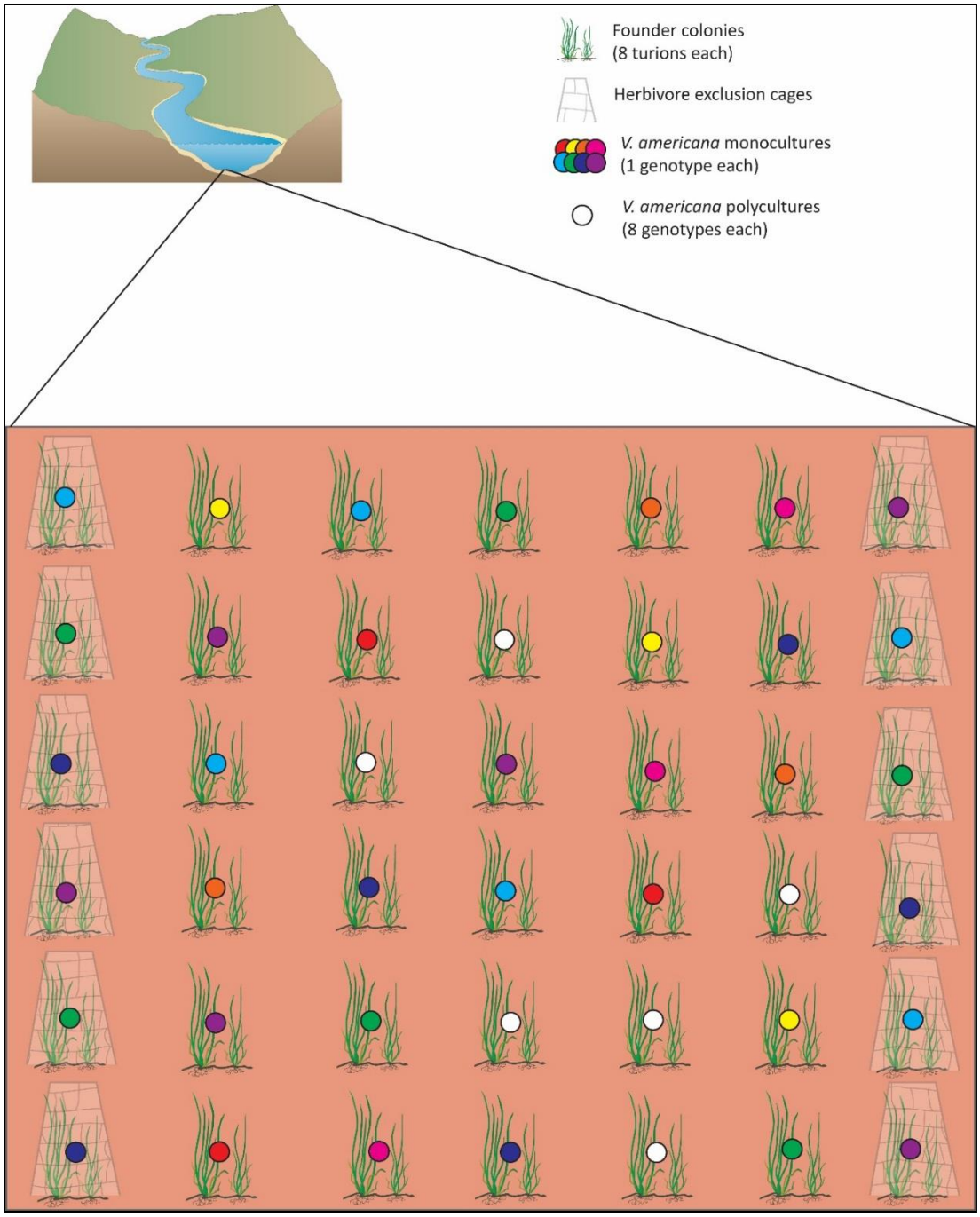


Figure 2.2. Representation of a single field site. Eight genotypes were planted at each site. Each founder colony received 8 turions from just one genotype or a mixture of all genotypes. Six transects (rows) were planted with seven founder colonies each for a total of 42 founder colonies and 336 turions at each of three field sites. Placement of genotypes within and among transects was randomized. Icons courtesy of the Integration and Application Network (IAN).

Table 2.1. Summary of environmental variables at the three field study sites. Minimum and maximum values for each variable are in parentheses. Data are from the Hudson River Environmental Conditions Observing System (HRECOS), collected at 15 minute intervals for the dates indicated. Data from the time frame of the experiment (summer 2017) are not available.

*calculated using this formula: specific conductance (mS/cm)$^{1.0878}$ * 0.4665

Planting site	**HRECOS station (dist. to site)**	**Available dates**	**DO (% air saturation)**	**pH**	**Turbidity (NTU)**	**Water temp. (°C)**	**Salinity (ppt)***
Stuyvesant	Shodack Is. (~11.3 km)	6/1/16 – 8/31/16	83.78 (60.3 – 115.5)	7.72 (7.4 – 8.3)	7.31 (0 – 97.3)	25.15 (19.5 – 28.3)	0.11 (0.1 – 0.13)
Esopus Meadows	Norrie Pt. (~5.3 km)	6/1/16 – 8/31/16	87.71 (60 – 105.7)	7.64 (7.3 – 8.0)	5.56 (0 – 167)	25.45 (20.8 – 28.5)	0.11 (0.1 – 0.13)
Iona Island	West Point (~9.4 km)	7/14/16 – 8/31/16	80.88 (74.4 – 92.9)	7.62 (7.5 – 7.8)	21.99 (7.7 – 110.3)	27.20 (25.2 – 28.8)	1.57 (0.57 – 3.36)

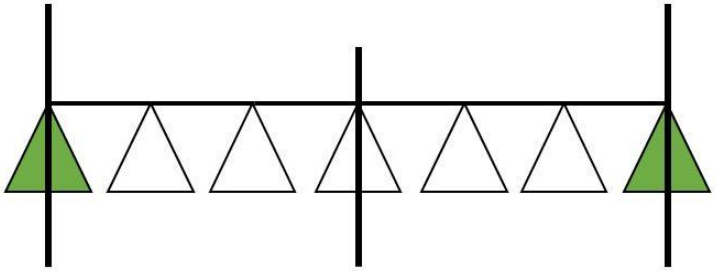
a.  b. 

Figure 2.3. Representation of a single field transect (a). Each triangle represents a biodegradable mesh bag of 8 turions, attached 1 m apart from another with string and stretched out in the river in a straight 6 m transect. The vertical lines represent PVC poles (center pole is 1.2 m, terminal poles are 1.5 m), used to anchor and mark the location of transects. Bags without poles were marked and anchored with pin flags. Green triangles indicate colonies that were enclosed in plastic mesh herbivory exclosures (b).

I monitored the founder colonies in the field for the first time in late July 2017, after nearly six weeks of growth. The turbidity of the water prevented precise measurements, but I recorded survival and approximated plant height using a PVC pipe marked off in 10cm intervals.

I harvested the founder colonies at the end of August (approximately ten weeks of growth). Harvested colonies were placed in cotton mesh bags and then placed on ice for transport to the lab. The cotton mesh bags were sufficiently intact that the plants were still entwined in them; thus, I pulled up the bags and all attached ramets as a measure of colony survival and growth. At Esopus Meadows, the density of the surrounding *V. americana* growth, which had not been previously observed, made this difficult, and it seemed likely that I harvested plants which were not part of my founder colonies but rather were already present.

Because the founder colonies may have been populated by naturally occurring plants at Esopus Meadows, I extracted (Synergy™ 2.0 Plant DNA Extraction Kit) *V. americana* DNA to identify multi-locus genotypes (MLGs) of harvested colonies using nine microsatellites (Burnett *et al.* 2009). Ramets connected by intact stolons (an "individual") were the sample unit, because connected ramets are genetically identical. There were two rounds of extractions. In the first round, I extracted all individuals (complete census) from at least one monoculture per transect, and randomly sampled one plant from all other monocultures, excluding those that were caged. This sampling design provided a compromise between a few full censuses and

a broad spatial subsampling. In addition, I extracted DNA from all individuals from all polycultures (complete census) to determine whether the 8 planted genotypes differed in persistence in polyculture (n=57 individuals), and the few surviving Stuyvesant colonies to learn which genotypes were the rare survivors (n=3 individuals; Table 2.2). In the second round of genotyping, I was able to extract from the remaining Esopus Meadows monocultures to obtain a near-census of the harvested plants. By this time, some of the plants had begun to decay, and so the number of individuals genotyped does not always represent all individuals harvested (Table 2.2). Overall, I extracted DNA from 87 individuals for genotyping (Table 2.2). DNA was stored at -20C until samples were genotyped at the University of Maryland College Park.

From extracted DNA, four polymorphic loci were amplified using the primers and protocols established by Burnett *et al.* (2009). PCR products were separated and measured on an ABI 3730xl DNA Analyzer with GeneScanTM-500 with a 500 LIZTM Size Standard (Applied Biosystems). Peak data were analyzed using GeneMapper v3.7 (Applied Biosystems) and all allele calls were visually inspected and confirmed following standards set by Marsden (2015) and used in genotyping the original samples from the Hudson River (Neel, *pers. comm.*). Ambiguous calls were re-genotyped and if the call remained ambiguous after 2 – 3 attempts, the alleles were coded as missing. The resulting multilocus genotypes were compared to genotypes of planted individuals to determine the genotypic identity of the harvested plants. If the

genotype did not match a planted genotype it was assigned to a new genotype code after being compared to all other known genotypes from the Hudson River.

After harvest, I weighed each colony (wet and dry weights) and counted individuals (single ramets or strings of ramets), individual ramets, and turions. I randomly selected four ramets from each colony and measured the length of their longest leaves. Additionally, I took photographs of the plants with a camera equipped with a near-infrared (NIR)-red-green filter (Figure 2.4). Terrestrial vegetation is uniquely suited to analysis via remote sensing due to its unique spectral signature; however, these methods are difficult to replicate with submersed aquatic vegetation due to the high absorption and scattering rates of the water column and its contents (Cho *et al.* 2008). Because I photographed the plants after harvest (*i.e.*, out of the water), the effects of the water column were removed. The plants were kept in water until they were processed, to minimize water loss for weights and photographs. The photographs were loaded into ENVI (Exelis Visual Information Solutions, Boulder, Colorado), and I clipped the area immediately around the plants to define regions of interest. I analyzed the regions for wavelength reflectance and calculated Normalized Differences Vegetation Index ($NDVI = (\rho NIR - \rho Red)/(\rho NIR + \rho Red)$) (Yoder and Waring 1994; Cho *et al.* 2008). NDVI is a popular index to assess vegetation qualities including biomass, water content, and chlorophyll content (Cho *et al.* 2008; Yoder and Waring 1994). Chlorophyll content is correlated with leaf nitrogen, which increases photosynthetic ability (Cho *et al.* 2008). Therefore, I used NDVI as an indicator of "greenness" and overall plant health.

Table 2.2. Extracted founder colonies to assess the proportion of the colony that was a planted MLG at Esopus, the identity of genotypes that survived in polyculture, and the identity of genotypes from disturbed colonies at Stuyvesant. "Colony ID" indicates the site (Iona (I), Esopus Meadows (E), or Stuyvesant (S)), transect number, and colony position along the transect. "Expected genotype(s)" indicates which genotypes were planted (Figure 2.1), or "polyculture" for colonies planted with 8 genotypes. The genotype "CTB" was harvested at three locations: Cheviot, Tivoli, and Brandow Point. Colonies were often subsampled, so the number of ramets extracted was less than the number of ramets harvested.

Colony ID	**Expected genotype(s)**	**# ramets harvested**	**# ramets genotyped**	**Proportion of colony sampled**
I-T5-2	Polyculture	5	5	1.00
E-T2-2	Polyculture	14	8	0.57
E-T4-5	Polyculture	10	10	1.00
E-T4-6	Polyculture	5	5	1.00
E-T5-3	Polyculture	26	20	0.77
E-T5-6	Polyculture	7	7	1.00
E-T6-2	Polyculture	12	12	1.00
S-T4	Unknown	1	1	1.00
S-T5	Unknown	2	2	1.00
S-T5	Unknown	1	1	1.00
E-T1-2	CTB-717	25	5	0.20
E-T1-3	RI-931	7	5	0.71
E-T1-4	Ch-952	9	2	0.22
E-T1-5	Ch-952	12	7	0.58
E-T1-6	TNB-779	6	2	0.33
E-T2-3	CTB-713	23	5	0.22
E-T2-4	RI-931	4	2	0.50
E-T2-5	TNB-989	12	8	0.67
E-T2-6	TNB-989	15	3	0.20
E-T3-2	Ch-951	5	3	0.60
E-T3-3	CTB-713	12	5	0.42
E-T3-4	Ch-952	3	1	0.33
E-T3-5	CTB-717	9	3	0.33
E-T3-6	RI-1008	5	2	0.40
E-T4-2	RI-1008	5	4	0.80
E-T4-3	Ch-951	11	4	0.36
E-T4-4	CTB-717	7	4	0.57
E-T5-2	RI-1008	20	5	0.25
E-T5-4	RI-931	6	6	1.00
E-T5-5	CTB-713	7	2	0.29
E-T6-3	TNB-779	7	3	0.43
E-T6-5	Ch-951	4	3	0.75
E-T6-6	TNB-989	9	6	0.67

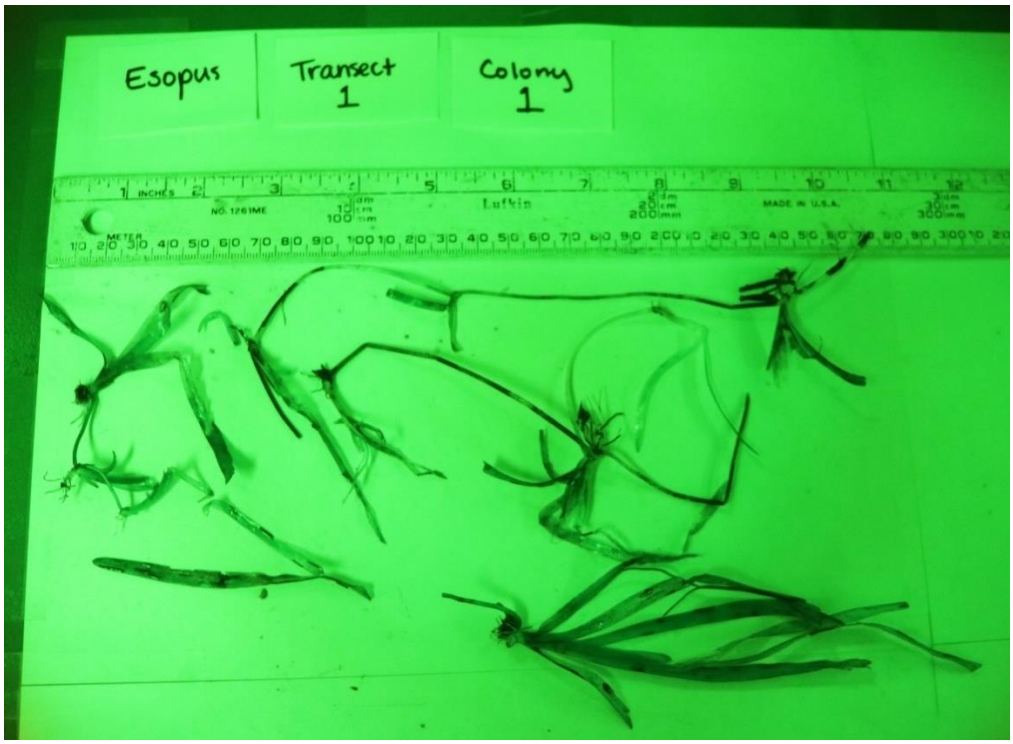


Figure 2.4. A photograph of a field sample (identified by the label at the top) taken using a NIR-red-green filter.

Greenhouse experiment

The greenhouse experiment, conducted in summer and fall 2017, employed a similar design as the field experiment. Three genotype groupings emulated the three-site field design: genotypes exclusively from Nutten Hooke, genotypes exclusively from Croton Point, and genotypes from across all sites (referred to as "cross-site") (Figure 2.1). The latter grouping allowed the incorporation of greater genetic breadth than the field plantings, which only planted genotypes local to a field site.

Experimental units (2.5 gallon buckets, 26 cm diameter, 24 cm deep) were filled ca. 3 cm deep with sterilized estuarine sediment (silt sand), and filled to the brim with dechlorinated tap water. All experimental units were planted with *V. americana.* Half (n=60 experimental units) were also planted with *Hydrilla verticillata* to test for invasibility of *V. americana* colonies that differ in genotypic diversity during establishment.

I assembled four genotype diversity treatments: one genotype (monoculture), two genotypes, four genotypes, and eight genotypes. Each experimental unit was planted with eight *V. americana* turions, mirroring the 8 turions planted per founder colony in the field experiment. Each experimental unit planted with multiple genotypes contained a unique combination of genotypes that was randomly selected from all available genotypes. Thus, the growth response of specific genotypes to the

environment cannot be tested except in cases when a genotype was selected multiple times for the monoculture treatment. Each of the four diversity treatments was replicated ten times for each of the three genotype groupings (n=40 per group, 120 founder colonies total), with half of the experimental units also planted with *H. verticillata* (n=60 founder colonies).

As with the field experiment, I measured length of each turion and collectively weighed the turions of each experimental unit prior to planting. Each turion was individually inserted into the sediment as opposed to planted in bags in the field. The *V. americana* was given a week to sprout before the addition of *H. verticillata* to half of the experimental units. *H. verticillata* was introduced as shoot tips (10 – 15 cm) that are known to root and propagate if fragmented and dispersed (Steward and Van 1987). Shoots were inserted 1 cm deep into the sediment.

I measured the maximum leaf length of *V. americana* and *H. verticillata* ramets at 6 weeks, mirroring the field experiment. The greenhouse experiment was harvested after 12 weeks of growth at the end of November 2017. At that time, I measured, weighed, photographed (Figure 2.5), and dried the colonies in the same fashion as the field experiment colonies.

Statistical Analyses

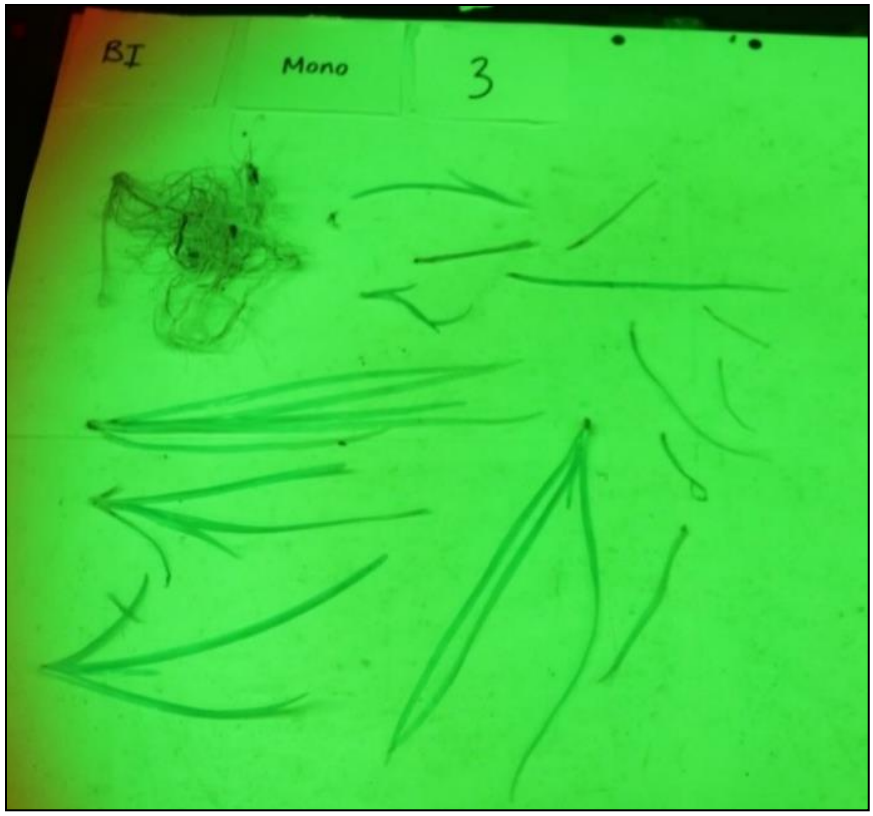
Field and greenhouse experiments tested the effects of genotypic identity and diversity on survival, growth, and invasibility of *V. americana* founder colonies and

how turion size influenced relationships. Because not all plants harvested from Esopus Meadows were identified as planted genotypes (Table 2.3a, 2.3b), data needed to be adjusted prior to statistical analysis to account for the proportion of the colony that had been naturally colonized. Specifically, I reduced the values of plant weight and ramet count using the proportion of genotyped ramets identified as the correct genotypes for each individual colony (Table 2.3a, 2.3b; Figure 2.7). The caged colonies were not genotyped, so the proportions of the adjacent positions across all transects were averaged; these averages were used to adjust caged colony data (Figure 2.7). For example, a colony with 0.50 harvested ramets identified as planted genotypes would have its ramet count and biomass reduced by 50 percent. This correction was more appropriate than using a blanket correction for the entire site because the proportion of unplanted genotypes varied widely across founder colonies (from 0 to 1). Natural population density was not measured and so the underlying spatial structure of the population could not be used to account for competitive pressure experienced by planted founder colonies at Esopus Meadows. To account for potential spatial variability in competitive pressure at the planting site, I tested whether proportion of colony that was planted differed across transects and across positions along each transect (two orthogonal blocks; Figure 2.2) in an ANCOVA. Genotype was included as an additional categorical factor to understand whether genotype identity contributes to ability of a founder colony to withstand the competitive pressure from the naturally emerging population.

I used correlation analyses to quantify the effects of initial turion size on early growth parameters (midsummer and harvest plant height, plant weight), as well as to quantify the effects of various growth parameters on each other (harvest plant height, plant weight, ramet count, NDVI). I used analyses of variance (ANOVA), and analyses of covariance (ANCOVA) to quantify the effects of continuous explanatory variables (initial turion size; random effect) and categorical explanatory variables (genotype, population, diversity level, and presence of an non-native invader; fixed effects) on plant height, above- and belowground biomass, ramet and turion number, and greenness of leaf tissues.

I performed an initial ANOVA test to determine if *V. americana* genotypes differed in turion size at planting. I expected to find that some genotypes and source populations had larger turions, such that turion size would need to be used as a continuous covariate in analyses of growth (ANCOVA). I compared the growth of *V. americana* plants by genotype, population, planting site, and diversity level (greenhouse experiment only) using ANCOVA. Although genotypes are grouped by population, no nesting was incorporated into the models because genotypes of the same population often varied as much as genotypes from different populations (Figure 2.8a, 2.9). I expected to find that genotypes with larger turions at planting produced larger plants, and that increased genotypic diversity increased overall colony plant growth. To test whether larger turions produce larger and more vigorous plants, I tested for correlation between turion size and three growth-related variables (plant height, biomass, and greenness).

I used ANCOVA to compare growth of *V. americana* and *H. verticillata* at different levels of *V. americana* diversity, using *V. americana* turion size as a covariate. I expected to find less *V. americana* growth in the presence of *H. verticillata,* but that the decrease would be lessened by greater genotypic diversity in *V. americana.* I also used correlation analyses to examine growth trade-offs within and between species; I expected that biomass of the two species would be negatively correlated, and that above- and belowground biomasses would be negatively correlated within species because of tradeoffs in resource use.

a. 

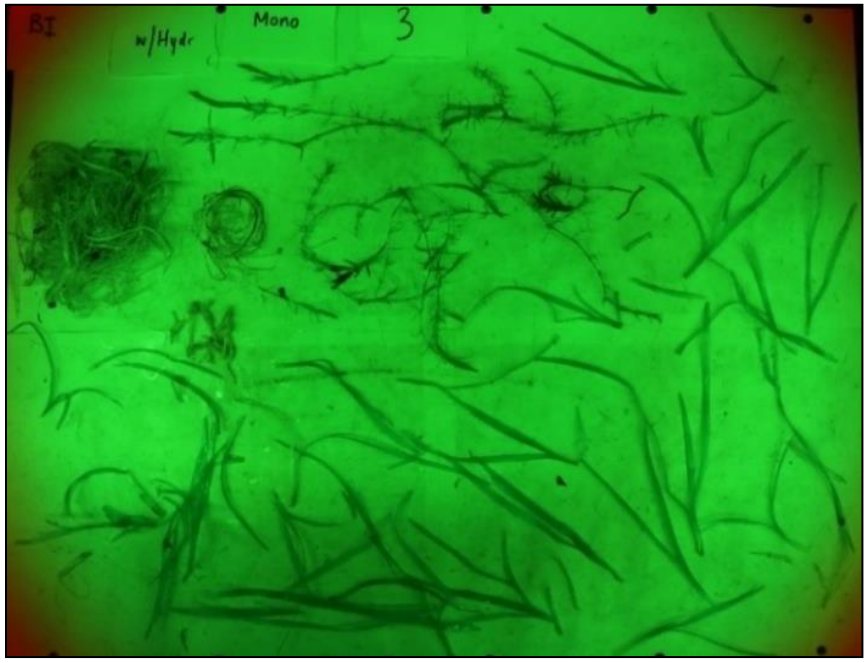
b. 

Figure 2.5. Photos of greenhouse colonies a) planted only with *V. americana* and b) planted with both *V. americana* and *H. verticillata* taken using the NIR-red-green filter.

RESULTS

Field Experiment

Survival of founder colonies (*i.e.*, colonies in which any *V. americana* growth was detected) was >90% at Esopus Meadows and Iona Island field sites at the six-week midsummer monitoring. Survival decreased to 55% at Iona Island by the end of the summer but remained >80% at Esopus Meadows (Figure 2.6). Only three colonies at Stuyvesant survived to the end of the experiments, but genetic analysis identified them: two colonies were planted MLGs, and the third was a known MLG that was planted at the site in error (Table 2.3c). Stuyvesant is removed from any subsequent analyses of plant growth due to lack of growth.

The herbivory exclosures had no effect on growth, nor did genotypic diversity (8 versus 1 genotype/s per founder colony) in the field experiment. Thus, these variables were not included in statistical models of founder colony performance.

Across Iona Island and Esopus Meadows, colonies supported from 0 to 26 ramets at harvest and had an average 4.8 ramets, which is 40% fewer ramets than the 8 potential ramets that could have emerged from 8 planted turions. Ramet count at Esopus Meadows increased from 8 potential ramets by 16 percent, whereas ramet count decreased by about half (54 percent) from 8 potential ramets at Iona Island. Although all sites were observed to be devoid of vegetation prior to the beginning of the field experiment, a natural *V. americana* bed emerged at Esopus Meadows in 2017 and intermingled with the planted founder colonies to confound survivorship

and ramet count estimates (Table 2.3a, 2.3b). Genotyped ramets from Esopus Meadows were 57% planted MLGs. Ramets in harvested founder colonies ranged from 0 – 100% planted genotypes (Table 2.3a, 2.3b). These proportions were applied to Esopus Meadows ramet count and biomass data to account for ramets and biomass that were estimated to come from the natural bed. The proportions for herbivory colonies were approximated by averaging the proportions of the adjacent colonies (Figure 2.7). After this correction was applied to Esopus Meadows ramet and biomass data, ramet count decreased, on average, by 48 percent.

The genotyped colonies did not have equal numbers of ramets, and I did not always genotype all ramets harvested from each colony (Table 2.2). However, there was no correlation between the proportion of colony genotyped and the proportion of the sample identified as the correct genotype ($p=0.28$). Therefore, survey effort (proportion of colony genotyped) did not appear to drive the outcome of the genotyping. Similarly, number of ramets harvested was not correlated with the proportion of the sample correct ($p=0.10$) even though four colonies with a high ramet count (>20 ramets; Table 2.2) after harvest supported a lower proportion of planting genotypes. A Type II ANCOVA model ($F=8.84$, $p=0.001$) examining the effects of spatial position of founder colonies at the Esopus Meadows planting sited (transect and position within a transect; Figure 2.2) and genotype identity found that genotype ($p=0.008$) and a colony's position within a transect ($p=0.001$) affected the proportion of the sample identified as the correct genotype. Transect had no effect in the model. All six colonies planted with either of two genotypes (Tiv-989 and Ch-

952) were completely uninvaded by the naturally occurring plant bed (Figure 2.7). All other genotypes were colonized by the naturally occurring bed twice or all three times they were planted in founder colony monocultures (Figure 2.7). However, because location of the founder colony was significant, it is unclear if by chance Tiv-989 and Ch-952 were planted outside the native bed.

Initial turion weight differed among genotypes and populations (Table 2.5b; Figure 2.8a) and was positively correlated with midsummer plant height (Table 2.5a; Figure 2.8b). For example, the two Turning Point (TP) genotypes had high initial turion weights (Figure 2.8a), which translated into high midsummer plant height (Figure 2.9a). In contrast, Nutten Hooke (H) and Croton (Cr) genotypes had small turions (Figure 2.8a). These genotypes also had low survival and height was relatively low in colonies that did survive (Figure 2.9a, 2.9b). Given this initial turion size difference, turion weight was included as a covariate in subsequent ANCOVA models (Table 2.5b).

The genotypes that survived in the polyculture colonies (Table 2.3b, 2.4) did not have small turions (Table 2.4, Figure 2.8a) but were not necessarily the highest-performing in monoculture (Figure 2.9, 2.10a). The most persistent genotypes in polyculture were genotypes CTB-713 and CTB-717 (Table 2.4) that can be found at multiple sites, specifically Cheviot, Tivoli, and Brandow Point. Other persistent genotypes included RI-931, Ch-952, and Tiv-989.

Midsummer (6 week) overall plant height varied across genotypes, source population of genotypes, and planting sites (Table 2.5b; Figure 2.9a). For example, plants that grew from Turning Point (TP) genotypes, which had larger turions (Figure 2.8a), were much taller than other plants after six weeks (Figure 2.9a). Variation in plant height lessened by the end of the summer but still differed across genotypes and sites at harvest (Table 2.5b; Figure 2.9b). The Turning Point (TP) genotypes, for example, were closer to the median height of all genotypes by the time of harvest, whereas the George's Island (GI) genotypes were tall both at midsummer and at time of harvest (Figure 2.9a, 2.9b), although GI did not have exceptionally large turions (Figure 2.8a). Overall plant height also varied by planting site; the plants at Iona Island tended to be shorter than the plants at Esopus Meadows, especially by the time of harvest (Figure 2.9a, 2.9b). Colonies that were taller at midsummer tended to be taller at harvest (Table 2.5a).

Harvest weight differed only between planting sites (Table 2.5b) but was positively correlated with midsummer height and harvest height (Table 2.5a). Initial turion weight accounted for some of the plant height variation at midsummer (Table 2.5b) but had no effect on the height and weight of harvested plants (Table 2.5b). Some of the site variation can be explained by the presence of extremely tall plants at Iona Island (Figure 2.9a).

The number of ramets produced by each founder colony varied by genotype and planting site (Table 2.5b; Figure 2.9c), with colonies at Esopus Meadows

producing 77 percent more ramets on average than Iona Island colonies. Number of ramets at harvest was positively correlated with harvest wet weight (Table 2.5a).

Plant greenness, as measured by NDVI, was positively correlated with plant height and weight at harvest (Table 2.5a; Figure 2.10b, 2.10c). NDVI differed among planting sites (Table 2.5b; Figure 2.10a), with Iona Island having slightly higher (~2%) NDVI values than Esopus Meadows and showing a much wider range of NDVI values (Figure 2.10a). The relationship between height and NDVI seemed to be stronger in certain genotypes: for example, Cheviot (Ch) 951 had mid-size plants with mid-range NDVI values, while Tivoli (Tiv) 989 had high NDVI values but mid-size plants (Figure 2.9b, 2.10a).

Greenhouse Experiment

All greenhouse experimental units survived for the duration of the experiment. Across all experimental units, 8 planted *V. americana* turions yielded on average 18 ramets, although ramet production ranged from 3 to 38 ramets). *H. verticillata* produced an average of 10 ramets (0 – 31 ramets) across 60 colonies. Ramet counts for either species did not differ between *V. americana* diversity treatments.

Initial *V. americana* turion weight did not differ among diversity treatments ($F=0.42$; $p=0.88$); however, just as in the field experiment, initial turion weight was correlated with plant height (Table 2.6a) at 6 weeks and at harvest and differed

among *V. americana* genotypes ($F=6.52$; $p<0.001$). *V. americana* turion weight was therefore used as a covariate in subsequent ANCOVA models (Table 2.6b).

Genotype groupings (Nutten Hooke, Croton, and cross-site genotypes) did not differ; therefore, this experimental factor was not included in subsequent analyses of *V. americana* performance.

V. americana plant height increased with diversity and decreased in the presence of *H. verticillata* (Table 2.6b; Figure 2.11a). As time passed, the presence of *H. verticillata* had a greater effect on *V. americana* plant height than did the diversity treatment, and initial turion weight remained important (Table 2.6b). In the monoculture treatment, the presence of *H. verticillata* had no effect on overall plant height (Figure 2.11a), whereas the relative difference between treatments with and without the invader increased with increasing diversity.

Total wet weight of *V. americana* at harvest was greater in the presence of *H. verticillata* but was unaffected by genetic diversity (Table 2.6b; Figure 2.11b). Although aboveground biomass of *V. americana* was unaffected by either the presence of *H. verticillata* or genetic diversity, root-to-shoot ratio of *V. americana* increased in the presence of *H. verticillata* (Table 2.6b; Figure 2.11c) and was negatively correlated with *V. americana* harvest height (Table 2.6a). *V. americana* greenness, when measured as NDVI, was positively correlated with *V. americana* wet

weight (Table 2.6a) but was unaffected by initial turion weight, genetic diversity, or *H. verticillata* presence (Table 2.6b).

Ramet production in *V. americana* was unaffected by genetic diversity or *H. verticillata* presence but was positively correlated with *V. americana* wet weight (Table 2.6a). The same was true for *H. verticillata* (Table 2.6a). Both species also showed a positive correlation between wet weight and turion production (Table 2.6a). Neither genetic diversity nor the presence of *H. verticillata* affected the production of either ramets or turions in *V. americana*; again, the same was true in *H. verticillata.*

The height of *H. verticillata* was unaffected by *V. americana* initial turion weight or diversity throughout the duration of the experiment (Table 2.6b). Harvest height of *H. verticillata* was positively correlated with *H. verticillata* wet weight at harvest (Table 2.6a), and *H. verticillata* wet weight at harvest was negatively correlated with *H. verticillata* NDVI (Table 2.6a). Although the ANCOVA test showed variation in *H. verticillata* wet weight by diversity treatment (Table 2.6b; Figure 2.12), a pairwise comparison showed that the variation is driven solely by the difference between the four- and eight-genotype treatments ($p=0.03$; Figure 2.12).

Table 2.3. Genotyping results from uncaged Esopus Meadows monocultures (a), all surviving polycultures from Esopus Meadows and Iona Island (b), and surviving Stuyvesant colonies (c). "Proportion of colony sampled" indicates the proportion of harvested ramets that were genotyped (Table 2.2).

a) Esopus Meadows monocultures

Colony ID	Planted genotype	Ramets genotyped	Proportion of colony sampled	Ramets correct	Proportion of sample correct	# new genotypes
E-T1-2	CTB-717	5	0.20	2	0.40	2
E-T1-3	RI-931	5	0.71	0	0.00	1
E-T1-4	Ch-952	2	0.22	2	1.00	0
E-T1-5	Ch-952	7	0.58	7	1.00	0
E-T1-6	Tiv-779	2	0.33	1	0.50	1
E-T2-3	CTB-713	5	0.22	2	0.40	1
E-T2-4	RI-931	2	0.50	1	0.50	1
E-T2-5	Tiv-989	8	0.67	8	1.00	0
E-T2-6	Tiv-989	3	0.20	3	1.00	0
E-T3-2	Ch-951	3	0.60	2	0.67	1
E-T3-3	CTB-713	5	0.42	1	0.20	2
E-T3-4	Ch-952	1	0.33	1	1.00	0
E-T3-5	CTB-717	3	0.33	1	0.33	1
E-T3-6	RI-1008	2	0.40	2	0.40	0
E-T4-2	RI-1008	4	0.80	3	0.75	1
E-T4-3	Ch-951	4	0.36	0	0.00	2
E-T4-4	CTB-717	4	0.57	1	0.25	1
E-T5-2	RI-1008	5	0.25	0	0.00	2
E-T5-4	RI-931	6	1.00	6	1.00	0
E-T5-5	CTB-713	2	0.29	2	1.00	0
E-T6-3	Tiv-779	3	0.43	1	0.33	1
E-T6-5	Ch-951	3	0.75	3	1.00	0
E-T6-6	Tiv-989	6	0.67	6	1.00	0

b) Surviving polycultures from Esopus Meadows and Iona Island

Colony ID	Planted genotypes	Ramets genotyped	Prop. colony sampled	Ramets correct	Prop. sample correct	Surviving genotype(s)
E-T2-2	CTB-713,	8	0.57	0	0.00	2 new genotypes
E-T4-5	CTB-717,	10	1.00	8	0.80	CTB-713 & 717, 1 new
E-T4-6	RI-931, RI-1008,	5	1.00	4	0.80	CTB-713 & 717, 1 new
E-T5-3	Ch-951, Ch-952,	20	0.77	5	0.25	CTB-713, RI-931, Tiv-989
E-T5-6	Tiv-779,	7	1.00	6	0.86	Ch-952, 1 new
E-T6-2	Tiv-989	12	1.00	12	1.00	RI-931, Ch-952, CTB-717
IT5-2	polyculture	5	1.00	5	1.00	TP-24

c) Surviving Stuyvesant colonies

Colony ID	Planted genotype(s)	Ramets genotyped	Proportion of colony sampled	Ramets correct	Proportion of sample correct	Surviving genotype(s)
S-T4	Unknown	1	1.00	1	1.00	CTB-713
S-T5	Unknown	2	1.00	2	1.00	SP-863
S-T5	Unknown	1	1.00	0	0.00	Cr-650*

*Cr-650 should have been planted at Iona Island

Table 2.4 Genotypes that were harvested at least once in polyculture at Esopus Meadows. Eight genotypes were planted per polyculture; 3 did not show up in harvest. "# times harvested" indicates the number of polycultures that contained the genotype at harvest; "# ramets harvested" indicates the total ramets harvested of that genotype across all polycultures. The average turion size from all genotypes is 1.28 g, and the average plant height from all genotypes is 19.51 cm.

Genotype	**# times harvested**	**# ramets harvested**	**Average turion size (min – max)**	**Average height (min – max)**
CTB-713	4	10	1.18 (0.57 – 1.88)	18.48 (10.38 – 28.78)
CTB-717	3	16	1.56 (1.19 – 2.06)	24.84 (14.47 – 38.60)
RI-931	2	8	1.99 (1.65 – 2.32)	14.53 (2.80 – 32.85)
Ch-952	2	7	1.26 (1.21 – 1.33)	10.20 (4.80 – 16.03)
Tiv-989	1	1	1.17 (1.06 – 1.37)	10.88 (10.23 – 11.95)

Table 2.5. Correlation analyses (a) and ANCOVA tests, with turion weight as a covariate (b), performed on field experiment data. Stuyvesant data are not included growth analyses due to low survival. Harvest height data were normalized using a square root transformation. Esopus Meadows data were adjusted for local invasion (Table 2.3). "Proportion of sample correct" (a) indicates the proportion of the genotyped individuals identified as the planted genotype (Table 2.3).
*p<0.05; **p<0.01; ***p<0.001

a) Correlation Analyses

Explanatory variable	Response variable	Correlation coefficient (r)
Turion weight	Midsummer height	0.40***
	Harvest height	0.18
	Harvest wet weight	0.17
	Harvest ramet count	0.08
	Proportion of sample correct	-0.63**
Midsummer height	Harvest height	0.41***
	Harvest wet weight	0.36**
	Harvest ramet count	0.08
Harvest height	Harvest wet weight	0.77***
	Harvest ramet count	0.14
	Harvest NDVI	0.40**
Harvest wet weight	Harvest ramet count	0.41***
	Harvest NDVI	0.52***

b) ANOVA/ANCOVA

Factor	Response	F_{model}	F_{factor}	$F_{turion\ weight}$
ANOVA				
Genotype	Average turion weight	14.68***		
Population	Average turion weight	13.42***		
Planting site	Average turion weight	8.78***		
ANCOVA				
Genotype	Midsummer height	4.51***	3.08**	25.93***
	Harvest height	2.16*	2.10*	3.12
	Harvest wet weight	1.45	1.44	1.71
	Harvest ramet count	2.86**	3.01**	0.29
	Harvest NDVI	0.84	0.86	0.55
Population	Midsummer height	5.92***	4.22***	21.17***
	Harvest height	1.33	1.25	2.01
	Harvest wet weight	1.38	1.36	1.59
	Harvest ramet count	1.12	1.21	0.18
	Harvest NDVI	0.91	0.94	0.59
Planting site	Midsummer height	19.25***	19.38***	19.13***
	Harvest height	3.41*	4.76*	2.06
	Harvest wet weight	4.84**	6.26**	2.02
	Harvest ramet count	1.29	1.73	0.44
	Harvest NDVI	4.45*	8.23**	0.67

Table 2.6. Correlation analyses (a) and ANCOVA tests, with turion weight as a covariate (b), performed on greenhouse experiment data. Species are denoted by genus only. Non-significant correlations are not included in Table 2.6a.
*p<0.05; **p<0.01; ***p<0.001
[+]data outliers were removed

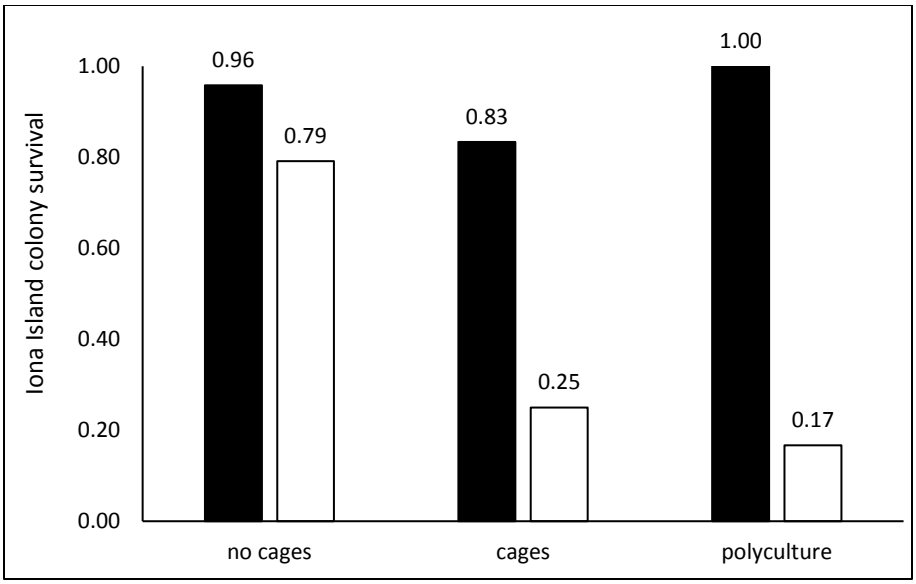
a) Correlation Analyses

Control	Response	Correlation coefficient (r)
Vallisneria average turion weight	*Vallisneria* 6-week height	0.34***
Vallisneria average turion weight	*Vallisneria* harvest height	0.31***
Vallisneria root-to-shoot (wet)[+]	*Vallisneria* harvest height	-0.41***
Vallisneria harvest height	*Vallisneria* harvest weight (wet)	0.29**
Vallisneria harvest weight (wet)	*Vallisneria* NDVI	0.19*
Vallisneria harvest weight (wet)	*Vallisneria* harvest turion count	0.43***
Vallisneria harvest weight (wet)	*Vallisneria* harvest ramet count	0.64***
Hydrilla harvest height	*Hydrilla* harvest weight (wet)	0.70***
Hydrilla harvest weight (wet)	*Hydrilla* NDVI	-0.36**
Hydrilla harvest weight (wet)	*Hydrilla* harvest turion count	0.40***
Hydrilla harvest weight (wet)	*Hydrilla* harvest ramet count	0.43***

b) ANCOVA

Response	F_{model}	$F_{diversity}$	$F_{Hydrilla}$	$F_{turionweight}$
Vallisneria 6-week height	6.95***	5.61**	0.32	17.59***
Vallisneria harvest height	6.96***	3.01*	11.61***	14.14***
Vallisneria harvest wet weight	2.99*	2.16	8.30**	0.20
Vallisneria root-to-shoot ratio (wet)	3.56**	2.17	11.25**	0.001
Vallisneria NDVI	2.08	2.58	2.27	1.77
Hydrilla 6-week height	1.53	1.64		1.20
Hydrilla harvest height	2.40	2.64		1.67
Hydrilla harvest wet weight	2.67*	2.81*		2.22
Hydrilla root-to-shoot ratio (wet)	0.88	0.96		0.62
Hydrilla NDVI	1.28	1.31		1.11

a.



b.

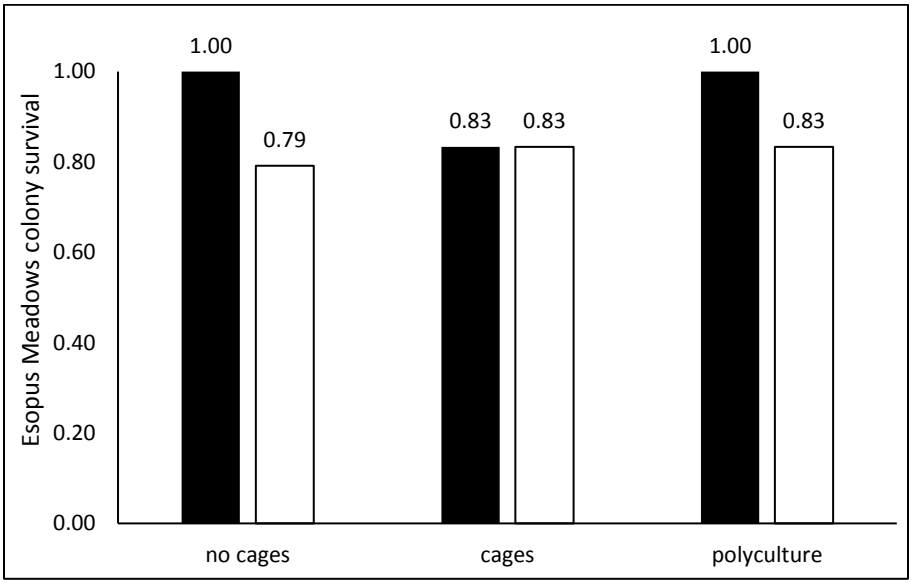


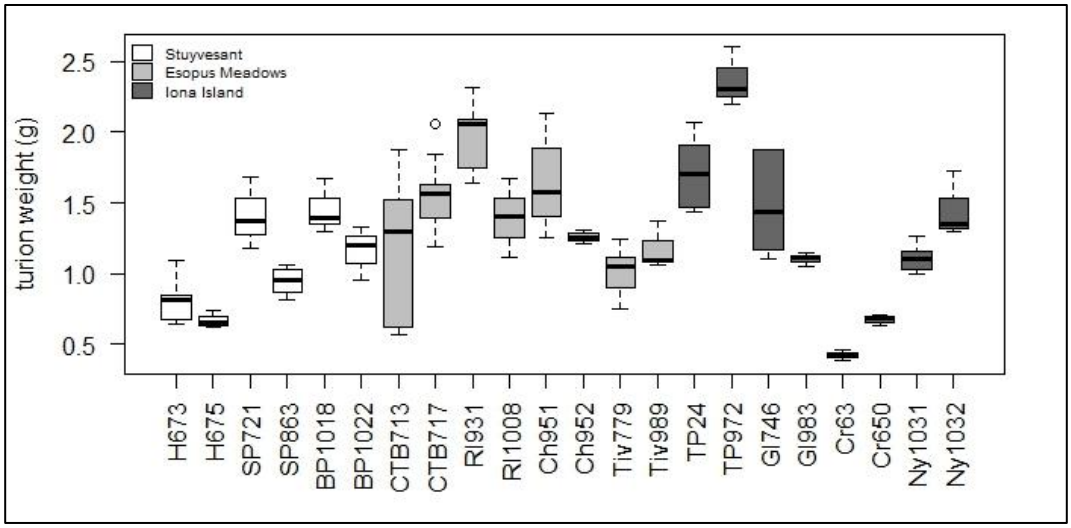
Figure 2.6. The proportion of colonies surviving at two field sites, Iona Island (a) and Esopus Meadows (b) after six weeks of growth (black bars) and at the time of colony harvest at ten weeks (white bars). Only three of 42 founder colonies survived at Stuyvesant. Colonies at Esopus Meadows that were completely invaded by unplanted MLGs are considered to have not survived.

T1-1 (cage) 0.47	T1-2 (CTB-717) 0.40	T1-3 (RI-931) 0	T1-4 (Ch-952) 1.00	T1-5 (Ch-952) 1.00	T1-6 (Tiv-779) 0.50	T1-7 (cage) 0.76
T2-1 (cage) 0.47	T2-2 (poly) 0	T2-3 (CTB-713) 0.40	T2-4 (RI-931) 0.50	T2-5 (Tiv-989) 1.00	T2-6 (Tiv-989) 1.00	T2-7 (cage) dead
T3-1 (cage) 0.47	T3-2 (Ch-951) 0.67	T3-3 (CTB-713) 0.20	T3-4 (Ch-952) 1.00	T3-5 (CTB-717) 0.33	T3-6 (RI-1008) 0.40	T3-7 (cage) 0.76
T4-1 (cage) 0.47	T4-2 (RI-1008) 0.75	T4-3 (Ch-951) 0	T4-4 (CTB-717) 0.25	T4-5 (poly) 0.80	T4-6 (poly) 0.80	T4-7 (cage) 0.76
T5-1 (cage) 0.47	T5-2 (RI-1008) 0	T5-3 (poly) 0.25	T5-4 (RI-931) 1.00	T5-5 (CTB-713) 1.00	T5-6 (poly) 0.86	T5-7 (cage) 0.76
T6-1 (cage) 0.47	T6-2 (poly) 1.00	T6-3 (Tiv-779) 0.33	T6-4 Tiv-779 dead	T6-5 (Ch-951) 1.00	T6-6 (Tiv-989) 1.00	T6-7 (cage) dead

Proportion of sample identified as correct genotype(s)	
	0 – 0.25
	0.26 – 0.50
	0.51 – 0.75
	0.76 – 1.00

Figure 2.7. Spatial representation of the colonies at Esopus Meadows. Each cell represents a colony, identified by transect number, position number, and genotype composition. Numbers indicate the proportion of the genotyped ramets identified as the planted genotype. Proportions used for the caged colonies, which were not genotyped, were averages of the proportions in the adjacent positions (*i.e.*, all proportions in position 2 were averaged for position 1 and all proportions in position 6 were averaged for position 7).

a.



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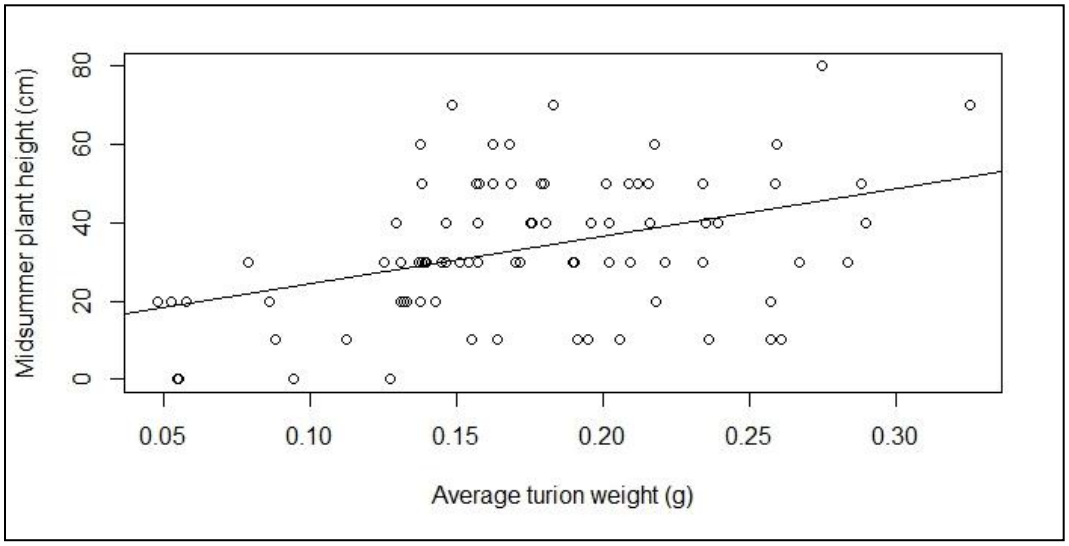


Figure 2.8. Turion weight varied across genotypes (2.8a; $p<0.001$) and is correlated with midsummer height (2.8b; $r=0.40$). In Figure 2.8a, genotypes are labeled by population (*i.e.*, TP for Turning Point) and multilocus genotype (MLG) number. Stuyvesant data are removed from Figure 2.8b due to low survival. Midsummer plant height was measured in 10cm intervals.

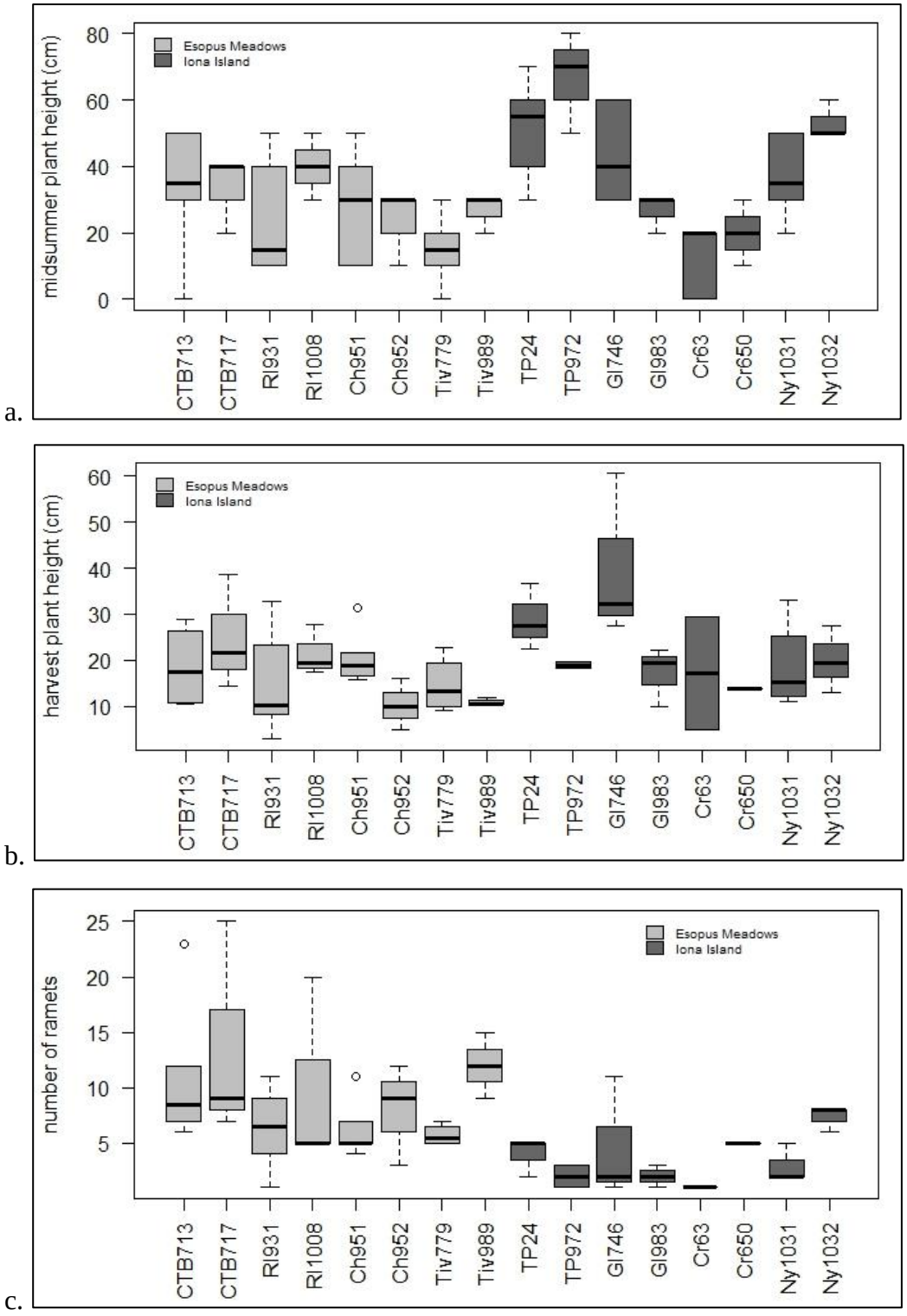
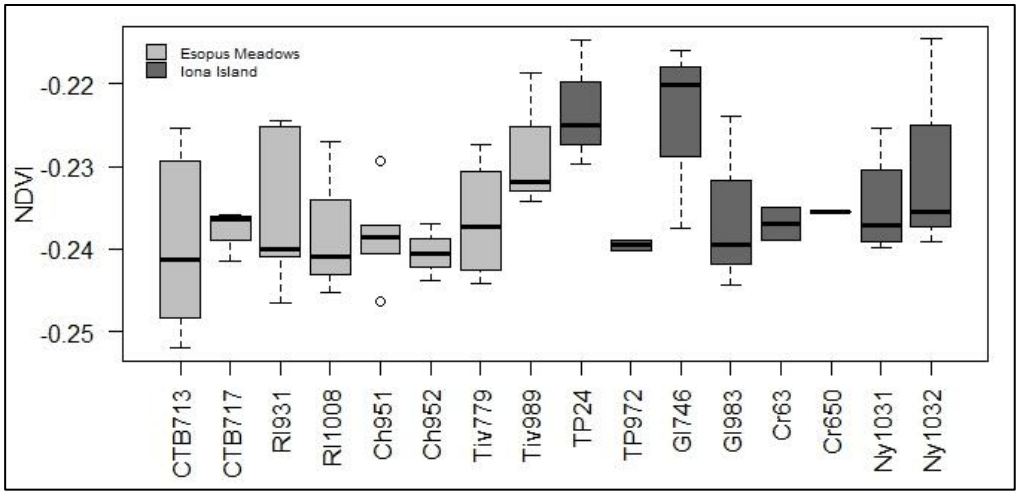


Figure 2.9. At midsummer, plant height (2.9a) varied by genotype ($p<0.01$) and planting site ($p<0.001$), but height variation was less at harvest (2.9b; $p<0.05$). The number of ramets produced by harvest (2.9c) varied by genotype ($p<0.05$) and planting site ($p<0.05$). Multilocus genotypes (MLGs) are labeled by population (*i.e.*, TP for Turning Point) and identifying number. Stuyvesant genotypes are not included because of low site survival.

a.



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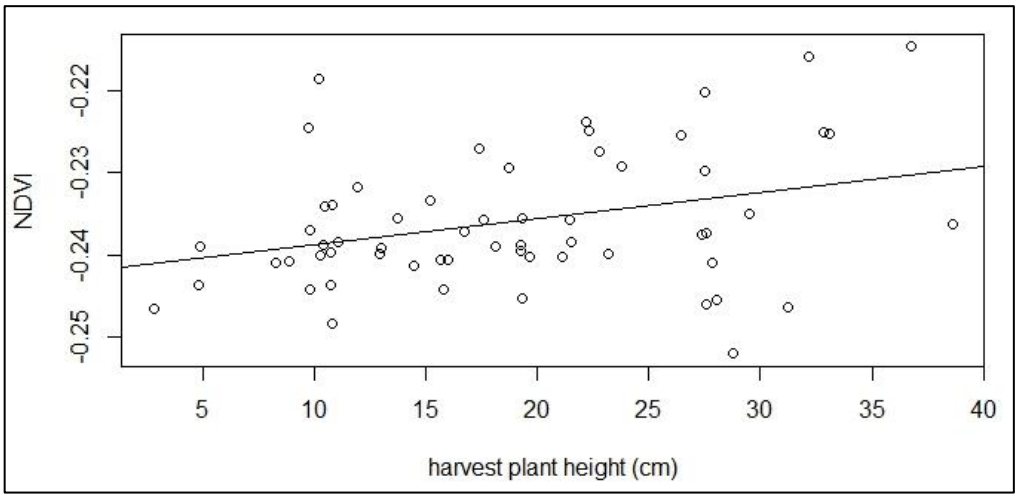


c.

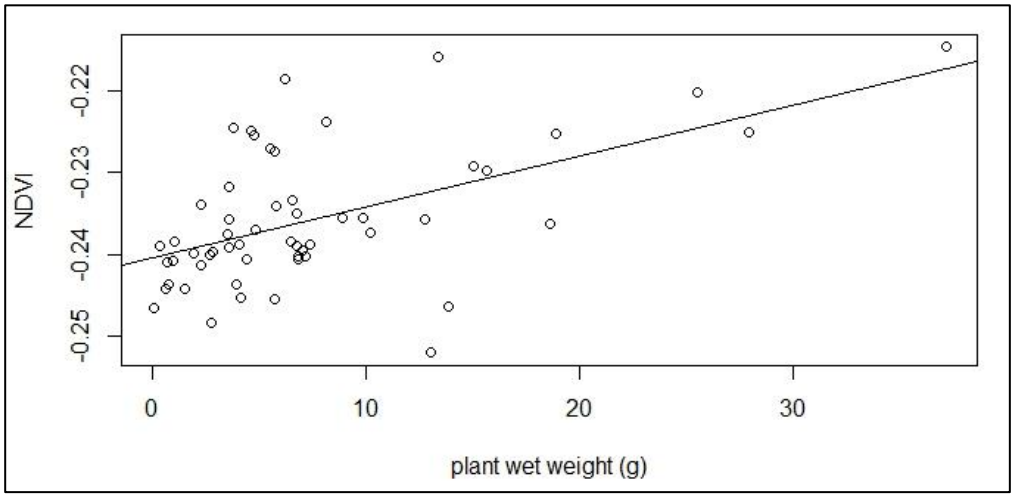
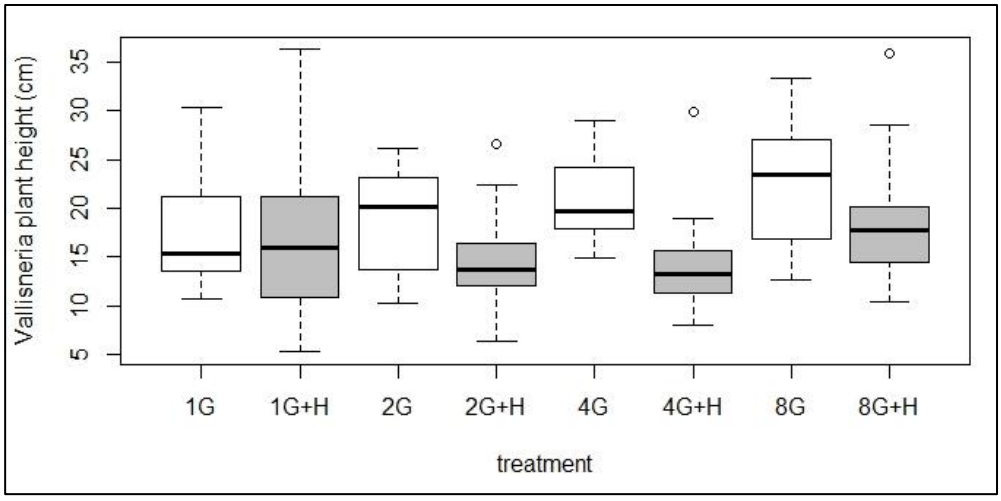
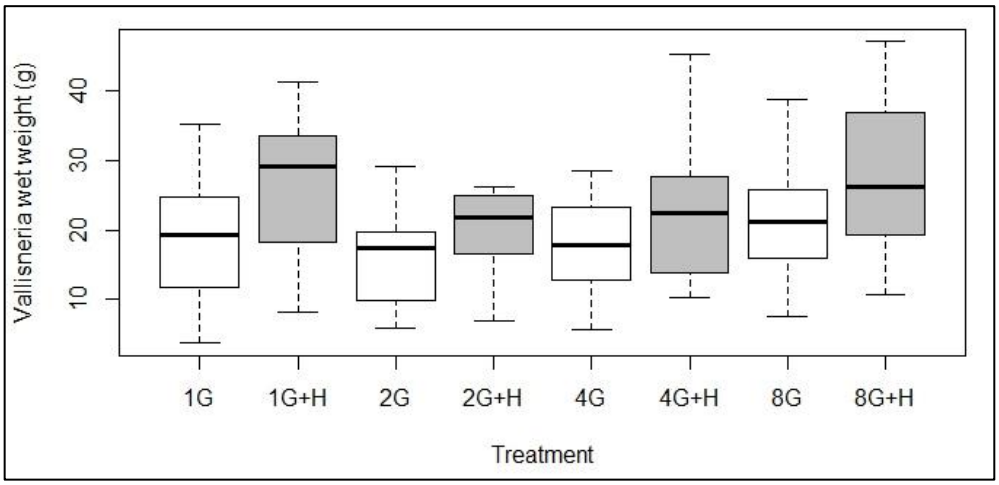


Figure 2.10. Plant greenness measured as NDVI varied by planting site (2.10a; $p<0.01$) and was correlated with harvest plant height (2.10b; $r=0.40$) and harvest wet weight (2.9c; $r=0.53$). Data outliers were removed from harvest plant height (b) and wet weight (c). Genotypes are labeled by population (*i.e.,* TP for Turning Point) and multilocus genotype (MLG) number. Stuyvesant genotypes are not included because of low site survival.

a.



b.



c.

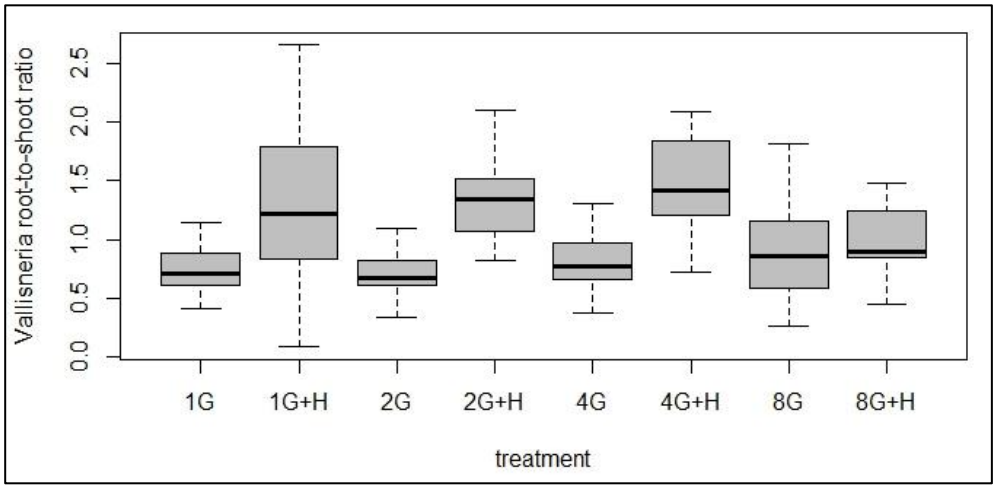


Figure 2.11. In the greenhouse, *V. americana* plant height (2.11a; $p_{diversity}<0.05$, $p_{Hydrilla}<0.001$), wet weight (2.11b; $p_{Hydrilla}<0.01$), and root-to-shoot ratio (2.11c; $p_{Hydrilla}<0.01$) varied among diversity and/or *Hydrilla* treatments. G = number of genotypes. H = *Hydrilla*. White boxes are *Vallisneria*-only treatments; gray boxes are *Hydrilla* treatments. Data outliers were removed from wet weight (b) and root-to-shoot ratio (c) to ease visualization.

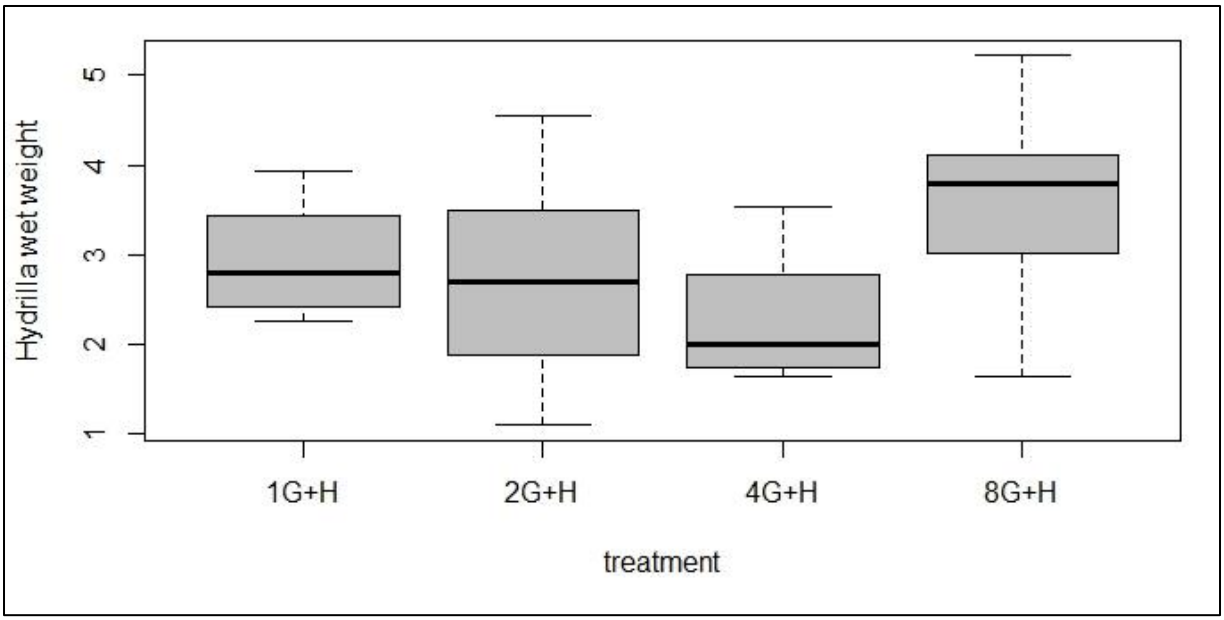


Figure 2.12. *H. verticillata* harvest wet weight differed between the four- and eight-genotype diversity treatments ($p_{diversity}<0.05$). G = *V. americana* genotype (planted in monoculture = 1, biculture = 2, and 4 and 8 genotypes).

DISCUSSION

Parallel field and greenhouse experiments suggest that survival and first-year growth of *V. americana* founder colonies are affected at least in part by founder colony genetics. Turion size varied among genotypes (Table 2.5b; Figure 2.8a), and this size advantage persisted through the growing season in the field by allowing individuals to grow taller and access light resources (Table 2.5b; Figure 2.8b, 2.9). Greater plant height was associated with greener plants and higher biomass, which was associated with higher ramet and turion production, suggesting that an early size advantage may have lasting and potentially inter-generational effects. However, the genetically-determined size advantage disappeared in the presence of the invader *H. verticillata* in greenhouse mesocosms and plants in the field were subject to pressure from local genotypes and hydrologic stress, suggesting that environmental factors may interfere with early gains. Although the height of *V. americana* plants increased with genotypic diversity (Table 2.6b, Figure 2.11a), invasion resistance did not, contrary to predictions. *V. americana* plant size varied by field planting site (Table 2.5; Figure 2.9, 2.10). I propose that the genetically-determined advantage in early growth conferred by *V. americana* turions may be a mechanism by which restored populations can establish rapidly and gain access to resources to increase survival, growth, and clonal expansion. However, restoration sites need to be carefully selected to ensure that hydrologic stress is minimized, and competitors are not present.

Propagule Size Advantage

The size of *V. americana* turions varied among multi-locus genotype (MLGs) source populations. At the population level, Turning Point (TP) genotypes produced large turions, whereas genotypes from Croton (Cr) and Nutten Hooke (H) produced smaller turions (Figure 2.8a). Even within a population, where genotypes tend to be genetically more similar, differences in turion size among genotypes were often just as pronounced as differences among populations, suggesting that "genotype" is an appropriate level of organization for understanding how genetics influences function. Similar intraspecific size variation in propagules and seeds has been observed in both terrestrial (Stanton 1984) and aquatic species (Lin and Sternberg 1995; Idestam-Almquist and Kautsky 1995; Doyle and Smart 2001), highlighting fitness consequences of larger propagules. For example, larger plant offspring have a higher chance of survival despite adverse environmental conditions (Spencer 1987; Idestam-Almquist and Kautsky 1995). A greater biomass reserve allows plants to grow faster and produce more leaves early on (Lin and Sternberg 1995; Doyle and Smart 2001), which enables them to overcome sediment burial (Spencer 1987) and resist mechanical disturbance (Idestam-Almquist and Kautsky 1995). Furthermore, aquatic plants that can grow quickly and produce larger leaves have a greater chance of thriving even in low light conditions; the increased height enables plants to better reach available light, and larger leaves with greater surface area enable them to intercept more of that light (Doyle and Smart 2001).

A turion size advantage in early growth was apparent in both the field and greenhouse experiments: *V. americana* plants that sprouted from larger turions were taller after six weeks (Table 2.5a, Table 2.6a). Rapid early growth in *V. americana* is likely to provide a competitive advantage, because plants are better positioned to access limiting light resources in the early growing season or when water is turbid (Lin and Sternberg 1995; Doyle and Smart 2001). These traits lead to increased plant survival and productivity (Lin and Sternberg 1995; Doyle and Smart 2001; Idestam-Almquist and Kautsky 1995).

Adverse environmental conditions, such as high turbidity or wave action, favor the survival and propagation of larger turions (Doyle and Smart 2001; Idestam-Almquist and Kautsky 1995). Similarly, turions that are buried deeply in the sediment must be larger to survive and reach the surface of the sediment to grow (Spencer 1987). Thus, an alternative explanation to a genetically-driven size advantage could be that the observed turion size variation is a legacy from prior field conditions. This alternative explanation can be rejected because genotypes used in the experiments had all been growing in the same greenhouse conditions for several generations. However, under natural field conditions or in a restoration setting, a legacy effect could be interacting with a genetically-driven size advantage to influence colony establishment.

The observed height advantage conferred by larger turion size disappeared by the end of the season in the field (Table 2.5b) but was still strong to the end of the

greenhouse experiment (Table 2.6b). Thus, the early size advantage may have a lasting effect under the optimal environmental conditions of the greenhouse but may be offset by other less ideal environmental factors (*e.g.* water currents, herbivores, competitors) in the field. Still, a height advantage was associated with higher harvest biomass and NDVI (Table 2.5, 2.6), suggesting that taller plants, in general, may have a lasting growth advantage that translates into greener plants and more biomass. NDVI is positively correlated with chlorophyll content and can be used as an indicator of photosynthetic potential and overall plant health (Yoder and Waring 1994; Cho *et al.* 2008). Although a positive correlation between plant size (measured either as biomass or leaf area index (LAI)) and NDVI (a measure of plant health and photosynthetic potential) is often observed, it is not universal (Yoder and Waring 1994; Cho *et al.* 2008). And I saw mixed evidence of relationships in my experiments. In the parallel field and greenhouse experiments, a positive correlation between plant size (height and biomass) and NDVI was observed in the field experiment, but the greenhouse experiment only showed a positive correlation between plant height and NDVI (with plant height, measured as length of leaves, acting as a stand-in for LAI). The biomass influence on NDVI in the field experiment suggests that plants growing in the field were healthier overall. This difference could be explained by the closed nature of the greenhouse colonies (*i.e.* lack of water flow and nutrient input), or by intra- and interspecific competition in closed greenhouse colonies.

Although plant height was not directly correlated with either clonal growth (ramet and turion production) or plant health (NDVI), a strong positive correlation emerged between plant height and harvest biomass in both experiments (Table 2.5a, 2.6a), and further, between biomass and ramet and turion production. Although a direct link between turion size and the next generation of plants (turions) was not observed, the significant correlations between plant height and harvest biomass, and between harvest biomass and the number of ramets and turions, suggest that turion size may be a catalyst of growth in *V. americana* plants which, at least initially, is genetically driven. However, this initial growth may be altered as plants interact with the physical environment and undergo inter- and intraspecific competition such that other genotypes with different growth traits may ultimately contribute as much, if not more, to ecosystem functioning than the initially highest-performing genotypes.

Genotypes that produce larger turions may ultimately not be the most successful in nature. If they were, common genotypes would produce larger turions and rare genotypes would have smaller turions. Similarly, the genotypes that persisted most frequently in polyculture were not necessarily those with the largest turions (Table 2.4; Figure 2.8a). Some of the most persistent genotypes, CTB-713 and CTB-717, are found at multiple collection sites in the Hudson River (Cheviot, Tivoli, and Brandow Point). Because of their comparatively wide geographic spread, these genotypes are potentially adapted to a wider suite of environmental conditions or are plastic in their responses to environmental conditions (Engelhardt *et al.* 2014). Alternatively, the widespread genotypes may have become widespread through

neutral processes such as random dispersal and demographic stochasticity (Rafajlović *et al.* 2017). Although the genotyped polycultures did not exhibit the effects of a propagule size advantage, the persistence of widespread genotypes highlights the potential importance of genotypic identity in the survival and establishment of *V. americana* plants.

Genetic Diversity Advantage

Previous studies have found that plant community productivity increases with species diversity (Tilman *et al.* 2001; Hooper *et al.* 2005) and explain that diversity increases the chance that a highly productive species will be present ("selection probability effect;" Crutsinger *et al.* 2006; Tilman *et al.* 2001), or that individuals use resources in complementary ways ("complementarity effect;" Crutsinger *et al.* 2006; Tilman *et al.* 2001). Some studies suggest that the presence of highly productive species may confound a true diversity effect based on complementarity (Wardle 1999, 2003). In response, Loreau and Hector (2001) suggest that the two effects can be differentiated by comparing biomass yield in monoculture compared to mixed cultures that differ in diversity. The yield of a mixed culture will exceed the yield of any single monoculture when complementarity is the dominant process, whereas the selection probability effect would result in equal yields between the mixed cultures and the most productive monocultures (Loreau and Hector 2001). Height of *V. americana* populations in greenhouse mesocosms clearly increased with genotypic diversity (Table 2.6b, Figure 2.11a). Here, a selection probability effect seems likely. Height in mixed culture did not exceed height in monoculture, a clear selection

probability effect pattern. In addition, because height was associated closely with turion size, the diversity effect on height was most likely driven by the initial size advantage of genotypes with large turions as opposed to complementarity among genotypes that allowed plants to grow taller when grown in mixed culture.

Genotypes that survived in mixed culture to the end of the field experiment were not necessarily the best performers is monoculture (Table 2.4), which may explain why genotypic diversity of *V. americana* did not affect biomass yield in both field and greenhouse experiment. This pattern suggests that, although an initial size advantage is evident, the size effects may be ephemeral and replaced by other short- and long-term processes that structure populations, such as adaptation to local conditions, lateral expansion of ramets, and flowering. This pattern in polyculture persistence could also indicate the importance of transect placement (Figure 2.7), which was done randomly, or random events, such as the colonies being washed away at Stuyvesant.

Plant height can be a measure of performance at the scale of individual ramets because taller ramets are likely to produce greater leaf biomass. However, in clonal species such as *V. americana,* lateral expansion of ramets can also be a measure of performance. Thus, the same biomass may be reached with a few tall ramets as with many small ramets. Engelhardt *et al.* (2014) observed a negative correlation between plant height and ramet count; if this were true in my experiments, it may explain why biomass did not increase with genetic diversity in the greenhouse experiment because

production of more, shorter ramets would have similar biomass to fewer, taller ramets. A trade-off between clone size (number of ramets) and plant height, however, was not observed, nor was a relationship between genetic diversity and number of ramets in the field and in the greenhouse. Thus, clonal expansion during establishment appears to be less genetically driven in the short term than plant height, suggesting that vertical growth is a priority in early establishment and that longer-term effects may emerge later.

Competitive Advantage

H. verticillata is a strong competitor for above-ground resources and an effective invader in shallow aquatic ecosystems (Langeland 1996; Van *et al.* 1998) owing to its aggressive, canopy-forming growth pattern. In the presence of the competitor, *V. americana* plants were substantially shorter and shifted growth to root production, leading to an overall increase in biomass in the presence of *H. verticillata* (Figure 2.11). Owens *et al.* (2008) found similar results when they added *H. verticillata* fragments to established *V. americana* colonies. At the same time, they found that *H. verticillata* monocultures were more productive than *H. verticillata* in biculture with *V. americana* (my greenhouse experiment lacked a *H. verticillata* monoculture). Similarly, other studies have found that *V. americana* and *H. verticillata* can coexist in suitable habitats, although *V. americana* does better in poor sediment and *H. verticillata* is more competitive in low-light conditions (Rybicki and Landwehr 2007; Rybicki and Carter 2002). This shift in resource use by *V. americana* may lower accessibility to light but provides *V. americana* greater resistance to

hydrological disturbances such as storm surges (Idestam-Almquist and Kautsky 1995) and allows greater access to nutrients in the sediments (Titus and Adams 1979). However, this shift means that the genetically-driven size advantage of *V. americana,* as observed in the field experiment, is lessened by the presence of a competitor.

H. verticillata height was not influenced by genotypic diversity in *V. americana* (Table 2.6b), which contrasts with the prediction that genetic diversity confers invasion resistances. Communities with greater species richness tend to resist invasion because the existing plant community uses resources more fully (Kennedy *et al.* 2002, Levine *et al.* 2004), which leaves less for potential invaders to exploit (Hooper *et al.* 2005). Studies have shown that genotypic diversity performs a similar function to species diversity in some ecosystems (Kotowska *et al.* 2010, Crutsinger *et al.* 2006). That this is not the case in mesocosms that are invaded by *H. verticillata* may be because *V. americana* was still establishing and not using resources fully, or that *V. americana* utilized the environment differently in the presence of *H. verticillata.*

Site Advantage

Survival was extremely low at the Stuyvesant site, high at Iona Island, and very high at Esopus Meadows, suggesting that external site factors were at play in plant survival. In the field, I observed that the site at Stuyvesant was subjected to extreme hydrological stress in the form of ships' wakes. Such high wave energy is detrimental to plant establishment (Koch 2001) and may have prevented the

Stuyvesant plants from even establishing. Therefore, it is important to select planting sites with low hydrologic stress when designing a restoration project (Cho and May 2006; Boustany 2003).

The other two field sites, Iona Island and Esopus Meadows, had relatively high survival (Figure 2.6) and showed no variation in overall plant height, biomass, or greenness (Table 2.5b), suggesting that both sites were well-suited for *V. americana* growth. The turbidity at Iona Island was much higher than at Esopus Meadows (HRECOS 2017 data); this could explain the lower survival of Iona Island plants because turbidity impedes plant growth (Moore and Wetzel 2000; Batiuk *et al.* 2000). Lower dissolved oxygen content at Iona Island (HRECOS 2017 data) could be indicative of algal growth caused by increased turbidity (*i.e.*, higher water column nutrient content), which would also contribute to *V. americana* plant survival and growth (Moore and Wetzel 2000; Batiuk *et al.* 2000; Kemp *et al.* 2004). Low light conditions could also explain the greater height and greenness (NDVI) of Iona Island plants in comparison with the Esopus Meadows plants (Figure 2.9b, 2.10a). Increased height would confer a competitive advantage in reaching available light, and heightened chlorophyll levels—illustrated by elevated NDVI—increase photosynthetic potential to better utilize available light.

Higher intraspecific competition could explain why Esopus Meadows and Iona Island plants showed comparable overall growth despite Esopus Meadows being the more favorable site because of its light and oxygen availability. Not all harvested

plants were identified as planted genotypes; the local *V. americana* population contaminated founder colonies at Esopus Meadows (Table 2.3a, 2.3b). Although it is ultimately desirable to have a submersed aquatic plant community present in the estuary, competition from the local *V. americana* population likely impeded the survival and establishment of planted founder colonies, and hampered monitoring efforts. When selecting sites for potential restoration, the surrounding plant community should be taken into consideration because the competition, both within and between species, will decrease the survival and growth of the restoration planting. Sites that already have native plant communities present may not require intense restoration, although bolstering the population with a variety of genotypes could increase the overall performance and resilience of the community (Evans *et al.* 2017).

Founder colonies at Esopus Meadows produced far more ramets than their counterparts at Iona Island, even after accounting for the local bed, although the Iona Island plants tended to be taller (Table 2.5b; Figure 2.9b). Turbidity at Iona Island was higher than at Esopus Meadows; therefore, plants at Iona Island likely dedicated their energy reserves to vertical expansion in order to reach available light. Esopus Meadows plants showed more horizontal expansion, though apparently to the slight detriment of their vertical growth.

Many of the colonies harvested from Esopus Meadows were at least partially colonized by unplanted local genotypes (Table 2.3, Figure 2.7). Colonization by the naturally occurring bed was uneven across the planting site, with transect positions 4

through 6 (located closer to the shore and in slightly shallower water) being less colonized, on average, by the natural bed. The colonies of the two genotypes, Ch-952 and Tiv-989, that did not include any local volunteers were all located entirely on the halves of the transects that were less colonized by the natural bed, even though the planting locations of genotypes in monoculture were randomized (Figure 2.7). Because larger turions, on average, produce larger plants (Figure 2.8b, 2.9), it was expected that genotypes with larger turions would be less invaded by local genotypes. The opposite turned out to be true; Ch-952 and Tiv-989 both have small turions (Figure 2.8a). This suggests that observed genotypic variation in proportion of colony colonized by the natural bed may be driven by the random positioning of certain genotypes in areas that are subjected to higher competitive pressure from the underlying natural bed, enforcing the importance of planting position and suggesting spatial variation in the existing local plant community.

Implications for Restoration

The size advantage of certain genotypes should be taken into consideration when planning restoration projects despite the influence of other factors because of the establishment advantage conveyed by larger turions. Genotypes that produce and sprout from larger turions are more likely to survive and establish, and those plants will exhibit more rapid and prolific early growth. This could allow them to use available resources before potential competitors can arrive and establish (Lin and Sternberg 1995; Doyle and Smart 2001).

Genotypes with an early size advantage may be analogous to pioneer species in communities, which establish rapidly but are ultimately replaced by other species with slower growth. Therefore, it is not enough to just plant genotypes with large turions assuming that large plants will develop from them. It is equally important to include genotypic diversity in restoration plantings, because diversity ensures the presence of genotypes that are adapted to the site conditions (Crutsinger *et al.* 2006). In addition, the long-term survival and vitality of the population is increased by genotypic diversity (Hughes and Stachowicz 2004) when conditions change. Successful restoration of ecosystems requires a holistic approach with long-term aims (Cho and May 2006). Aquatic systems will continue to face disturbance threats from biotic invasion and physical disturbance (*i.e.*, storm surges and wave action). Combatting these threats in the future requires ensuring population resilience in the present.

Successful restoration projects account for the habitat needs of the species being restored (Cho and May 2006; Boustany 2003). *V. americana* is tolerant of low light conditions, which is beneficial given the prevalence and persistence of anthropogenic turbidity (Batiuk *et al.* 2000; McFarland and Shafer 2008), but past restoration projects have failed because young plants are easily ripped out of the sediment by current or wave action (Boustany 2003; Cho and May 2006). Therefore, it is important to select restoration sites for their physical traits as well as water quality. At Esopus Meadows, the presence of an existing SAV community seemed favorable because it indicated the site's suitability. However, at Stuyvesant, the cove

in which I planted my founder colonies was subjected to intense flow and wave action from ships' wakes, and the founder colonies could not survive and establish, even though there had been a SAV bed at Stuvesant at one time (Findlay *pers. obs.*).

In conclusion, different genotypes of *Vallisneria americana* have varying levels of performance, and environmental factors can either enhance or degrade plant growth. Therefore, it is vital that future SAV restoration projects incorporate both high-performing genotypes and high genotypic diversity and account for local environmental conditions, ensuring that restoration sites are not prone to disruption by physical or biological factors, in order to ensure the success and long-term survival of SAV communities.

Chapter 3: Restoration Implications

The duration and magnitude of anthropogenic disturbance in natural systems necessitates the development of restoration ecology as a separate field. The complexity of many natural systems is not fully understood, and the effects of human disturbance on those systems adds another dimension to this complexity. Therefore, effective and enduring restoration requires an adaptive, holistic approach that accounts for many different factors that could enhance the outcome of restoration. With this in mind, I propose that future submersed aquatic vegetation (SAV) restoration plantings incorporate the meticulous selection of planting sites and plant genotypes. Furthermore, I encourage the use of "founder colonies" in restoration plantings to facilitate easy deployment and reduce the risk of planting failure.

Reducing Hydrological Stress: the Bag Method

Previous SAV restorations have relied heavily on transplantation of young seedlings or ungerminated seeds (Boustany 2003; Cho and May 2006). Many of these plantings have limited success, because wave action or strong currents can easily uproot small plants, which have shallow and sparse root systems. Transplant stress is also a major concern. Transplanted *Vallisneria americana* typically loses most of its above-ground tissues and requires ca. 3 weeks to grow new leaves from the base of the plant (Engelhardt *pers. obs.*). Owing to the immense disturbance caused by transplantation, submersed plants are less able to resist the stresses of their new environment (Cho and May 2006; Boustany 2003). Alternative methods that account

for these stresses have been more successful. For example, Boustany (2003) pre-established young plants in a vegetative mat before anchoring that mat in the riverbed, which greatly reduced both hydrological and transplanting stress. However, all of these methods are labor-intensive and require a viable source of young plants; in the case of Boustany (2003), nontrivial amounts of greenhouse space was required for establishment in the vegetative mats.

The planting methods employed in this study aimed to reduce hydrological and transplanting stress by planting unsprouted vegetative propagules, called turions, in biodegradable cotton mesh bags. Each bag contained eight turions from different genotypes and is referred to as a "founder colony." To ensure that the founder colonies remained in the substrate long enough to sprout and establish, each bag was weighted down with gravel and anchored in the sediment with a pin flag or PVC pipe. These anchoring points also served as markers to ease colony monitoring. Tying the baggies together at regular intervals along a string further served to attach the colonies to each other and keep them rooted in the desired location. Furthermore, the combination of string, pin flags, and PVC made the colonies easier to locate and facilitated effective monitoring. I was able to feel along the string in the riverbed and find the founder colonies, still entangled in their bags, to measure the plants' growth. The pin flags were similarly helpful in locating colonies, especially when turbidity was heightened by my movements in the water.

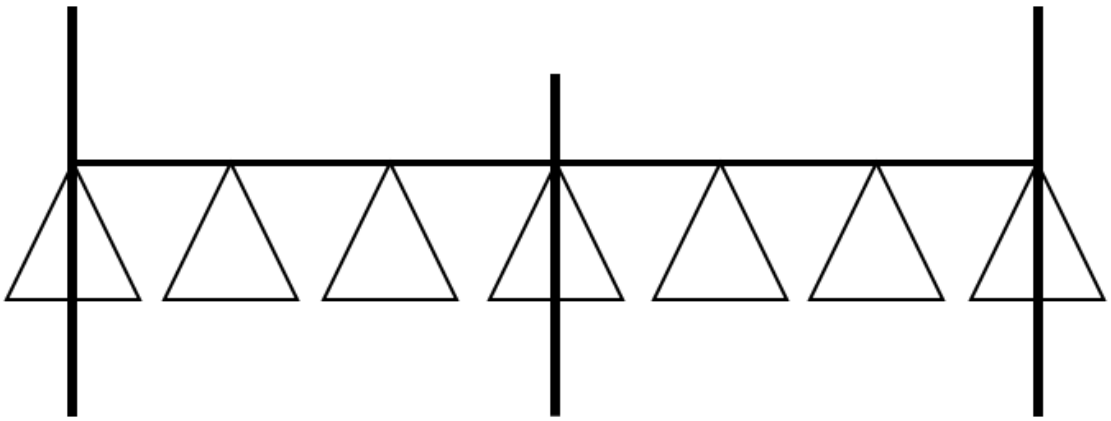


Figure 3.1. A single field transect. Each triangle represents a biodegradable cotton mesh bag containing eight *V. americana* turions, all of the same genotype, and some gravel for weight. Bags were tied together with string (horizontal black line) and stretched out in the riverbed in a straight transect for monitoring and stability. Vertical black lines represent PVC poles (center pole is 1.2 m, terminal poles are 1.5 m), used to anchor and mark the location of transects. Bags without poles were marked and anchored with pin flags.

The bag method is far less labor-intensive than other SAV restoration methods because it does not require digging to plant seedlings, nor is there a long period of plant establishment in the greenhouse prior to planting. Deployment of the bags does not require the use of divers and expensive diving equipment and support. Bag deployment could be further simplified, if close monitoring is not required, by dropping the bags into the river, rather than carefully placing them along strings into the sediments as I did. Bags, however, should always be anchored to ensure that they are not washed away or disturbed during establishment. Even so, bags I placed at one of my sites were washed away owing to high wave energy from ships' wakes. The use of turions eliminates the transplanting stress that has caused problems in past restoration efforts (Cho and May 2006) because turions contain stored energy that is used as plants first start growing and are not able to access light resources owing to their small stature. The use of turions in biodegradable mesh bags is also highly beneficial because turions can be stored in cold storage for several months and can be assembled into bags in the lab prior to deployment in the field. Bags are easily transferred in coolers into the field and do not require a lot of space. However, obtaining the turions for planting in the first place requires access to a sizable colony of SAV. My turions were obtained from greenhouse populations which had been grown in a controlled setting for several growing seasons. Lacking this resource, digging turions out of an existing SAV bed in the river would have been labor-intensive as well as disruptive to the plants. Additionally, the genotypic diversity of turion colonies would be lower than that of colonies established from sexually produced seeds (McFarland and Shafer 2008).

The Importance of Restoration Site Selection

Despite the various anchoring methods used in planting, most of the founder colonies at one of the three field sites were washed away by intense hydrological disturbance. This emphasizes the importance of selecting suitable sites for planting. Past restoration projects and studies have discussed the importance of site selection (Boustany 2003; Cho and May 2006), highlighting variables such as light availability (as relates to depth and water turbidity), salinity, and hydrology (Cho and May 2006; Boustany 2003; McFarland and Shafer 2008). Some water flow is desirable, to bring in new organic material and sediment, but high flow can scour nutrients from the sediment and uproot young plants (Koch 2001). The survival rates of my founder colonies at three different field sites highlighted the importance of site selection for inter- and intraspecific competition as well as suitable hydrology.

In selecting my field sites, I sought locations that were historical SAV sites and not subject to much disturbance. The most saline site, at Iona Island in Bear Mountain State Park, New York, had previously been home to beds of *Vallisneria americana,* but had not recovered from the 2011 storm season (Hamberg *et al.* 2017). The site was well away from the main shipping channel and tucked into a small cove near the shoreline of the island. When I planted my founder colonies in June 2017, patches of *Myriophyllum spicatum* (Eurasian watermilfoil) and *Trapa natans* (water chestnut)—both invasive species—were present, but no *V. americana* was evident. By the end of the summer, survival (*V. americana* presence) of my founder colonies

was about 50%, and *M. spicatum* grew around the planting site. *M. spicatum* is a highly competitive SAV species that produces allelochemicals that inhibit competitors' photosynthesis (Leu *et al.* 2002). Therefore, it is possible that *V. americana* survival and growth may have been negatively affected by the presence of *M. spicatum.* The results of the greenhouse experiment further showed that *V. americana* growth—specifically, plant height—was negatively affected by *H. verticillata* that I planted in half of the experimental units. Therefore, future restorations should seek sites that have little to no nonnative invasion at the time of planting.

Competition within species can be as inhibiting to colony growth and survival as competition between species, as illustrated by the Esopus Meadows site, which was located near the Esopus Meadows Preserve in Ulster Park, New York. Again, the site was selected because of its historic SAV beds, which had not fully recovered six years after the 2011 storms (Hamberg *et al.* 2017). Although this site was not as physically sheltered as the Iona Island site, the river was wide enough that the planting site was well removed from the shipping channel and largely unaffected by wave action from ships. At the time of planting in June 2017, I noticed some *M. spicatum* and *T. natans* growing nearby, as well as sparse *V. americana.* By the end of the summer, however, *V. americana* that was present at the site had grown and spread enormously, filling the spaces between my planting transects and—in many cases—mingling with my founder colonies, as revealed when the harvested plants were genotyped. Although it is undoubtedly desirable for SAV to grow so thickly in a

place where it has grown historically, the native bed of *V. americana* made it difficult to ascertain the success of my founder colonies. It is possible that the presence of my colonies increased site suitability by anchoring sediments and cleaning the water (Batiuk *et al.* 2000; Biernacki and Lovett-Doust 1997), thereby enabling the existing bed to grow and spread more successfully.

My third field site at Stuyvesant highlighted the importance of hydrology in SAV habitat suitability. At first glance, this site seemed as suitable as the other two: it was a historical SAV site, some distance from the shipping channel, and tucked into a cove like the Iona Island site. No SAV grew in the cove at the time of planting, and that remained true throughout the summer. When I returned to monitor the founder colonies after six weeks, I observed no detectable growth, and the pin flags were all gone. A large ship steamed by in the main river channel, its wake changing the water level in the cove by several feet and leaving waves (up to 1 m high) behind. Clearly, the founder colonies had been washed away. The small cove I planted in, which seemed sheltered at the outset, was a place where water disturbed by ships was funneled into a smaller space, increasing its power. Furthermore, the river at Stuyvesant is much narrower than at the other two sites, so the wake does not lose as much power over distance. When I returned to harvest at the end of the summer, I found only three tiny colonies. The colonies were genotyped to determine what they were.

Turion Size Advantage and Genetic Identity

By planting a variety of genotypes, I sought to test the importance of genotypic identity and diversity on founder colony survival and productivity. The initial size of turions varied between genotypes, and genotypes which sprouted from larger turions had produced taller plants by the end of the growing season. This variation illustrates the advantage provided by larger propagules and emphasizes the importance of planting a genetically diverse SAV community during restoration.

Before planting, I measured the length of each individual turion and weighed each founder colony (8 turions). By examining the turion size data from monoculture founder colonies, I determined that some genotypes have larger turions than others. Previous studies in both terrestrial and aquatic ecosystems (Stanton 1984; Lin and Sternberg 1995; Idestam-Almquist and Kautsky 1995; Doyle and Smart 2001) have found that plants which sprout from larger propagules—whether those propagules are produced sexually (seeds) or asexually (turions, stolons)—have a competitive edge in initial establishment and growth (Lin and Sternberg 1995; Doyle and Smart 2001). Because they are drawing from larger biomass reserves, young plants sprouting from larger propagules are able to grow quicker than their competitors, pre-empting valuable resources such as light, space, and nutrients (Lin and Sternberg 1995; Doyle and Smart 2001). Larger propagules are also better able to resist adverse establishment conditions, such as low light or sediment burial (Doyle and Smart 2001; Spencer 1987).

Given that larger turions provide an establishment advantage, and that some genotypes have larger turions than others, it is important to plant such genotypes in restoration projects to ensure their success in early establishment. Equally important, however, is ensuring the sustainability of the restored population by including high genotypic diversity that is locally adapted, even if some of those genotypes do not have the desirable large turions. The results of my field and greenhouse experiments show that the effect of large turion size is strong within the first weeks of growth but diminishes as plants mature. This suggests that smaller turions can catch up in growth through time. Even though large turions facilitate establishment, the genotype may not be optimally adapted to the local environment. A genotype with small turions may in the end have higher fitness. Thus, selecting only for large turion size in restoration design would be short-sighted, because initial establishment is only one of several life stages that determine a plant's survival, growth, reproduction, and vegetative expansion.

Biodiversity, whether at the community level or the species level, increases productivity (Tilman *et al.* 2001; Hooper *et al.* 2005) via the selection probability effect (the increased likelihood that a productive species or genotype will be present; Crutsinger *et al.* 2006; Tilman *et al.* 2001) or by the complementarity effect (overall resource use increases because species or genotypes use resources in different ways; Crutsinger *et al.* 2006). Increased diversity speeds up population recovery from disturbance via the sampling effect by increasing the likelihood that a species or genotype well-adapted to the new conditions, or more resistant to disturbed

conditions, is present in the population (Hughes and Stachowicz 2011; Kettenring *et al.* 2014). The long-term effects of biodiversity are particularly desirable for restored populations; otherwise, multiple restorations might become necessary, especially as human disturbance continues to increase (Sala *et al.* 2000; Batiuk *et al.* 2000).

Conclusions

Many factors need to be considered when planning the restoration of *Vallisneria americana* and other SAV communities. The importance of site selection cannot be overstated: restoration sites should have suitable environmental conditions as well as a lack of nonnative species. Although the presence of a native plant community is ultimately desirable, it can make restoration monitoring difficult. A variety of genotypes should be planted in restoration projects, with an emphasis on including genotypes with large propagules to ensure rapid early establishment. Finally, the methods of restoration should be carefully considered to minimize cost and labor intensity and reduce transplanting stress on young plants. If all these factors are taken into consideration, initial success and long-term sustainability of restored SAV populations is much more likely.

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