FACTORS AFFECTING THE GROWTH AND ESTABLISHMENT OF *VALLISNERIA AMERICANA* IN THE HUDSON RIVER, NY

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

The ecological importance of aquatic vegetation is due to its ability to attenuate waves, sequester nutrients, and provide habitat. One such aquatic macrophyte species is *Vallisneria americana,* whose genetic diversity in the Hudson River has been quantified. In the Hudson River Estuary, there has been a decline in aquatic vegetation exacerbated by large storm events in 2011. Because recovery has been slow, it is beneficial to restoration ecologists to know which genotypes of *V. americana* are more suited for restoration purposes due to their higher productivity. This study answered this question by planting a variety of *V. americana* genotypes in the Hudson River and allowing them to grow over the summer. Environmental conditions in the field affected survival and performance of plants, and certain genotypes established more successfully. There is a relationship between genotypic identity and tuber size, and a relationship between tuber size and summer growth that is also tied to genotypic identity. This information will allow managers to select productive, location-appropriate genotypes for restoration planting.

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INTRODUCTION

Expanding human populations are putting pressure on natural systems (Sala et al. 2000). Coastal aquatic systems are especially at risk, given the high human population density in these areas, and in need of conservation and restoration (Sala et al. 2000). Understanding the processes that allow ecosystems to function as naturally as possible, given continuing human disturbance, is an important line of scientific inquiry with applications in restoration ecology and natural resources management. This experiment focuses on the restoration of submerged aquatic macrophytes in a tidal river, which affect ecosystem functioning locally, and whose health and function affects downstream reaches.

Submerged aquatic vegetation (SAV) performs many important ecosystem functions, including wave attenuation, sediment capturing, nutrient sequestration, and habitat provision (Biernacki and Lovett-Doust 1997; Rybicki and Landwehr 2007). Many of these functions improve water quality (Biernacki and Lovett-Doust 1997), but plant survival is impeded by high turbidity and reduced light availability (Moore et al. 2010; Batiuk et al. 2000; Rybicki and Landwehr 2007). Nutrient loading in aquatic systems leads to excess nutrients and sediments in the water column, thereby increasing turbidity (Moore et al. 2010; Batiuk et al. 2000) and causing widespread declines of SAV populations in the twentieth century (Batiuk et al. 2000; Orth and Moore 1984). The recovery of SAV populations is impeded by large storms that flood rivers with nutrients and sediment (Ralston et al. 2013; Orton et al. 2012; Orth and Moore 1984) as well as by continued chronic light limitation (Lefcheck et al. 2018). Recovery is further impeded by the establishment of invasive species of SAV, such as *Trapa natans* or *Myriophyllum spicatum*, in sites historically occupied by native species (Findlay et al. 2006;

Rybicki et al. 2001; Orth and Moore 1984). Grazing by fishes and waterfowl inhibit species' growth and ability to propagate (Van Onsem and Triest 2018; Van Donk and Otte 1996), and the prevention of grazing via the use of herbivory "exclosures" can increase plant growth throughout the season (Van Donk and Otte 1996). Given these environmental stresses and the negative consequences that SAV loss has on ecosystems, understanding processes that facilitate the reestablishment and restoration of these populations is important for estuarine management.

An important component of ecological restoration is the maintenance of inter- and intraspecific diversity. Higher biodiversity, at both the community and species level, increases productivity and facilitates ecosystem recovery following a disturbance (Tilman et al. 2001; Hughes and Stachowicz 2011). Within species, genotypes can differ in their phenotypic expression and resource use (Bischoff et al. 2009; Engelhardt et al. 2014) so that populations with higher genotypic richness are higher performing in disturbed or undisturbed ecosystems (Hughes et al. 2008). This experiment focuses on the idea of genotypic identity and diversity in restoration, using the submerged aquatic macrophyte *Vallisneria americana* as the 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, each one sprouting a shoot referred to here as a "ramet," 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). Its productive cloning enables the collection of large numbers of genetically identical plants, which is ideal for experimenting on the genetic variation within a species.

The Hudson River Estuary (HRE) is home to an SAV community historically dominated by *V. americana*, although there has been a decline in coverage areas (Findlay et al. 2006). In 2011, tropical storms Irene and Lee inundated the river with sediments, as well as a 70-year high flow event (Ralston et al. 2013; Orton et al. 2012). This water and sediment influx washed away many existing macrophyte communities, and increased turbidity and sediment deposition have inhibited reestablishment (Hamberg et al. 2017). In addition to these catastrophic events, communities of *V. Americana* in the HRE face competition from *Trapa natans* (water chestnut) and *Myriophyllum spicatum* (Eurasian watermilfoil), invasive species that have colonized vacated sites (Findlay et al. 2006). These conditions provide an ideal setting in which to test the effect of *V. americana* genotypic identity on establishment and growth.

This study sought to identify whether there was a relationship between *V. americana* genotypic identity and establishment success at restoration sites in the Hudson River. The hypothesis was tested that some genotypes are more productive than others, and therefore better suited to restoration purposes. Additionally, herbivory exclosures similar to those used by Van Donk and Otte (1996) aimed to examine the relationship between grazing and establishment success. An additional hypothesis was tested that those plants protected by the exclosures would not be grazed, and therefore more successful in establishment and growth.

METHODS

Twenty-two genotypes of *V. americana* were selected from a repository of 187 genotypes that were sampled from across the salinity range within the Hudson River Estuary: 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). The repository has been cloned since 2015 in the University of Maryland Center for Environmental Science Appalachian Laboratory greenhouse and since 2011 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.

Three field sites were selected in different sections of the tidal Hudson River: Iona Island, Esopus Meadows, and Stuyvesant (Figure 1). Sites were ca. 75 km apart to test restoration growth under different environmental conditions. Publicly available data from the Hudson River Environmental Conditions Observing System (HRECOS) showed that factors such as dissolved oxygen, turbidity, and salinity varied between the three sites (Table 1).

Each of the three planting sites received 42 founder colonies planted with eight turions each. Eight location-appropriate genotypes were planted at each site, consisting of two genotypes from each of four collection sites. Selection of genotypes for each study site was based on genetic similarity, turion availability, and geographic proximity to the planting sites. One genotype from each collection site was used for the herbivory experiment. Each genotype monoculture was replicated three times (eight genotypes x three replicates = 24 colonies), with another set of 3 replicates for the herbivory experiment (four genotypes x three replicates = 12 colonies), and six polycultures containing one turion from each of the eight genotypes (six colonies).

Prior to field implementation, the turions of each founder colony were weighed, and the individual turions' lengths were measured. 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 (Figure 2). This created six transects of seven founder colonies each.

In the field, the transect strings were spaced approximately 2 m apart and placed parallel to each other. The first, fourth, and seventh founder colony on each transect was marked with a PVC pipe anchored in the riverbed; all other colonies were marked with pin flags (Figure 2). For the herbivory experiment, 12 of the colonies were enclosed by small, cone-shaped cages of plastic mesh. These were intended to exclude herbivores and facilitate increased growth.

The field plantings were monitored for the first time after about six weeks of growth. The turbidity of the water prevented precise measurements, plant height was approximated using a PVC pipe marked off in 10 cm intervals. Additionally, notes were taken on the nature of the surrounding plant community, if any existed.

After ten weeks, the colonies were harvested. The cotton mesh bags were intact enough that the plantings were still entwined in them; thus the bags and all attached ramets were used to assess colony survival and growth. At Esopus Meadows, the density of the surrounding *Vallisneria* growth made this difficult, and it seems likely that plants were harvested which were not part of the founder colonies. Back in the lab, each colony was weighed (wet and dry); each colony's ramets were counted, and four leaves were measured and average together to estimate colony height.

Correlation analyses were used to quantify the effects of initial turion size on 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). Analyses of variance (ANOVA) and analyses of covariance (ANCOVA) were used to quantify the effects of continuous explanatory variables (initial turion size; random effect) and categorical explanatory variables (genotype, population, planting site; fixed effects) on plant height, biomass, and ramet and turion production.

Table 1: **Summary of environmental variables at the three 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. Salinity (ppt) was calculated using this formula: specific conductance (mS/cm)$^{1.0878}$ * 0.4665**

Planting Site	HRECOS station (dist. to site)	Available dates	DO (% air saturation)	Turbidity (NTU)	Salinity (ppt)
Stuyvesant	Shodack Is. (~11.3 km)	6/1/16 – 8/31/16	83.78 (60.3 – 115.5)	7.31 (0 – 97.3)	0.11 (0.1 – 0.13)
Esopus Meadows	Norrie Pt. (~5.3 km)	6/1/16 – 8/31/16	87.71 (60 – 105.7)	5.56 (0 – 167)	0.11 (0.1 – 0.13)
Iona Island	West Pt. (~9.4 km)	7/14/16 – 8/31/16	80.88 (74.4 – 92.9)	21.99 (7.7 – 110.3)	1.57 (0.57 – 3.36)

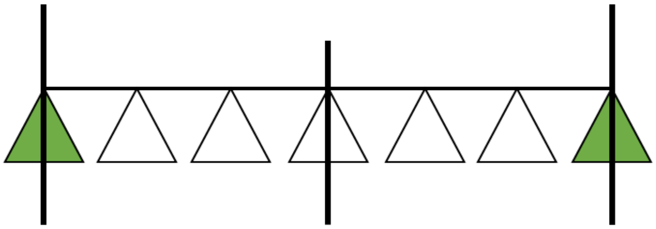

Figure 1: Representation of a single transect in the field. Vertical lines represent PVC pipes anchoring the bags to the riverbed. Green triangles represent caged colonies.

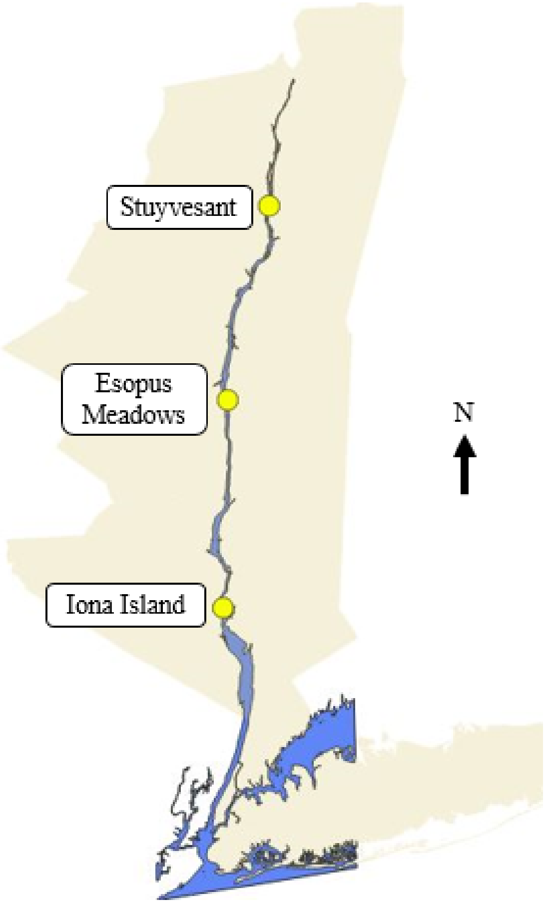

Figure 2: Locations of the three study sites in the Hudson River Estuary. Only the tidal portion of the river is pictured, which terminates at the Hudson River Lock & Dam in Troy, New York.

RESULTS

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 (Figure 3, Figure 4). Survival decreased to 55% at Iona Island by the end of the summer (Figure 3) but remained >80% at Esopus Meadows (Figure 4). No colonies survived at Stuyvesant; therefore, Stuyvesant is removed from any subsequent analyses.

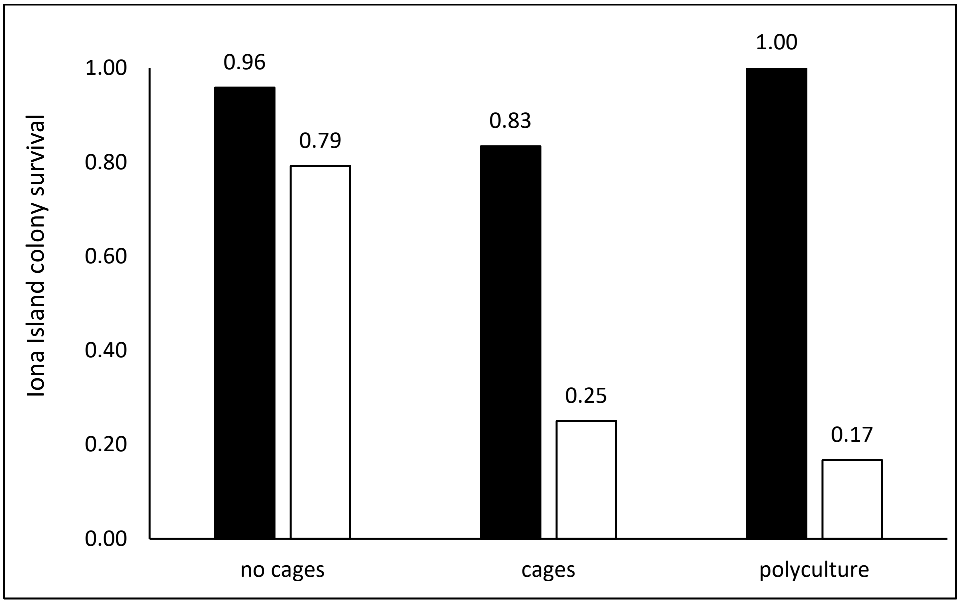

Figure 3: The proportion of colony survival at Iona Island, by treatment, at the time of monitoring (black bars) and the time of harvest (white bars).

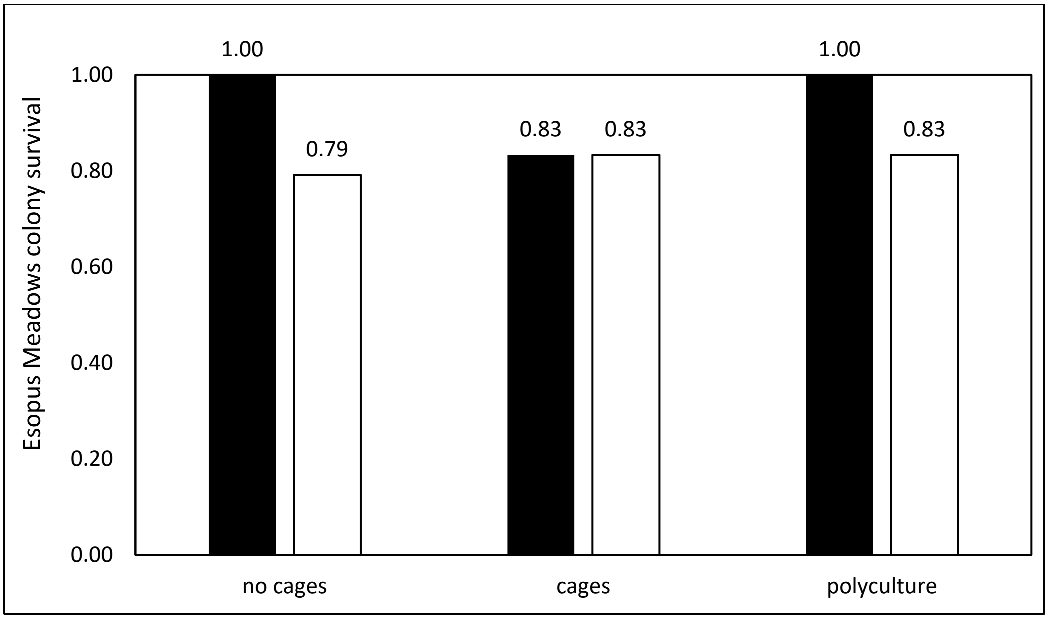

Figure 4: **The proportion of colony survival at Esopus Meadows, by treatment, at the time of monitoring (black bars) and the time of harvest (white bars).**

The herbivory exclosures had no effect on growth, nor did genotypic diversity (eight versus one genotype/s per founder colony). Therefore, these colonies are removed from any subsequent analyses.

The total turion weight of each founder colony differed between genotypes and populations (Table 3; Figure 5), and initial turion weight was positively correlated with midsummer plant height (Table 2). For example, the two Turning Point (TP) genotypes had relatively large turions (Figure 5) and relatively tall plants at midsummer (Figure 6). In contrast, Croton (Cr) genotypes had relatively small turions (Figure 5); these genotypes also had low survival and produced shorter plants in those colonies that did survive (Figure 6; Figure 7). Given this initial variation in turion size between genotypes, turion size was included as a covariate in subsequent ANCOVA models (Table 3).

Table 2: **Correlation analyses performed on field experiment data. Stuyvesant data are included in turion weight analyses but not in growth analyses due to low survival. Harvest height data were normalized using a square root transformation.**

*** p<0.05; **p<0.01; ***p<0.001**

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 wet weight	Harvest ramet count	0.41***

Table 3: **Analysis of variance (ANOVA) and analyses of covariance (ANCOVA) performed on field experiment data. Turion weight is the covariate in ANCOVA analyses. Harvest height data was normalized using a square root transformation.**

***p<0.05; **p<0.01; ***p<0.001**

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
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
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

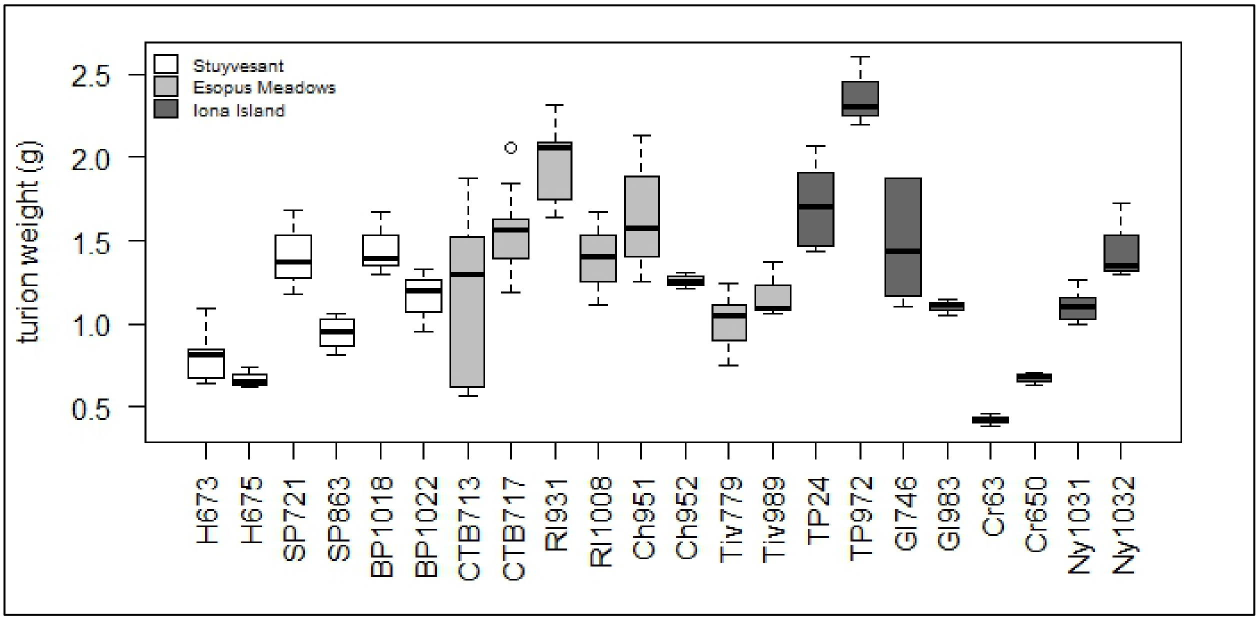

Figure 5: **The turion size of genotypes planted in the Hudson River Estuary. Genotypes are labeled by population (*i.e.*, TP for Turning Point) and identifying number.**

Midsummer (six week) plant height, a categorical estimate made using a PVC pipe marked in ten-centimeter increments, varied between genotypes, source populations, and planting sites (Table 3; Figure 6). The variation in plant height lessened by the end of the summer but still differed between genotypes and planting sites (Table 3; Figure 7). The plants at Iona Island, the most saline and turbid of the three sites (Table 1) tended to be shorter than the plants at Esopus Meadows (Figure 7). Colonies that were taller at midsummer tended to be taller at harvest (Table 2).

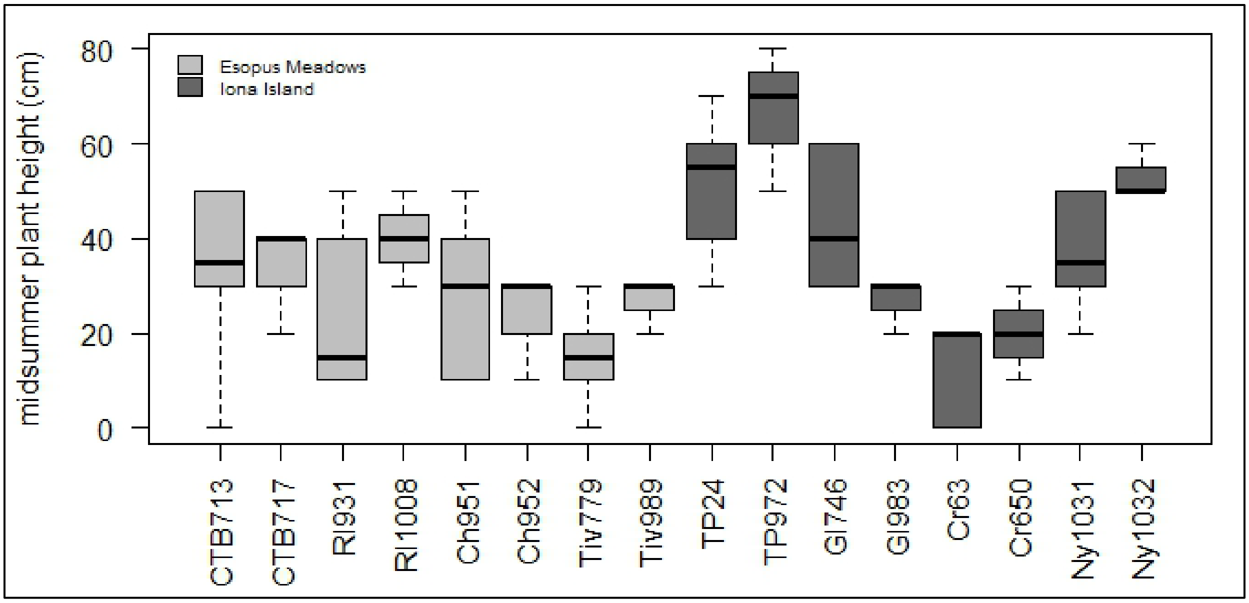

Figure 6: **The approximate maximum height of plants after six weeks of growth. Genotypes are labeled by population (*i.e.,* TP for Turning Point) and identifying number. Stuyvesant data has been removed due to lack of survival.**

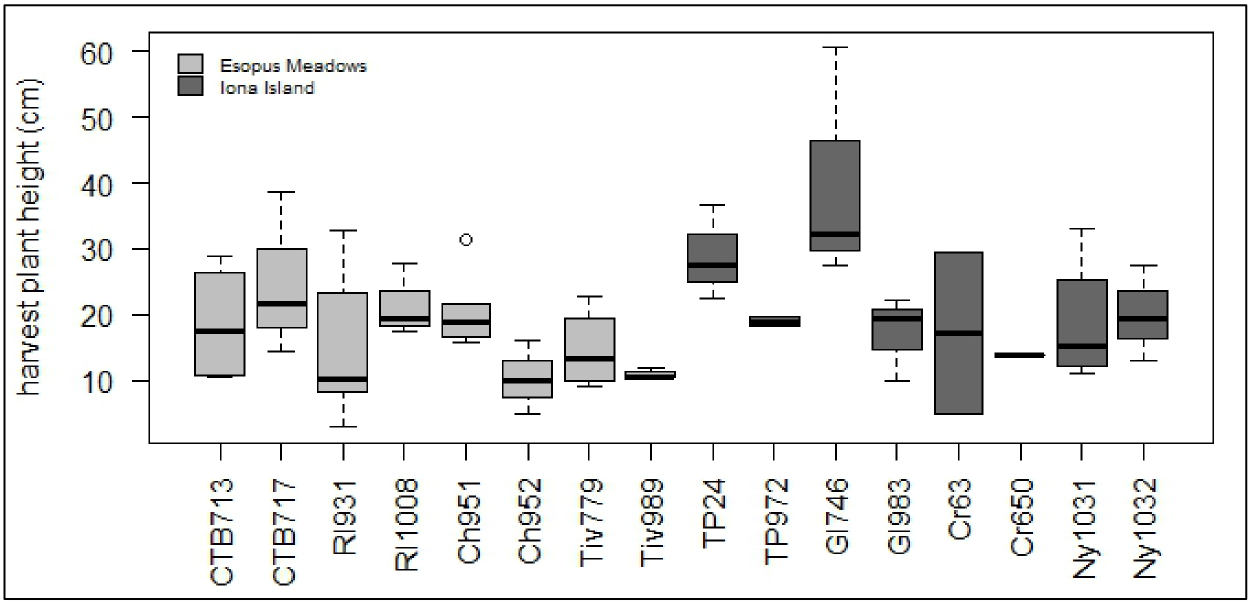

Figure 7: **The average height of plants after ten weeks of growth (the time of harvest). Genotypes are labeled by population (*i.e.,* TP for Turning Point) and multilocus genotype (MLG) number. Stuyvesant data has been removed due to lack of survival.**

The total wet weight of harvested founder colonies varied only between planting sites (Table 3) but was positively correlated with both midsummer and harvest height (Table 2). Initial turion weight accounted for some of the height variation at midsummer (Table 3) but had no effect on the height and weight of harvested plants (Table 3).

The number of ramets (individual shoots, sometimes connected by underground stolons and therefore part of the same genetic individual) produced by harvested founder colonies varied by genotype and planting site (Table 3; Figure 8). Harvested colonies at Esopus Meadows produced 77 percent more ramets on average than the colonies harvested at Iona Island. The number of ramets was positively correlated with total wet weight (Table 2).

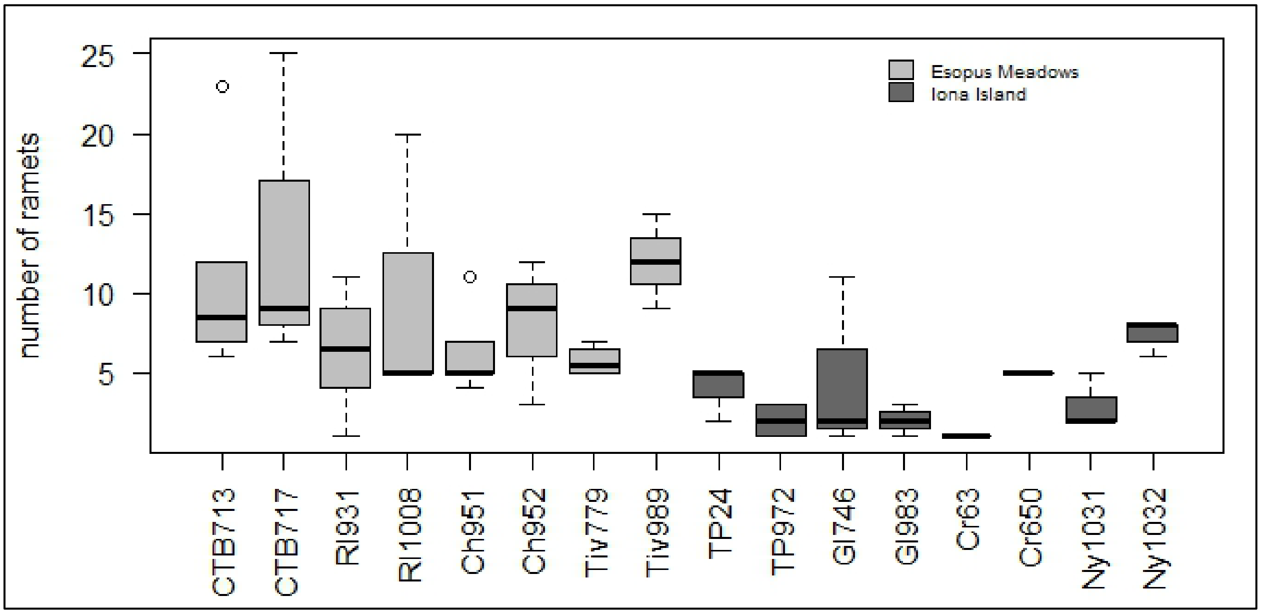

Figure 8: **The number of ramets produced after ten weeks of growth (the time of harvest). Genotypes are labeled by population (*i.e.,* TP for Turning Point) and identifying number.**

DISCUSSION

The results of this experiment suggest that that survival and first-year growth of *V. americana* is influenced by genetics and habitat characteristics. Turion size varied among genotypes (Table 3, Figure 5), and larger turions produced taller plants through the duration of the growing season (Table 3, Figure 6, Figure 7). Greater plant height was associated with greater biomass and increased ramet and turion production (Table 3, Figure 8), suggesting that an early growth advantage may have lasting and potentially inter-generational effects; however, planting sites need to be selected carefully for optimal conditions.

The herbivory cage treatment showed very low survival (Figure 3, Figure 4). Plants that managed to establish in the cages were sheared off by ships' wakes and tidal currents when they cleared the mesh. By the end of the summer, few of these colonies had survived, so no conclusions can be drawn about the impact of herbivory. The polyculture treatment showed similarly low survival (Figure 3, Figure 4); however, there were only six polyculture plantings per site, so these results do not illustrate the effect of genetic diversity well.

There were environmental differences between sites that may have affected their survival and growth. Colony survival was highest at Esopus Meadows (Figure 4), where there was already an established bed of *V. Americana* with some invasive species (*Trapa natans, Myriophyllum spicatum).* The colonies planted near Iona Island have moderate survival (Figure 3), but there was no other native vegetation in the area and some invasive species (mostly *M. spicatum*). At Stuyvesant, there was no other vegetation growth of any kind, and only two colonies survived the entire summer. This site was in a small inlet not far from the main shipping channel; it is likely that the pull of ships' wakes uprooted plants that managed to establish. High wave action or current velocity can uproot aquatic plants, with young plants being particularly vulnerable (Koch 2001; Madsen et al. 2001).

The size of *V. americana* turions varied among genotypes (Table 3, Figure 5), and larger turions produced larger plants throughout the growing season (Table 3, Figure 6, Figure 7). Intraspecific size variation in vegetative propagules (such as shoots or tubers) and seeds has been observed in both terrestrial (Stanton 1984) and aquatic plant species (Lin and Sternberg 1995; Idestam-Almquist and Kautsky 1995; Doyle and Smart 2001), illustrating the benefits of larger propagules. In plants, larger offspring have a higher chance of survival in adverse conditions (Idestam-Almquist and Kautsky 1995). Greater biomass in larger propagules allows plants to grow faster and produce more leaves early on (Lin and Sternberg 1995; Doyle and Smart 2001), enabling them to overcome sediment burial and resist mechanical disturbance (Idestam-Almquist and Kautsky 1995). Aquatic plants that sprout quickly and produce large leaves have a greater chance of surviving and thriving in low light conditions, because their height and high leaf surface area better enable them to intercept available light (Doyle and Smart 2001).

In conclusion, different genotypes of the aquatic plant *Vallisneria americana* have varying levels of establishment 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 account for local environmental conditions to ensure the success and long-term survival of SAV communities.

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