ASSESSING MODE OF REPRODUCTION IN *VALLISNERIA AMERICANA* OF THE HUDSON RIVER, NY AND THE CHESAPEAKE BAY, MD

A Final Report of the Tibor T. Polgar Fellowship Program

Carrie E. Perkins

Polgar Fellow

Plant Sciences
University of Maryland-College Park
College Park, MD 20742

Project Advisor:

Dr. Maile C. Neel
Plant Sciences
University of Maryland-College Park
College Park, MD 20742

Perkins, C.E. and M.C. Neel. 2020. Assessing mode of reproduction in *Vallisneria americana* of the Hudson River, NY and the Chesapeake Bay, MD. Section VI:1-59 pp. *In* S.H. Fernald, D.J. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2018. Hudson River Foundation.

ABSTRACT

Vallisneria americana, a dioecious macrophyte native to eastern North America, is capable of both sexual and asexual reproduction. Mode of reproduction plays a role in determining amounts and structure of genetic diversity at multiple scales, potential for dispersal, and resilience to disturbances. Observations from 2015 indicated that sexual reproduction in the Hudson River was lower than in the Chesapeake Bay. Mode of reproduction was assessed at 14 sites on the Hudson River representing tidal-saline, tidal-fresh, and non-tidal environments. Three tidal Chesapeake Bay sites were sampled for comparison. Owing to previous findings that high salinity and low temperature inhibit flowering in *V. americana,* it was hypothesized that flowering would be positively correlated with large plant size, high water temperature, low salinity, high genotypic diversity, and high bed density. In July, male and female inflorescences were counted using shoot-level transect sampling and the size of each plant was recorded. In August, female flowers were counted and the presence of male flowers was noted using surface transect sampling. Chesapeake Bay sites had an average of 312.3 inflorescences in shoot-level sampling and 1137.3 female inflorescences in surface sampling, whereas the averages for the Hudson River were 15.64 and 431.5, respectively. The highest-producing Hudson River sites had statistically similar numbers of inflorescences to Chesapeake Bay sites. Plants with long leaves and many ramets were more likely to flower. Chesapeake Bay plants had longer leaves and Hudson River plants had more ramets. Spatial isolation of sexes was evident at both the estuary scale and transect scale. The results of this study give managers a new way to evaluate the resilience of *V. americana* beds throughout the Hudson River, informing restoration decisions.

TABLE OF CONTENTS

LISTS OF FIGURES AND TABLES

INTRODUCTION

Like many native aquatic plants worldwide, *Vallisneria americana* Michx. in the Hudson River Estuary is threatened by chronic poor water quality and increasingly extreme and unpredictable weather events. Because this species grows submersed in the water column, it is especially vulnerable to conditions that limit light, such as excessive suspended sediment and high nutrient levels in the water (Orth and Moore 1983: Davis 1985). This dependence is strong enough that often submersed species are biological sentinels, alerting us to declines in water quality (Orth et al. 2017). Chronic light limitation (e.g., Davis 1985) and rare but extreme weather events (Bayley et al. 1978) have caused massive, decades-long declines in *V. americana* across eastern North America (Brush and Hilgartner 2000; Findlay et al. 2014). Most recently, intense flooding, scouring, and sediment deposition from Hurricane Irene and Tropical Storm Lee in 2011 caused major losses throughout the Hudson River (Hamberg et al. 2017). Before those storms, *V. americana* was the dominant submersed aquatic species in the Hudson River (Nieder et al. 2004, 2009), typically forming single-species or low diversity beds through a combination of vegetative and sexual reproduction.

Both long-term declines and catastrophic losses are of great concern to managers because submersed aquatic plants such as *V. americana* are foundation species and ecological engineers (Orth et al. 2006). They play critical roles in providing habitat for fish and crustaceans (Heck and Thoman 1984) and sustenance for waterfowl (Lubbers et al. 1990), oxygenating water (Findlay et al. 2006), stabilizing sediments, and removing excess nutrients from the water (Sand-jensen 1998). As such, potential for resilience in *V. americana* is of great interest to managers and scientists.

Populations are said to be resilient in the face of disturbance if they are able to absorb change while maintaining the same function, regain essential functions after a loss, or acclimate in response to altered environmental conditions (Holling 1973; Sgrò et al. 2011). Resilience can take many forms, and could include habitat patches persisting year-to-year, patches shifting while maintaining the same core location, patches continuing to provide a constant amount of ecosystem services, and patches being lost but regained through colonization. Each of these forms of resilience fundamentally requires that plants reproduce and that propagules reach suitable habitat in which they can grow and ultimately reproduce themselves. The ability to grow requires environmental conditions within the range of tolerance for the species. Reproduction and dispersal strongly affect the amounts and distribution of genetic diversity which increases the potential for tolerance of and acclimation to a broader range of conditions, and for adaptation as conditions change (e.g., Procaccini and Piazzi 2001; Hughes and Stachowicz 2004; Sgrò et al. 2011). For clonal species such as *V. Americana,* relative amounts of asexual versus sexual reproduction are particularly important for determining the structure of genetic diversity (Eckert and Barrett 1993). Thus, without any reproduction even short-term resilience is not possible, and longer-term resilience depends on the mode of reproduction (asexual versus sexual) and dispersal.

Dramatic recovery since the 2011 storms provides good evidence for resilience in *V. americana*; however, the species is still absent from some locations that supported large beds, indicating recovery is not complete. Of more concern for the future is that genetic sampling in 2015 indicates that most sites have relatively low genotypic diversity (i.e., they consist of many copies of one or a few genetic individuals) compared to most

of the Chesapeake Bay and tidal Potomac River. Further, more genotypes are found to occur at multiple sites. These patterns suggest that recovery was accomplished largely through asexual reproduction and dispersal of vegetative propagules rather than via sexual reproduction.

Because *V. americana* is dioecious (each genotype is either male or female), spatial isolation of the sexes can hinder sexual reproduction. For pollen to reach the female flowers that float on the water's surface, male flowers must rise to the surface after breaking free from the inflorescence that grows from the base of shoots. They contact the female either as complete flowers or as floating pollen. Pollen movement is extremely localized, typically to 2 to 5 m (Lloyd et al. 2018). In the extreme, spatial isolation can arise from chance Allee effects if only one sex reaches a site during founder events or if one sex is lost due to population bottlenecks (Eckert and Barrett 1993). If isolated beds consist of only one sex, sexual reproduction is impossible. Even when both sexes are present, low genotypic diversity can limit reproduction through sex bias (Engelhardt et al. 2014) and extensive clonal growth that isolates males and females. This reduced sexual reproduction may limit resilience to future change or disturbance if low genotypic and genetic diversity yields low potential to acclimate or adapt.

Even more fundamental than the spatial distribution of male and female inflorescences, is flowering itself. Preliminary observations during field sampling for genetic analysis in 2015 indicated that many *V. americana* beds in the Hudson River produced fewer inflorescences than those in the Chesapeake Bay and Potomac River. Female flowers were observed at only a few sites. Few male inflorescences (composed of hundreds of highly reduced flowers) were noted on collected plants, and almost no

male flowers were seen on the water surface in the field. Not surprisingly, given the lack of male flowers, very few mature fruits were seen even where female flowers were present.

Although intriguing, these observations were anecdotal and better understanding of the capacity for sexual reproduction in the Hudson River was needed. In summer 2018, two field studies were conducted to quantify inflorescence production and potential for pollination in Hudson River plants and compare them to the Chesapeake Bay. In July, fine-scale sampling was conducted to count female and male inflorescences on collected plants. Although extremely informative, this detailed sampling represented only a small area of each sampled bed. To estimate flowering and reproduction over a larger area at each site, this detailed sampling was conducted in August when flowers and fruits visible at the surface of the water on longer transects were counted.

To test hypotheses for possible mechanisms for limited flowering, the field sampling was augmented with a mesocosm study and with genetic and greenhouse data collected previously in a related project. Five hypotheses for low levels of flowering were tested: 1) Plants that had colonized after the 2011 storms had not reached reproductive size in 2015, given known effects of plant size on flowering (Titus and Hoover 1991; Engelhardt et al. 2014); 2) Salinity or temperature were not conducive to flowering. Flowering is negatively affected when salinity is above 5 to 10 ppt (French and Moore 2003). Although studies have suggested that water temperature is important for flowering (Titus and Stephens 1983; Titus and Hoover 1991; Best and Boyd 2001), there are no data on specific temperature requirements; 3) Genotypic diversity was too low to yield flowering (Engelhardt et al. 2014); 4) Density is too low or beds are too

patchy to support flowering for either of two possible reasons. First, positive feedbacks in dense beds result in heightened local water clarity (Gurbisz and Kemp 2014). Second, higher density beds may reflect more resource rich environments that support both more vegetative growth and more sexual reproduction; 5) Plants in the Hudson River have lower capacity to flower due to an intrinsic shift towards asexual reproduction.

Beyond flowering, potential for pollination was assessed by quantifying proximity of male and female individuals at and within sites, and whether these proximities differed in relation to salinity and genotypic diversity was tested. The results of this assessment of reproduction give managers an indication of both short- and long-term resilience of sites spanning the salinity gradient of the Hudson River, and highlight the specific risks. Quantifying the number of inflorescences and their sex ratios makes it possible to assess the potential for sexual reproduction and for future genotypic diversity among sites. Sites that produce many flowers of both sexes have the potential to generate new genotypes resulting in higher genotypic diversity and the ability to acclimate and adapt to changing environmental conditions (e.g., Procaccini and Piazzi 2001; Hughes and Stachowicz 2004; Sgrò et al. 2011). By contrast, sites that persist through vegetative reproduction and primarily amplify existing genotypes by cloning may have limited capacity under changing conditions (Eckert et al. 2016). Determining if, and specifically, how flowering or pollination is limited provides direction to managers who may seek to increase reproduction to increase diversity.

METHODS

Sampling sites were chosen to represent the non-tidal (n=2), tidal-fresh (n=7), and tidal-saline (n=5) environments of the Hudson River (Figure 1). Three tidal Chesapeake Bay sites were selected to provide comparison (Figure 2). All sites were sampled twice in 2018. During small-scale intensive sampling in July, flowering on individual shoots was assessed and flowering was associated with characteristics of those shoots, as well as of each site. These detailed samples represented only a small part of each site. In August, sampling took place on longer transects to get a more comprehensive estimate of flowering at each site, but flowering could not be related to characteristics of individuals. All sites had previously been sampled for genetic analysis in 2015 and 2016 at the University of Maryland College Park. Resulting unpublished data were used to estimate genotypic diversity at each site. From those same studies, plants from 30 samples from each of 11 of the Hudson River sites and 15 samples from each of two Chesapeake Bay sites had been propagated at the University of Maryland Center for Environmental Science Appalachian Laboratory. Variation in flowering was quantified among genotypes from a subset of sampled sites in a mesocosm experiment planted with turions from these propagated individuals. Further, data on flowering from the greenhouse samples were summarized to provide an additional assessment of capacity for flowering of Hudson River genotypes.

Shoot-Level Sampling

In July, flowering was estimated on all *V. americana* shoots collected from 20 to 25, ~0.04 m^2 samples taken every ~4 m along a transect line at each site (Table 1). The path of each transect and location of each sample were recorded using a handheld Garmin

Etrex 30 GPS unit. If a sample frame had no plants, the absence was noted and sampling continued until at least 20 samples with plants were obtained.

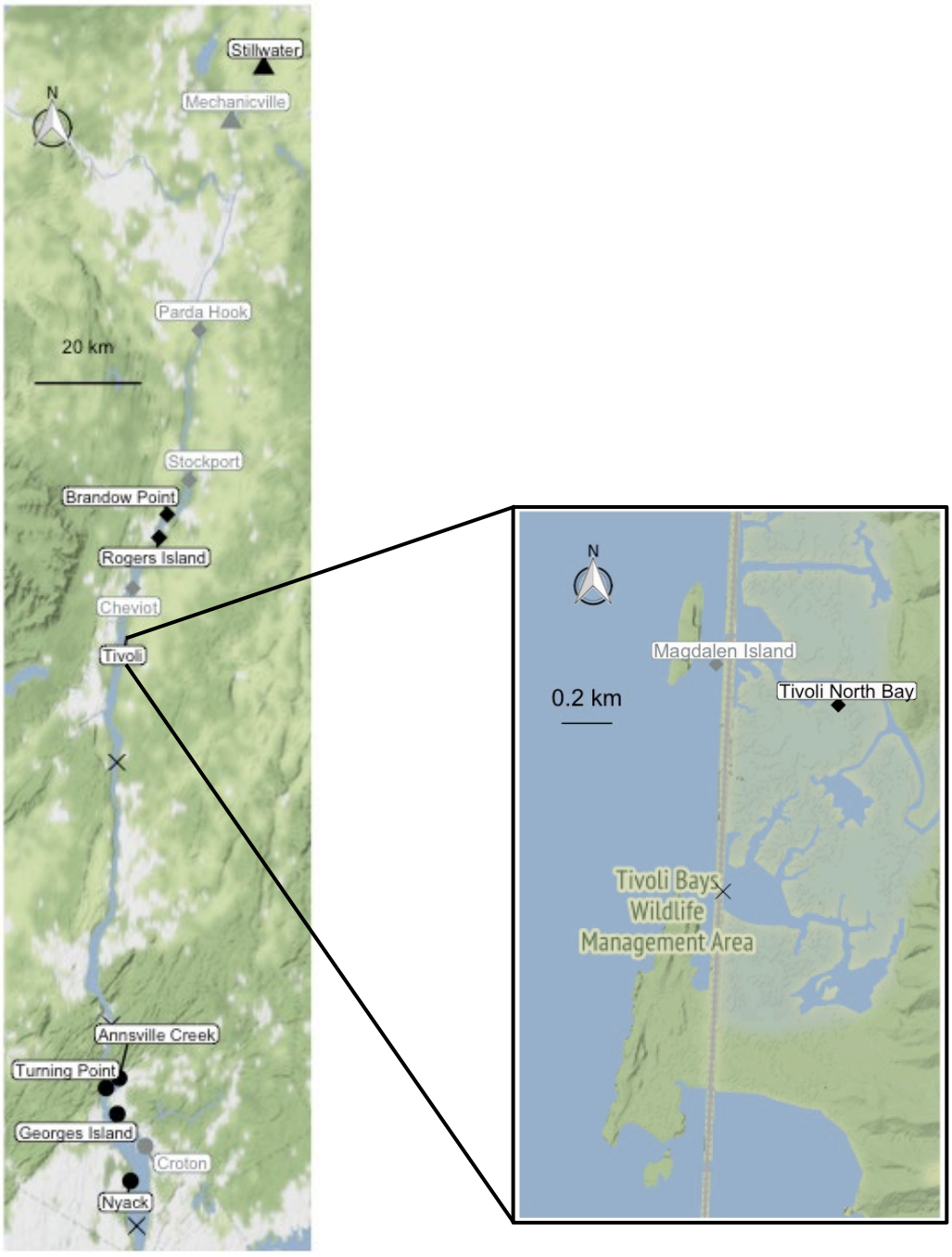

Figure 1. **Map of Hudson River sampling sites showing tidal regime (▲= non-tidal, ♦= tidal-fresh, and ● = tidal-saline) for each site and whether the site was included (gray) in the mesocosm experiment or not (black). HRECOS monitoring station sites (×) are also noted.**

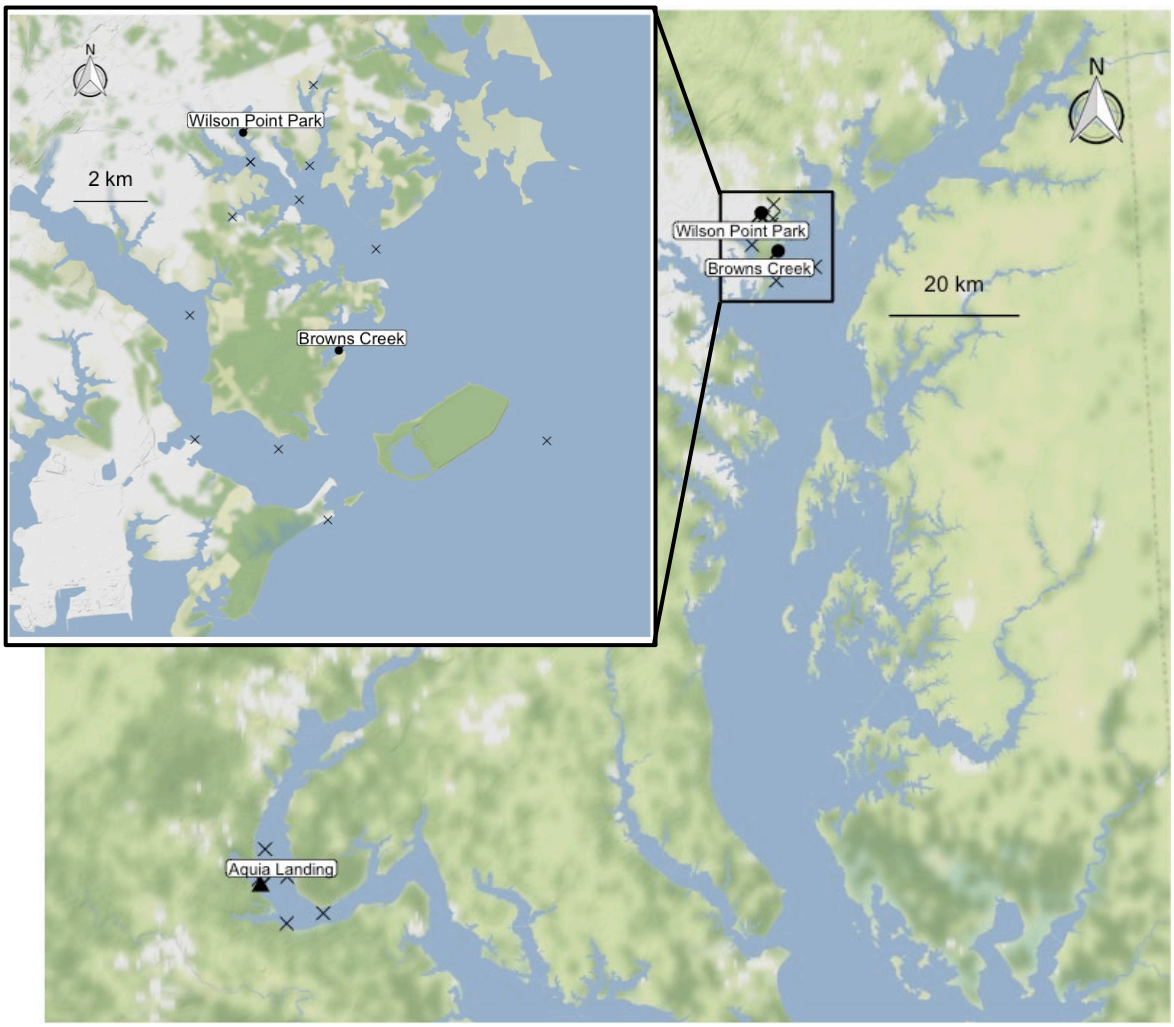

Figure 2. Map of Chesapeake Bay sampling sites showing region (●=Central Bay and ▲=Potomac River). Water quality monitoring station sites (×) are also noted.

All single ramets and ramets connected by stolons in each sample were counted, as were stolons that were connected to undeveloped ramets or that had been connected to shoots but were broken. To avoid confusing them with undeveloped ramets, the term *shoots* is used to refer to ramets on which leaves were expanded and green such that they could photosynthesize and potentially support inflorescences. Each separate shoot or set of connected shoots is referred to as an individual; however, many of them could be the same genetic individual.

All female flowers and male inflorescences were recorded on each ramet (hereafter collectively called inflorescences for convenience). Sexual reproductive status of each shoot was noted as male, female, or non-flowering based on presence or absence of inflorescences. Individuals were noted as male or female if any shoot possessed inflorescences of the respective sex, and were coded as non-flowering if there were no inflorescences. The number of inflorescences on each shoot was counted. The number of shoots that were female, male, and non-flowering, and the number of inflorescences were summarized at the individual, sample, and site levels.

Plant size was measured in three ways. First, the number of connected shoots and the number of stolons that were broken or attached to undeveloped shoots (hereafter referred to collectively as total number of ramets) were summed per individual. Second, the length of longest leaf of each shoot was measured to the nearest centimeter. Third, for each single shoot or connected set of shoots, the width of the widest leaf was measured to the nearest millimeter. The maximum shoot leaf length, leaf width per individual, and number of ramets per individual were used to analyze the effect of plant size on flowering.

Although it was not feasible to collect detailed environmental data at each site, sampling from three broad tidal and salinity environments in the Hudson River made it possible to categorically assess the effects of salinity on flowering. Salinity data from three Hudson River Environmental Conditions Observing System (HRECOS) monitoring stations - Norrie Point, NY (lower to mid tidal-fresh environment), West Point (near the upper end of the tidal-saline), and Piermont Pier, NY (lower tidal-saline environment) were used to describe different sampling sites within the River (HRECOS 2018).

Temperature data from the aforementioned Hudson River stations as well as the station at Tivoli, NY, and from 16 monitoring stations in the Chesapeake Bay were used to compare minimum daily water temperature in the early growing season between the regions (Data Download | Chesapeake Bay Program 2018).

Overall bed density was estimated using the mean and coefficient of variation (CV) of the number of shoots per sample at each site, including samples that had no plants. Density within patches was also calculated by excluding empty buckets for the calculations. Patchiness was estimated as the ratio of samples with plants to the total number of samples needed to obtain ≥20 samples with plants. Dense, continuous beds will have a high average number of shoots with a low CV, and a ratio of occupied to total samples near 1.

Surface Sampling

In August, all female flowers at the water surface were counted and presence of floating male flowers was noted along two transect lines that were mapped using Garmin GPS units. Transects were a minimum of 500 m long and observed from kayaks, and the sampling area was limited to 0.9 m on either side of the boats. The maximum length depended on the extent of plants and amount of flowering at each site (Table 1). More search effort was expended when no or few flowers were found. The size was standardized for the statistical analysis described below to density of female flowers found per 1000 m^2 to allow comparison across sites. Waypoints were used to identify the start and end of *V. americana* patches along the transect and to identify line segments with which to quantify spatial distribution of flowers along the transects. The method of marking line segments depended on the number of flowers at a site. When many female

flowers were found, waypoints were recorded after counting every ~100 inflorescences. In addition to quantifying spatial variation in presence and density, intermediate increments also helped with accuracy of counts. If single flowers were found, their precise location was marked.

Presence of *V. americana* and abundance of flowers associated with the marked waypoints were aligned to tracks using a full outer join of the two datasets using the base R, version 3.5.1 (R Core Team 2018) *merge* function with latitude and longitude as the common joining columns. This process yielded a list of georeferenced points, appropriately coded with the features identified at specific waypoints (e.g., beginning and end of the transect, beginning and end of patches of *Vallisneria*, and beginning and end of segments with a specified density of inflorescences). Lists of coordinates bounded by transect, patch, and flowering segment start and end waypoints were transformed into spatial line segments in a SpatialLinesDataFrame using the *sp* package, version 1.3-1 (Pebesma and Bivand 2018). Finally, the length of each line segment was determined using *lineLength* in the SDraw Package, version 2.1.3 (McDonald 2016). From these coded line segments, the total length of each transect line, the length of the transect occupied by *V. americana*, and the length of the transect in which female and male flowers were found were calculated. The number of gaps between patches was also calculated.

Bed density was estimated by calculating the proportion of total distance of each transect containing plants, and patchiness was estimated by summing the number of gaps between beds at each site.

Mesocosm Study

Propagules of 16 genotypes from 6 of the field sampling sites were planted in May 2018 in mesocosms at the Appalachian Lab in Frostburg, MD, to determine whether flowering differs among genotypes when plants are grown in ambient light conditions. Sites were selected based on turion availability, but at least one site from each of the three Hudson River environments was represented. A female genotype (CRV7A3) from a *V. americana* bed in Croton River, just upstream from the CRO sampling site, was also planted. In total, 16 genotypes (11 females and 5 males) were planted, with 8 replicate pots per genotype and 4 turions per pot. Longest leaf, number of shoots, and number and sex of inflorescences in each bucket were recorded monthly from June to September. When plants bloomed, the longest leaf of each flowering shoot was recorded.

Flowering in Greenhouse Grown Plants

To assess more broadly whether Hudson River genotypes have reduced capacity to flower, flowering in genotypes that have been propagated at the Appalachian Laboratory since their collection from 11 Hudson River sites in 2015 and two Chesapeake Bay sites in 2016 was quantified. The number of male genotypes and number of female genotypes from each site were calculated and used to compute sex ratios within sites.

Table 1. Sampling dates, number of samples, transect lengths, genotypic diversity, and inflorescences and flowers seen at each site.

Site	Code	Date Sampled	# of July Samples with plants/ Total # of Samples Taken	Total Shoot-Level Transect Length (m)	# Shoots	# Inflorescences	Genotypic [1] Diversity	Date Sampled	August patch distance/ total distance searched (m)	August total female flowers
Hudson River										
Stillwater	STW	07/13	22/24	48.6	110	10	0.24	08/11	1090/1141	786
Mechanicville	MEC	07/13	22/67	199.1	175	22	0.42	08/11	657/1760	128
Parda Hook	PHK	07/12	22/35	79.5	166	7	0.55	08/11	1246/3554	121
Stockport	STK	07/19	21/26	59.3	117	9	0.12	08/09	1080/1080	0
Brandow Point	BPT	07/18	22/22	117.8	153	49	0.35	08/13	761/898	2661
Roger's Island	RIS	07/18	22/23	59.6	231	39	0.43	08/13	1491/3567	554
Cheviot	CHV	07/17 & 07/19	22/22	67.4	303	25	0.27	08/09	2471/2480	112
Tivoli North Bay	TNB	07/16	23/30	129.0	148	17	0.53	08/10	1146/2516	1411
Magdalen Island	MIS	07/16	23/23	47.8	155	13	0.24	08/10	752/820	264
Annsville	ACR	07/14	20/25	65.8	65	1	0.79	08/10	107/5661	0

Creek										
Turning Point	TPT	07/15	22/31	133.2	79	12	0.29	08/15	1363/1372	83
George's	GIS	07/10	21/21	66.9			0.18	08/09	1771/2084	4
Island					119	8				
Croton	CRO	07/20	22/42	115.5	114	3	0.63	08/08	1468/1650	3
Nyack	NYK	07/11	25/26	132.6	240	7	0.10	08/08	1240/1274	6
Chesapeake										
Bay										
Wilson Point		08/07						07/28		
Park	WPP		23/23	65.0	219	337	0.59		645/696	2581
Brown's Creek	BC	07/28	23/23	34.5	213	276	0.62	07/28	217/546	817
Aquia Landing	AL	08/06	23/25	94.6	250	324	1.00	08/06	683/699	14

[1]Genotypic diversity is calculated based on the number of genotypes found at a site (G) relative to the number of samples taken (N) in 2015 in the Hudson River and 2016 in the Chesapeake Bay using the formula: (G-1)/(N-1).

Analysis - Flowering

In shoot-level sampling, the number of inflorescences was summarized at three main levels: site, sample, and individual. The number of inflorescences was also summarized at the region level, but unequal sample sites in the Chesapeake Bay and Hudson River require that comparisons be interpreted with caution. Number of inflorescences per site estimates the total amount of flowering, whereas the number per sample standardizes across sites with different sample numbers. Summarizing at the individual level controls for variation in number of individuals and shoots in samples. In addition to numbers of inflorescences, the number of flowering shoots and number of flowering individuals at each site and in each sample were calculated. Inflorescences were measured if they were at least 2 cm.

Significance of region-level differences in number of inflorescences, number of flowering shoots, and number of flowering individuals per site and per sample was assessed using nested ANOVAs, with Site as a random effect nested within Region as a fixed effect, as implemented by the *stats* package in R, version 3.5.1 (R Core Team 2018).

In surface sampling, the total number of female flowers and density per 1000 m^2 at each site were computed. The site-level densities were used in an ANOVA with Site as a random effect nested within Region as a fixed effect to determine whether the Chesapeake Bay sites had higher density of female flowers than the Hudson River. The distance (m) of patch-only transect containing female flowers and distance not containing female flowers were computed for each site, and the ratio of patch-only transect containing flowers to not containing flowers was calculated. The number and length of

transect segments containing male flowers were computed to estimate the presence of males.

The number of flowers per genotype in the replicated mesoscosm experiment was noted. Presence and sex of flowers were noted for the field collected samples of known genotype that had been growing in the greenhouse. Reproductive status was summarized to the genotype level as male, female, or non-flowering.

Testing Hypotheses for Levels of Flowering

Plant Size

Distributions of maximum leaf length, leaf width, and number of ramets for flowering versus non-flowering individuals from the July shoot-level sampling were plotted. Differences were tested for in these three size variables between the Hudson River and Chesapeake Bay using separate nested ANOVAs, with Sample as a random effect nested within Site, and Site as a random effect nested within Region as a fixed effect. A multivariate logistic regression with backwards stepwise variable entry was used to quantify the effect of maximum leaf length, leaf width, and total number of ramets per individual on the probability that the individual was flowering. After examining regression predictions of flowering at varying leaf lengths, leaf widths, and numbers of ramets, the size below which each individual has a 95% chance of not flowering was determined. Individuals with leaves that had been broken off by the sampling frame were excluded from these analyses.

Environmental Conditions

Salinity

The maximum daily salinity (ppt) at three HRECOS monitoring stations, Piermont Pier, West Point, and Norrie Point, was calculated for comparing salinity levels along the gradient of the river. These maximum daily values were averaged across 2008 to 2018 for each station and plotted by day of year. The tidal-saline sites were located between the Piermont Pier and West Point stations so these stations bracket salinities at the sampled sites. Tidal-fresh sites were all upstream from Norrie Point. Differences in ratio of flowering to total individuals (from the July shoot-level sampling) and site-level density of female flowers (from the August surface sampling) among tidal salinity categories were formally tested for using separate one-way ANOVAs.

Temperature

The minimum daily temperatures (°C) at Hudson River and Chesapeake Bay monitoring stations were averaged across all stations within each region and across all years from 1984 to 2018 in the Bay, and from 2008 to 2018 in the Hudson River. The differences in the minimum values on each day were compared, and then the first day of the year each minimum temperature (based on whole °C) was observed in each region was determined.

Genotypic Diversity

To understand if sites with more genotypes flowered more, a logistic regression was used to determine the degree to which site-level genotypic diversity predicted the ratio of flowering to non-flowering individuals per site measured in the July shoot-level

sampling. This analysis included only Hudson River sites to prevent confounding genotypic and environmental differences of the Chesapeake Bay samples.

Bed Density

The effect of shoot density and degree of patchiness at sites was estimated using logistic regression to predict the ratio of flowering to non-flowering individuals per site as a function of the mean and coefficient of variation (CV) of the number of shoots per sample and the proportion of samples with plants.

In the surface sampling, density was estimated as the proportion of total distance of each transect containing plants and as number of gaps between patches. The proportion of patch-only transect containing flowers was also computed. To analyze effects of bed density in surface sampling, a logistic regression was used to test the effect of the number of gaps between patches and proportion of total distance of each transect containing plants on the proportion of patch-only transect containing flowers to patch-only transect lacking flowers.

Overall Capacity for Flowering

The percentages of greenhouse-grown Hudson River genotypes that flowered were compared in a nested ANOVA, with Site as a random effect nested within Region as a fixed effect.

Analysis – Pollination Potential

Potential limitations to pollination were assessed by examining isolation of sexes among and within sites. First, in both July shoot-level and August surface sampling, presence versus absence of each sex at each site was noted.

Next, in the shoot level sampling, when both sexes were present at sites the sex ratio of reproductive shoots was calculated, where an equal sex ratio (0.5) is maximal and increasing dominance by either sex goes to 0. A chi square test was also used to compare sites in terms of the number of individuals that were assigned to each reproductive category (male, female, non-flowering).

The spatial distribution of males and females within sites was then quantified. In the shoot-level samples the minimum Euclidean distance between samples containing each of the sexes was calculated. If male and female shoots were found together in a sample, the distance was set to 0 m and the number of times this occurred in each site was noted.

On surface sampling transect lines spatial proximity of the sexes was assessed by counting the total number of segments containing both male and female inflorescences. The small number of male flowers precluded formal statistical tests.

Whether potential for pollination at Hudson River sites was predicted by genotypic diversity or salinity was also tested. Chesapeake Bay samples were excluded from this analysis to prevent confounding genotypic and environmental differences that exist between Maryland and New York. The effect of site-level genotypic diversity on the site-level sex ratio above was assessed using a linear regression. It was then asked whether the magnitude of deviation from an equal sex ratio differed among the tidal-saline, tidal-fresh, and non-tidal sites in a one-way ANOVA.

RESULTS

Flowering

The July shoot-level sampling yielded 219 inflorescences on 2,175 shoots representing 1,515 individuals in the Hudson River and 937 inflorescences on 682 shoots representing 587 individuals in the Chesapeake Bay. This stark difference in flowering in the two regions carried through to averages of 15.64 (sd=13.78) versus 312.33 (sd=32.13) inflorescences per site and of 0.71 (sd=1.28) versus 13.58 (sd=9.00) inflorescences per sample (Figure 3) in the Hudson River and Chesapeake Bay, respectively. Differences in number of inflorescences per site between regions (F=719.8, df=1, p=4.31e-14), per sample between regions (F=695.3, df=1, p=5.57e-14), and per individual between regions (F= 554.7, df=1, p= 2.92e-13) were significant based on nested ANOVAs. Even the sites with the most inflorescences per sample in the Hudson River (BPT and RIS) had far fewer inflorescences than samples at Chesapeake Bay sites (Figure 3).

Differences in number of inflorescences per site between the regions were partially accounted for by larger numbers of shoots at Chesapeake Bay sites ($\bar{x}$=227.3, sd=19.8) than at most Hudson River sites ($\bar{x}$=155.4, sd=65.5; F= 3.393, df=1, p= 0.0853; Figure 4). But more importantly, a much larger number of shoots were flowering at each Chesapeake Bay site ($\bar{x}$=120.0 versus $\bar{x}$=12.4 in the Hudson River; F=285.7, df=1, p=3.56e-11; Figure 4) and per sample at each site (F=289.9, df=1, p=3.21e-11). In the Chesapeake Bay >45% of shoots at each site were flowering ($\bar{x}$=53.1%) whereas no Hudson River site had more than 23% of shoots flowering ($\bar{x}$=7.7%).

All inflorescences at all Hudson River sites were <2 cm long at the time of sampling. In the Chesapeake Bay, 2.2% (BC) -21% (WPP) of flowers sampled were ≥2

cm. At AL, the longest male inflorescence was 13 cm (n=26) and the longest female was 9 cm (n=2). At BC the longest female inflorescence was 25 cm (n=22) and the longest male was 3 cm (n=5). At WPP the longest female inflorescence was 94 cm (n=9) and the longest male was 11 cm (n=37). Although they were not included in samples, many mature female flowers were observed on the water surface at BC and AL during sampling. In the Hudson River, only one mature female flower was observed outside of the sampling area at BPT on the day the shoot-level samples were collected.

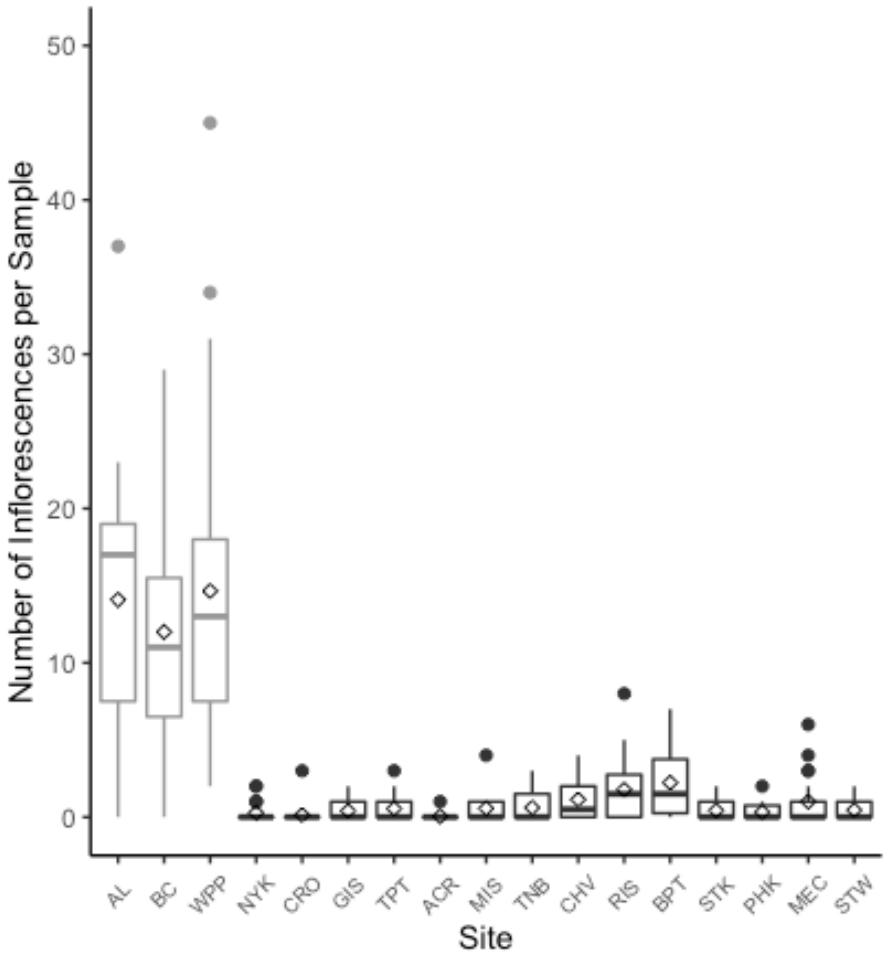

Figure 3. Distribution of the number of inflorescences per 0.04 m^2 sample at each site. Lines indicate median number of inflorescences and open diamonds are mean numbers. Regions (MD=light gray; NY=dark gray) were significantly different based on a nested ANOVA.

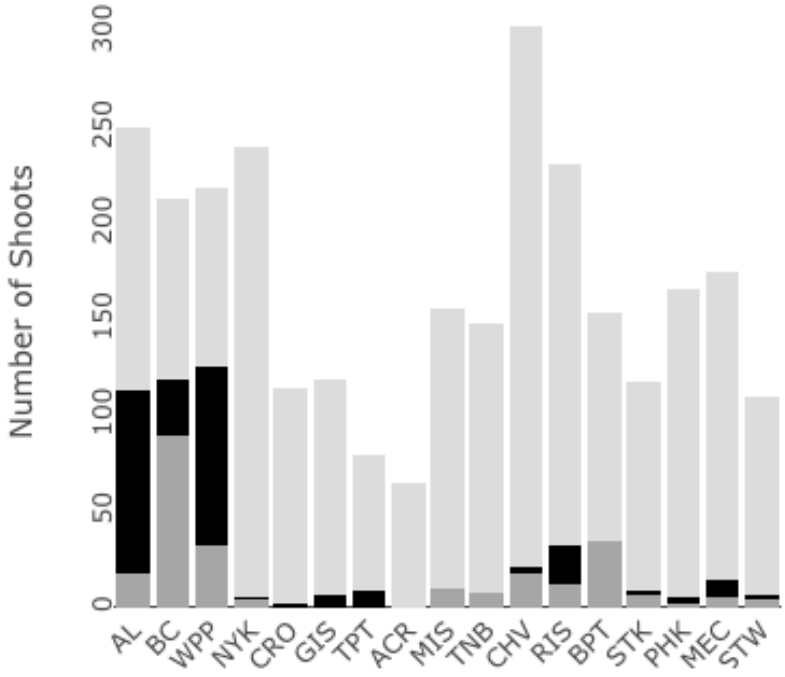

Figure 4. Total number of male (black), female (medium gray), and non-flowering shoots (light gray) per site.

In the August surface sampling, the number of female flowers detected at sites ranged from 0 (STK and ACR) to 2,661 (BPT) and averaged 561. Despite extensive survey ≤6 female flowers were found at AL, NYK, CRO, and GIS (Table 1). Although the number of flowers found in some Hudson River sites exceeded what was found in the Chesapeake Bay, the search effort required to find those flowers was much higher (Table 1). When standardized to 1000 m^2, density of female flowers was significantly higher in the Chesapeake Bay ($\bar{x}$= 0.97) than the Hudson River ($\bar{x}$= 0.19) based on a nested ANOVA (F=4.859, df=1, p=0.0435; Figure 5) despite finding very few female flowers at Aquia Landing. The distance (m) of patch-only transect containing female flowers averaged 412.2 m and ranged from 0 m (STK) to 876.1 m (STC). The distance of patch-only transect not containing female flowers averaged 581.4 m and ranged from 0 m (BC

and WPP) to 2009 m (CHV). Translating these data into the ratio of patch-only transect containing flowers to not containing flowers, the average was 0.47 and ranged from 0 (STK) to 1.0 (BC and WPP).

Male flowers were observed at all sites except CHV, NYK, STW, STC, and STK. Through this broader sampling, males at three sites that had been entirely female in the shoot-level sampling (BPT, STC, MIS), and females at two sites that had been only male (GIS and TPT) were documented. Males were also found in the surface sampling at ACR, a site that had been without flowers in July; however, failure to find any female flowers made ACR the only site at which both sexes were not detected. Further, although both sexes were found at STK in July, none were seen during surface sampling. Within transects, WPP and BC had the most segments containing male flowers (20 segments totaling 497.3 m and 18 segments totaling 139 m, respectively), whereas AL had 2 segments (160 m) and the Hudson River sites that contained males had an average of 3.78 segments or 84.0 m.

Only 11 fruits were found across all sites in the Hudson River (1 at MEC and 10 at BPT). Although many female flowers that were past anthesis were seen, no others were forming fruit, indicating lack of pollination. By contrast, most flowers in the Chesapeake Bay were maturing into fruits. The mesocosm study provided little additional information on flowering because only two genotypes, Stockport 864 (2 male inflorescences) and Croton CRV7A3 (2 female flowers), flowered during the study.

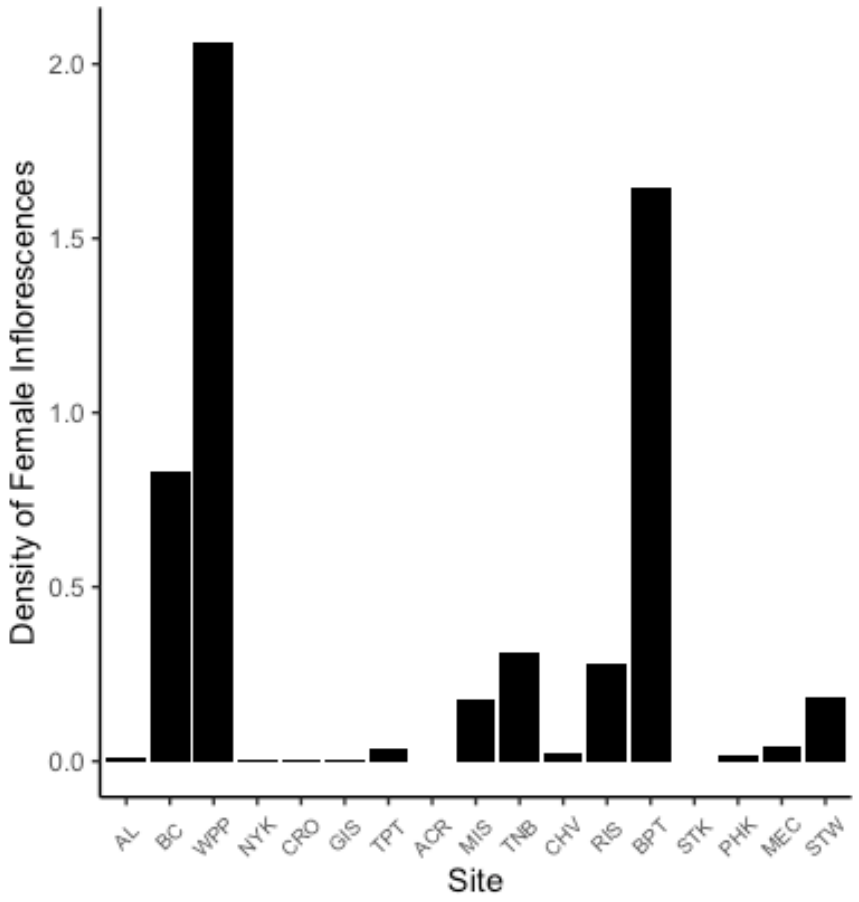

Figure 5. Density of female inflorescences (per 1000m^2) at each site based on the area of the full transect.

Testing Hypotheses for Levels of Flowering

Plant Size

The Chesapeake Bay had longer maximum leaf lengths per individual than the Hudson River ($\bar{x}$=68.1 cm, sd= 27.8 versus $\bar{x}$=40.66 cm, sd=24.8, respectively), and this difference was confirmed by a nested ANOVA (F=17.75, df=1, p=0.000753). In both regions, flowering individuals tended to be near the upper end of the distribution of maximum leaf lengths (Figure 6) with an overall average length of 74.5 cm (sd=25.0) for flowering individuals and 41.3 cm (sd=25.0) for non-flowering individuals. In the

Hudson River, maximum leaf lengths of flowering individuals were ~19 cm shorter than in the Chesapeake Bay ($\bar{x}$=59.3 cm [sd=22.4] and $\bar{x}$=78.5 cm [sd=24.1], respectively). Maximum leaf lengths on non-flowering individuals averaged 39.2 cm (sd=24.4) in the Hudson River and 52.0 cm (sd=25.3) in the Chesapeake Bay. Beyond the average values, the differences in the distributions of leaf lengths in the two regions is striking, with values for Hudson River individuals being right skewed (Figure 6).

Flowering individuals also fell near the upper end of the distribution of leaf widths with an overall average maximum width of 7.70 mm (sd=1.37) for flowering individuals versus 6.06 mm (sd=1.68) for non-flowering individuals (Figure 6). The overall average leaf width was slightly higher in the Chesapeake Bay ($\bar{x}$=6.67 mm, sd=1.75) than the Hudson River ($\bar{x}$=6.36 mm, sd=1.75), but a nested ANOVA revealed no statistically significant difference (F= 0.481, df=1, p= 0.499). The mean leaf width for flowering individuals in the Hudson River ($\bar{x}$=8.11 mm, sd=1.37) was higher than the Chesapeake Bay ($\bar{x}$=7.60 mm, sd=1.35). The average leaf width for non-flowering individuals on the Hudson River was 6.22 mm (sd=1.71), and the mean leaf width for non-flowering individuals in the Chesapeake Bay was 5.24 mm (sd=1.25; Figure 6).

Despite having shorter leaves, there were more ramets overall on Hudson River individuals ($\bar{x}$=3.22, sd=1.72; F= 6.98, df=1, p= 0.0185) compared to Chesapeake Bay individuals ($\bar{x}$=2.49, sd=1.10). In both the Hudson River and the Chesapeake Bay, flowering individuals had more ramets ($\bar{x}$=4.36, sd=1.67 and $\bar{x}$=2.64, sd=1.10, respectively) than non-flowering individuals ($\bar{x}$=3.13, sd=1.70 and $\bar{x}$=2.25, sd=1.06, respectively; Figure 6).

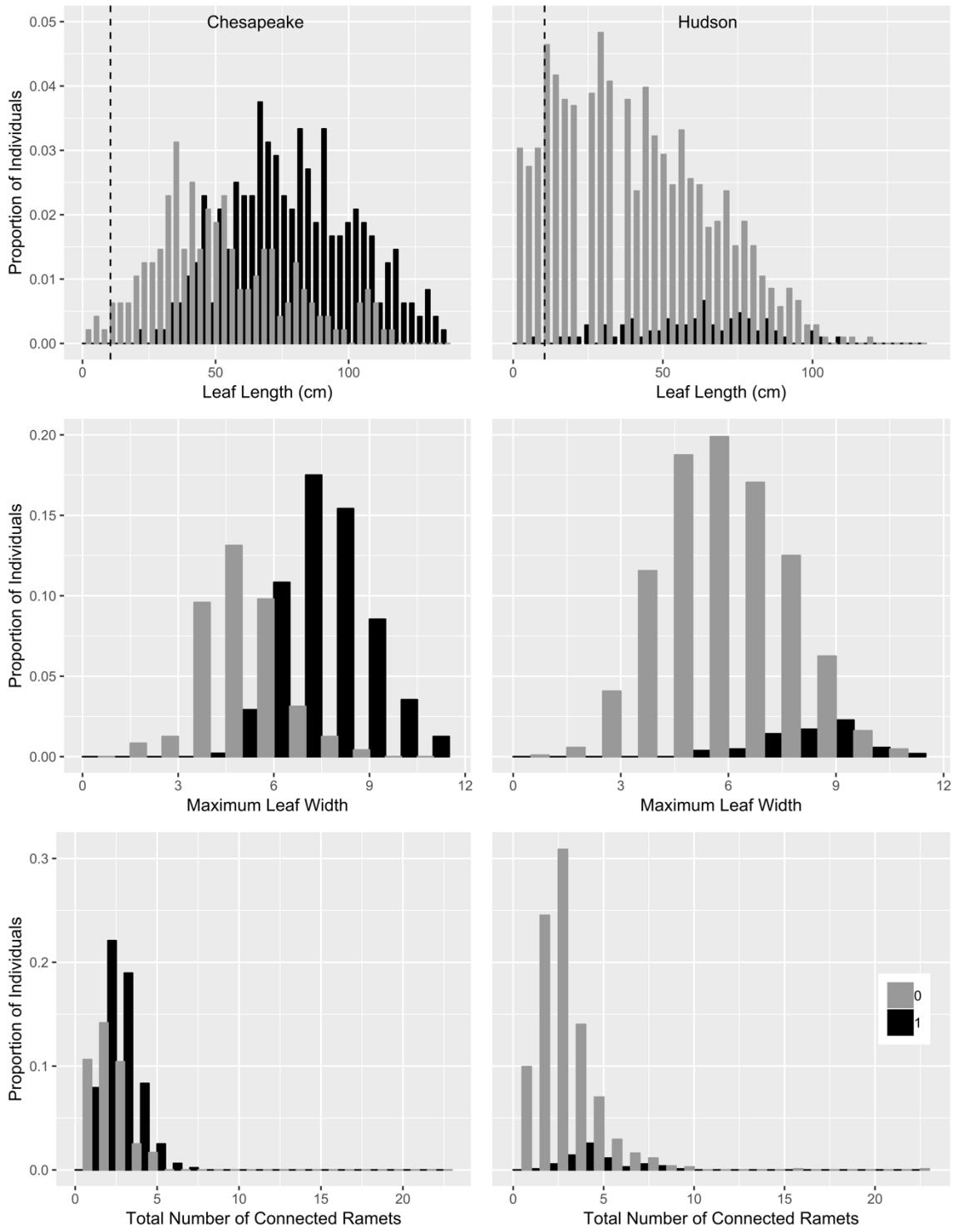

Figure 6. **Maximum leaf length, leaf width, and number of ramets distribution for flowering (black; 1) vs. non-flowering (gray; 0) individuals in Chesapeake Bay (left) and Hudson River (right). Dashed lines mark the leaf length at which an individual has a 95% chance probability of not flowering (10.5 cm) based on logistic regression predictions.**

A multivariate logistic regression (pseudo r^2= 0.28) revealed that as the maximum leaf length (p value=2e-16), leaf width (p value=2e-16), and total number of ramets (p value= 0.0449) per individual increased, so did the probability of an individual flowering. Individuals of maximum leaf length 10.5 cm, paired with leaf width 6.5 mm (average) and number of ramets 2.8 cm (average) had a 95% chance of not flowering (Figure 7). For any given leaf length, varying leaf width had a large effect on the chance of flowering. For example, a leaf width of 8.2 mm (mean+1sd) increased the chance of flowering from 5% to 13.3%, whereas 4.7 mm wide leaves (mean-1sd) paired with the same leaf length (10.5 cm) and number of ramets (2.8) had only a 2.1% chance of flowering (Figure 7). The number of ramets had a smaller effect on the probability of flowering. Probability of flowering at a leaf length of 10.5 cm only rose to 6.1% when the number of ramets was increased from 2.8 (mean) to 4.2 (mean+1sd) and only dropped to 4.7 % for plants with 1.5 ramets (mean-1sd) (Figure 8). Individuals with a maximum leaf length ≤ 10.5 cm were all from the Hudson River, further emphasizing the smaller stature of plants from this region as compared to the Chesapeake Bay (Figure 6).

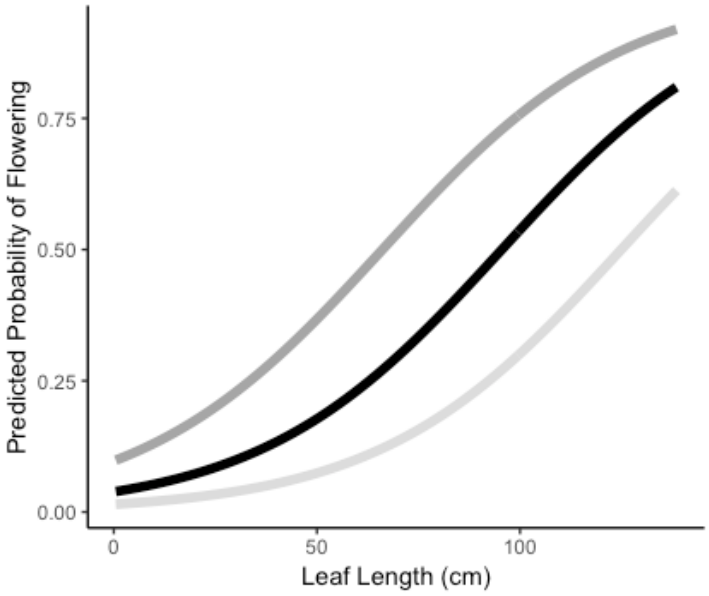

Figure 7. **Probability of an individual flowering given varying leaf lengths paired with mean 2.8 ramets and three different leaf widths: mean-1sd =4.7 mm (light gray line); mean=6.5 mm (black line); mean+1sd=8.2 mm (dark gray line)**

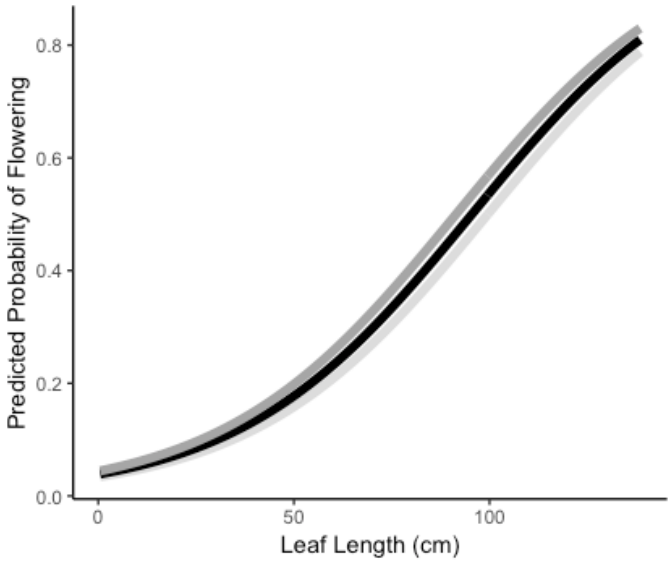

Figure 8. **Probability of an individual flowering given varying leaf lengths (X axis) paired with mean 6.5 mm leaf width and three different numbers of ramets: mean-1sd =1.5 (light gray line); mean=2.8 (black line); mean+1sd=4.2 (dark gray line).**

Environmental Conditions

Salinity

Maximum daily salinity at Piermont Pier (tidal-saline) ranged from 3.10 ppt to 11.3 ppt and at West Point it ranged from 0.1 ppt to 3.56 ppt (Figure 9). The tidal-saline sampling sites would have maximum daily salinities between these values. At Norrie Point, maximum daily salinities were low and had a narrow range, from 0.10 ppt to 0.15 ppt. All remaining sites sampled would be below these values.

There was no significant difference among the Hudson River sites nested in the three environments spanning the river's tidal and salinity gradient in terms of either the proportion of individuals that were flowering ($F=1.562$, $df=2$, $p=0.253$) in July or the density of female flowers ($F=1.364$, $df=2$, $p=0.296$) in August.

Temperature

Year-round minimum daily temperature ranged from 1.3 °C to 29.1 °C in the Chesapeake Bay, and from -0.068 °C to 26.0 °C in the Hudson River. Between the end of March and early June, minimum temperature each day was on average of 4.5 °C warmer in the Chesapeake Bay and any given minimum temperature was reached first, on average 29.1 days earlier (Figure 10). This differential continues through mid-summer, at which time the differences decrease. Temperatures become increasingly similar from September to November.

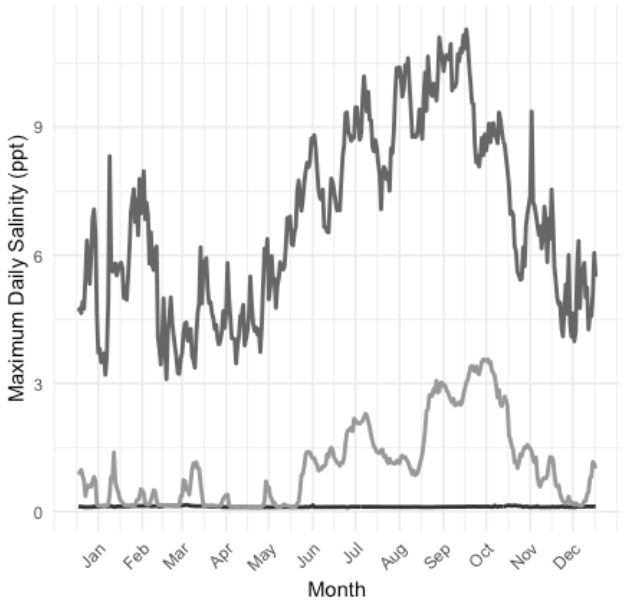

Figure 9. **Maximum daily salinity (ppt) at Norrie Point (black), West Point (light gray), and Piermont Pier (dark gray) HRECOS monitoring stations, averaged across years from 2008 to 2018.**

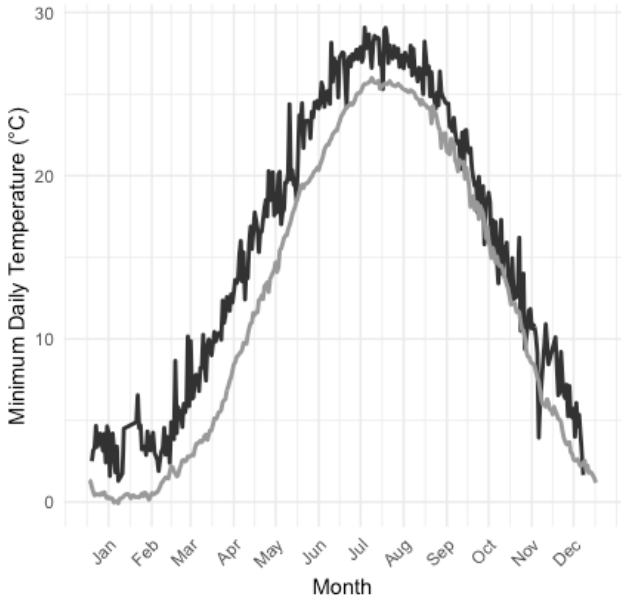

Figure 10. **Mean of the minimum daily temperature (°C) at four Hudson River monitoring stations (gray) and 16 Chesapeake Bay monitoring stations (black) averaged across years from 1984 to 2018.**

Genotypic Diversity

Genotypic diversity was not a significant predictor of flowering (success) versus not flowering (failure) at sites in the Hudson River when tested with a logistic regression (pseudo r^2= 0.01544292, p value = 0.318).

Bed Density

A low proportion of sample frames containing plants at MEC (0.33) and CRO (0.55) indicates low patch density that contrasts with BC, WPP, GIS, MIS, CHV, and BPT where all samples supported plants (Table 1). The overall mean number of shoots per sample at sites, including samples that contained no plants, ranged from 2.6 to 13.8, and averaged 6.5. When empty samples were excluded, densities within patches ranged from averages within sites of 3.2 to 13.8 shoots per sample, with an overall average of 7.5 shoots per sample. Bed patchiness, measured by CV of numbers of shoots among samples, was lowest at CHV (0.34) and highest at TPT (1.06) and CRO (1.0) (Figure 11). In a logistic regression, a higher proportion of sample frames containing plants (pseudo r^2= 0.2366674, p=0.027709) and a higher CV (p=0.000136) predicted a higher ratio of flowering to non-flowering individuals, but mean number of shoots per sample was insignificant (p= 0.488260).

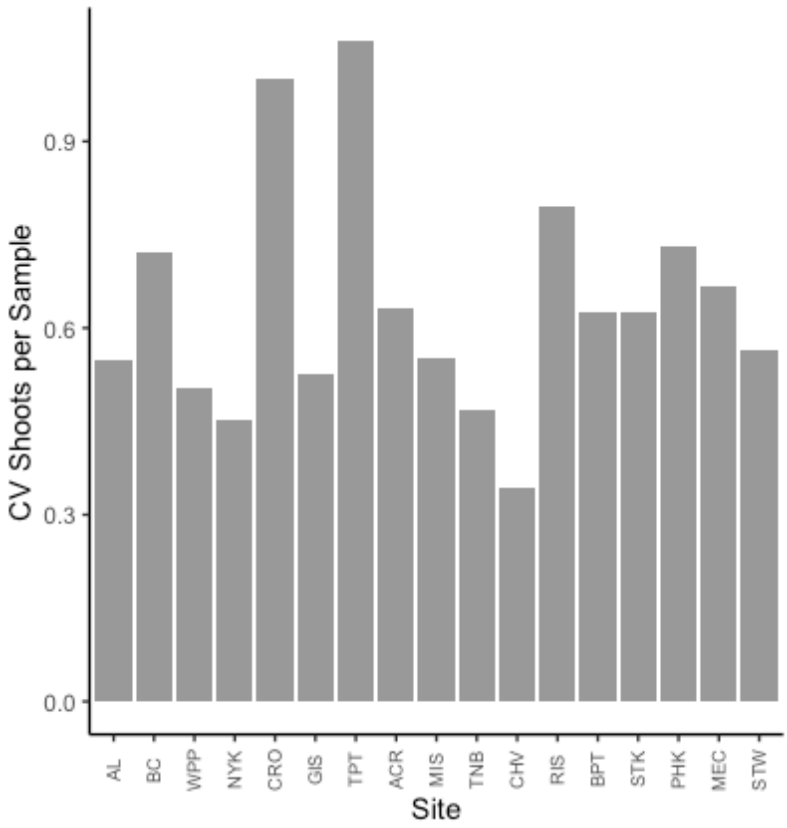

Figure 11. Coefficient of Variation (CV) of numbers of shoots among buckets. High CVs indicate greater variation in *Vallisneria* density within sites.

In surface sampling, the proportion of total distance of each transect containing plants was highest at BC, BPT, CHV, GIS, MIS, and WPP (1.0) and lowest at MEC (0.33) and CRO (0.52). The number of gaps between patches averaged 6.65, and ranged from 0 (STK) to 25 (CRO). A lower proportion of total transect distance containing plants ($p=9.36e-12$) and more gaps between beds ($p < 2e-16$) predicted a higher ratio of patch-only transect containing flowers to not containing flowers in a logistic regression (pseudo $r^2= 0.05922251$).

Overall Capacity for Flowering

The mean number of multilocus genotypes per Hudson River site grown at the Appalachian Laboratory was 12 and ranged from 4 (NYK) to 19 (BPT); 9 genotypes

from WPP and 10 from BC were planted (Table 2). Variation in number of genotypes reflects genotypic diversity at the sample sites. From 50 to 100% of genotypes from the 11 Hudson River sites flowered. For 6 of those sites, the percentage was >85% (Table 2). In the two Chesapeake Bay sites, 70% (BC) and 44% (WPP) genotypes flowered. A nested ANOVA revealed no significant difference between percentage of flowering genotypes between the Chesapeake Bay and Hudson River, although the sample sizes were highly unbalanced regions ($F= 3.373$, $df=1$, $p= 0.0934$).

Table 2. Number of multilocus genotypes from each site that flowered in the greenhouse at the Appalachian Laboratory. Sex and resulting deviation from a 50:50 sex ratio based on genotypes flowering are indicated.

Site	# of MLGs Grown	Proportion Flowered	# Female	# Male	Deviation from 50:50 Sex Ratio
Hudson River					
MEC	14	1.00	9	5	0.36
PHK	17	1.00	10	7	0.41
STK	6	1.00	3	3	0.50
BPT	19	0.63	12	0	0.00
RIS	17	0.65	8	3	0.27
MIS	11	1.00	9	2	0.18
CHV	6	1.00	4	2	0.33
TPT	13	0.85	6	5	0.45
GIS	9	0.67	2	4	0.33
CRO	19	0.89	3	14	0.18
NYK	4	0.50	2	0	0.00
Chesapeake Bay					
WPP	9	0.44	2	2	0.50
BC	10	0.70	3	4	0.43

Pollination Potential

Isolation of males and females was seen in the Hudson River that contrasted with patterns in the Chesapeake Bay. Females and males were found at all three Chesapeake Bay sites, whereas only one sex was detected in the fine-scale shoot sampling at five

Hudson River sites. At TPT and GIS only males were found and at MIS, STC, and BPT only females were found. AL in the Chesapeake Bay and four Hudson River sites with both sexes had strongly biased sex ratios, deviating from the maximum value of 0.5 by at least 20% (Figure 12). In the Hudson River, only CRO, PHK, RIS, and MEC had relatively balanced numbers of males and females at the scale of the sampling transect (i.e. sex ratios close to 0.5; Figure 12). Neither genotypic diversity (r^2= 0.05386, p value= 0.425) nor salinity/tidal category (F= 2.826, df=2, p= 0.102) explained variation in sex ratios.

A chi squared test comparing sites in terms of the total number of male, female, and non-flowering individuals was significant (χ^2=891.98, p-value < 2.2e-16). More females than expected were found at BPT and there were more males than expected at AL and TPT. BC and WPP had both more males and females than expected relative to non-flowering individuals. All Hudson River sites except for BPT had more non-flowering individuals than expected.

Differences between regions were also assessed in terms of isolation of sexes among samples within the fine-scale transects. All Chesapeake Bay sites had multiple samples with both males and females (AL, n=4; BC, n=7; WPP, n=12). By contrast, only one sample each at CRO and MEC had both sexes. In the other six Hudson River sites in which both males and females were found, the minimum distance to the opposite sex ranged from 1.16 m (PHK) to 17.13 m (STK) and averaged 7.1 m. Thus, more isolation of males and females was seen at the scale of the whole transect and within transects in the Hudson River than in the Chesapeake Bay.

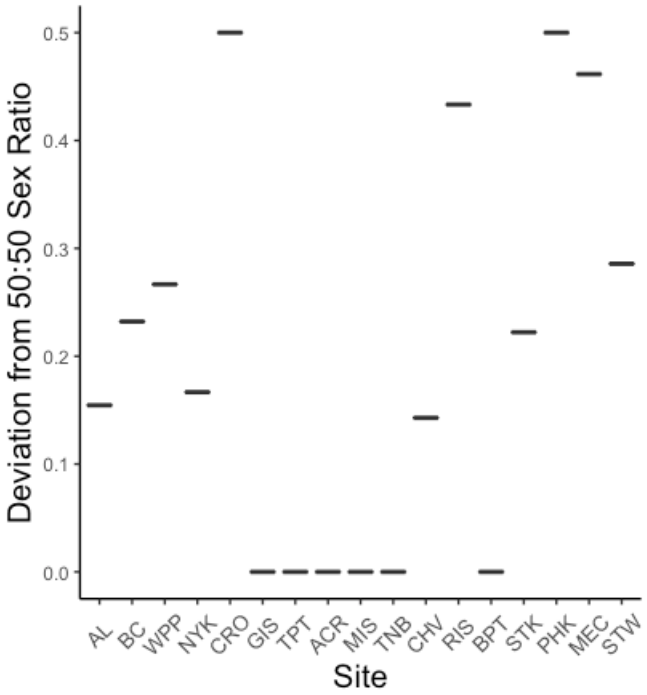

Figure 12. Site-level deviation from 50:50 sex ratio. Values closer to 0 indicate dominance by either sex.

In surface sampling, flowers of both sexes were found at all sites except ACR. The total number and length of segments containing both male and female flowers were highest at BC (9 segments totaling 113.1 m) and WPP (20 segments totaling 497.3 m). Males and females were found in the same segment at only six of the nine Hudson River sites at which both sexes were present on the surface transect. At these sites, the sexes were never found together in more than two segments (Figure 13) and the total length of these segments never exceeded 66.0 m.

Among the Appalachian lab greenhouse plants, deviation from a 50:50 sex ratio was most extreme in genotypes from NYK and BPT where all flowering genotypes were female. By contrast, PHK, STK, TPT, WPP and BC had relatively even sex ratios (Table 2).

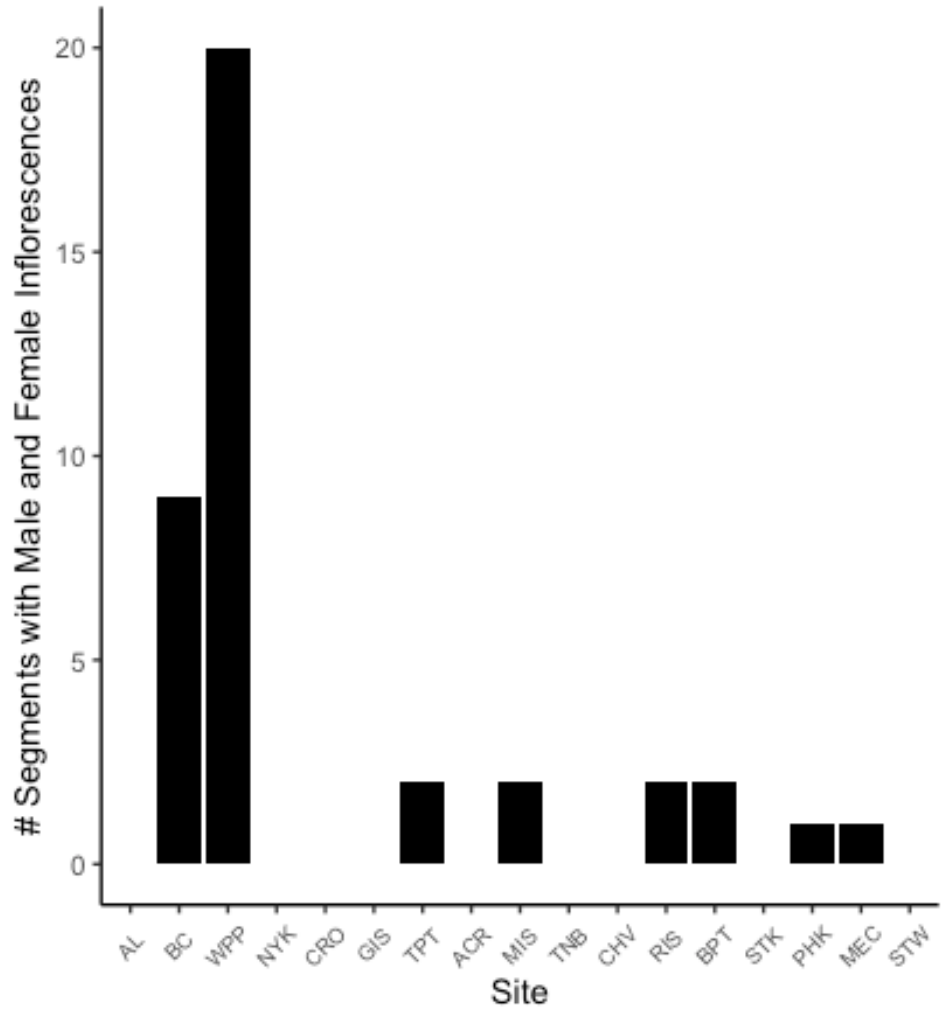

Figure 13. Number of segments containing both male and female inflorescences based on full area surveyed.

DISCUSSION

The capacity for asexual reproduction is ubiquitous in aquatic angiosperms and is considered a key feature of their evolutionary and ecological success (Philbrick and Les 1996). The balance between asexual and sexual reproduction is a dominant factor in structuring genetic diversity. Sexual reproduction generates genetically unique individuals with variation that fuels natural selection, whereas asexual reproduction generates many copies of the same genotype, potentially leaving populations and species without the diversity needed to respond to environmental change (Eckert et al. 2016). As such, the paucity of sexual reproduction in *Vallisneria americana* in the Hudson River is concerning. Sexual reproduction is first compromised by significantly fewer flowers (Figure 3) and flowering shoots (Figure 4) in fine-scale samples, as well as lower densities of female flowers and fewer encounters with male flowers along large-scale transects (Table 1, Figure 5) than in Chesapeake Bay sites. Of even greater importance is lack of fruiting by the vast majority of flowers in the Hudson River. On the positive side, the fact that Hudson River plants have not lost the inherent capacity to flower (Figures 3 and 4, Table 2) implicates environmental factors in driving the observed differences.

Neither low genotypic diversity nor high salinity were explanatory factors for limited flowering. Although there was variation across sites (Figures 3 and 4), low flowering throughout the Hudson River likely precluded finding patterns related to environmental and genotypic factors within the river if any existed. The lack of effect of high salinity was surprising given that ≥5 - 9 ppt is known to limit vegetative growth and reproduction (Doering et al. 1999; French and Moore 2003; Boustany et al. 2010) and these salinities were likely seen at high tides in the tidal saline sites (Figure 9). Bed

density had a small effect on probability of flowering, but in the opposite direction than was hypothesized, as measured by the CV of the number of shoots per sample, the proportion of samples with plants, the number of gaps between beds, and the proportion of each surface transect containing plants. This result was surprising. Higher density was originally hypothesized to yield more flowering due to positive feedback loops between establishment and expansion of SAV beds and water clarity (Gurbisz and Kemp 2014). Water clarity yields increased reproduction in *V. americana* (e.g., Carter et al. 1996; Doyle and Smart 2001), generating larger, denser beds that can further slow water movement (Koch 2001; de Boer 2007). In slow moving water, more suspended particles can sink, further increasing water clarity (Ward 1985; Gacia and Duarte 2001; de Boer 2007); however, effects of density on water quality were not tested and if they did exist, they did not manifest in terms of more flowering in this study.

The most important proximate predictor of flowering was plant size (Figures 6, 7, and 8), which was not surprising given the well-known importance of biomass (Titus and Hoover 1991) and leaf length (Engelhardt et al. 2014) in flowering in *Vallisneria*. Water temperature differences between regions (Figure 10) coincide with the differences in leaf length, ramet production, and flowering. Higher water temperatures in the spring and early summer in the Chesapeake Bay may result in increased vegetative growth earlier in the year, leading to plants reaching reproductive size more quickly than in the Hudson River. The link between temperature and size was demonstrated by Marsden 2015 in a growth chamber experiment in which Chesapeake Bay plants were smaller when grown at Hudson River temperatures and Hudson River plants were larger when grown at Potomac River temperatures. Further development of flowers was more advanced in the

Chesapeake Bay fine scale samples, in that none of the Hudson River plants had flowers that had reached 2 cm at approximately the same time Chesapeake Bay female flowers were open at the water's surface. Temperature could also have contributed to lack of flowering in the mesocosm experiment. Based on previous experiments that were conducted on benches in the same greenhouse, flowering was expected. In this case, plants were grown on the greenhouse floor, which may have kept water temperatures cooler than they would have been on the benches.

Effects of temperature on growth and phenology are broadly known (e.g., Roy and Sparks 2000) and are a function of enzyme kinetics (Trudgill et al. 2005). The relationship is so strong that a number of days above a species-specific base temperature is needed for phenological phenomena (e.g., germination, bud break, flowering, hatching of insects, etc.) to occur, termed Growth Degree Days (GDD). GDD is calculated as the daily difference between the midpoint of the maximum (T_{max}) and minimum (T_{min}) temperature and the base temperature (T_{base}) for a species:

$$GDD = \frac{T_{max} + T_{min}}{2} - T_{base.}$$

Although *V. americana* flowering is known to coincide with increases in temperature (Titus and Stephens 1983; Carter and Rybicki 1985; Best and Boyd 2001), the GDD needed to initiate flowering in *V. americana* is not known. McFarland (2006) suggested temperatures needed for flower initiation based on the aquatic species *Lobelia dortmanna* in which the peduncle supporting the female flowers begins elongating at a threshold temperature of 19.1 °C (Szmeja 1987); however the idea of GDD has not been employed. Elements of temperature requirements are known, but the picture is incomplete. Rybicki and Carter 2002 found that >92% of turions planted in sediment germinated at all tested

temperatures from 13 °C to 22 °C. Further, leaf extension at the higher temperatures was substantially higher (Rybicki and Carter 2002). Temperatures from 28 °C to 32 °C are optimal for vegetative growth in the field (Titus and Adams 1979; Barko et al. 1982, 1984) and in the growth chamber (Marsden 2015).

Given the importance of temperature for development and phenology, the observed daily differences in minimum temperatures of 4.5 °C with warming in the Chesapeake Bay preceding the Hudson River by ~29 days in the spring (Figure 10) could easily generate the observed growth differences. It is possible that the lower water temperatures in the Hudson River limit the growing season compared to the Chesapeake Bay, affording plants little time to reach reproductive size, and causing flowers to bloom when there is little time for fruits to mature.

Such latitudinal differences in terrestrial environments are known as Hopkins' Bioclimatic Law (Hopkins 1918) which predicts a four day delay in phenology for each poleward degree of latitude. These predictions have not been extensively tested in aquatic environments but they have been found to be accurate for *Chara hispida* (Calero et al. 2017) and *Hydrilla verticillata* (Spencer and Ksander 2001). Determining GDD needed for flowering and fruiting in *Vallisneria* would be extremely useful for predicting reproductive potential given annual temperatures.

If phenology is delayed by ~29 days, estimates of flowering from the fine-scale sampling would undoubtedly have been higher if flowers on individual ramets were measured in August; however, flowers developing that late will not reliably have sufficient time to develop mature fruits before the growing season ends in September due

to declining photoperiod (Dawson 1980) and temperatures (Titus and Stephens 1983; Titus and Hoover 1991).

Although temperature differences appear to account for the observed difference in flowering, the explanation for higher number of total ramets per individual in the Hudson River (Figure 6) is not clear. The larger number of ramets is suggestive of increased investment in asexual reproduction. Turion production was not quantified and, thus, it is not certain if the ramets have an impact beyond the current growing season. Turion production is typically correlated with number of ramets (Titus and Hoover 1991) and genetic data indicate high levels of clonality (Table 1). It is possible that increased turion production simply results at lower temperatures; however, there is evidence for adaptive differences in ramet production. Marsden (2015) found Hudson River plants produced more turions than Potomac plants even in Potomac River growing conditions, and Potomac plants did not produce more turions in Hudson River conditions. It is easy to see the fitness benefit of leaving more asexual propagules, especially in environments in which sexual reproduction is compromised.

The fact that Hudson River plants retain the capacity to flower yields optimism for future resilience – sexual reproduction is not precluded. That optimism is tempered by the fact that the vast majority of the female flowers that did bloom were not pollinated and failed to form fruits – in fact only 11 fruits were found in the >31 km that were surveyed within Hudson River *Vallisneria* beds (Table 1).

Abortion of flowers indicates pollen limitation, which can arise from myriad factors in a dioecious plant with complicated water pollination. Pollination is limited by deep water and high current, wind and waves (Sullivan and Titus 1996). Although depth

does not vary systematically between all Chesapeake Bay and Hudson River sites, the differences in tidal range (0.6-0.8 m in the Chesapeake Bay and 1.2-1.8 m in the Hudson River) will place female flowers at the surface where pollination takes place for less time each day in the Hudson River. Synchrony between males and females is also known to be critical for pollination (Titus and Hoover 1991).

Beyond these environmental factors, lack of pollination is likely related to strongly skewed sex ratios (Figures 4 and 12) and spatial isolation of male and female plants (Figure 13), which were much greater at more spatial scales in the Hudson River than in the Chesapeake Bay (although AL was highly male biased). Only two sites on the Hudson River had any fine-scale samples with both male and female inflorescences in shoot-level sampling, whereas all three Chesapeake Bay sites had both sexes within the same sample. Presence of only one sex on fine-scale transects at five sites indicates males and females can be isolated by ~100 m. Later in the season, many segments along large-scale transects contained both male and female flowers in the Bay whereas this occurred in no more than 2 segments at each of six Hudson River sites. Biased sex ratios have been found in other *Vallisneria* populations (Doust and Laporte 1991; Lokker et al. 1994, 1997) so the Hudson River is not unique, but the number of sites throughout the river that are strongly biased is concerning.

Extensive asexual reproduction can be self-reinforcing, perpetuating more asexual reproduction while limiting future sexual reproduction (Barrett 2015). In locations with the predominance of one sex, asexual reproduction will predominate. As individual genotypes become more extensive, flowers of the opposite sex will become more and more isolated from one another, further reducing the potential for sexual reproduction. In

populations with prolonged clonal reproduction and barriers to sexual reproduction, patches may ultimately consist of just one or a few large clones (Eriksson 1989; Honnay and Bossuyt 2005).

Clonal diversity is likely to interact with the spatial distribution of the species. Dominance by a limited number of genotypes will be more likely in sites that are isolated such that dispersal is limited and rescue by genotypes is less likely. The source of the propagules also matters. Dispersal of sexual propagules leads to increased genotypic diversity at neighboring sites and genetic rescue for nearby small, low-diversity populations, whereas dispersal of asexual propagules amplifies the clonal extent of few genotypes across multiple sites. Thus, it is important to consider the degree to which the balance of clonality versus sexual reproduction represents long-term patterns in the Hudson River and the degree to which it is the consequence of increased isolation from the 2011 storms. If *Vallisneria* has survived in the Hudson River for millennia with low genotypic diversity and low sexual reproduction, it is hard to argue that there have been dire consequences for resilience. By contrast, if relatively few genotypes came to dominate recently and persist (and even thrive) in the current environmental conditions, they may be at risk for reduced resilience in the future if acclimation or adaptation is precluded.

Conservation Implications

Reproduction is an essential component of resilience because it facilitates the growth and expansion of beds. Demographic consequences of the lack of sexual reproduction at so many sites on the Hudson River appear to be offset by asexual reproduction. The immediate demographic benefits thus may have longer term

consequences by precluding generation of new genotypes through recombination. Thus, two key elements needed for natural selection to be effective, genetic diversity and sexually produced progeny, are at low frequency or absent.

A key unknown is the amount of sexual reproduction necessary to generate sufficient genetic diversity and for resilience. Clearly sexual reproduction is low, but it does occur. Unpublished data (Neel and Engelhardt, Table 1) indicate that on average, ~37% of samples at sites were generated by sexual reproduction; sites varied from ~10-79% with a standard deviation of ~18%. To begin to understand the amount of diversity needed, one has to know about the breadth of environmental tolerances of individuals. If individual genotypes have broad tolerances that allow acclimation, extensive clonality will have little negative impact on resilience. By contrast, if individual genotypes vary in their tolerance and persistence at a site depends on tolerances present in an array of individuals, low genotypic diversity will create high risk. Thus, it is of particular interest to determine whether extensive highly clonal genotypes have the same breadth of environmental tolerance as arrays of genotypes generated through sexual reproduction.

Three factors lessen concern for *Vallisneria* in the Hudson River. First, aquatic angiosperms are typically broadly tolerant of a range of environmental conditions and abundant asexually produced individuals are potentially suited to conditions beyond what currently exist (Philbrick and Les 1996). At the same time aquatic environments are considered to be more stable than terrestrial environments (Philbrick and Les 1996). It is common to see limited sexual reproduction and low genotypic diversity at higher latitudes at edges of species geographic ranges (Yakimowski and Barrett 2014; Eckert et al. 2016), and yet many of these species are often ecologically successful in these

situations. Clearly, *Vallisneria* in the Hudson River is an ecological success story having recovered rapidly after devastating storms. Genetic data collected in 2015 (Neel and Engelhardt unpublished data, Table 1) indicate that much of this recovery can be attributed to asexual reproduction. Thus, increasing extent through stoloniferous growth within seasons that is translated across seasons through production of turions compensates for a lack of seed production.

Second, the temperature increases predicted under climate change are likely to benefit *Vallisneria* by generating warmer temperatures earlier in the season (Badeck et al. 2004) that allow sufficient growth for flowering and thus lengthening the growing season. Such beneficial effects on temperate seagrasses have been noted as a contrast to tropical species (Short et al. 2016). Although climate change also brings risks of storms of increased magnitude and sea level rise, in the particular case of seasonality of temperature there is a rare bit of good news.

Third, sampling each site only twice in one season provides a useful but incomplete picture of reproductive potential. In particular, the large proportion of non-flowering ramets may yield misestimates of the true sex ratios in populations. The proportions of ramets observed are within ranges documented elsewhere (5% (Lokker et al. 1994), to 24% (Titus and Stephens 1983), to 42% (Doust and Laporte 1991), to 69% (Lokker et al. 1997)). Further, females have been found to have a higher proportion of ramets with flowers (Doust and Laporte 1991). Thus, if large expanses of apparently non-reproductive plants found in the August sampling included males that did not release flowers exactly at the right time to be observed, perhaps prospects for pollination are higher than it might appear.

Management Options

If managers decide that increasing sexual reproduction and generating new genotypes is a desired goal, a relatively easy management solution would be to bring males and females into closer proximity to see if fruit set increases. All but one site (ACR) supported both sexes so such movement could be accomplished with no introduction of genotypes across sites. One could hand pollinate females with mature male flowers to generate seeds. Alternatively, moving different numbers of shoots of the opposite sex into extensive single sex patches could provide longer-term benefit. If plantings were done at different densities, information on spatial distribution of sexes needed to facilitate pollination could be gained. A downside of this approach is that siring by few fathers can yield many seeds that are full or half sibs. In the Chesapeake Bay, female fruits on average contain seeds sired by seven fathers and even so there is significant biparental inbreeding (Lloyd et al. 2018). The potential implications of many seeds being sired by one or a small number of fathers would need to be weighed in planning such an endeavor. If it was deemed necessary to increase genetic diversity beyond an individual bed, introducing males from neighboring sites to female-skewed sites and introducing female plants from neighboring sites to male-skewed sites might be viable options for increasing the potential for sexual reproduction; however, transplanting between sites should be considered with an abundance of caution. Care should be taken to only transplant between proximal sites, given the risk of out-breeding depression (Marsden et al. 2013) and transplanting should commence only after looking more closely at genetic relationships between populations.

Future Studies

The present study provided a first look at mode of reproduction in *V. americana* in the Hudson River; however, additional studies are needed to provide a comprehensive picture of the potential of *V. americana* to reproduce sexually. Assessing flowering in July and August provided information on the flower production and proximity of flowers for pollination, but how widely pollination actually occurred and led to fruit set by the end of the growing season is still unknown. Assessing flowering through to senescence in the fall and counting how many fruits are set throughout the growing season would more accurately assess the contribution of sexual reproduction in the river. Also, developing the relationship between GDD and plant and flower development by sampling more thoroughly through the early growing season would allow prediction of annual flowering potential based on water temperatures monitored by HRECOS. It is possible that sufficient sexual reproduction occurs in years with warmer spring temperatures. GDD predictions could provide great insight into long-term potential for the balance of sexual and asexual reproduction in the Hudson River. Further, determining if the relationship varies among *Vallisneria* genotypes, across the salinity gradient, or among geographic areas would provide insight into how different individuals might contribute differently to resilience and about local adaptation that might affect restoration choices. Beyond variation in GDD, assessing whether these genotypes vary in breadth of environmental tolerance will increase understanding of the risks associated with extensive clonal reproduction. Specifically, it would be instructive to ask if the extensive clones identified in the Hudson River genetic diversity sampling in 2015 have broader environmental tolerances than clones with limited distributions and to compare the tolerances of widespread versus rare genotypes to environmental conditions projected

under climate change. Examining the balance between flowering and vegetative growth in widespread versus rare genotypes across a range of growing season water temperatures would be fruitful. Taken together, these future studies would build on the intriguing results of this Polgar Fellowship to help predict the potential for sexual reproduction of *V. americana* in the Hudson River.

ACKNOWLEDGMENTS

We thank the Hudson River Foundation for providing funding through the Tibor T. Polgar fellowship, and the Cary Institute of Ecosystem Studies for hosting us in August. Special thanks to lab member Natalia Noyes, 2018 Polgar Fellow Virginia Caponera, and Patrick Gardullo, who assisted with field sampling, and David K. Thulman who assisted with measuring plants. Thanks also to Katia Engelhardt, who provided data on flowering on plants she propagated at the Appalachian Laboratory.

REFERENCES

Badeck, F.W., A. Bondeau, K. Bottcher, D. Doktor, W. Lucht, J. Schaber, and S. Sitch. 2004. Responses of spring phenology to climate change. New Phytologist 162:295–309.

Barko, J.W., D.G. Hardin, and M.S. Matthews. 1982. Growth and morphology of submersed freshwater macrophytes in relation to light and temperature. Canadian Journal of Botany 60:877–887.

Barko, J.W., D.G. Hardin, and M.S. Matthews. 1984. Interactive influences of light and temperature on the growth and morphology of submersed freshwater macrophytes. Technical Report A-84-3. U.S. Army Corps of Engineers, Aquatic Plant Control Research Program, Vicksburg, MS.

Barrett, S.C.H. 2015. Influences of clonality on plant sexual reproduction. Proceedings of the National Academy of Sciences 112:8859–66.

Bayley, S., V.D. Stotts, P.F. Springer, and J. Steenis. 1978. Changes in submerged aquatic macrophyte populations at the head of Chesapeake Bay, 1958-1975. Estuaries 1:171.

Best, E.P.H., and W.A. Boyd. 2001. A simulation model for growth of the submersed aquatic macrophyte American Wildcelery (*Vallisneria americana*). Final Report ERDC/EL TR-01-5. U.S. Army Corps of Engineers, Aquatic Plant Control Research Program, Vicksburg, MS.

de Boer, W.F. 2007. Seagrass–sediment interactions, positive feedbacks and critical thresholds for occurrence: a review. Hydrobiologia 591:5–24.

Boustany, R.G., T.C. Michot, and R.F. Moss. 2010. Effects of salinity and light on biomass and growth of *Vallisneria americana* from Lower St. Johns River, FL, USA. Wetlands Ecology and Management 18:203–217.

Brush, G.S., and W.B. Hilgartner. 2000. Paleoecology of submerged macrophytes in the Upper Chesapeake Bay. Ecological Monographs 70:645.

Calero, S., D. Auderset Joye, A. Rey-Boissezon, and M.A. Rodrigo. 2017. Time and heat for sexual reproduction: comparing the phenology of *Chara hispida* of two populations at different latitudes. Aquatic Botany 136:71–81.

Carter, V., and N.B. Rybicki. 1985. The effects of grazers and light penetration on the survival of transplants of *Vallisneria americana* Michx. in the tidal Potomac River, Maryland. Aquatic Botany 23:197–213.

Carter, V., N.B. Rybicki, and M. Turtora. 1996. Effect of increasing photon irradiance on the growth of *Vallisneria americana* in the tidal Potomac River. Aquatic Botany 54:337–345.

Chesapeake Bay Program. 2018. https://www.chesapeakebay.net/what/downloads/cbp_water_quality_database_1984_present.

Davis, F.W. 1985. Historical changes in submerged macrophyte communities of Upper Chesapeake Bay. Ecology 66:981–993.

Dawson, F.H. 1980. Flowering of *Ranunculus penicillatus* (Dum.) Bab. var. calcareus (R.W. Butcher) C. D. K. Cook in the river piddle (Dorset, England). Aquatic Botany 9:145–157.

Doering, P.H., R.H. Chamberlain, K.M. Donohue, and A.D. Steinman. 1999. Effect of salinity on the growth of *Vallisneria americana* Michx. from the Caloosahatchee Estuary, Florida. Quarterly Journal of the Florida Academy of Sciences 62:89–105.

Doust, J.L., and G. Laporte. 1991. Population sex ratios, population mixtures and fecundity in a clonal dioecious macrophyte, *Vallisneria Americana*. The Journal of Ecology 79:477.

Doyle, R.D., and R.M. Smart. 2001. Impacts of water column turbidity on the survival and growth of *Vallisneria americana* winterbuds and seedlings. Lake and Reservoir Management 17:17–28.

Eckert, C.G., and S.C.H. Barrett. 1993. Clonal reproduction and patterns of genotypic diversity in *Decodon verticillatus* (Lythraceae). American Journal of Botany 80:1175–1182.

Eckert, C.G., M.E. Dorken, and S.C.H. Barrett. 2016. Ecological and evolutionary consequences of sexual and clonal reproduction in aquatic plants. Aquatic Botany 135:46–61.

Engelhardt, K.A.M., M.W. Lloyd, and M.C. Neel. 2014. Effects of genetic diversity on conservation and restoration potential at individual, population, and regional scales. Biological Conservation 179:6–16.

Eriksson, O. 1989. Seedling dynamics and life histories in clonal plants. Oikos 55:231.

Findlay, S.E.G., W.C. Nieder, E.A. Blair, and D.T. Fischer. 2006. Multi-scale controls on water quality effects of submerged aquatic vegetation in the tidal freshwater Hudson River. Ecosystems 9:84–96.

Findlay, S.E.G., D.L. Strayer, S.D. Smith, and N. Curri. 2014. Magnitude and patterns of change in submerged aquatic vegetation of the tidal freshwater Hudson River. Estuaries and Coasts 37:1233–1242.

French, G.T., and K.A. Moore. 2003. Interactive effects of light and salinity stress on the growth, reproduction, and photosynthetic capabilities of *Vallisneria americana* (wild celery). Estuaries 26:1255–1268.

Gacia, E., and C.M. Duarte. 2001. Sediment retention by a Mediterranean *Posidonia oceanica* meadow: the balance between deposition and resuspension. Estuarine, Coastal and Shelf Science 52:505–514.

Gurbisz, C., and W.M. Kemp. 2014. Unexpected resurgence of a large submersed plant bed in Chesapeake Bay: analysis of time series data. Limnology and Oceanography 59:482–494.

Hamberg, J., S.E.G. Findlay, K.E. Limburg, and S.A.W. Diemont. 2017. Post-storm sediment burial and herbivory of *Vallisneria americana* in the Hudson River estuary: mechanisms of loss and implications for restoration. Restoration Ecology 25:629–639.

Heck, K.L., and T.A. Thoman. 1984. The nursery role of seagrass meadows in the Upper and Lower reaches of the Chesapeake Bay. Estuaries 7:70.

Holling, C.S. 1973. Resilience and stability of ecological systems. Annual Review of Ecology and Systematics 4:1–23.

Honnay, O., and B. Bossuyt. 2005. Prolonged clonal growth: escape route or route to extinction? Oikos 108:427–432.

Hopkins, A.D. 1918. Periodical events and natural law as guides to agricultural research and practice. Washington, Govt. print. off.

Hudson River Environmental Conditions Observing Network (HRECOS). 2018. Water Quality Monitoring Historical Data. http://www.hrecos.org/index.php?option=com_content&view=article&id=143&Itemid=54. Accessed 2018.

Hughes, A.R., and J.J. Stachowicz. 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proceedings of the National Academy of Sciences of the United States of America 101:8998–9002.

Koch, E.W. 2001. Beyond light: physical, geological, and geochemical parameters as possible submersed aquatic vegetation habitat requirements. Estuaries 24:1.

Lloyd, M.W., H.R. Tumas, and M.C. Neel. 2018. Limited pollen dispersal, small genetic neighborhoods, and biparental inbreeding in *Vallisneria americana*. American Journal of Botany 105:227–240.

Lokker, C., L. Lovett-Doust, and J. Lovett-Doust. 1997. Seed output and the seed bank in *Vallisneria Americana* (Hydrocharitaceae). American Journal of Botany 84:1420–1428.

Lokker, C., D. Susko, L. Lovett-Doust, and J. Lovett-Doust. 1994. Population genetic structure of *Vallisneria americana*, a dioecious clonal macrophyte. American Journal of Botany 81:1004.

Lubbers, L., W.R.W. Boynton, and W.M.W. Kemp. 1990. Variations in structure of estuarine fish communities in relation to abundance of submersed vascular plants. Marine Ecology Progress Series 65:1–14.

Marsden, B.W. 2015. Enhancing *Vallisneria americana* restoration using knowledge of genotypic and phenotypic diversity. University of Maryland College Park.

Marsden, B.W., K.A.M. Engelhardt, and M.C. Neel. 2013. Genetic rescue versus outbreeding depression in *Vallisneria americana*: implications for mixing seed sources for restoration. Biological Conservation 167:203–214.

McDonald, T. 2016. SDraw: spatially balanced sample draws for spatial objects.

Nieder, W.C., E. Barnaba, S.E.G. Findlay, S. Hoskins, N. Holochuck, and E.A. Blair. 2004. Distribution and abundance of submerged aquatic vegetation and *Trapa natans* in the Hudson River Estuary. Journal of Coastal Research 10045:150–161.

Nieder, W.C., S. Hoskins, S.D. Smith, and S.E.G. Findlay. 2009. Distribution and spatial change of Hudson River Estuary submerged aquatic vegetation: implications for coastal management and natural resource protection. Pages 259–277 *in* X. Yang, editor. Remote Sensing and Geospatial Technologies for Coastal Ecosystem Assessment and Management. Springer Berlin, Heidelberg.

Orth, R.J., T.J.B. Carruthers, W.C. Dennison, C.M. Duarte, J.W. Fourqurean, K.L. Heck, A.R. Hughes, G.A. Kendrick, W.J. Kenworthy, S. Olyarnik, F.T. Short, M. Waycott, and S.L. Williams. 2006. A global crisis for seagrass ecosystems. BioScience 56:987–996.

Orth, R.J., W.C. Dennison, J.S. Lefcheck, C. Gurbisz, M. Hannam, J. Keisman, J.B. Landry, K.A. Moore, R.R. Murphy, C.J. Patrick, J. Testa, D.E. Weller, and D.J. Wilcox. 2017. Submersed aquatic vegetation in Chesapeake Bay: sentinel species in a changing world. BioScience 67:698–712.

Orth, R.J., and K.A. Moore. 1983. Chesapeake Bay: an unprecedented decline in

submerged aquatic vegetation. Science 222:51–3.

Pebesma, E., and R. Bivand. 2018. sp: Classes and Methods for Spatial Data.

Philbrick, C.T., and D.H. Les. 1996. Evolution of aquatic angiosperm reproductive systems. BioScience 46:813–826.

Procaccini, G., and L. Piazzi. 2001. Genetic polymorphism and transplantation success in the Mediterranean seagrass *Posidonia oceanica*. Restoration Ecology 9:332–338.

R Core Team. 2018. R: a language and environment for statistical computing. Vienna, Austria.

Roy, D.B., and T.H. Sparks. 2000. Phenology of British butterflies and climate change. Global Change Biology 6:407–416.

Rybicki, N.B., and V. Carter. 2002. Light and temperature effects on the growth of wild celery and hydrilla. Journal of Aquatic Plant Management 40:92–99.

Sand-jensen, K. 1998. Influence of submerged macrophytes on sediment composition and near-bed flow in lowland streams. Freshwater Biology 39:663–679.

Sgrò, C.M., A.J. Lowe, and A.A. Hoffmann. 2011. Building evolutionary resilience for conserving biodiversity under climate change. Evolutionary applications 4:326–37.

Short, F.T., S. Kosten, P.A. Morgan, S. Malone, and G.E. Moore. 2016. Impacts of climate change on submerged and emergent wetland plants. Aquatic Botany 135:3–17.

Spencer, D.F., and G.G. Ksander. 2001. Field evaluation of degree-day based equations for predicting sprouting of hydrilla (*Hydrilla verticillata*) turions and tubers. Journal of Freshwater Ecology 16:479–486.

Sullivan, G., and J.E. Titus. 1996. Physical site characteristics limit pollination and fruit set in the dioecious hydrophilous species, *Vallisneria americana*. Oecologia 108:285–292.

Szmeja, J. 1987. The structure of a population of *Lobelia dortmanna* L. along a gradient of increasing depth in an oligotrophic lake. Aquatic Botany 28:1–13.

Titus, J.E., and M.S. Adams. 1979. Coexistence and the comparative light relations of the submersed macrophytes *Myriophyllum spicatum* L. and *Vallisneria americana* Michx. Oecologia 40:273–286.

Titus, J.E., and D.T. Hoover. 1991. Toward predicting reproductive success in

submersed freshwater angiosperms. Aquatic Botany 41:111–136.

Titus, J.E., and M.D. Stephens. 1983. Neighbor influences and seasonal growth patterns for *Vallisneria americana* in a mesotrophic lake. Oecologia 56:23–29.

Trudgill, D.L., A. Honek, D. Li, and N.M. Straalen. 2005. Thermal time - concepts and utility. Annals of Applied Biology 146:1–14.

Ward, L.G. 1985. The influence of wind waves and tidal currents on sediment resuspension in Middle Chesapeake Bay. Geo-Marine Letters 5:71–75.

Yakimowski, S.B., and S.C.H. Barrett. 2014. Clonal genetic structure and diversity in populations of an aquatic plant with combined vs. separate sexes. Molecular Ecology 23:2914–2928.