ABSTRACT

Title of Document: **ENHANCING VALLISNERIA AMERICANA RESTORATION USING KNOWLEDGE OF GENOTYPIC AND PHENOTYPIC DIVERSITY**

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Vallisneria americana Michx. (Hydrocharitaceae) is an ecologically important submersed aquatic plant that once dominated freshwater to oligohaline environments in eastern North America. After dramatic declines it is the target of many restoration initiatives. To increase knowledge of the capacity of remaining populations to either adapt through natural selection or acclimate to emerging environmental conditions, I combined genetic data and common environment experiments to quantify *V. americana* genetic diversity and differentiation at local to regional scales, evaluate evidence of local adaptation to different climate conditions, and assess evidence of inbreeding or outbreeding depression.

I quantified the structure of genetic diversity in five sites from the tidal Potomac, Hudson, and Kennebec Rivers, and 33 sites across the species' distribution in the Potomac. Genotypic (0.1-1.0) and allelic diversity (1.5-5.5), observed

heterozygosity (0.34-0.72), and relatedness (-0.06-1.00) varied greatly along rivers and across latitude. Hudson *V. americana* had the lowest genetic diversity and Potomac had the highest. Differentiation and network analysis of relatedness revealed no common genetic diversity distribution patterns within rivers. Major differences in genetic structure were observed across the tidal and non-tidal Potomac.

Common environment experiments evaluating growth and reproductive performance of Potomac, Hudson, and Kennebec *V. americana* grown in different temperature and photoperiod conditions only found evidence of local adaption in Potomac plants. Few overall differences in morphological and life history traits were observed between local and foreign plants. Plants grown under global warming conditions had reduced performance. Limited evidence of local adaptation and high acclimation to different conditions suggest that populations have high potential for resilience in the face of climate change, so long as temperatures do not exceed thermal tolerances. Climate change mitigation strategies that involve transplanting individuals may also be successful.

To investigate consequences of restoration strategies that translocate individuals, I evaluated seed production and germination success of controlled reproductive crosses between *V. americana* within and among genetically differentiated populations in the Chesapeake Bay. There were no consistent patterns of inbreeding or outbreeding depression in crosses. Effects of mixing sources were site-specific and not predicted by levels of relatedness among individuals, genetic diversity within, or differentiation among populations.

ENHANCING VALLISNERIA AMERICANA RESTORATION USING KNOWLEDGE OF GENOTYIC AND PHENOTYPIC DIVERSITY

By

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Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2015

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Preface

This dissertation contains an introduction, four chapters, a concluding chapter, and one supporting appendix. Each chapter is presented in manuscript form; therefore, background and methods may be repeated, pronouns reflect manuscript authorship, and tables and figures appear at the end of each chapter. A single reference section occurs at the end of the dissertation for literature cited throughout. Copyright clearance has been obtained as required.

Dedication

To my husband, Jesse, whose support and steadfast love is my foundation.

To my parents, Linda and Page, whose endless encouragement and humbling words of advice give me the bravery to pursue my dreams.

Acknowledgements

This research would never have come together if was not for the tremendous support I have received over the years from an incredible network of people. I would like to start by thanking Dr. Maile Neel, who first encouraged me to transition into the doctoral program and has supported me during all stages of my education and research. She continually challenged me to grow as a scientist and pushed me to consider the larger impacts of my work. She has also offered generous professional and personal support. I also thank the members of my committee: Dr. Katia Engelhardt for imparting her valuable insight into submersed aquatic vegetation, providing tremendous technical greenhouse support, and writing many letters of support; Dr. Matt Fitzpatrick for broadening my exposure to spatial and landscape ecology and providing instrumental instruction in R; Dr. Joseph Sullivan for advancing my understanding of plant physiology and making sure he pushed me to take a seat at 'the big kid's table;' and Dr. Lora Harris for stepping in at the final hour to provide new perspectives and ensure completion of my dissertation.

Past and present members of the Neel lab deserve acknowledgement and much of my appreciation. Dr. Mike Lloyd was my unofficial mentor and trained me in all of the core functions in our lab. His guidance and example taught me how to work productively and his mentoring got me though my qualifying exams. Shanie Gal-Edd assisted me on many long days and nights in the greenhouse and lab. As a friend she has celebrated with me during my research highs and helped curve my insecurities during my research lows. I would also like to acknowledge Chris Frye, Dr. Judy Che-Castaldo, and Dr. Sara Zeigler for their support of my work. Finally,

the assistance of several undergraduate students has been both invaluable to my research and the highlight of my graduate career. Hayley Tummas, Lessley Peterson, Sergio Correa, Audrey Simmons, Jordan Luber, Vera Pervitsky, Paul Widmeyer, Deirdre Griffin, and Ryan Blaustein assisted me on incredible amounts of field, greenhouse, and lab work that I could not have accomplished on my own and mentoring them made me a better scientist.

My doctoral research would not have been possible without being partially supported by teaching assistantships, but more importantly my graduate school experience would have been incomplete without the opportunity to teach undergraduate students at the University of Maryland. I would like to especially thank my TA coordinators, Dr. Hans Lemke, Dr. Jeffery Firestone, and Dr. Edgar Moctezuma, for training me, supporting me, and giving me the opportunity to teach in a variety of capacities.

I would like to thank the additional sources funding that supported my graduate school education and research, including the EPA Science to Achieve Results (STAR) Fellowship, the Washington Biologists' Field Club, NOAA Sea Grant Maryland, the Maryland Agricultural Experiment Station, the University of Maryland Center for Environmental Science Appalachian Laboratory, and the University of Maryland College Park.

I am grateful for the assistance I received in conducting my research. In particular I thank the many local organizations and individuals that helped me plan and coordinate my field work in Florida, South Carolina, Maryland, New York, and Maine. In particular I'd like to thank Dr. Richard Bartleson with the Sanibel Captiva

Conservation Foundation Marine Laboratory in Florida for aiding in the identification of collection locations, coordinating permitting, and collecting preliminary samples; Scott Lamprech and Larry McCord with the South Carolina Department of Natural Resources for aiding in the identification of collection locations and providing free accommodations at the SCDNR's Dennis Wildlife Center Dorm; and Dr. Stuart Findlay with the Cary Institute of Ecosystem Studies in New York for aiding in the identification of collection locations, providing accommodations, and generously assisting in the field. I would also like to thank Dr. Robert Hilderbrand and Dr. Frank Siewerdt for consultation and assistance with statistical analysis and Amy Beaven for support at the University of Maryland Genomics Core.

Last but definitely not least I want to thank my friends and family. The enduring friendship of Katharine, Meghan, Jessica, and Lindsay carried me through many difficult days and allowed me to continue to evolve my life during graduate school. I am grateful for my two rescue dogs, who save me every day with their de-stressing puppy kisses. Growing up with G.P., Allison, Derek, and Jennifer made me the person I am today and the addition of Morgan, Jim, Gretchen, Keith, and Dianne has further enriched my life and my mind. I want to thank my dad, Page, for always advising me to chase my interests and supporting me in every decision that followed. I am thankful that my mom, Linda, would lock me outside as a child and let me play in the mud – it created in me a sense of curiosity about nature that I still pursue. And I thank Jesse because this dissertation would not have been possible without the unwavering support of my husband. He inspires me to be a better person and wholeheartedly embraces the journey we are on together.

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Introduction

General Objectives

Globally, large-scale losses in submersed aquatic vegetation (SAV) have occurred over relatively short time periods, and with increasing frequency (Short and Wyllie-Echeverria 1996, Orth et al. 2006). Conservation of remaining SAV as well as restoration of areas devoid of this valuable resource have occurred at local scales with the goal of slowing and reversing declines in SAV coverage. Concomitantly, the threats of global climate change continue to stress aquatic ecosystems, so natural resource managers are looking beyond local restoration approaches to develop new strategies aimed at maximizing long-term persistence in the face of rapid environmental change. Without more complete understanding of the degree of genetic diversity and variation in phenotypic responses, developing strategies to manage SAV resources so that they are resilient in the face of climate change will be challenging at best, and deconstructive at worst. Furthermore, despite the urgency to develop novel restoration strategies, basic ecological risks of new strategies must be evaluated, especially the risks associated with moving and mixing sources of genetic material, as is often done in restoration practice.

The objective of my dissertation research was to provide a scientific foundation to inform restoration and management practices for the submersed aquatic plant species *Vallisneria americana* (wild celery) in the context of a globally changing environment. Additional knowledge of the risks of inbreeding and outbreeding depression in wild populations as well as the capacity of extant

populations to either adapt or acclimate to climate change is urgently needed. I evaluated the potential resiliency of *V. americana* populations located along three rivers spanning a broad latitudinal gradient by first quantifying the genetic diversity and differentiation within and among populations found along each river and comparing the patterns across latitude. Second, I assessed phenotypic variation and evidence of local adaptation of populations to provide insight into potential for future persistence through acclimation. Finally, I examined the impact of moving and mixing individuals from different populations by evaluating the reproductive success of crosses between individuals of *V. americana* collected from different sites and populations.

The results of my research provide information on how current and historic conditions have affected diversity within and connectivity among *V. americana* populations and offer insight into the potential resiliency of the species across latitudinal regions. Using *V. americana* dominated SAV beds a model system, these data provide a scientifically sound basis for choosing appropriate management options aimed at slowing and reversing declines in SAV.

Theoretical Motivation

Submersed aquatic vegetation (SAV) provides essential ecosystem services, including provision of food, shelter, and nursery habitat for nearshore fisheries, sediment and nutrient sequestration, primary production, physical stabilization, and erosion reduction (Fonseca and Cahalan 1992, Orth et al. 2006). Major factors contributing to large-scale losses of SAV include light limitations due to increased turbidity (e.g., nutrient and sediment loading), physical disturbances, competition

with non-native species, and loss of habitat connectivity. In response to these declines, protected areas that include SAV have been established, and SAV monitoring and restoration projects have been implemented worldwide (Orth et al. 2006).

To date, restoration efforts have primarily been implemented at small, local scales (Broadhurst et al. 2008) and used information on SAV sensitivity to water quality and light availability to guide selection of appropriate revegetation sites (Kemp et al. 2005). Unfortunately, many of these restoration efforts have had marginal success, with only small increases in SAV coverage since the 1980s (Moore et al. 2000). Although restoration typically includes planting locally sourced material (Lloyd et al. 2012), little attention is paid to the genetic and phenotypic diversity of the restored SAV stock.

Even as current stresses such as pollution and nutrient loading continue, the effects of global climate change are anticipated to dramatically alter SAV distribution and abundance (Oviatt 2004). Future projections reported by the IPCC (2014) predict an increase in global mean surface temperature between 0.3°C to 0.7°C by 2035, with temperature increases likely to exceed 2°C by 2100. Likewise, global projections for sea level rise estimate that the rate of sea level rise will increase to a rate of 8-16 mm/year by 2100. There is also strong likelihood that extreme weather events will become more intense and frequent (IPCC 2014). As a result, ecosystems around the globe will face novel disturbance regimes with increasingly greater differences from historical conditions (Carpenter et al. 2011, Scheffer et al. 2012). Altered timing of life history traits, geographical shifts in species ranges, and modified ecosystem

interactions have already been documented in natural populations as a response to global climate change (Root et al. 2003, Parmesan 2006). In SAV populations, declines along the western Atlantic have already been associated with periods of warming (Oviatt 2004). The potential overarching effects of climate change may make current, local restoration strategies insufficient in the long-term.

A species' capacity to adapt to a changing environment (i.e., its evolutionary potential) is determined by the amount of genetic and phenotypic variance on which natural selection can act (Frankham 2005). There are four possible responses of SAV to rapid environmental changes imposed by local and global anthropogenic threats: 1) populations persist under the altered conditions because many genotypes have sufficient phenotypic plasticity to acclimate to novel conditions; 2) populations are sufficiently diverse such that one or a few genotypes are able to persist and the population then adapts to new conditions through the process of natural selection; 3) genotypes currently at a site will not survive, but their offspring can disperse to locations that are more suitable; 4) genotypes currently at a site cannot survive and natural dispersal is not possible.

Managed relocation (MR) is emerging as a potential strategy to address biodiversity management and restoration when acclimation, adaptation, and dispersal are not possible (Richardson et al. 2009). MR involves the intentional movement of populations of species or genotypes from current areas of occupancy to locations where their probability of future persistence is predicted to be higher (Richardson et al. 2009). Advocates of MR claim that climate constrains the distribution of most taxa and that the rapid habitat shifts associated with future climate change will limit

dispersal capabilities and thus warrant human assistance (McLachlan et al. 2007). Officially, MR has been used sparingly to date, but its importance as a restoration strategy is likely to grow as changes in climate become more pronounced (Richardson et al. 2009). Research needs to address not only the feasibility of such strategies, but also the risks associated with this approach. MR would involve the mixing of genotypes from disparate populations. Short-term negative consequences of such plantings may arise if individuals are poorly adapted to the planting site and are not able to survive and establish populations, while long-term negative consequences of MR may manifest if offspring from mating between local and foreign individuals result in low fitness due to outbreeding depression (e.g. Montalvo and Ellstrand 2001).

Mounting evidence regarding changing climate and increased climate variability highlight the importance of maintaining or restoring resiliency to ensure the future persistence of natural populations and communities. The ultimate goal of ecological restoration is to reestablish self-sustaining ecosystems that will be resilient to future perturbation without ongoing human input (e.g. Procaccini and Piazzi 2001, Rice and Emery 2003, Ramp et al. 2006, Broadhurst et al. 2008, Liu et al. 2008). Resilience refers both to the ability of populations to persist in their current state and to undergo evolutionary adaptation in response to changing environmental conditions (Sgrò et al. 2011). We need to know if SAV species have sufficient genetic and phenotypic variability to acclimate or adapt to novel environmental conditions such that they are resilient into the future.

Research Objectives and Dissertation Format

The goals of my dissertation were to (1) quantify the genetic structure of *Vallisneria americana* Michx. (Hydrocharitaceae) within and across multiple rivers spanning a broad latitudinal gradient, (2) determine if there are patterns in the structure of genetic diversity within rivers that might be applicable to rivers where genetic information is not known, (3) evaluate the acclimation potential of *V. americana* to different regional temperature and photoperiod profiles, and (4) assess the fitness tradeoffs associated with moving and mixing *V. americana* from different populations or regions.

My first chapter quantified the spatial patterns of genetic variation of *V. americana* across the tidal portions of three major rivers in the Northeastern United States. For this work I collected *V. americana* from the Potomac River in Maryland, the Hudson River in New York, and the Kennebec River in Maine. In chapter 1, I quantified the range of *V. americana* genotypic and genetic diversity within and among the three rivers and commented on how this information can inform management decisions related to persistence and resilience of *V. americana* populations. Moreover, I evaluated if diversity within each river was structured similarly so that information from one location could inform restoration and management decisions for locations for which genetic diversity data are not available.

For my second chapter, I performed a more fine scale analysis on *V. americana* genetic diversity within a single river, quantifying the spatial patterns of genetic variation across the entire range of the species within the Potomac River, Maryland. I focused on evaluating major differences that were previously observed

in the structure of genetic diversity within the non-tidal versus tidal portions of the river (Lloyd et al. 2011).

In my third chapter, I assessed the potential for *V. americana* from each of the three rivers to acclimate to temperature and photoperiod conditions representative of each river region as well as to a future global warming scenario. I quantified evidence of *V. americana* local adaption using common environment experiments. The objective of this chapter was to determine whether or not *V. americana* have the acclimation potential to remain *in situ* in response to global warming, or if future restoration strategies that translocate populations across broad regions, like MR, are even feasible.

For my forth chapter, I evaluated reproductive success in terms of fruit size, seed number, seed size, and germination in controlled-environment crosses of *V. americana* sourced from within versus among genetically defined populations in the Chesapeake Bay. Results from the crosses were used to assess the relative risks and benefits associated with restoration strategies that either advocate for the local sourcing of material or promote mixing of stock sourced from multiple populations.

I summarized my overall conclusions in my fifth chapter as well as comment on ongoing and future projects that have evolved from the foundation of this work.

Chapter 1: **A comparison of the structure of *Vallisneria americana* genetic diversity & relatedness in three tidal rivers distributed across the northeastern United States: Implications for restoration**

Abstract

Coastal aquatic systems are expected to be disproportionately affected by changes in sea surface temperature and sea level rise. Thus, resilience in these communities is of paramount importance. *Vallisneria americana* Michx. (Hydrocharitaceae) is an important and broadly distributed submersed aquatic plant that has undergone dramatic declines in abundance and is the target of many restoration initiatives. To appropriately manage for resilience of this species in the face of climate change, additional knowledge on the capacity of remaining populations to either adapt through natural selection or acclimate to emerging environmental conditions is urgently needed. To this end, I quantified the structure of genetic diversity in *V. americana* populations sampled from five sites from the tidal portions of each of three major rivers spanning a broad latitudinal gradient – the Potomac River in Maryland, the Hudson River in New York, and the Kennebec River in Maine. Sampled sites varied in terms of genotypic diversity (0.27-1.00), allelic diversity (2.8-5.5), and observed heterozygosity (0.36-0.60). The Potomac River had significantly higher allelic diversity than the Hudson River, but significantly lower

observed heterozygosity than either the Hudson or Kennebec Rivers. *V. americana* from the Hudson River also has significantly higher levels of pairwise relatedness than individuals from the Potomac River. Moreover, the Hudson River had two multilocus genotypes (MLGs) that were found across multiple collection sites and the Kennebec River had three widespread MLGs found across multiple collection sites. Measures of population differentiation, STRUCTURE analysis, and individual network analysis of relatedness revealed that there were no common patterns in the distribution of genetic diversity within rivers. Therefore, understanding the range of *V. americana* genotypic and genetic diversity of one river cannot be used to inform restoration and management decisions in other rivers.

Introduction

As society continues to document the effects of anthropogenic disturbances on natural ecosystems, it is becoming increasingly clear that genetic factors affect population persistence and resilience (Sgrò et al. 2011). Resilience refers both to the ability of populations to persist or to undergo evolutionary adaptation in response to changing environmental conditions (Sgrò et al. 2011). In general, the potential for resilience depends upon whether or not extant populations have 1) phenotypic variation or plasticity to acclimate to changing environmental conditions, 2) heritable phenotypic and genetic variation for adaptation through natural selection, or 3) the ability to relocate through dispersal to more suitable locations. Genetic variation is essential for resilience in that it is associated with increased fitness (Williams 2001, Leimu et al. 2006), enhanced growth and productivity (Williams 2001, Reynolds et al. 2012a), species diversity (Booth and Grime 2003, Vellend 2006, Lankau and

Strauss 2007), and rapid response to disturbances (Hughes and Stachowicz 2004, Reusch et al. 2005). Furthermore, in an evolutionary context, genetic diversity enhances reproductive success (Ellstrand and Elam 1993, Crnokrak and Roff 1999, Amos et al. 2001) and enables adaptation of individuals to local environments (Montalvo and Ellstrand 2000, Joshi et al. 2001, Montalvo and Ellstrand 2001, Hammerli and Reusch 2002, Hufford and Mazer 2003). Genetic diversity is fundamental to resilience because it is necessary for adaptation to environmental change and evolution over the longer term (Sgrò et al. 2011).

Mounting evidence that climate is changing directionally and increasing in variability highlights the importance of maintaining or restoring resiliency to ensure the future persistence of natural populations and communities. Future projections reported by the Intergovernmental Panel on Climate Change (IPCC) predict an increase from the current global mean surface temperature between 0.3°C to 0.7°C by 2035, with temperature increases likely to exceed 2°C by 2100 (IPCC 2014). Likewise, global mean sea level rise will likely surpass the currently observed rate of 2.0 mm/year, with some projections estimating a rise of 8-16 mm/year by 2100. There is also a strong likelihood that extreme weather events will become more intense and frequent (IPCC 2014). As a result, ecosystems around the globe will face novel disturbance regimes with greater and greater differences from historical conditions (Carpenter et al. 2011, Scheffer et al. 2012). Altered timing of life history traits, geographical shifts in species ranges, and modified ecosystem interactions have already been documented in natural populations as a response to global climate change (Root et al. 2003, Parmesan 2006).

Coastal aquatic ecosystems are already among the most threatened in the world due to the prevalence of stressors related to chemical and organic pollution, hydromorphological changes from land development, and invasive species (Branch 1999, Kennish 2002). In addition to degradation brought on by current and historic anthropogenic land-use changes that cause increased nutrient and sediment runoff, nearshore aquatic communities will also be disproportionally affected by changes in surface temperature and sea level rise (Kennish 2002). The IPCC (2014) synthesis report notes that a large fraction of freshwater and marine species face increased extinction risk due to the current and future impacts of climate change.

Submersed aquatic vegetation (SAV) is an important component of aquatic ecosystems that is already being negatively impacted by warmer water temperatures (e.g., Oviatt 2004), sea-level rise and salt water intrusion (Quammen and Onue 1993, French and Moore 2003), and large-scale disturbances (Kemp et al. 1983, Orth and Moore 1983, 1984, Fernald et al. 2012). Both marine and freshwater rooted angiosperm communities promote healthy and diverse benthic communities (Orth et al. 2006) by providing shelter and nursery habitat to nearshore fish communities (Killgore et al. 1989, Orth et al. 2006) and acting as a primary source of food for waterfowl (Perry and Deller 1996), fish, and invertebrate communities (Strayer and Malcom 2007). SAV also provide critical ecosystem services, like improvement in water quality through sediment and nutrient sequestration (Brix and Schierup 1989, Takamura et al. 2003, Moore 2004, Gu 2008), physical sediment stabilization (Sand-Jensen 1998, Madsen et al. 2001), and erosion reduction (Fonseca and Cahalan 1992).

Globally, large-scale losses in SAV have occurred over relatively short time periods, and repeated reductions are occurring with increasing frequency (Walker and McComb 1992, Short and Wyllie-Echeverria 1996, Orth et al. 2006, Waycott et al. 2009). The most recent review found that areal extent of SAV worldwide is declining at a mean rate of 1.5% per year (Waycott et al. 2009). Major factors contributing to the declines include light limitation due to increased turbidity (from nutrient and sediment loading), physical disturbances (e.g., dredging), competition with non-native species, herbivory, and loss of habitat connectivity (Quammen and Onue 1993, Lokker et al. 1997, French and Moore 2003, Oviatt 2004, James et al. 2006, Orth et al. 2006).

In response to these global declines, marine protected areas that include SAV have increased and major SAV monitoring and restoration projects have been proposed and implemented worldwide (Orth et al. 2006). Although the need to include processes that maintain genetic diversity and adaptive potential in restoration planning and management has been advocated for some time (Pressey et al. 2007, Mace and Purvis 2008), assessments of genetic diversity are often not included in plans because this information is typically lacking and it is expensive to obtain (Lloyd et al. 2011, 2012). Further, issues arising from low levels of genetic diversity are often seen as being secondary to more immediate threats. However, in addition to affecting population persistence in dynamic environments (e.g., Lande and Shannon 1996), genetic diversity also increases the chances for successful establishment and persistence of restored populations (Williams 2001, Reynolds et al. 2012a, Reynolds et al. 2012k).

While there are several non-exclusive hypotheses about the broad-scale distribution of genetic diversity, freshwater angiosperms have received little attention. Across latitudes, many plants and animals show signs of geographic parthenogenesis, the broader and higher-latitude geographic distribution of asexually reproducing individuals compared to their sexual counterparts (Bierzychudek 1985, Thompson and Whitton 2006, Verhoeven and Biere 2013). Two common hypotheses for this trend are that asexually reproducing individuals have higher phenotypic plasticity due to efficient selection for general-purpose genotypes (Lynch 1984) and/or better colonizing abilities that facilitate range expansion (e.g., into previously glaciated areas; Bierzychudek 1985, Verhoeven and Biere 2013). Genetic structuring in marine systems, however, is often weak or random as a consequence of stochastic connectivity (Johnson and Black 1984, Becheler et al. 2010, Selkoe et al. 2010, Sinclair et al. 2014). More regionally, it is hypothesized that unidirectional gene flow in riverine systems will lead to erosion of genetic diversity in upstream river stretches and accumulation of genetic diversity in downstream stretches (Ritland 1989, Barrett et al. 1993). However, such associations have rarely been described (Gornall et al. 1998, Lundqvist and Andersson 2001, Liu et al. 2006, Pollux et al. 2007, Smith et al. 2015).

Our intent was to quantify the spatial patterns of genetic variation of the SAV species *Vallisneria americana* Michx. (Hydrocharitaceae) across the tidal portions of three major rivers spanning a latitudinal gradient – the Potomac River in Maryland (~39°N latitude), the Hudson River in New York (~41°N latitude), and the Kennebec River in Maine (~44°N latitude). We compared genotypic and genetic diversity

within each river to test if diversity was structured similarly both within rivers and across latitude. If genotypic and genetic diversity has similar patterns in structure within and across rivers, then information from one location could inform management decisions related to persistence and resilience of *V. americana* populations in locations where genetic diversity data are not available.

Methods

Study Species

The submersed aquatic plant *V. americana* is broadly distributed and exhibits extensive phenotypic plasticity and morphological variation (Les et al. 2008). *Vallisneria americana* is native to eastern North America and extends from southern Canada along the Atlantic coast to Florida and along the Gulf coast to Texas (McFarland and Shafer 2008). It is a perennial, dioecious, freshwater angiosperm that reproduces both sexually and vegetatively (Wilder 1974). Species within the genus *Vallisneria* exhibit extensive phenotypic plasticity in their morphological traits (Les et al. 2008), and *V. americana* has genotypically based variation in growth characteristics observed both within the scale of the Chesapeake Bay (Engelhardt et al. 2014a) and across its range (Les et al. 2008).

This once-dominant species has declined in abundance and distribution across the eastern United States (e.g., Brush and Hilgartner 2000, Shafer and Bergstrom 2010) but it remains locally dominant in freshwater and oligohaline waters. It is vitally important because of its ability to perform many of the functions widely documented for SAV, including maintaining dissolved oxygen and serving as habitat for fishes and invertebrates (Kemp et al. 2005, Findlay et al. 2006, Strayer and

Malcom 2007, Findlay et al. 2014). Dramatic declines in *V. americana* cover and extent coupled with its important ecological role have led to targeted efforts to conserve this species and to restore it in historic but currently unoccupied areas (Rybicki et al. 2001, Schloesser and Manny 2007, Lloyd et al. 2012).

Collection Locations

Vallisneria americana samples were collected from five sites in the tidal regions of each of three major rivers in the Northeastern United States – the Potomac River, MD, the Hudson River, NY, and the Kennebec River, ME (Figure 1.1).

The Potomac River originates at Fairfax Stone on the Allegheny Plateau of West Virginia and flows northeastward toward Cumberland, MD before turning southeast and ultimately discharging into the Chesapeake Bay at Point Lookout, MD. The 486 km long river drains approximately 38,000 km^2 and the tidal influence extends approximately 188 km from the mouth to the vicinity of Washington, DC. The tidal portion of the Potomac can be divided into three segments based on salinity in parts-per-thousand (ppt); the upper freshwater (<0.5 ppt) reach ranges from Washington, DC to the Indian Head peninsula, the middle oligohaline (0.5-5 ppt) reach continues downstream to Morgantown, MD, and the lower mesohaline (5-18 ppt) reach stretches to Point Lookout, MD (Mason and Flynn 1976). The mean tidal range is approximately 0.88 m in the upper tidal region near Washington, DC, and approximately 0.43 m near the mouth at the Chesapeake Bay (Cronin 1971, Mason and Flynn 1976). Mean annual temperature in the Potomac River estuary is about 13°C (Mason and Flynn 1976) and the surface water temperature ranges from about 18.4°C in the spring, to 25.9°C in the summer, 11.2°C in the fall, and 1.8 °C in the

winter (Carter and Rybicki 1986) (USGS Water Resources). The Potomac River is the second largest tributary of the Chesapeake Bay and water quality is characterized by high nutrient and suspended sediment concentrations (Mason and Flynn 1976). Coverage of SAV has been highly variable in the tidal freshwater reach of the Potomac River, but from 1988 to 2005 it has been fairly consistent in the middle reach and has even steadily increased in the lower reach since 1992 (Karrh et al. 2007). Species composition varies annually (Karrh et al. 2007, Rybicki et al. 2007), but the most common SAV species include the native *Vallisneria americana* (wild celery) and *Zannichellia palustris* (horned pond weed), and the invasive *Hydrilla verticillata* and *Myriophyllum spicatum* (milfoil; Karrh et al. 2007). A third exotic, *Najas minor* (naiad), has been documented but has minimal coverage in the Potomac River (Rybicki et al. 2007). Other SAV species that are commonly documented at low abundance include *Najas quadalupensis* (southern naiad), *Elodea canadensis* (waterweed), and *Heteranthera dubia* (stargrass) as well as the alga *Chara vulg*aris (muskgrass; Rybicki et al. 2007).

The Hudson River begins in the Adirondack Mountains and enters the Atlantic Ocean just south of New York City after travelling 507 km. The Hudson River watershed drains approximately 33,800 km^2 and the tidal influence extends north ~250 km to Troy, NY. The tidal reach of the river is broadly divided into four reaches and, while salinity is temporally variable, freshwater generally stretches from Troy, NY to Newburgh, NY, oligohaline continues downstream to Peekskill, NY, and mesohaline continues until just north of New York City, NY, where the polyhaline reach (18-30 ppt) begins (Yozzo et al. 2005). The average tidal range of the Hudson

River estuary is 1.4 meters and the mean tidal velocities are around 0.4 m/s (Limburg et al. 1986). The mean annual temperature in the Hudson River estuary is around 4°C, ranging from about -4°C during the winter to 24°C during the summer (Yozzo et al. 2005, Blumberg and Hellweger 2006). Dissolved oxygen in the estuary fluctuates with season and location (Hummel and Findlay 2006) and, while suspended matter also varies annually, water is generally turbid with high levels of inorganic nutrients (Findlay et al. 1999). Submersed aquatic vegetation is almost exclusively composed of *V. americana* (Strayer and Malcom 2007) with the non-native *Trapa natans* being locally abundant (Yozzo et al. 2005). *Hydrilla verticillata* has been noted only since 2013 (NYSDEC 2013). Another non-native species, *Trapa natans* (water chestnut), was purposefully introduced to New York State in the late 1800s (Hummel and Kiviat 2004).

The Kennebec River in Maine originates at Moosehead Lake (the state's largest lake) and flows 270 km to the Gulf of Maine in the North Atlantic Ocean, just south of Phippsburg. The Kennebec River watershed drains approximately 15,500 km^2 and the tidal influence extends north ~55 km to Augusta (Flynn 1978). The Kennebec River estuary is a narrow, glacially carved river valley characterized by temporally and spatially variable flow because of its non-uniform channel geometry, large tidal prism of freshwater toward its head, and highly variable freshwater discharge (Mayer et al. 1996, Fenster et al. 2001). Near the town of Richmond, the Kennebec River joins with six other rivers to form Merrymeeting Bay, the largest freshwater tidal bay on the eastern seaboard north of the Chesapeake Bay (Maine Department of Conservation 1982). The outlet of Merrymeeting Bay is the division

between the oligohaline and mesohaline reaches of the Kennebec River (Moore and Reblin 2008), whereas the inlet to Merrymeeting Bay is the division point between freshwater and oligohaline reaches of the river (Wong and Townsend 1999). However, due to the highly variable freshwater discharge, salinity levels are characterized by major temporal and spatial variability. For example, at Bath measured salinities near the surface were < 2 ppt at low tide during a "high-flow" (700 m^3/s) event in April, 1997 versus 11-12 ppt at high tide during a "low flow" (125 m^3/s) event in September, 1995 (Kistner and Pettigrew 2001). The semidiurnal tides have a mean range of 2.5 m and a maximum spring range of 3.5 m during proxigean spring tides (Fenster et al. 2001). The mean annual temperature in the region is around 7°C, ranging from about -6°C during the winter to nearly 20°C during the summer (Dionne et al. 2006). Common aquatic macrophytes in the Kennebec River include *V. americana*, *Potamogeton* spp., and *Elodea* spp. (Casperl et al. 2006). No invasive aquatic species have been observed in the Kennebec River (MEDEP 2014).

Collections

Samples of *V. americana* were collected across similar spatial scales from five sites in each of the three rivers (Figures 1.1, 1.2, 1.3, 1.4). Samples from the Hudson River and Kennebec River were collected during the summer of 2011; samples from the Potomac River were collected in 2008 by Lloyd et al. (2011). Collection sites were identified with the help of natural resource managers familiar with each river. Within each site we collected up to 30 shoots, each approximately 5–10 m apart, to be consistent with the sampling protocol of Lloyd et al. (2011). Generally, shoots were

collected along two transects parallel with the river and distances among samples were kept as consistent as possible given the natural variation in densities within and between sites. We recognize the limitations in detecting clonal extent within sites when sampling along transects (e.g., Arnaud-Haond et al. 2007), but our goal in sampling was to estimate the genotypic and allelic diversity at sites so that we could examine the spatial distribution of diversity within and across rivers, not to document the exact spatial extent of clones within sites. Latitude and longitude coordinates were taken for each sampled shoot using a handheld GPS unit. Shoots were placed on ice within one hour of field collection. They were transported to the University of Maryland College Park, where they were frozen at -20°C until DNA extraction.

DNA Extraction and Genotyping

Shoots from the Potomac River had previously been extracted and genotyped at 10 microsatellite loci (Lloyd et al. 2011) using robust primers with specific amplification that were developed for the species (Burnett et al. 2009). DNA from newly collected shoots from the Hudson and Kennebec Rivers was isolated using two different extraction protocols. First, DNA was extracted from all samples using a modified Chelex Bead^{TM} (Bio-Rad Laboratories) extraction method where a 1 cm^2 fragment of frozen leaf tissue was manually ground with a sterilized glass tamp in 200 µl of a 10% Chelex slurry. Samples were then boiled at 100°C for 10 minutes on an MJ Research PTC-200 Peltier Thermal Cycler. Supernatant containing DNA was then removed and diluted 1:2 in sterilized deionized water for subsequent genotyping. DNA was also extracted from leaf tissue of all samples using LGC sbeadx plant maxi DNA extraction kits (LGC) following the manufacturer's instructions.

Newly extracted DNA was amplified using the same ten loci used for the Potomac samples (Burnett et al. 2009). Polymerase chain reactions (PCR) were performed on an MJ Research PTC-200 Peltier Thermal Cycler using fluorescent labeled 500 LIZ™ forward primers (Applied Biosystems) and reagents in the TopTaq DNA Polymerase Kit (QIAGEN). Reaction conditions for all loci followed the protocols described by Burnett et al. (2009), with the modifications described by Lloyd et al. (2011). PCR products were separated and measured on an ABI 3730xl DNA Analyzer with GeneScan™-500 with the 500 LIZ™ Size Standard (Applied Biosystems). Peak data were then analyzed using GENEMAPPER v3.7 (Applied Biosystems) and all allele calls were visually inspected and made consistent with the Potomac River data by following the standards previously set by Lloyd et al. (2011).

For quality control purposes we genotyped DNA isolated from both extraction protocols for all sampled shoots and allele scoring in GENEMAPPER was done blind to sample number and site origin. Every ambiguous call was run a third time, and if the call was still ambiguous after three attempts, the alleles were coded as missing. Our final data set contained 0.02% missing data spread across all 10 loci in 51 individuals from 13 sites.

Genotypic Diversity

We assigned individual sampled shoots to unique multilocus genotypes (MLGs) using the program GENODIVE v2.0b17 (Meirmans and Van Tienderen 2004). Because mutation and scoring errors can lead to individuals originating from the same sexual reproductive event being assigned to different genotypes, we compared MLG assignments based on complete multilocus genotype matches with assignments based

on individuals that only differed by the minimum number of mutation steps needed to transform one genotype into another genotype (three mutation steps for tri-nucleotide repeat microsatellites). The latter method would group individuals with clearly distinct allele profiles in GENEMAPPER into one MLG. To prevent underestimating genotypic diversity we required complete multilocus matches to assign individual shoots to MLGs. This approach originally overestimated the number of MLGs because individual shoots with missing allele data were assigned to new MLGs. Therefore, we manually checked all shoots that had missing data and assigned them to unique MLGs only if their mutilocus genotype was unique despite missing loci (this occurred 32 times). If shoots with missing data were ambiguous we didn't assign them to any MLG and we discarded them from all subsequent analyses, even though they matched another MLG at all remaining loci (this occurred 19 times).

Within sites, the proportion of unique genotypes was calculated as (G - 1)/(N - 1), where G is the number of unique genotypes and N is the total number of sampled shoots assigned to MLGs (Arnaud-Haond et al. 2007). Differences in genotypic diversity among rivers were examined using one-way Analysis of Variance (ANOVA) in R v3.0.1 (R Core Team 2013).

Measures of Genetic Diversity

For each of the sampled sites, a suite of genetic diversity measurements was calculated. For all measures, each MLG was represented by only one shoot within each sampling site. The average number of alleles per locus (A), number of private alleles (A_p), percentage of polymorphic loci (P), and the mean observed (H_o) and expected (H_e) heterozygosity within each of the 15 sampling sites spanning three

latitudinal regions was calculated using GENALEX 6.5 (Peakall and Smouse 2006). Differences in all measures of genetic diversity among the three rivers were assessed in R using either one-way ANOVA or Kruskal-Wallis tests when data didn't meet the assumption of normality under the Shapiro-Wilk test. Evidence of geographic structure of genetic diversity along river gradients within regions was analyzed using Spearman rank correlation analysis of each measure of genetic diversity against latitude using R. We used latitude within each river to represent upstream versus downstream locations because the three rivers run mostly north to south within our sampled ranges.

Wright's F_{is} was calculated for the full dataset and for each sampling site using the estimator *f* (Weir and Cockerham 1984) in GDA (Lewis and Zaykin 2002) to test for site-level deviations from Hardy–Weinberg equilibrium. Significance of F_{is} for each locus was obtained using Fishers Exact tests in GDA with 3200 randomizations (Zaykin et al. 1995), and was assessed at the Bonferroni-adjusted $\alpha = 0.005$ for 10 comparisons. Significance of F_{is} for each sampled site was tested by obtaining confidence limits around each estimate generated by 1000 bootstraps in GDA. Significant departures from Hardy–Weinberg equilibrium can indicate a departure from random mating.

To detect recent bottlenecks, we determined if expected heterozygosity exceeded levels expected at equilibrium using Wilcoxon's sign rank test in BOTTLENECK v1.2.02 (Cornuet and Luikart 1996). A two-phase mutation model (TPM) run for 1000 iterations was used because it provides results intermediate between an infinite allele model and a stepwise mutation model, which are considered

to be most appropriate for microsatellites (Di Rienzo et al. 1994). Significance of the one-tailed Wilcoxon's sign rank test for heterozygosity excess was assessed at Bonferroni-adjusted $\alpha = 0.0033$ ($n = 15$ comparisons).

Estimation of Regional Genetic Structure and Differentiation

Analysis of molecular variance (AMOVA) was used to partition the genetic variation within and between sampling sites and also between latitudinal regions (Potomac River, Hudson River, and Kennebec River). AMOVA was conducted using GENALEX with population differentiation based on genotypic variance. This option produces an estimate of Φ_{PT}, an analogue of $F_{ST.}$ The program interpolated missing locus information and was run for 999 permutations to evaluate significance.

The distribution of diversity among sampling sites within rivers and among rivers was analyzed using three measures: Wright's F_{st} (Weir and Cockerham 1984), G'_{st} (Hedrick 2005), and D_{est} (Jost 2008). Even though genetic differentiation among populations is widely measured by calculating Wright's F_{st} statistic or its analogue for multiple alleles, G_{st} (Nei 1977), there are assumptions that complicate the interpretation of genetic divergence and gene flow among populations and these assumptions are almost always violated in natural systems (e.g., Bossart and Pashley Prowell 1998, Neigel 2002). When individual populations have high allele richness such as is found at hypervariable microsatellite loci, G_{st} underestimates differentiation because it measures the amount of variation among populations relative to the total variation without taking into account the identity of the alleles (Hedrick 2005). One simple method to account for allelic richness and overcome the dependence on levels of heterozygosity is to scale G_{st} by the maximum G_{st} possible for the observed

amount of heterozygosity (Hedrick 2005). The resulting statistic, $G'_{st,}$ varies from 0–1 and better reflects the underlying patterns of genetic diversity, but remains fundamentally based on heterozygosity. To overcome the issues associated with using heterozygosity as a means to describe genetic differentiation, Jost (2008) developed a summary statistic, $D_{est,}$ based on effective numbers of alleles (Jost 2008, Meirmans and Hedrick 2011). All three measures of population differentiation were calculated using GENALEX and significance was assessed using 1000 permutations. Differences in F_{st}, G'_{st}, and D_{est} estimated between sites within rivers were compared with measures estimated for sites between different rivers using independent two-way t-tests in R. Likewise, significance of differences in measures among sites within rivers was assessed using one-way ANOVAs in R.

The relationship between geographic distance and genotypic distance were considered using an isolation-by-distance analysis. Euclidean geographic distances were derived from the GPS coordinates of each sample. Euclidean distances were adequate to reflect the distances among sites because the sampled reaches of each river are relatively straight. Linearized genotypic distances were estimated with missing locus data interpolated as the average genetic distance calculated across all non-missing pairwise individual distances for the relevant population contrast. Significance was analyzed using a Mantel test with 999 permutations, as implemented by GENALEX.

We used the program STRUCTURAMA v2.0 (Huelsenbeck and Andolfatto 2007) to identify theoretical a posteriori 'populations' from our global collection based on minimal deviations from both Hardy–Weinberg and linkage equilibrium as

described by Pritchard et al. (2000). STRUCTURAMA differs from the program STRUCTURE (Pritchard et al. 2000) in that the number of theoretical populations is included as a random variable in a Dirichlet process model (Pella and Masuda 2006) and is estimated from a posterior distribution for the probabilities of each number. Because Huelsenbeck and Andolfatto (2007) suggest that the estimation of the number of populations can suffer when the aggregation parameter of the Dirichlet process model (α) is misspecified, we ran STRUCTURAMA under a range of α values. First, we set the prior mean of the number of populations to three (for the number of regions we sampled) and six (to represent a scenario that had more structure within sampled regions). The resulting estimated number of populations was sensitive to the α input, therefore we also let α act as a random variable represented by a gamma probability distribution with shape $\kappa=3$ and scale $\theta=2$. These parameter values allowed the Dirichlet process to test a variety of possible numbers of populations based on a range of α values from ~1 to 12. The sampler was run using four heated chains for 1,000,000 generations, and samples were taken every 25 generations for a total of 40,000 samples. Data were summarized after discarding 10,000 burn-in samples. We chose the mean partition value (K) containing the highest posterior probability as the number of theoretical populations.

Because STRUCTURAMA lacks interpretable visualization of individual assignments, we used STRUCTURE to assess distinctiveness of theoretical populations (Berryman 2002) by assigning individuals to the number of populations inferred by STRUCTURAMA. Following the recommendations of Onogi et al. (2011) for unbalanced sample sizes, STRUCTURE was run assuming no prior admixture and no

correlation of alleles, with 1,000,000 steps in the Markov Chain Monte Carlo sampler, using a burn-in of 50,000 steps.

Estimates of Relatedness

Variation in degree of genetic relatedness among individuals can either be a source of variation upon which natural selection can act, or a source of unaccounted for similarity among otherwise distinct genotypes. In absence of known pedigree information, a relatedness estimator can quantify the degree to which individuals share alleles and estimate the probability that the genes are identical by descent based on population level allele frequencies. Relatedness ranges from 0 (unrelated) to 1 (identical clones). For instance, first degree relatives (e.g., parent-offspring, full-sibs) average a relatedness coefficient of 0.5, second degree relatives (e.g., half-sibs) 0.25, third degree relatives (e.g., first cousins) 0.125, and unrelated individuals average a relatedness coefficient of 0.

We used the program COANCESTRY v1.0 (Wang 2011) to calculate the Wang (2002) estimator of pairwise relatedness among all collected individuals using both 1) region-specific allele frequencies (r_R) and 2) global allele frequencies (r_G). Using allele frequencies from a larger set of samples increases the accuracy in relatedness estimation (Bink et al. 2008), but also tends to increase local levels of relatedness. The global *V. americana* allele frequencies were calculated from data collected from all 15 sites in this study. We chose Wang's estimator because previous Monte-Carlo simulations (Marsden et al. 2013) indicated it had the lowest variance and minimal bias across various relationship categories (Van de Casteele et al. 2001). We used Pearson correlation analysis in R to assess the relationship between pairwise

relatedness coefficients calculated from region-specific allele frequencies and global allele frequencies.

We also sought to determine whether or not MLGs from each river were genetically more related to one another than expected from a randomly mating, panmictic population. Therefore, we compared the observed mean and variance of pairwise relatedness estimates within each river against their expected distribution under the null hypothesis of panmixia using 1000 Monte Carlo permutations of the same number of alleles, as implemented in the program IDENTIX v1.1 (Belkhir et al. 2002). Briefly, 2 *N* alleles were randomly sampled without replacement, independently for each of 10 loci, and assigned at random to the number of individuals within each river. The pairwise relatedness estimates for the observed data were compared with 1000 random permutations to evaluate significance. Even when the mean pairwise relatedness estimate does not differ from the null expectation of panmixia, a significantly high variance in the observed pairwise relatedness estimate can indicate that the sample is composed of groups of related individuals that are unrelated to each other (Belkhir et al. 2002). For this analysis, pairwise relatedness estimates were calculated with the Lynch and Ritland (1999) estimator using region-specific allele frequencies.

To understand the spatial distribution of relatedness within each river, we created spatially explicit individual-based networks of relatedness (based on regional-level allele frequencies, r_R) for each region at relatedness thresholds of r_R = 1.0, 0.5, and 0.25 in ArcMap v10 (ESRI 2011). Network nodes represent individual sampled

shoots (including duplicate MLGs) in their geographic location, and edges represent connections between shoots that were at or above each relatedness threshold value.

Finally, we created individual-based, spatially implicit networks of relatedness at a threshold of $r_R \geq 0.5$ and visualized them using the *igraph* package in R (Csardi and Nepusz 2006). In contrast to the spatially explicit networks, only one copy of each MLG was included in the spatially implicit network. Networks for each river were created based on Wang (2002) pairwise relatedness estimates using region-specific allele frequencies. To quantify connectivity between MLGs within rivers we calculated degree centrality (Freeman 1978, Wasserman and Faust 1994), closeness centrality (Freeman 1978), and eigenvector centrality (Bonacich 1987) for each MLG within each region using the *igraph* package in R. Degree centrality is a count of the number of adjacent edges of each node (MLG) in a network. MLGs with high degree centrality are directly related at $r_R \geq 0.5$ to many other MLGs within the river. Closeness centrality is a measure of how close a node is to all other nodes in a network, measured as the reciprocal of the sum of the distances to all other nodes in a connected network. MLGs with high closeness centrality have more and shorter paths to other MLGs within a network, and thus are more closely related to other MLGs. Finally, eigenvector centrality measures the influence of a node in a network based on the influence of nodes to which it is connected. Therefore, MLGs that are closely related to many other MLGs that are in turn closely related to many other MLGs have higher scores. Evaluating centrality metrics allowed us to determine if particular MLGs contribute disproportionately to sexual reproduction. Differences between measures of centrality among the three rivers were assessed using non-

parametric Kruskal-Wallis tests with subsequent post-hoc Nemenyi tests in R using the *PMCMR* package (Pohlert 2014). We also compared networks across rivers by quantifying the total number of edges and nodes within each network, the total number of components in each network, and the normalized graph level centrality indices using the *igraph* package in R.

Results

Genotypic Diversity

Out of 440 sampled shoots, 421 were successfully assigned to one of 314 unique MLGs. Missing data precluded unambiguous assignment of the remaining 19 individuals to MLGs. From the Potomac River, 129 MLGs (86%) were identified from 150 genotyped shoots (Figure 1.2), 73 MLGs (54%) were identified from 135 genotyped shoots from the Hudson River (Figure 1.3), and 106 (78%) MLGs were identified from 136 genotyped shoots from the Kennebec River (Figure 1.4). Genotypic diversity within sampling sites ranged from 0.27 to 1.00, with a mean of 0.73 (Table 1.1). Overall regional differences in genotypic diversity approached significance (ANOVA; $F_{2,12} = 3.74$; $p = 0.055$), with the largest differences occurring between the Potomac River and the less genotypically diverse Hudson River.

To visually demonstrate the range in genotypic diversity across sites and the extent of MLGs within sites, each site within each river was graphed in ArcMap and samples of the same MLG were connected with lines (Figures 1.2-1.4). In the Potomac River, 34 shoots (22.7% of those genotyped) were assigned to one of the 13 MLGs that were identified multiple times, accounting for 7-17% of the shoots genotyped within a single site. In the Hudson River, 82 shoots (60.7% of those

genotyped) were assigned to one of the 20 MLGs that were identified multiple times. These MLGs comprised 3-67% of the shoots within a site. In the Kennebec River, 43 (31.6%) of the genotyped shoots were assigned to one of 13 MLGs that were identified multiple times and comprised 4-29% of the shoots within a site.

Five MLGs were found across multiple sites; two were in the Hudson River (Figure 1.3) and three were in the Kennebec River (Figure 1.4, Table 1.2). No MLGs were shared across sites in the Potomac River (Figure 1.2) or across rivers. The two MLGs detected in multiple sites in the Hudson comprised 3-14% of the shoots genotyped within a site. The three MLGs that spanned multiple sites across the Kennebec comprised between 4-29% of the shoots genotyped within a site (Table 1.2).

The magnitude and direction of correlations between genotypic diversity and river location were not consistent across the three sampled rivers (Figure 1.5). Genotypic diversity in the Potomac and Hudson Rivers tended to be higher in downstream than upstream locations; the opposite pattern was seen in the Kennebec River (i.e., genotypic diversity decreased in an upstream to downstream direction). However, none of the correlations between genotypic diversity and latitude were significant (Figure 1.5).

Genetic Diversity

All 10 loci were polymorphic. The proportion of polymorphic loci (*P*) averaged across sites was $\bar{x}$ = 0.91 (*SD* = 0.06). Overall, MLGs from these study rivers did not differ in their proportion of polymorphic loci (Kruskal-Wallis; H = 0.49, df = 2, p = 0.78). In the full data set, eight loci departed significantly from

Hardy–Weinberg equilibrium (Table 1.3). When rivers were tested separately, two loci departed significantly from Hardy–Weinberg equilibrium in the Potomac River, one locus in the Hudson River, and six loci in the Kennebec River (Table 1.3).

The average number of alleles per locus (*A*) in the full data set was 7.5 (range 4-14) across all sampled MLGs and loci from all 15 sample sites, and within sites the average was 3.88 (*SD* = 0.77; Table 1.1). The Potomac River had a total of 62 alleles across all 10 loci, the Hudson River had 44, and the Kennebec had 58. There were significant differences across rivers in the average number of alleles per site (ANOVA; $F_{2,12} = 7.94$; $p = 0.006$), where the Potomac River had significantly higher allelic diversity than the Hudson River (Tukey HSD; $p = 0.005$). All rivers displayed a negative correlation between allelic richness and river location (Figure 1.5), but the correlation was only significant in the Hudson River ($\rho_S = -0.90$; n = 5; $p = 0.04$).

Across the 15 sample sites from all three rivers, eight private alleles occured at frequencies of 0.02 to 0.12 (Table 1.4). Every river contained at least one private allele. Two private alleles were found in the Potomac River in two sites (LSP and AL). One private allele was found at a Hudson River site (PEK). Five private alleles were found in the Kennebec River, including one at the SID site and four at the BTC site (Table 1.4). Private alleles tended to occur in more downstream locations (Figure 1.5), but correlations were not significant.

Average observed heterozygosity (H_o) of genets within all sample sites was 0.50 (*SE* = 0.02). H_o differed across rivers (ANOVA; $F_{2,12} = 18.40$; $p < 0.001$), with the Potomac River having lower H_o than either the Hudson (Tukey HSD; $p < 0.001$)

or the Kennebec (Tukey HSD; $p = 0.004$). H_o and river location were not correlated (Figure 1.5).

The Potomac River showed signs of heterozygote deficit ($f = 0.130$; 95% CI 0.08 to 0.20), whereas heterozygote excess was detected in the Hudson River ($f = -0.149$; 95% CI -0.21 to -0.60) and Kennebec River ($f = -0.089$; 95% CI -0.20 to -0.02). Within the Potomac, three sampled sites showed signs of heterozygote deficit (Table 1.1): GWP ($f = 0.181$; 95% CI 0.07 to 0.27), LSP ($f = 0.204$; 95% CI 0.13 to 0.30), and AL ($f = 0.193$; 95% CI 0.09 to 0.28). Based on analysis with the program BOTTLENECK, none of the sites sampled in the Potomac River showed evidence of a recent bottleneck based on H_e exceeding H_{eq} (heterozygosity expected at equilibrium). Within the Hudson River, three sites showed heterozygote excess (Table 1.1): NBB ($f = -0.341$; 95% CI -0.47 to -0.18), GAR ($f = -0.258$; 95% CI -0.42 to -0.01), and CRO ($f = -0.145$; 95% CI -0.24 to -0.07). No sites in the Hudson River had evidence of a recent bottleneck, but three of the five sites sampled approached significance at the unadjusted $\alpha = 0.05$ level: BNR ($p = 0.064$), PEK ($p = 0.064$), and CRO ($p = 0.064$). Finally, within the Kennebec River, three sites also showed signs of heterozygote excess (Table 1.1): WAT ($f = -0.300$; 95% CI -0.55 to -0.06), SID ($f = -0.199$; 95% CI -0.40 to -0.003), and RCH ($f = -0.075$; 95% CI -0.12 to -0.02). None of the Kennebec sites had evidence of a recent bottleneck, but the BTC site ($p = 0.007$) approached significance at the unadjusted $\alpha = 0.05$.

Estimation of Regional Genetic Structure and Differentiation

AMOVA indicated that 61% of molecular variance was within sampling sites ($\Phi_{PT} = 0.387$; $p = 0.001$), 14% among sampling sites within regions ($\Phi_{PR} = 0.183$; $p =$

0.001), and 25% among regions (Φ_{RT} = 0.250; p = 0.001). Measures of F_{st}, G'_{st}, and D_{est} supported the AMOVA findings of high genetic differentiation among regions in that all three measures were significantly different from zero (Table 1.5), and measures among sites within rivers were lower than measures among sites from different rivers (F_{st}: $t_{(57.96)}$ = 8.68, p < 0.001; G'_{st}: $t_{(46.95)}$ = 8.61, p < 0.001; D_{est}: $t_{(50.56)}$ = 9.97, p < 0.001; (Figure 1.6).

Among all sites combined, median F_{st} was 0.121 ($\bar{x}$ = 0.127; SD = 0.06), median G'_{st} was 0.331 ($\bar{x}$ = 0.321; SD = 0.15), and median D_{est} was 0.246 ($\bar{x}$ = 0.242; SD = 0.12). Although they varied in total magnitude (F_{st}: 0.006-0.282; G'_{st}: -0.010-0.621; D_{est}: -0.006-0.480), measures of F_{st}, G'_{st}, and D_{est} were consistent with one-another (Table 1.5). For example, pairwise estimates between GWP samples and SWP samples had the lowest F_{st}, G'_{st}, and D_{est} values (Table 1.6). Within the Potomac River, the mean pairwise values of F_{st}, G'_{st}, and D_{est} were 0.027, 0.046, and 0.031, respectively. Within the Hudson River, the mean pairwise values of F_{st}, G'_{st}, and D_{est} were 0.077, 0.174, and 0.124, respectively. Within the Kennebec River the mean pairwise values of F_{st}, G'_{st}, and D_{est} were 0.092, 0.226, and 0.163, respectively (Table 1.6). These measures differed among rivers: F_{st} (ANOVA; $F_{2,27}$ = 9.03; p = 0.001); G'_{st} (ANOVA; $F_{2,27}$ = 9.09; p = 0.001); and D_{est} (ANOVA; $F_{2,27}$ = 8.72; p = 0.001; Figure 1.7). Sites within the Potomac River were less differentiated than sites within either the Hudson (Tukey HSD; F_{st} p = 0.011; G'_{st} p = 0.017; D_{est} p = 0.021) or Kennebec (Tukey HSD; F_{st} p = 0.001; G'_{st} p < 0.001; D_{est} p = 0.001) Rivers (Figure 1.7).

Geographic distance and genetic distance were positively related ($R^2 = 0.226$, $p = 0.001$; Figure 1.8) when all sites were combined. Mantel tests also demonstrated that the relationships between geographic distance and linearized genotypic distance were positive, albeit weaker, within the Potomac ($R^2 = 0.043$, $p = 0.001$), Hudson ($R^2 = 0.073$, $p = 0.001$), and Kennebec ($R^2 = 0.155$, $p = 0.001$).

Bayesian clustering analysis implemented by STRUCTURAMA revealed four distinct genetic clusters among the 15 geographically separated sampling sites when the prior mean of the number of populations was set to three ($Pr[K = 4|X] = 0.92$). When the prior mean of the number of populations was set to six, STRUCTURAMA analysis supported five distinct genetic clusters ($Pr[K = 5|X] = 0.87$). Because the estimation of the number of populations appeared to be sensitive to the α parameter in the Dirichlet process, we also let α act as a random variable with a gamma distribution. Setting the shape (κ) and scale (θ) parametersto $\kappa=3$ and $\theta=2$ resulted in STRUCTURAMA randomly testing α values ranging from ~1-12. Under this model, the Bayesian clustering analysis found five distinct genetic clusters among the 15 geographically separated sampling sites ($Pr[K = 5|X] = 0.87$).

Visualization of the five genetic clusters with STRUCTURE revealed geographic structuring within rivers (Figure 1.9). When STRUCTURE was run assuming $K = 5$, two alternative groupings were found. Five of 10 runs supported a distribution with two genetic populations in the Potomac River, one in the Hudson River, and two in the Kennebec River (Figure 1.9, Table 1.7). The other 5 runs indicated one genetic population in the Potomac River and two populations in each of the Hudson and Kennebec Rivers (Figure 1.9, Table 1.7). The highest likelihood scores were

associated with the former distribution (Table 1.7). In either case, the partitioning of *V. americana* into genetic clusters based on minimal deviations from Hardy-Weinberg and linkage equilibrium revealed strong differentiation among rivers and weaker evidence of differentiation within rivers.

Estimates of Relatedness

V. americana pairwise relatedness coefficients, calculated using region-specific allele frequencies (r_R) and global allele frequencies (r_G; r = 0.971; df = 18917; $p < 0.001$), were positively correlated. Use of global allele frequencies consistently increased the relatedness coefficient estimate relative to estimates based on regional allele frequencies (Figure 1.10). Alleles restricted to one river appear rarer in the full dataset, such that all MLGs that share these less frequent alleles look more related to one another when global allele frequencies were used. These results are intuitively pleasing because it logically follows that alleles shared among MLGs within a hydrologically connected river are more likely to be identical-by-descent than alleles from different rivers. However, even though our results using the two different initial allele frequencies were highly correlated (Figure 1.10), the discrepancy between the relatedness estimates support findings from studies that have noted the limitations of pairwise relatedness estimates and their dependency on initial allele frequency input (e.g., Van de Casteele et al. 2001).

In the Potomac River, the average pairwise relatedness across all MLGs was 0.140 (*SD* = 0.229) based on global allele frequencies (r_G) and -0.105 (*SD* = 0.295) based on regional allele frequencies (r_R; Table 1.8). The average pairwise relatedness across all MLGs in the Hudson River was 0.158 (*SD* = 0.280) based on global allele

frequencies (r_G) and -0.017 (SD = 0.340) based on regional allele frequencies (r_R; Table 1.8). In the Kennebec River, the average pairwise relatedness across all MLGs was 0.105 (SD = 0.340) based on global allele frequencies (r_G) and -0.026 (SD = 0.390) based on regional allele frequencies (r_R; Table 1.8). Pairwise relatedness between all three rivers differed for both r_G (ANOVA; $F_{2,18916} = 49.2$; $p < 0.001$) and r_R (ANOVA; $F_{2,18916} = 131.8$; $p < 0.001$). Pairwise relatedness based on global allele frequencies (r_G) within the Hudson was higher than the Potomac (Tukey HSD; $p = 0.009$) and Kennebec (Tukey HSD; $p < 0.001$). Pairwise relatedness between MLGs from the Potomac were also higher than pairwise relatedness between MLGs from the Kennebec (Tukey HSD; $p < 0.001$). When pairwise relatedness was based on region-specific allele frequencies (r_R), individuals in the Potomac River were less related than either the Hudson River (Tukey HSD; $p < 0.001$) or Kennebec River (Tukey HSD; $p < 0.001$). MLGs from the Kennebec River were no more or less related to one another than MLGs from the Hudson River (Tukey HSD; $p = 0.444$).

The average estimate of relatedness between MLGs from within a sample site was consistently higher than the average estimate of relatedness between MLGs from an entire river (Table 1.8). The average within site relatedness also differed for both r_G (ANOVA; $F_{2,3843} = 131.1$; $p < 0.001$) and r_R (ANOVA; $F_{2,3843} = 324.8$; $p < 0.001$). For both measures, within site relatedness on the Potomac was lower than within site relatedness on the Hudson (r_G Tukey HSD; $p < 0.001$; r_R Tukey HSD; $p < 0.001$) and Kennebec (r_G Tukey HSD; $p < 0.001$; r_R Tukey HSD; $p < 0.001$). All three rivers had a positive correlation between both measures of within site relatedness and river

location (Figure 1.5), but the correlations were only significant in the Potomac River (r_G ρ_S = 0.90; n = 5; p = 0.04; r_R ρ_S = 0.90; n = 5; p = 0.04).

According to permutation tests implemented in the program IDENTIX, MLGs were not, on average, more related to one another than expected from a null hypothesis of panmixia in the Potomac River (p = 0.895), Hudson River (p = 0.849), or Kennebec River (p = 0.992). However, the variance in pairwise estimates of relatedness was higher in all three rivers (Potomac p = 0.001; Hudson p = 0.001; Kennebec p = 0.001). High variance in relatedness indicates pairwise comparisons involved a combination of highly related and unrelated individuals (Belkhir et al. 2002).

Spatially explicit networks of relatedness calculated from r_R show the distribution of relatedness in the three rivers (Figure 1.11). At a relatedness threshold of r_R = 1.0, network edges connect samples that were assigned to the same MLG. Thus, these networks represent all clones that were sampled multiple times. Such clones accounted for 0.3% of pairwise comparisons among sampled shoots in the Potomac River, 3.7% of comparisons in the Hudson River, and 1.2% of comparisons in the Kennebec River (Figure 1.11A). As previously noted, connections between like MLGs in the Potomac River occur exclusively within sites (Figure 1.11A and Figure 1.2). Connections among shoots that were related at $r_R \geq 0.5$ (e.g., first degree relatives up to and including clones) were found within and among all five sites in each of the three rivers (Figure 1.11B). These connections accounted for 3.5%, 11.6%, and 11.7% of all pairwise comparisons within the Potomac, Hudson, and Kennebec Rivers, respectively. Finally, at $r_R \geq 0.25$ (e.g., second degree relatives up

to and including clones), the number of connections nearly doubled in the Hudson and Kennebec Rivers, and increase almost six fold in the Potomac River, relative to the number of connections at the $r_R \geq 0.5$ level (Figure 1.11C). Connections at $r_R \geq 0.25$ account for 19.1%, 24.4%, and 20.7% of all pairwise comparisons between shoots from the Potomac, Hudson, and Kennebec Rivers, respectively.

To understand relationships among individual MLGs and groups of MLGs, we created spatially implicit networks of relatedness within each river at a relatedness threshold of $r_R \geq 0.5$ (Figure 1.12). Overall, the Potomac River had the lowest proportion of MLGs included in the network (93/129 = 0.72), and the Kennebec River had the highest (99/106 = 0.93; Table 1.9). The Hudson River had the smallest total number of MLGs (nodes) included in the network (65/73 = 0.89), the smallest number of edges, and the smallest number of components (Table 1.9). The Kennebec River had the most total nodes, the most edges, and the most components (Table 1.9). Rivers were different from one another in the degree centrality (Kruskal-Wallis; $H = 51.03$, $df = 2$, $p < 0.001$) and closeness centrality (Kruskal-Wallis; $H = 111.71$, $df = 2$, $p < 0.001$), but not in eigenvector centrality (Kruskal-Wallis; $H = 4.82$, $df = 2$, $p = 0.090$) calculated for each MLG node. Post-hoc Nemenyi tests reveal that degree centrality of MLGs from the Kennebec River are higher than MLGs from either the Potomac River (Nemenyi Tukey-Kramer; $p < 0.001$) or Hudson River (Nemenyi Tukey-Kramer; $p < 0.001$). Likewise, all three rivers are different from one another in closeness centrality scores for each MLG, such that closeness centrality is lower in the Kennebec River than either the Potomac River (Nemenyi Tukey-Kramer; $p < 0.001$) or Hudson River (Nemenyi Tukey-Kramer; $p < 0.001$), and closeness

centrality is lower in the Hudson River than the Potomac River (Nemenyi Tukey-Kramer; $p < 0.001$).

Within all three rivers, the five MLGs that had the highest degree centrality scores also tended to have the highest eigenvector centrality scores (Table 1.10), with the exception of MLG 359 from the LSP site, MLG 279 from GWP, and MLG 73 from CRO. MLG 93 from the Kennebec River was ranked in the top five within the Kennebec for all three measures of centrality. Interestingly, MLG 93 was one of the clones that was sampled more than once across multiple sites in the Kennebec River (Figure 1.4). The other MLGs that demonstrated clonal expansiveness by occurring in multiple sites varied in their within river ranking for each measure of centrality (Table 1.11). From the Kennebec River, MLG 93 and MLG 149 were directly related to many other MLGs in the Kennebec River (high degree centrality), were closely related to all other MLGs in the Kennebec (high closeness centrality), and were related to many other highly related MLGs (high eigenvector centrality; Table 1.11). MLG 80 was evenly related to all other MLGs in the Kennebec River with a high closeness centrality score (Table 1.11). However, MLG 1 and MLG 24 from the Hudson River ranked lower in centrality measures than the MLGs that were found across multiple sites from the Kennebec (Table 1.11). In general, the expansive MLGs from the Hudson River ranked relatively low when compared to the expansive MLGs from the Kennebec River.

Discussion

Despite accumulating evidence that genetic variation is fundamental to the resilience of a population, genetic data are often not included in conservation and

restoration planning because they are time consuming and expensive to obtain. For these reasons, managers often turn to the use of surrogates for genetic information, including general rules or extrapolation of patterns from limited data to inform their decisions. They hope that information learned from one location can inform decisions for locations for which genetic diversity data are not available. Variation in spatial patterns of genotypic and genetic variation in *V. americana* across tidal portions of three major rivers spanning a broad latitudinal gradient indicate limited ability to generalize. Patterns in the distribution of genotypic and genetic diversity were not consistent within rivers or across latitudes. Although there was some evidence for the downstream accumulation of genetic diversity in the Potomac and Hudson Rivers, this was not observed in the Kennebec River. Moreover, genotypic and genetic diversity differed among the three rivers in this study, with the lowest levels of genotypic and genetic diversity occurring in the Hudosn River at the center of the studied latitudinal range. Therefore, the environmental and hydrological processes that influence genetic structure of populations appear to be region specific. Our site specific results highlight the potential inability to manage populations across latitudes or rivers, even those distributed across similar spatial scales, in the same way.

Diversity and Relatedness of Multilocus Genotypes (MLGs)

We found a large range in the measures of genotypic and genetic diversity of *V. americana* across the species' distribution both within rivers and across latitudinal regions (Table 1.1).

Genotypic diversity was high for each site (Table 1.1) compared to the mean values observed for clonal terrestrial species (Genotypic Diversity = 0.17; Ellstrand and Roose 1987), and the broad range in genotypic diversity is a phenomenon seen in other clonal aquatic macrophytes (Genotypic Diversity: 0.20 to 0.71; Chen et al. 2007, Serra et al. 2010, Kamel et al. 2012, Sinclair et al. 2014). The broad range in genotypic diversity also suggests that some sites range from having very little detectable sexual reproduction to very little detectable asexual reproduction (Arnaud-Haond et al. 2010). Sites in the Hudson River had the lowest within river genotypic diversity and the largest range in genotypic diversity across sites with a river (Table 1.1).

Five of the *V. americana* MLGs span multiple sites (Table 1.2; Figures 1.3, 1.4) and account for up to 27% of the sampled shoots within sites. There are three possible ways that an MLG may come to be found across multiple sites within a river and to dominate within a site. First, a dominant MLG may have some phenotypic advantage through which it outperforms other MLGs in terms of vegetative growth. Higher vegetative growth could allow a clone to spread within a site during a single growing season and to produce more turions than other clones at that site at the end of a growing season. Over time, a clone with a cycle of faster vegetative growth and greater turion production could come to dominate. It is also possible that there are differences in plasticity between MLGs, such that the ones found across multiple sites have increased plasticity while other MLGs have a more restricted niche. Such clones could become expansive across sites because of their range of tolerances, as well as dominate within sites if the sites experience frequent disturbances or broad

variation in environmental conditions. Second, the expansive and dominant MLGs may have arisen by chance after recent bottleneck or disturbance events. Although borderline insignificant, there was some evidence of such bottlenecks in two of the Hudson sites where the expansive MLGs were found (BNR and PEK). Finally, the MLGs may have been found across multiple sites due to human-mediated transfer. Recreational fishing and boating are prominent in all three rivers and boaters may inadvertently transfer dislodged shoots (e.g., Rothlisberger et al. 2010) between boat ramps along each river.

To evaluate these alternative hypotheses, additional growth studies must be performed. Growth studies comparing the dominant MLGs with more rare MLGs can be used to understand the ecological importance of these genotypes. Greenhouse diversity experiments can assess why some MLGs dominate within sites, and under what environmental conditions. Such experiments will also give us better insight into the acclimation potential for each MLG. Acclimation in addition to genetic diversity will ultimately influence the resiliency of *V. americana* populations.

The variation in allelic diversity across all 15 sites from the three rivers (2.8 to 5.5 alleles/locus) fell within the range of *V. americana* allelic diversity previously reported for the Chesapeake Bay (1.5 to 5.8 alleles/locus; Lloyd et al. 2011), and more broadly within the range of other aquatic plant species from around the world (2.3 to 10.5 alleles/locus; Reusch et al. 1999e, 2000, Rhode and Duffy 2004, Pollux et al. 2007, Kornelis van Dijk et al. 2009). The site with the lowest allelic diversity, GAR in the Hudson (A = 2.9), also supported the fewest MLGs (Table 1.1). Allelic variation is important for long term resiliency because previous studies have observed

that it is associated with increased fitness (Williams 2001, Leimu et al. 2006) and enhanced growth and productivity of individuals (Williams 2001, Reynolds et al. 2012a).

Within individuals, levels of heterozygosity are related to the effects of inbreeding and influence probabilities of survival and reproductive success (Dudash 1990, Barrett and Kohn 1991, Ellstrand and Elam 1993, Fenster and Dudash 1994). Therefore, sites with low heterozygosity may have reduced resiliency due to diminished reproductive success and low offspring fitness (Ellstrand and Elam 1993, Crnokrak and Roff 1999, Amos et al. 2001). In fact, a recent study evaluating the ecological importance of different *V. americana* MLGs found that individuals with higher levels of heterozygosity produced more turion biomass (Engelhardt et al. 2014b). As previously mentioned, clones that produce more turions will influence the composition of MLGs within populations and overtime can come to dominate within sites. Across all sites within a river, the Potomac River showed signs of heterozygote deficit while the Hudson and Kennebec Rivers showed signs of heterozygote excess.

Because populations in need of conservation often have complex pedigree structures and high levels of fragmentation, isolation, and inbreeding, knowledge of relatedness can inform conservation strategies (Oliehoek et al. 2006). Increased levels of relatedness between individuals within a population have been associated with diminished reproductive success (Amos et al. 2001) and decreased offspring fitness (Crnokrak and Roff 1999, Amos et al. 2001). As a dioecious species, small populations of *V. americana* have increased risks of inbreeding due to lack of compatible mates. Therefore, understanding how individual MLGs of *V. americana*

are related to one another within sites and across rivers is essential to identifying and managing regions that may suffer the effects of mating among relatives.

It is of note that *V. americana* from the Potomac River had the highest genotypic diversity compared to the other rivers, yet significantly lower observed heterozygosity and evidence of inbreeding at three sites (GWP, LSP, AL; Table 1.1). Likewise, sites within the Potomac River contained MLGs that were significantly less related to one another than sites within the other two rivers. It is possible that the greater genotypic diversity in *V. americana* in the Potomac was due to a larger proportion of reproduction being sexual than plants collected from the other rivers. On the other hand, four of the five Hudson sites had among the lowest genotypic diversities of any sampled site, and had some of the higher observed heterozygosities. Hudson River *V. americana* also had significantly lower allelic diversity than the Potomac River (Table 1.1) and significantly higher relatedness between MLGs within a site (Table 1.8). The composition of genetic diversity in the Hudson River could potentially be the result of a heterozygote advantage that enabled a few MLGs with some advantageous gene combination to persist in their local environments. Heterosis is predicted to be high in small or highly structured populations (Whitlock et al. 2000, Theodorou and Couvet 2002, Coutellec and Caquet 2011). Stressful environments may also increase the incidence of heterosis (Armbruster and Reed 2005). With such different compositions of genetic diversity, and the wide range of factors that might influence this variation, these two rivers will need different management approaches to ensure long term resiliency.

Regional Genetic Structure and Population Differentiation

Quantifying genetic differentiation between sites that may or may not be a part of a continuous, natural population is difficult and each method for assessing structure in genetic diversity has its limitations. Moreover, differences in the genetic composition of different sites, especially in linear systems like rivers, may be more strongly driven by isolation-by-distance rather than any actual physical barrier to gene flow. For example, when gradients of genetic variation are created by neighbor mating, STRUCTURE tends to force continuous variation into genetic clusters (Schwartz and McKelvey 2009, Kalinowski 2011). Estuarine systems with tidal pulsing will have more complicated distributions of genetic diversity. Likewise, some assumptions complicate the interpretation of population differentiation because they are almost always violated in natural systems (Bossart and Pashley Prowell 1998, Neigel 2002). Measures of F_{st} and G'_{st}, which are based on heterozygosity, tend to depress overall estimates of differentiation and don't take into account the identity of the alleles (Hedrick 2005). Because of the limitations imposed by the use of any one way of assessing population differentiation, we used a variety of methods including standard measures of population differentiation, STRUCTURE analysis, and relatedness networks to gain multiple perspectives on the patterns of *V. americana* genetic variation observed across the length of each river. Overall, the distribution of genetic diversity varied greatly across rivers and there were no consistent patterns.

Calculation of F_{st}, G'_{st}, and D_{est} revealed significant differentiation between samples from the Potomac, Hudson, and Kennebec Rivers (Table 1.5). Despite the limitations of F_{st}, several previous studies on species of *Vallisneria* also measured

differentiation among sites using F_{st}, providing us with broader context for the observed levels of *V. americana* differentiation within each of the three study rivers. Calculations of F_{st} across the three hydrologically isolated rivers ($0.086 \leq F_{st} \leq 0.104$) are slightly lower than that reported for *V. americana* collected from genetically and geographically distinct populations in the Chesapeake Bay ($F_{st} = 0.114$; Lloyd et al. 2011). Likewise, measures of F_{st} between the three rivers in this study are lower than those estimated for other species of *Vallisneria* collected from isolated water bodies (e.g., Fst = 0.132–0.202; Wang et al. 2010). Measures of genetic differentiation among rivers in another SAV species, *Zostera marina* (eelgrass), sampled across a similar latitudinal gradient extending from North Carolina to Maine, had a broader range in F_{st} than the samples collected in this study ($F_{st} = 0.093$–0.363; Campanella et al. 2010a). The mean value of population differentiation for outcrossing species is $F_{st} = 0.146$ (Hamrick and Godt 1989) and a value of $F_{st} > 0.25$ can generally be regarded as indicating high population differentiation (Slatkin 1993). By this criterion, *V. americana* found in the Potomac, Hudson, and Kennebec Rivers showed moderate population differentiation. Population subdivision between *V. americana* from the Potomac, Hudson, and Kennebec Rivers is further supported by the AMOVA and STRUCTURE results. The AMOVA results revealed that 25% of the detected genetic variation was partitioned among regions and the STRUCTURE results display a strong division occurring between the three rivers when the *V. americana* MLGs are partitioned into genetic clusters (Figure 1.8).

AMOVA analysis also revealed that the lowest proportion of genetic variation (14%) was found among sample sites within rivers, while the highest proportion of

genetic variation (61%) was detected within sites. This pattern is most likely shaped by the outcrossing mating system of *V. americana*. Outcrossed species tend to have higher genetic diversity within populations and lower genetic differentiation among populations. Genetic drift and inbreeding, on the other hand, lead to lower genetic diversity within populations and higher differentiation among populations (Loveless and Hamrick 1984, Hamrick and Godt 1989). Potomac River *V. americana* (mean F_{st} = 0.006) samples had similar levels of genetic differentiation to other studies that sampled *Vallisneria* species along hydrologically connected rivers, including *V. americana* in the Detroit River (mean F_{st} = 0.025; Lokker et al. 1994) and *Vallisneria spinulosa* in the Yangtze River (F_{st} = 0.06; Chen et al. 2007). However, sites within the Potomac River had significantly less differentiation than sites within the Hudson or Kennebec River (Figure 1.7). Mean levels of *V. americana* differentiation in the Hudson (F_{st} = 0.077) and Kennebec (F_{st} = 0.089) were higher than those reported in the previous studies, indicating either reduced sexual reproduction or reductions in connectivity among sites in these two rivers.

Beyond assessing genetic differentiation between *V. americana* across rivers, we wanted to determine if there were similar patterns in the distribution of genetic diversity within rivers. Although the Bayesian clustering analysis implemented by STRUCTURAMA had high support for the genetic clustering of five populations, the STRUCTURE assignment of MLGs had mixed results (Figure 1.9), highlighting the limitations of using analyses like STRUCTURE along environmental and geographic gradients. The two conflicting STRUCTURE results suggest that each river had one or two genetically distinct populations, divided into upstream and downstream segments

with evidence of significant admixture between the two clusters (i.e., assignments of individual MLGs were divided between the two clusters). However, these trends can be attributed to the significant isolation-by-distance detected within each river.

Even though STRUCTURE results provided no strong support for discrete genetic populations within rivers, network analysis on related MLGs revealed distinctly different patterns in the distribution of genetic variation for each river (Figures 1.11, 1.12). The most notable pattern discovered from the spatially explicit networks was the fewer overall connections between MLGs in the Potomac, at all threshold levels, relative to MLGs from the Hudson and Kennebec Rivers, which had similar numbers of connections. Similarly, r_R was lower between MLGs from the Potomac than between MLGs from either the Hudson or Kennebec, indicating that most MLGs within the Potomac River are marginally related to one another at values of $r_R < 0.25$. By comparison, more $r_R \geq 0.25$ connections among MLGs within sites in the Hudson and Kennebec Rivers suggest they are highly related, but significantly higher measures of F_{st}, G'_{st}, and D_{est} indicate more isolation among sites.

The spatially implicit networks revealed that the nature of connectivity between related MLGs varied greatly across rivers (Figure 1.12). Nodes from the Potomac River network had significantly higher closeness centrality (Table 1.9), indicating that MLGs were more evenly related to one another relative to the Hudson or Kennebec networks. Likewise, Potomac River MLGs are just as related to MLGs within their own site as they are to MLGs from other sites (Figure 1.12). At the other extreme, MLGs from the Kennebec had significantly higher degree centrality, indicating that on average, Kennebec MLGs were more highly related ($r_R \geq 0.5$) than

MLGs from the other rivers. However, MLGs from the Kennebec also had significantly lower closeness centrality (Table 1.9). Therefore, MLGs from the Kennebec River were highly related to other MLGs from the same site, clustering tightly together, whereas MLGs from other sites were not as related (Figure 1.12). The Kennebec relatedness network even had six individual components represented from the five sampled sites (Figure 1.12). Likewise, the more distinct STRUCTURE divides in the Kennebec River reflect the highly clustered and subdivided relatedness network (Figures 1.8, 1.12).

Despite being more structured, the Hudson and Kennebec Rivers also had a few MLGs that were found multiple times across different sites within each river (Figures 1.3, 1.4). In general, the expansive MLGs from the Hudson River ranked relatively low in measures of centrality when compared to the expansive MLGs from the Kennebec River (Table 1.11). This might indicate differences in the asexual versus sexual contribution of these MLGs. For example, MLG 93 and MLG 149 from the Kennebec River may contribute disproportionately to the gene pool through both vegetative expansion (found multiple times within and across sites along the Kennebec River) and sexual reproduction (MLGs were closely and directly related at $r_R \geq 0.5$ to many other MLGs in the Kennebec). Increased sexual reproduction of MLGs in the Kennebec relative to the Hudson might contribute to the greater observed levels of genotypic diversity in the Kennebec (Table 1.1). However, the differences in the centrality measures for each MLG that was found multiple times across sites indicates that spatial dominance does not necessarily lead to cascading opportunities for sexual reproduction.

Lack of Common Patterns Found Across Rivers

Levels of genetic variation, structure, and clonal dominance vary by region. Although there is some evidence suggesting a pattern of upstream to downstream accumulation of genetic diversity (Figure 1.4) and differentiation (Figure 1.8), there are no consistent patterns in the structure of genetic diversity found across the three rivers examined in this study. Sampling the same species at similar spatial scales from three different rivers revealed differences in the distribution of genetic diversity that are site/region dependent. Therefore, restoration and conservation practices suitable to maintain long term resiliency in one region may not be applicable to other rivers, even for the same species across similar scales. The range and distribution of genetic diversity in *V. americana* appears to be more influenced by local environmental context and landscape history than by common patterns of dispersal and gene flow in rivers.

Periodic or fluctuating disturbances often foster more genotypic diversity when the fitness of individual genotypes differ under varying environmental conditions (Hammerli and Reusch 2003). Previous studies have correlated higher genotypic diversity with increased resistance to periodic stressors and more resilience after climatic extremes in experimental settings (Hughes and Stachowicz 2004, Reusch et al. 2005, Hughes and Stachowicz 2009). But, this pattern depends on the magnitude and frequency of disturbances. Extreme levels of disturbance may exceed the physiological tolerances of most genotypes and could lead to low genotypic diversity. The *V. americana* samples from the Potomac, Hudson, and Kennebec Rivers analyzed in this study each experience different degrees of tidal and salinity

stress associated with tidal flux. Variation in the degree of tidal stress and disturbance might contribute to the observed differences in overall levels of genetic diversity. Even though *V. americana* were sampled at similar spatial scales along three rivers, the geography of each river differed such that this scale transected different salinity regimes. In the Potomac River, samples were only collected from tidal freshwater and oligohaline portions of the river (Figure 1.2; Mason and Flynn 1976). Sampled sites extended from the tidal fresh to oligohaline transition zone into mesohaline portions of the Hudson River (Figure 1.3; Yozzo et al. 2005). In the Kennebec River, *V. americana* samples were collected from freshwater to mesohaline tidal regions as well as from non-tidal reaches of the river (Figure 1.4; Wong and Townsend 1999, Moore and Reblin 2008).

The Potomac River discharges into the Chesapeake Bay and is thus more protected from variable, extreme tidal pulsing (Cronin 1971), which may be a contributing factor to the lower levels of genetic differentiation observed in the Potomac River (Figure 1.6, 1.12). In contrast, extreme tidal pulses of the Hudson extend far up the main stem of the river and have great annual and interannual variation (Limburg et al. 1986). It is possible that repeated tidal stress contribute to the occurrence of lower overall genotypic diversity (Table 1.1) and higher relatedness among MLGs within sites (Table 1.8). Because our sampling scheme crossed multiple salinity zones, it is also not surprising that *V. americana* genetic differentiation among sites was high within the Hudson (Figure 1.6) and that r_R was dramatically higher between MLGs within sites than between MLGs across all sites (Table 1.8). Tidal and salinity zone transitions correspond with genetic breaks in the

Kennebec River. For example, the non-tidal to tidal transition in the Kennebec occurs between the SID and GDR sites (Figure 1.4) and corresponds to a stark division in the STRUCTURE results for Kennebec MLGs (Figure 1.8) as well as with a break in the clustering of closely related MLGs in the spatially implicit relatedness network (Figure 1.12). Likewise, the BTC site near the mouth of Merrymeeting Bay is proximate to the oligohaline to mesohaline transition. Moreover, Merrymeeting Bay marks the confluence of six additional rivers (Maine Department of Conservation 1982) and it is possible that some MLGs from this site might be partially related to upstream MLGs from the other rivers, and thus more distinct from upstream Kennebec MLGs. STRUCTURE analysis grouped MLGs from the BTC site with MLGs from the non-tidal WAT and SID sites (Figure 1.8). This assignment was probably an artifact of the limitations of STRUCTURE because MLGs from the BTC site are actually less related and more genetically distinct from MLGs from the rest of the river. The spatially implicit relatedness network confirms that many MLGs from BTC are not connected to MLGs from the other sites (Figure 1.12).

In additional to variation in tidal and salinity ranges, it is possible that differences in the geographic location and exposure of each of the three rivers will cause variation in the frequency and degree of major storm events, like hurricanes. Even if regions are impacted with the same frequency by major storms, variation in history and timing of the last major storm will have lasting impacts and large effects on levels of genotypic and genetic diversity within a river. In addition to the short term consequences of bottlenecks from major storms, disturbances resulting in small population sizes can have long-lasting influence on overall genetic diversity, potential

for resiliency, and evolutionary potential (Ellstrand and Elam 1993). For example, during the 1960s and 1970s, populations of all SAV species in the Chesapeake Bay declined concomitantly with regional water quality degradation and Hurricane Agnes in 1972 (Kemp et al. 1983, Orth and Moore 1983, 1984). Natural resurgence of SAV in the tidal Potomac wasn't documented until 1983 and then it was associated with decreased concentrations of phytoplankton, increased water clarity, and favorable flow and weather conditions (Carter and Rybicki 1986, Rybicki et al. 2001). Since recovery there has been great variation in SAV coverage and species composition (Carter and Rybicki 1994, Rybicki et al. 2001), but more recent surveys indicate that SAV has been fairly consistent in the tidal Potomac since 1992 (Karrh et al. 2007). Minimal impact of other large scale disturbances since Hurricane Agnes in 1972 may be one contributing factor to the higher observed levels of genotypic and allelic diversity and lower levels of differentiation among sites in the Potomac relative to the other two rivers. Unfortunately, all three rivers may be at risk of increasing exposure to major disturbances as global climate models suggest that rising ocean sea-surface temperatures may increase the frequency and intensity of hurricanes across the northwest Atlantic Ocean (Knutson and Tuleya 2004, Michaels et al. 2006).

Although not associated with natural disasters, the structure of genetic variation in the Kennebec may have been impacted by historic manmade disturbances. The Kennebec River has an extensive hydroelectric history. As many as eleven dams were built on the main stem of the Kennebec River, including East Outlet Dam at Moosehead Lake, Harris Dam at the foot of Indian Pond, Wyman Dam in Moscow, Williams Dam in Solon, the Upper Anson and Lower Abenaki Dams at

Madison, Weston Dam in Skowhegan, Shawmut Dam in Fairfield, the Hydro Kennebec and Lockwood Dams in Waterville, and the Edwards Dam in Augusta (Didisheim 2002, Michor 2003). All of the dams except for Edwards Dam were upstream from our sampling sites and are still in operation today. Edwards Dam, which was removed in 1999, falls between the SID and GDR sites (Didisheim 2002, Michor 2003). In addition to the tidal to non-tidal transition, the construction of Edwards Dam in 1837 may have also attributed to the strong genetic structuring between the SID and GDR sites (Figure 1.8). The spatially implicit network of relatedness for the Kennebec River show low relatedness between the upstream WAT and SID sites and the downstream GDR and RCH sites (Figure 1.12), suggesting that gene flow is present, but minimal. Monitoring over time will be needed to assess whether or not there are signs of increasing gene flow across these two sites. Dams are also present on the main stems of the Potomac and Hudson Rivers, but they occur in non-tidal areas upstream from the areas sampled in this study (Yozzo et al. 2005, Southworth et al. 2008)

Knowledge that each river is independently structured means that restoration plans for aquatic macrophytes like *V. americana*, and the species that depend on them, must be evaluated within their regional context. Our current data lead us to several different predictions about the various factors that might be influencing the structure and extent of genetic diversity within each river. Relatively high levels of genotypic and allelic diversity in Potomac and low overall levels of relatedness between MLGs combined with little evidence of population structure and no detection of expansive MLGs suggests that *V. americana* from the tidal Potomac

experience only moderate levels of environmental disturbances and may have increased sexual reproduction. Meanwhile the low levels of genetic diversity and highly related MLGs that show structure across the Hudson River are likely frequently exposed to maximal tidal disturbances resulting in evidence of excess levels of heterozygosity. The relatively higher levels of observed heterozygosity in the Hudson may be the result of recent bottlenecks. On the other hand, excess heterozygosity may arise from locally adapted heterozygotic advantages that enables persistence of a few advantageous gene combinations that are then passed on and shared by related MLGs within disturbed sites. Finally, we predict that high degree of genetic structuring across sites in the Kennebec River, despite the apparent sexual reproduction of MLGs that were found across many sites, is driven by a combination of local adaptation to a wide range of salinities and tidal regimes in addition to long term physical barriers to gene flow cause by dam construction. Genetic monitoring of these sites over time and controlled greenhouse experimentation on the morphological responses of the MLGs collected in this study to varying environmental conditions are needed to more fully test these new hypotheses.

Implications for Restoration

The ultimate goal of ecological restoration is to reestablish self-sustaining ecosystems that will be resilient to future perturbation without ongoing human input (Procaccini and Piazzi 2001, Rice and Emery 2003, Ramp et al. 2006, Broadhurst et al. 2008, Liu et al. 2008). We emphasize that genetic diversity should be taken into consideration for future restoration efforts because of the accumulating evidence that genotypically-based variation in growth characteristics can ultimately affect

ecosystem functioning (e.g., Bolnick et al. 2003). Therefore, based on our results we currently recommend that if natural recovery is insufficient and sources of *V. americana* must be collected for restoration, they should be harvested from a few sites local to the restoration spot. We further recommend that *V. americana* not be transferred across rivers from different latitudinal regions.

Restoration has the capacity to capture levels of genetic diversity that are comparable to those naturally occurring in well-established beds (Reynolds et al. 2012k, Reynolds et al. 2013). For example, when comparing naturally recruited meadows of *Zostera marina* (eelgrass) along the North American Atlantic coast to sites that had been restored, Reynolds et al. (2013) found that restoration was successful at reestablishing meadows with high genetic diversity while naturally recruited meadows were less diverse and exhibited signs of genetic drift. However, restoration activities that capture similar levels of genetic diversity do not necessarily capture the same composition of that diversity relative to natural, local populations (e.g., Lloyd et al. 2012). Restoration activities can even lead to deleterious effects, like outbreeding depression, when source material has non-local adaptations (McKay et al. 2005).

Because restoration activities have the capacity to alter the genetic composition and structure of SAV populations relative to natural and/or historic conditions (e.g., Lloyd et al. 2012), the appropriate selection of restoration stock to minimize long-term risks and maximize resiliency is still highly debated and remains controversial. Some scientists debate that a restoration strategy with the potential to alter genetic diversity is beneficial because it can increase diversity and counteract

local inbreeding through the introduction and mixing of genotypes from multiple foreign source populations (Broadhurst et al. 2008, Weeks et al. 2011). Other managers maintain that local restoration stock is best because local stock can be well adapted to the environmental conditions of a site and will successfully establish and integrate into a new site with no risk of outbreeding depression (Montalvo and Ellstrand 2000, 2001, McKay et al. 2005). These two paradigms for selecting restoration stock represent two ends of a spectrum. In reality managers must make decisions, often without much background knowledge of the genetic context of their local populations or local repository stock, to balance the risks of inbreeding versus outbreeding depression.

Just as genetic differentiation among populations on a contiguous landscape is not discrete, but rather on a continuous scale, the range of restoration options also fall along a continuum. Selection of restoration stock can range from source selection within sites, among several local sites within regions, to selection of stock from among different regions. Selection of stock from local sites has the perceived benefit of reducing the risks of genetic dilution, maladaptation, and outbreeding depression (Montalvo and Ellstrand 2000, 2001, McKay et al. 2005), but risks decreasing in fitness if inbreeding depression is present and inhibiting future acclimation or adaptation if standing genetic diversity is too low (Fenster and Dudash 1994, Broadhurst et al. 2008, Hughes et al. 2008, Weeks et al. 2011). Therefore, we recommend local selection of stock for restoration when populations have relatively high within site genetic diversity, no signs of inbreeding depression, and relatively high differentiation from other sites or regions. Selection of stock from multiple sites

within a geographic or genetically defined region has the perceived benefit of increasing genetic diversity, leading to genetic rescue (Fenster and Dudash 1994, Broadhurst et al. 2008, Hughes et al. 2008, Weeks et al. 2011). However, mixing stock from multiple sources increases the risk of genetic dilution of locally adapted genotypes and potential outbreeding depression (McKay et al. 2005). Therefore, we recommend this stagey for populations that have relatively low within site genetic diversity with evidence of inbreeding depression. At the extreme, selection of stock from different regions might be beneficial for long-term resilience of a population if selection is able to match current local adaptations to expected future environmental conditions (McLachlan et al. 2007). This strategy is most closely akin to managed relocation (MR). Managed relocation (MR; Richardson et al. 2009) is emerging as a potential climate change mitigation strategy that involves the intentional movement of populations or appropriately adapted genotypes from currently occupied areas to locations where probability of future persistence is predicted to be higher (Richardson et al. 2009). However, few studies have thoroughly examined the feasibility of such strategies nor quantified the risks associated with them.

Choosing among source selection strategies is a major undertaking in restoration, especially because the degree of differentiation among populations and inbreeding within populations vary independently, making the overall risks of inbreeding versus outbreeding depression site specific (Marsden et al. 2013). But even beyond the ecological and evolutionary implications of selecting one restoration strategy over another, natural resource managers must also balance budget and time constraints and the interests of multiple constituents and stakeholders. Therefore, our

goal is to provide the most scientifically defensible and conservative recommendations we can based on our understanding of current data. This work shows that there are differences in the structure of *V. americana* genetic diversity between regions that is largely influenced by local conditions and region-specific history to which *V. americana* might be locally adapted. Therefore we recommend that genotypes from one river should not be transferred across broad latitudes to another river because they will be at increased risk of outbreeding depression when they mix with more local genotypes, assuming plants are even capable of acclimating to the new environmental conditions. This recommendation is in contrast to that proposed by Campanella et al. (2010a, 2010g), who suggested that *Z. marina* from either the Chesapeake Bay or northern Maine would serve as good donor sites for Barnegat Bay, NJ restoration stock. Bed coverage of *Z. marina* in Barnegat Bay has declined 62% over the last 25 years (Bologna et al. 2000) and restoration efforts have had varying levels of success (Reid et al. 1993, Bologna and Sinnema 2005, 2006). They characterized the genetic diversity of *Z. marina* at several sites ranging from North Carolina to Maine and found that beds from the Chesapeake Bay and northern Maine had relatively high levels of genetic diversity (Campanella et al. 2010a, Campanella et al. 2010g). However, because they only assessed one site within each region they were not able to examine the variability or distribution of genetic diversity within each region. Strategies like MR should only be considered as a last resort when there is insufficient phenotypic variation for acclimation, limited genetic variation for adaptation, and natural dispersal to suitable conditions is restricted due to human activities.

In addition, we found enough differences in the structure and degree of *V. americana* genetic diversity between the three rivers to warrant different recommendations for future restoration activities. Wang et al. (2010) suggested that any population of the *V. spinulosa* studied by Chen et al. (2007) could be used as stock for re-introduction along the Yangtze River in China because it had considerable genetic variation and low population genetic differentiation among sites along a well-connected river. Likewise, Reynolds et al. (2012k) demonstrated that *Z. marina* seeds harvested from nearby beds can preserve genetic diversity in restored sites. In the Potomac River, there was minimal evidence of structure and relatively high levels of genetic diversity. Therefore, stock could be sourced from any of the five sites for restoration of another tidal site located within the geographic scope of this study. However, because there was evidence of inbreeding in three sites (GWP, LSP, and AL), we do not recommend sourcing restoration stock from a single site, but rather from a mix of a few local sites. We stress that these recommendations are only for sites within the limited geographic range of this study because a previous work found evidence of local adaptation between *V. americana* sourced from the Potomac River and *V. americana* sourced from the central Chesapeake Bay (Engelhardt et al. 2014b).

There was evidence of population differentiation among sites within the Hudson and Kennebec Rivers as well as low levels of genotypic and allelic diversity relative to the Potomac. Therefore, each site within the Hudson and Kennebec should be evaluated independently. Unlike recommendations for the Potomac River, restoration stock cannot be sourced from just any of the five sites within each river.

Rather, we recommend sourcing material from the closest site or the most locally accessible site to minimize risks of outbreeding depression between genetically dissimilar genotypes. Although pairwise relatedness was higher and overall levels of genetic diversity were lower, there were no signs of inbreeding depression within sites on the Hudson and Kennebec. In fact, there was actually evidence of excess heterozygosity in sites along the Hudson. Therefore, experiments designed to evaluate the effect of individual levels of heterozygosity on growth and reproductive potential of Hudson MLGs should be performed prior to efforts to combine or supplement genotypes from two or more locations to increase standing genetic diversity. If heterozygosity is higher in the Hudson River because of a heterozygote advantage, then increasing genetic diversity within Hudson River sites may not be necessary, despite the high levels of relatedness. In fact, bringing in outside variation may disrupt locally adapted gene complexes that are successful at that site. On the other hand, if excess heterozygosity is the result of a bottleneck, evidence of which was only marginally insignificant in our analyses, then the sites may be at risk of future inbreeding depression and a combination of stock from a few local sites may be sufficient to increase levels of standing genetic variation.

Unfortunately, in August 2011, after collections for this study were complete, extensive losses of SAV habitat were documented in the tidal Hudson River due to runoff and suspended sediment from Tropical Storms Irene and Lee (Fernald et al. 2012; S. Findlay personal communication 2012, Wall and Hoffman 2012). In an effort to best to facilitate recovery of SAV in the Hudson that are resilient to future perturbations from extreme events, natural resource managers have been actively

evaluating potential restoration strategies. They are currently determining whether or not there are sufficient remnant populations of *V. americana* to facilitate natural recovery or if active restoration is needed to not only improve SAV coverage in the short-term, but also ensure persistence in the long-term. Based on this research, we know that *V. americana* in the Hudson River already suffered relatively fewer genotypes, lower allelic diversity, high pairwise relatedness within sites, and greater differentiation among sites prior to Tropical Storms Irene and Lee. Genetic diversity likely decreased further given the magnitude of the reduction in SAV. Information on the genetic diversity of remnant *V. americana* in the Hudson, the genetic composition and relatedness of upstream, non-tidal *V. americana* to tidal MLGs, and the performance and plasticity of the remaining MLGs is needed to further aid natural resource managers in making the decision to either actively restore or focus on promoting natural recolonization of devastated sites.

Conclusions

Even though the goal of restoration is to ultimately create self-sustaining and resilient systems, there will be ongoing need for restoration of aquatic ecosystems in the near future as changing climate and variability is disproportionally affecting these already threatened ecosystems (Branch 1999, Kennish 2002). Unfortunately, major differences in the range of genetic diversity, spatial distribution of genetic diversity, and level of relatedness among *V. americana* collected at similar spatial scales from three rivers spanning different latitudinal regions indicate that the environmental and hydrological processes that influence genetic structure of populations are region specific. Therefore, the use of detailed genetic information of one SAV population in

a tidal region has limited utility in informing management decisions for similar populations from another tidal region.

Table 1.1: Summary of multilocus genotypic and genetic diversity estimates for 15 *Vallisneria americana* sites sampled from the Potomac River (MD), Hudson River (NY), and Kennebec River (ME) based on 10 microsatellite loci

Site	Code	Location				N	G	Genotypic Diversity	A	A_p	P	H_o	H_e	f
Potomac River, MD														
George W Parkway	GWP	38.7303	°N	77.0416	°W	30	28	0.93	4.2	0	1.0	0.36	0.44	**0.18**
Piscataway Park	SWP	38.6849	°N	77.1019	°W	30	29	0.97	4.2	0	0.8	0.42	0.45	0.08
Gunston Manor	GM	38.6353	°N	77.1441	°W	30	17	0.55	4.1	0	0.9	0.51	0.49	-0.01
Leeslvania State Park	LSP	38.5835	°N	77.2583	°W	30	25	0.83	4.9	1	0.9	0.42	0.51	**0.20**
Aquia Landing	AL	38.3884	°N	77.3213	°W	30	30	1.00	5.5	1	1.0	0.42	0.50	**0.19**
	Potomac Average					*30.00*	*25.80*	*0.86*	*4.58*	*0.40*	*0.92*	*0.43*	*0.48*	***0.13***
	Potomac SE					*0.00*	*2.35*	*0.08*	*0.27*	*0.24*	*0.04*	*0.02*	*0.01*	*0.04*
Hudson River, NY														
Newburg-Beacon	NBB	41.5428	°N	73.9800	°W	20	11	0.53	2.8	0	0.9	0.55	0.40	**-0.34**
Breakneck Ridge	BNR	41.4532	°N	73.9872	°W	30	12	0.38	3.1	0	0.9	0.54	0.49	-0.05
Garrison	GAR	41.3782	°N	73.9497	°W	27	8	0.27	2.9	0	0.9	0.59	0.45	**-0.26**
Peekskill	PEK	41.2991	°N	73.9692	°W	29	17	0.57	3.5	1	0.9	0.54	0.52	-0.01
Croton	CRO	41.1809	°N	73.8785	°W	29	27	0.93	3.7	0	0.9	0.60	0.52	**-0.15**
	Hudson Average					*27.00*	*15.00*	*0.53*	*3.20*	*0.20*	*0.90*	*0.56*	*0.47*	***-0.15***
	Hudson SE					*1.82*	*3.33*	*0.11*	*0.17*	*0.20*	*0.00*	*0.01*	*0.02*	*0.06*
Kennebec River, ME														
Waterville	WAT	44.5331	°N	69.6439	°W	28	28	1.00	3.2	0	0.8	0.49	0.37	**-0.30**
Sidney	SID	44.4286	°N	69.7015	°W	28	18	0.63	3.5	1	0.9	0.52	0.42	**-0.20**
Gardiner-Randolph	GDR	44.2281	°N	69.7661	°W	28	23	0.81	4.7	0	1.0	0.53	0.53	0.01
Richmond	RCH	44.0879	°N	69.7953	°W	28	24	0.85	4.3	0	1.0	0.54	0.49	**-0.08**
Butler Cove	BTC	43.9721	°N	69.8441	°W	24	17	0.70	3.6	4	0.9	0.53	0.53	0.03
	Kennebec Average					*27.20*	*22.00*	*0.80*	*3.86*	*1.00*	*0.92*	*0.52*	*0.47*	***-0.09***
	Kennebec SE					*0.80*	*2.02*	*0.06*	*0.28*	*0.77*	*0.04*	*0.01*	*0.03*	*0.06*
	Global Average					28.07	20.93	0.73	3.88	0.53	0.91	0.50	0.47	-0.05
	SE					0.71	1.85	0.06	0.20	0.27	0.02	0.02	0.01	0.05

N number of genotyped shoots; *G* unique genets; *Genotypic Diversity* = (G - 1)/(N - 1); *A* average number of alleles per locus within a sampling site; A_p number of private alleles; P proportion of polymorphic loci; H_o observed heterozygosity; H_e expected heterozygosity; f Wright's inbreeding coefficient - the correlation of alleles within individuals within populations. f values in bold type are significantly different from zero at $p < 0.05$.

Table 1.2: Number of *Vallisneria americana* shoots for each multilocus genotype (MLG) that are shared among sites along the Hudson and Kennebec Rivers, and the proportion (in parentheses) of the MLG within each sampling site

MLG ID	Hudson River					Kennebec River				
	NBB (n=20)	**BNR (n=30)**	**GAR (n=27)**	**PEK (n=29)**	**CRO (n=29)**	**WAT (n=28)**	**SID (n=28)**	**GDR (n=28)**	**RCH (n=28)**	**BTC (n=24)**
1		2 *(0.07)*		4 *(0.14*)						
24		1 *(0.03)*	1 *(0.04)*							
80							8 *(0.29)*	2 *(0.07)*		
93						1 *(0.04)*	1 *(0.04)*	1 *(0.04)*		
149						1 *(0.04)*			1 *(0.04)*	

Sites are ordered within rivers from upstream (left) to downstream.

Table 1.3: Genetic diversity of individual loci averaged over all *Vallisneria americana* sampled sites and the results of Exact tests for Hardy-Weinberg Equilibrium on the global allele set and the alleles set from each river

Locus	% Missing Data	A	H_e	H_o	f	Global p	Potomac p	Hudson p	Kennebec p
atg002	0.32	5.40	0.69	0.80	0.09	**<0.001**	0.006	**<0.001**	**<0.001**
aagx051	3.18	6.07	0.69	0.69	0.20	**<0.001**	**<0.001**	0.112	0.091
aag002	0.96	2.87	0.40	0.39	0.34	**<0.001**	0.083	0.760	**<0.001**
aagx012	0.32	3.93	0.45	0.48	0.39	**<0.001**	0.097	0.235	**<0.001**
m13	5.10	4.47	0.64	0.61	0.22	**<0.001**	**<0.001**	0.113	**<0.001**
m16	0.32	1.33	0.03	0.03	-0.01	1.000	1.000	1.000	1.000
aagx071	3.82	5.80	0.67	0.70	0.17	**<0.001**	0.065	0.577	**<0.001**
m49	0.64	3.40	0.44	0.52	0.10	**<0.001**	0.819	0.242	**0.002**
aag004	1.27	3.27	0.52	0.66	0.08	**<0.001**	0.016	0.030	0.054
aagx030	0.32	2.27	0.14	0.16	0.01	0.463	0.014	1.000	1.000
Average	*1.62*	*3.88*	*0.47*	*0.50*	*0.16*				
SE	*0.55*	*0.49*	*0.02*	*0.02*	*0.04*				

A total number of alleles, H_o observed heterozygosity, H_e expected heterozygosity, f the inbreeding coefficient. *p*-values in bold type are significantly different from zero at the Bonferoni adjusted $p < 0.005$.

Table 1.4: Private allele frequency for 8 *Vallisneria americana* alleles found across 3 rivers and 5 sampled sites.

River	Code	Locus	Allele	Frequency
Potomac	LSP	aag004	388	0.021
	AL	atg002	172	0.083
Hudson	PEK	atg002	144	0.118
Kennebec	SID	aag004	373	0.056
	BTC	aagx071	245	0.107
	BTC	aagx071	248	0.036
	BTC	aagx071	250	0.036
	BTC	aagx071	256	0.036

Table 1.5: Summary of regional genetic differentiation measures for *Vallisneria americana* sampled in the Potomac (MD), Hudson (NY), and Kennebec (ME) Rivers based on 10 microsatellite loci. F_{st} (Weir and Cockerham 1984), G'_{st} (Hedrick 2005), and D_{est} (Jost 2008) were calculated using GENALEX 6.5 (Peakall and Smouse 2006). Population differentiation estimates are below the diagonal and p-values based on 1000 permutations are shown above the diagonal.

	Potomac River	Hudson River	Kennebec River
Potomac River	F_{st}	0.001	0.001
	G'_{st}	0.002	0.002
	D_{est}	0.001	0.001
Hudson River	0.095		0.001
	0.299		0.002
	0.227		0.001
Kennebec River	0.104	0.086	
	0.323	0.284	
	0.247	0.219	

Table 1.6: Summary of population genetic differentiation measures for all 15 *Vallisneria americana* sampled sites in the Potomac (MD), Hudson (NY), and Kennebec (ME) Rivers based on 10 microsatellite loci. F_{st} (Weir and Cockerham 1984), G'_{st} (Hedrick 2005), and D_{est} (Jost 2008) were calculated using GENALEX 6.5 (Peakall and Smouse 2006). Population differentiation estimates are below the diagonal and *p*-values based on 1000 permutations are shown above diagonal.

		Potomac River					Hudson River					Kennebec River				
		GWP	SWP	GM	LSP	AL	NBB	BNR	GAR	PEK	CRO	WAT	SID	GDR	RCH	BTC
Potomac River	GWP	F_{st}	0.883	0.087	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		G'_{st}	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		D_{est}	0.883	0.077	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	SWP	0.006		0.182	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		-0.010		0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		-0.006		0.171	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	GM	0.020	0.016		0.026	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		0.019	0.010		0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		0.012	0.006		0.021	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	LSP	0.029	0.031	0.025		0.202	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		0.052	0.059	0.034		0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		0.034	0.040	0.024		0.203	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	AL	0.048	0.046	0.035	0.014		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		0.107	0.105	0.070	0.009		0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		0.072	0.071	0.048	0.006		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Hudson River	NBB	0.191	0.191	0.139	0.132	0.132		0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		0.440	0.451	0.331	0.321	0.319		0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		0.320	0.332	0.236	0.232	0.229		0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	BNR	0.185	0.178	0.138	0.130	0.131	0.066		0.140	0.054	0.001	0.001	0.001	0.001	0.001	0.001
		0.477	0.469	0.366	0.355	0.356	0.128		0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		0.370	0.365	0.280	0.273	0.273	0.084		0.130	0.054	0.001	0.001	0.001	0.001	0.001	0.001
	GAR	0.202	0.200	0.163	0.145	0.161	0.107	0.037		0.003	0.001	0.001	0.001	0.001	0.001	0.001
		0.491	0.497	0.414	0.372	0.418	0.224	0.041		0.002	0.002	0.002	0.002	0.002	0.002	0.002
		0.378	0.385	0.316	0.282	0.322	0.149	0.027		0.002	0.001	0.001	0.001	0.001	0.001	0.001
	PEK	0.145	0.139	0.111	0.101	0.102	0.079	0.031	0.063		0.001	0.001	0.001	0.001	0.001	0.001
		0.384	0.376	0.303	0.282	0.286	0.175	0.042	0.128		0.002	0.002	0.002	0.002	0.002	0.002
		0.291	0.285	0.229	0.214	0.216	0.120	0.029	0.089		0.001	0.001	0.001	0.001	0.001	0.001
	CRO	0.133	0.135	0.121	0.097	0.111	0.098	0.101	0.134	0.056		0.001	0.001	0.001	0.001	0.001
		0.358	0.373	0.342	0.279	0.322	0.237	0.273	0.352	0.144		0.002	0.002	0.002	0.002	0.002
		0.267	0.282	0.261	0.210	0.245	0.165	0.204	0.265	0.105		0.001	0.001	0.001	0.001	0.001
Kennebec River	WAT	0.264	0.254	0.225	0.210	0.229	0.282	0.212	0.204	0.181	0.210		0.010	0.001	0.001	0.001
		0.610	0.599	0.550	0.528	0.575	0.621	0.512	0.466	0.453	0.536		0.002	0.002	0.002	0.002
		0.476	0.467	0.426	0.410	0.455	0.480	0.391	0.341	0.340	0.418		0.010	0.001	0.001	0.001
	SID	0.219	0.213	0.191	0.173	0.192	0.260	0.197	0.187	0.167	0.191	0.021		0.001	0.001	0.001
		0.534	0.530	0.492	0.455	0.506	0.606	0.502	0.448	0.443	0.517	0.029		0.002	0.002	0.002
		0.412	0.410	0.382	0.351	0.397	0.477	0.392	0.335	0.341	0.409	0.017		0.001	0.001	0.001
	GDR	0.108	0.108	0.094	0.074	0.085	0.150	0.135	0.135	0.095	0.098	0.106	0.073		0.002	0.001
		0.285	0.293	0.258	0.202	0.241	0.380	0.383	0.353	0.273	0.292	0.262	0.178		0.002	0.002
		0.208	0.216	0.193	0.150	0.180	0.283	0.299	0.269	0.208	0.223	0.182	0.124		0.002	0.001
	RCH	0.111	0.115	0.100	0.079	0.084	0.142	0.147	0.157	0.115	0.102	0.185	0.152	0.032		0.001
		0.281	0.300	0.266	0.211	0.225	0.343	0.400	0.401	0.324	0.289	0.453	0.389	0.067		0.002
		0.200	0.217	0.195	0.154	0.163	0.246	0.309	0.304	0.246	0.216	0.335	0.288	0.047		0.001
	BTC	0.139	0.139	0.128	0.103	0.118	0.154	0.121	0.121	0.098	0.103	0.114	0.087	0.058	0.089	
		0.370	0.381	0.360	0.293	0.341	0.389	0.331	0.305	0.275	0.302	0.278	0.213	0.150	0.244	
		0.280	0.292	0.279	0.224	0.264	0.291	0.254	0.228	0.210	0.232	0.195	0.151	0.111	0.181	

Table 1.7: Summary statistics for partitioning *Vallisneria americana* MLGs into five (K=5) Bayesian-modelled genetic clusters, as implemented in STRUCTURE (Pritchard et al. 2000). STRUCTURE was run assuming no prior admixture and no correlation of alleles, with 1,000,000 steps, using a burn-in of 50,000 steps. Results indicated that the five genetic clusters into which MLGs were partitioned followed one of two distributions across the Potomac River (PR), Hudson River (HR), and Kennebec River (KR).

Structure Run	Estimated Ln Probability of Data	Mean value of ln likelihood	Variance of ln likelihood	Genetic Cluster Distribution
1	-6376.9	-6244.8	264.0	1PR, 2HR, 2KR
2	-6347.8	**-6211.9**	271.7	2PR, 1HR, 2KR
3	-6348.4	**-6211.9**	273.0	2PR, 1HR, 2KR
4	-6347.6	**-6211.9**	271.3	2PR, 1HR, 2KR
5	-6374.5	-6244.8	259.4	1PR, 2HR, 2KR
6	-6350.5	**-6212.1**	276.8	2PR, 1HR, 2KR
7	-6346.9	**-6211.9**	270.1	2PR, 1HR, 2KR*
8	-6374.9	-6244.8	260.2	1PR, 2HR, 2KR
9	-6374.2	-6244.7	259.1	1PR, 2HR, 2KR*
10	-6374.6	-6244.8	259.5	1PR, 2HR, 2KR

Values in bold represent the highest likelihood scores from each of the STRUCTURE runs.
* denotes the STRUCTURE results displayed in Figure 1.9.

Table 1.8: Summary of *Vallisneria americana* pairwise relatedness estimates for multilocus genotypes (MLGs) sampled from 15 sites in the Potomac River (MD), Hudson River (NY), and Kennebec River (ME).

		Mean MLG Relatedness within Rivers		Mean MLG Relatedness within Sites	
	Site	r_G	r_R	r_G	r_R
Potomac River, MD					
	GWP	0.176	-0.063	0.228	0.002
	SWP	0.187	-0.048	0.258	0.041
	GM	0.163	-0.067	0.199	-0.015
	LSP	0.110	-0.144	0.097	-0.157
	AL	0.082	-0.176	0.091	-0.160
	Potomac Average	*0.140*	*-0.105*	*0.175*	*-0.058*
	Potomac SE	*0.003*	*0.003*	*0.034*	*0.042*
Hudson River, NY					
ND	NBB	0.260	0.106	0.582	0.502
NC	BNR	0.192	0.022	0.247	0.100
NB	GAR	0.193	0.023	0.538	0.439
NA	PEK	0.164	-0.012	0.206	0.048
NE	CRO	0.105	-0.082	0.322	0.208
	Hudson Average	*0.158*	*-0.017*	*0.379*	*0.259*
	Hudson SE	*0.005*	*0.006*	*0.080*	*0.091*
Kennebec River, ME					
MC	WAT	0.209	0.091	0.553	0.492
MB	SID	0.189	0.061	0.392	0.291
MA	GDR	0.071	-0.062	0.124	-0.001
MD	RCH	0.038	-0.096	0.279	0.191
ME	BTC	0.015	-0.131	0.211	0.112
	Kennebec Average	*0.105*	*-0.026*	*0.312*	*0.217*
	Kennebec SE	*0.004*	*0.005*	*0.074*	*0.084*
	Global Average	0.144	-0.039	0.288	0.140
	SE	0.017	0.009	0.040	0.039

Relatedness estimates use Wang's (2002) coefficient of relatedness based on global allele frequencies from the entire dataset (r_G) or local, region-specific allele frequencies (r_R). Sites correspond to the locations in Table 1.1.

Table 1.9: Summary of graph measures for individual *Vallisneria americana* multilocus genotypes (MLGs) and graph-level metrics from spatially implicit networks of relatedness created for the Potomac River, Hudson River, and Kennebec River. Networks were created using the *igraph* package (Csardi and Nepusz 2006) in R v3.0.1 (R Core Team 2013) at a relatedness threshold of $r_R \geq 0.5$.

Network Region	Total # of Nodes	Total # of Edges	Total # of Components	Degree Centrality		Closeness Centrality		Eigenvector Centrality	
				Mean (range)	Normalized Graph-Level	Mean (range)	Normalized Graph-Level	Mean (range)	Normalized Graph-Level
Potomac River	93	156	5	3.35 (1-10)	0.072	$6.68e^{-4}$ ($1.18e^{-4}$ to $8.01e^{-4}$)	0.0249	0.055 (0.00-0.381)	0.873
Hudson River	65	141	4	4.39 (1-14)	0.151	$5.68e^{-4}$ ($2.44e^{-4}$ to $7.19e^{-4}$)	0.0197	0.062 (0.00-0.387)	0.866
Kennebec River	99	640	6	12.93 (1-36)	0.235	$4.74e^{-4}$ ($1.04e^{-4}$ to $5.71e^{-4}$)	0.0194	0.062 (0.00-0.208)	0.716

The Mean columns summarize the mean graph measure across all individual nodes/MLGs in each network as well as the range of values for each graph measure (in parentheses).

Table 1.10: The five highest ranked *Vallisneria americana* multilocus genotypes (MLGs) for each graph measure calculated from spatially implicit networks of relatedness created for the Potomac River, Hudson River, and Kennebec River. Networks were created using the *igraph* package (Csardi and Nepusz 2006) in R v3.0.1 (R Core Team 2013) at a relatedness threshold of $r_R \geq 0.5$.

	Degree Centrality			Closeness Centrality			Eigenvector Centrality		
Region	MLG	MLG Site (# Samples/Site)	Score	MLG	MLG Site (# Samples/Site)	Score	MLG	MLG Site (# Samples/Site)	Score
Potomac River	309	SWP (1)	10	363	AL (1)	$8.01e^{-4}$	309	SWP (1)	0.381
	359	LSP (1)	9	302	SWP (1)	$7.89e^{-4}$	276	GWP (1)	0.325
	334	GM (1)	9	279	GWP (1)	$7.87e^{-4}$	334	GM (1)	0.320
	283	GWP (1)	9	340	LSP (2)	$7.86e^{-4}$	283	GWP (1)	0.276
	279	GWP (1)	9	344	LSP (1)	$7.84e^{-4}$	294	SWP (1)	0.246
Hudson River	73	CRO (1)	14	73	CRO (1)	$7.19e^{-4}$	45	NBB (2)	0.387
	45	NBB (2)	11	61	CRO (1)	$7.16e^{-4}$	40	NBB (4)	0.374
	40	NBB (4)	10	3	PEK (1)	$7.14e^{-4}$	47	NBB (1)	0.337
	47	NBB (1)	9	77	CRO (1)	$7.09e^{-4}$	43	NBB (1)	0.321
	43	NBB (1)	9	64	CRO (1)	$7.09e^{-4}$	42	NBB (4)	0.305
Kennebec River	122	WAT (1)	36	119	SID (1)	$5.71e^{-4}$	88	GDR (1)	0.208
	88	GDR (1)	35	93	WAT(1),SID(1),GDR(1)	$5.70e^{-4}$	122	WAT (1)	0.207
	138	WAT (1)	34	108	SID (1)	$5.69e^{-4}$	138	WAT (1)	0.200
	93	WAT(1),SID(1),GDR(1)	33	114	SID (1)	$5.68e^{-4}$	110	SID (2)	0.199
	110	SID (2)	33	80	SID (8), GDR (2)	$5.68e^{-4}$	93	WAT(1),SID(1),GDR(1)	0.196

Cells in white represent MLGs that only ranked in the top five for one graph measure, cells light grey represent MLGs that ranked in the top five for two graph measures, cells in dark grey represent MLGs that ranked in the top five for all three graph measures. The MLG Site column describes the sites where each MLG was found as well as the number of times it occurred at that site (in parentheses).

Table 1.11: The rank of three calculated graph centrality measures for the extensive *Vallisneria americana* multilocus genotypes (MLGs) from the Hudson River and Kennebec River spatially implicit networks of relatedness. The networks were created using the igraph package (Csardi and Nepusz 2006) in R v3.0.1 (R Core Team 2013) at a relatedness threshold of $r_R \geq 0.5$.

	Hudson River		Kennebec River		
	MLG 1	MLG 24	MLG 80	MLG 93	MLG 149
Degree Centrality	#21 *(top 35%)*	#25 *(top 40%)*	#43 *(top 45%)*	#4 *(top 1%)*	#7 *(top 1%)*
Closeness Centrality	#52 *(top 80%)*	#54 *(top 85%)*	#5 *(top 1%)*	#2 *(top 1%)*	#19 *(top 20%)*
Eigenvector Centrality	#50 *(top 80%)*	#55 *(top 85%)*	#44 *(top 45%)*	#5 *(top 1%)*	#10 *(top 2%)*

A total of 65 MLGs were included in the $r_R \geq 0.5$ spatially implicit network of relatedness for the Hudson River and a total of 99 MLGs were included in the Kennebec River network (see Table 1.9).

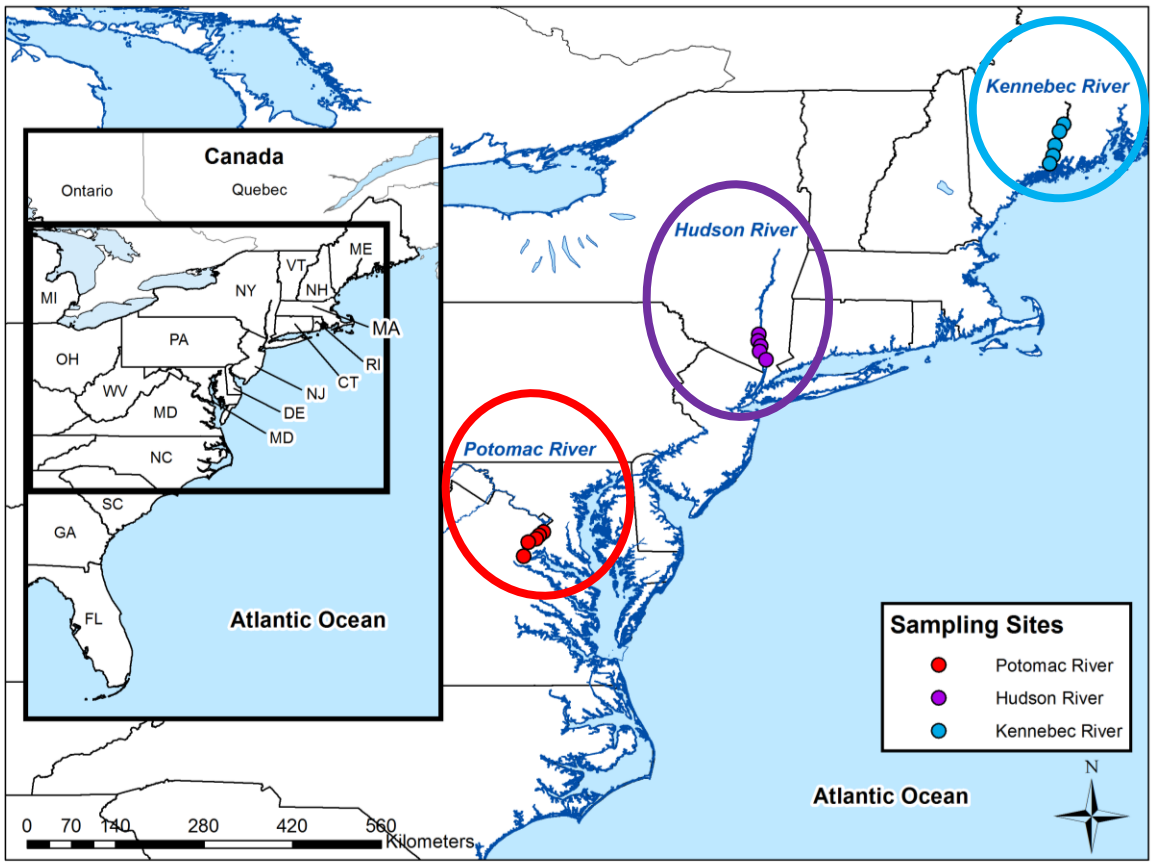

Figure 1.1: Map of the 15 *Vallisneria americana* sampling locations spanning three major rivers along the northeast coast of North America. The sampled rivers include the Potomac River in Maryland (red circles), Hudson River in New York (purple circles), and the Kennebec River in Maine (blue circles).

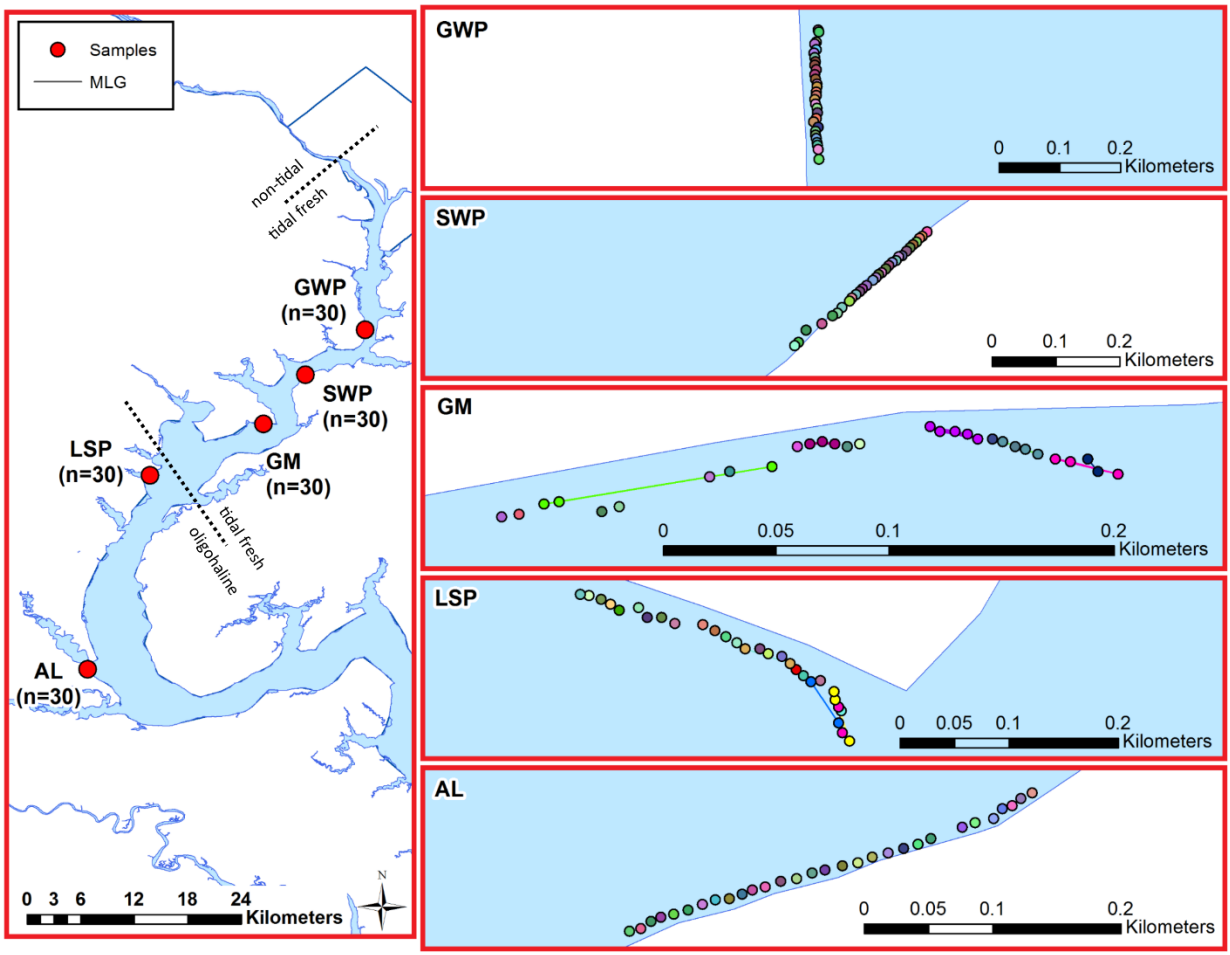

Figure 1.2: Map of the 5 *Vallisneria americana* sampling sites within the Potomac River in Maryland (**left**) as well as maps of sampled shoots from each of the five collection sites. Site names are defined in Table 1.1 and the number in parentheses corresponds to the number of samples that were genotyped from each site. Each multilocus genotype (MLG) is represented by a different colored circle. Shoots that were assigned to the same MLG share the same color and are connected by a line. No MLGs were found in multiple sites in the Potomac River. Dashed lines depict approximate salinity zone transitions.

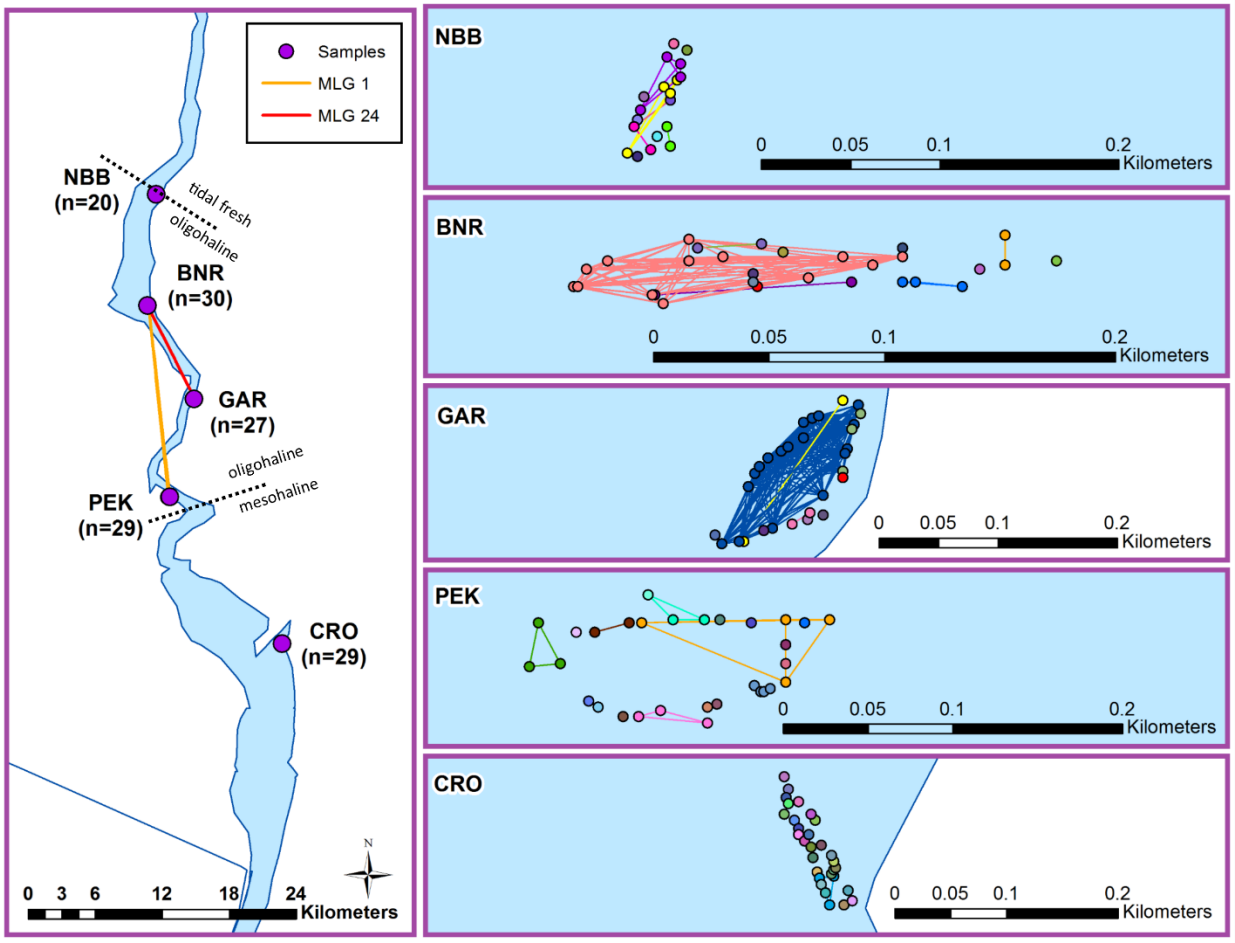

Figure 1.3: Map of the 5 *Vallisneria americana* sampling sites within the Hudson River in New York (**left**) as well as maps of sampled shoots from each of the five collection sites. Site names are defined in Table 1.1 and the number in parentheses corresponds to the number of samples that were genotyped from each site. Each multilocus genotype (MLG) is represented by a different colored circle. Shoots that were assigned to the same MLG share the same color and are connected by a line. Two MLGs, MLG 1 and MLG 24, were found in multiple sites in the Hudson River. Dashed lines depict approximate salinity zone transitions.

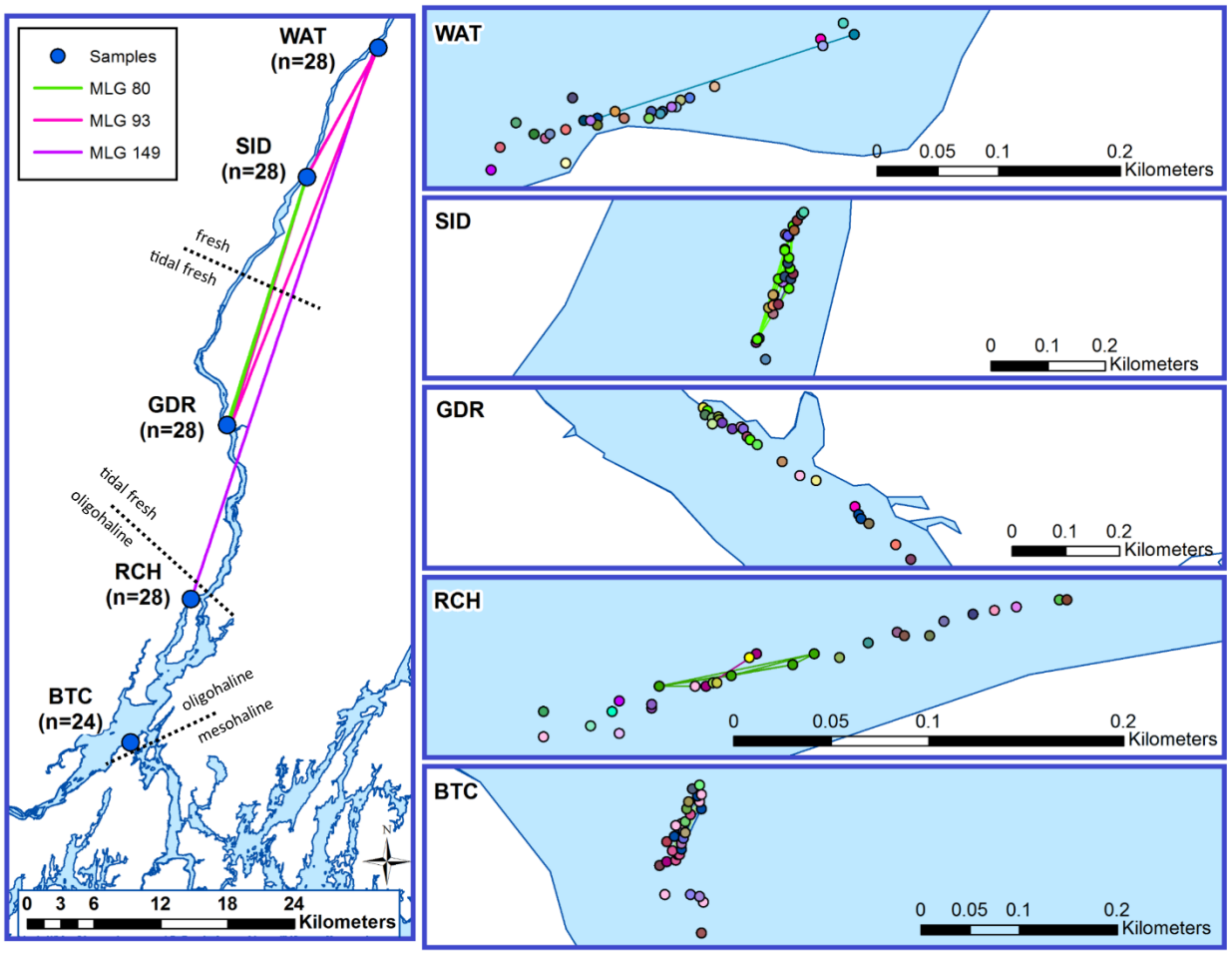

Figure 1.4: Map of the 5 *Vallisneria americana* sampling sites within the Kennebec River in Maine (**left**) as well as maps of sampled shoots from each of the five collection sites. Site names are defined in Table 1.1 and the number in parentheses corresponds to the number of samples that were genotyped from each site. Each multilocus genotype (MLG) is represented by a different colored circle. Shoots that were assigned to the same MLG share the same color and are connected by a line. Three MLGs were found in multiple sites in the Kennebec River. Dashed lines depict approximate salinity zone transitions.

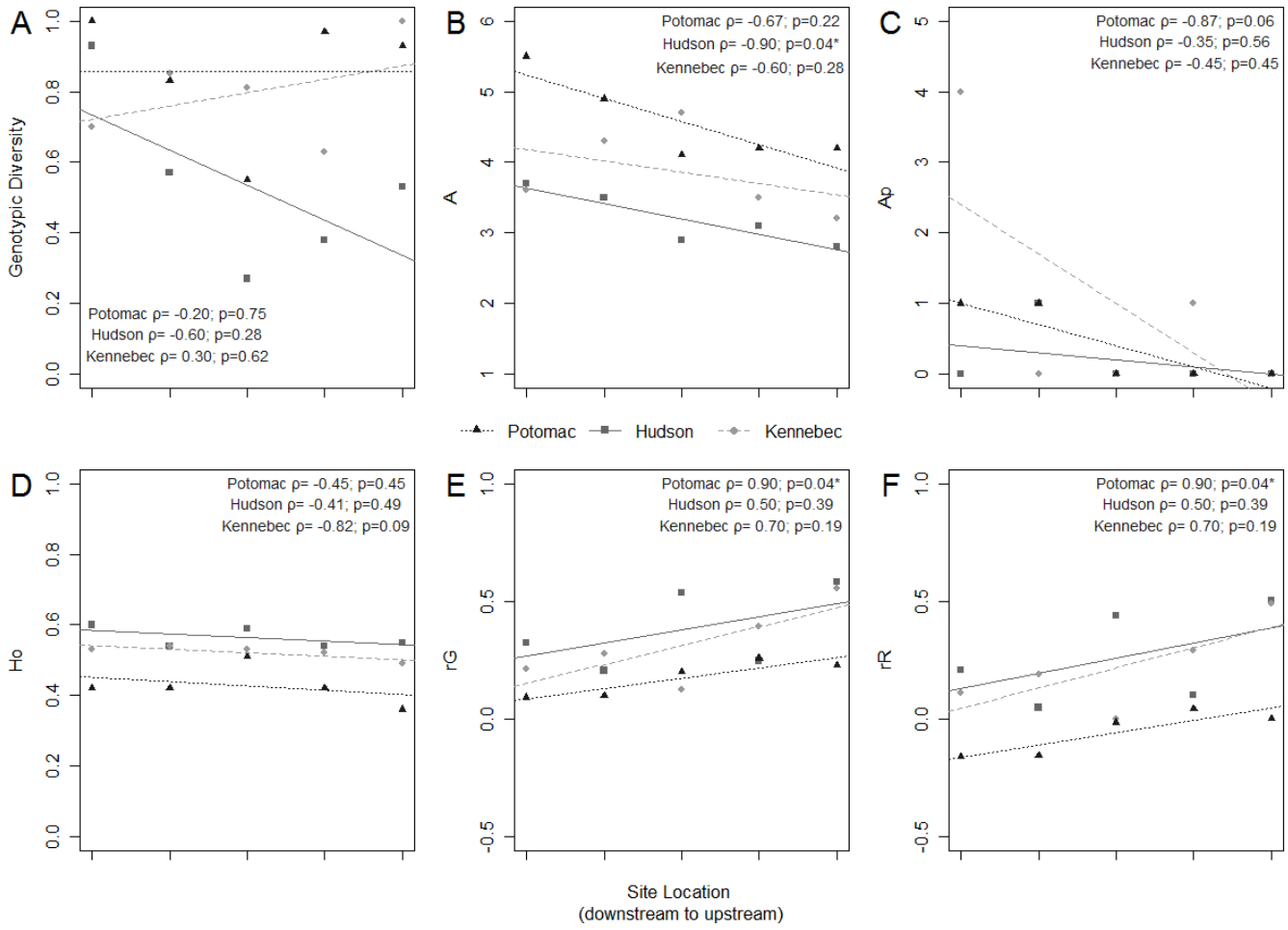

Figure 1.5: Scatterplots of measures of *Vallisneria americana* genotypic and genetic diversity along three rivers, including the Potomac River (triangles), Hudson River (squares), and Kennebec River (circles). The x-axis represents the five sampled sites from each river, moving in a downstream to upstream direction (AL, LSP, GM, SWP, GWP for the Potomac River; CRO, PEK, GAR, BNR, NBB for the Hudson River; BTC, RCH, GDR, SID, WAT for the Kennebec). Results of nonparametric Spearman's rank correlation (ρ) analysis and corresponding *p*-values are provided on the plots for (**A**) genotypic diversity, (**B**) allelic diversity (*A*), (**C**) the number of private alleles found within each sampled site (A_p), (**D**) observed heterozygosity (H_o), (**E**) average relatedness of unique multilocus genotypes (MLGs) within each sampled site calculated using the Wang (2002) relatedness coefficient with global allele frequencies (r_G), and (**F**) average relatedness of MLGs within each sampled site calculated using the Wang (2002) relatedness coefficient with regional allele frequencies (r_R). An * indicates significant rank correlations at $p < 0.05$.

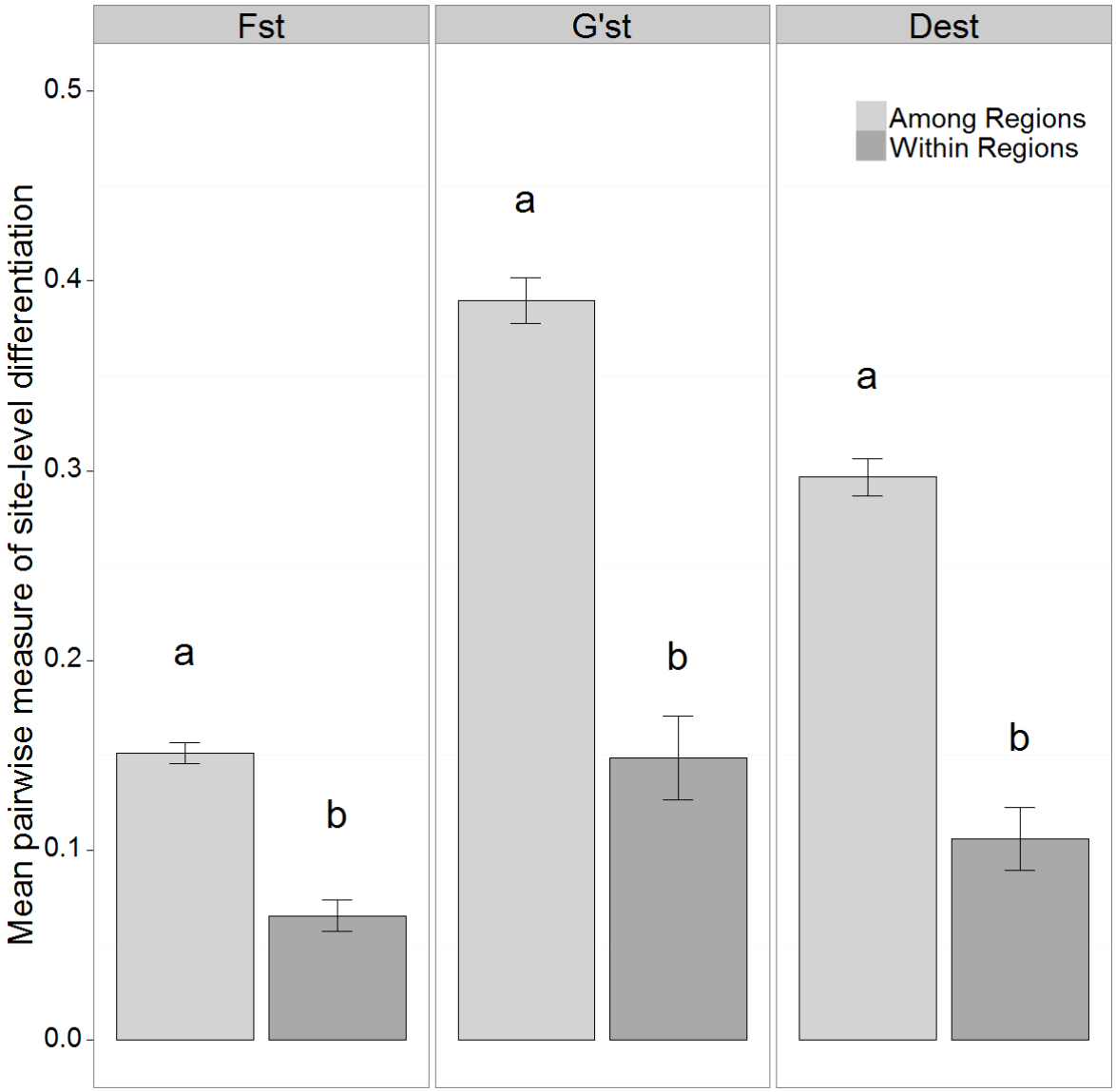

Figure 1.6: Mean measures of site-level *Vallisneria americana* differentiation between sites sampled within the same river region or among different river regions. Pairwise measures of differentiation between sites within rivers were significantly lower from pairwise measures of differentiation between sites sampled from different rivers for all three measures of differentiation, including F_{st} ($t_{(57.96)} = 8.68$, $p < 0.001$), G'_{st} ($t_{(46.95)} = 9.52$, $p < 0.001$), and D_{est} ($t_{(50.56)} = 9.97$, $p < 0.001$).

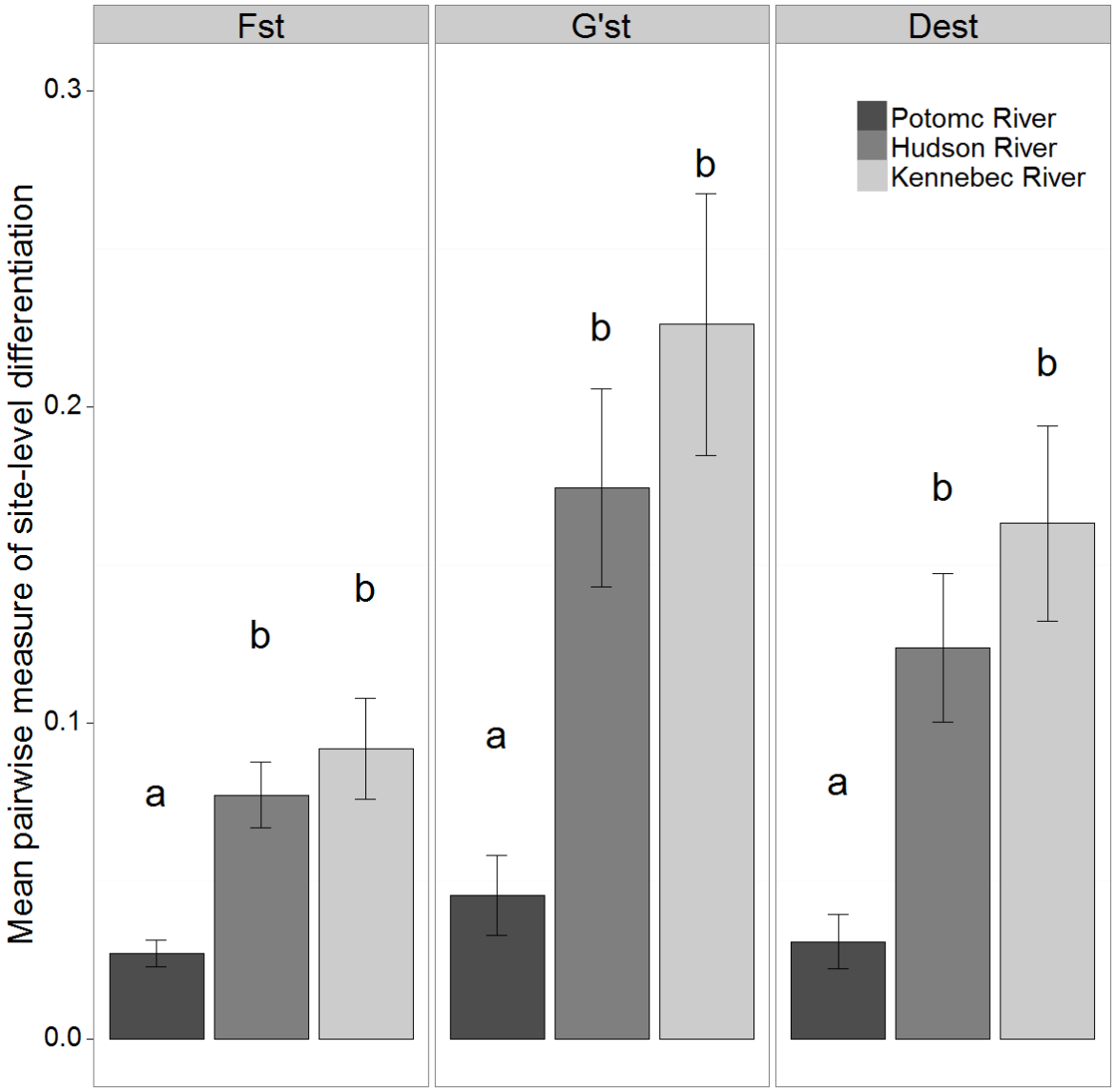

Figure 1.7: Mean measures of site-level *Vallisneria americana* differentiation between sites sampled within the Potomac River (darkgrey), Hudson River (grey), and Kennebec River (lightgrey). Pairwise measures of differentiation were significantly different across rivers for F_{st} (ANOVA; $F_{2,27} = 9.03$; $p = 0.001$); G'_{st} (ANOVA; $F_{2,27} = 8.33$; $p = 0.002$); and D_{est} (ANOVA; $F_{2,27} = 8.72$; $p = 0.001$).

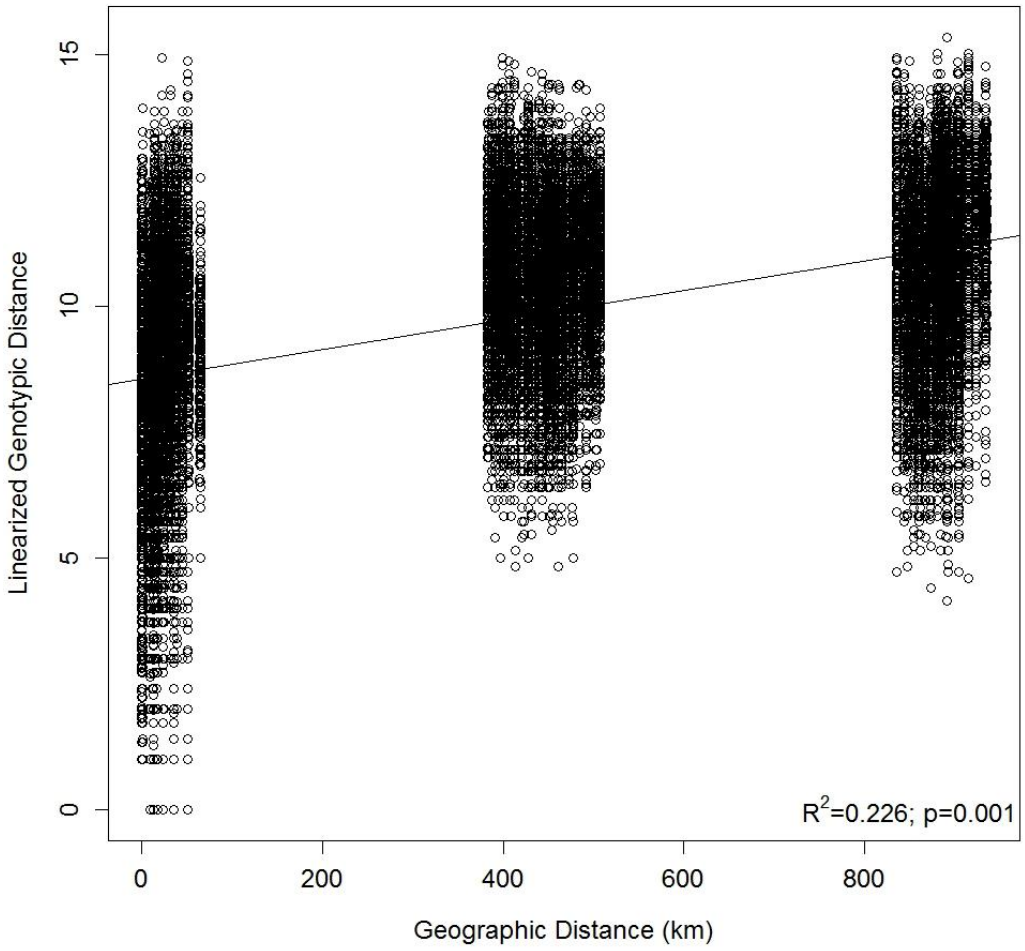

Figure 1.8: Linearized genotypic distance between all pairwise *Vallisneria americana* MLGs as calculated in GENALEX v6.5 (Peakall and Smouse 2006) regressed against Euclidean geographic distance. Relationships were assessed with a Mantel Test.

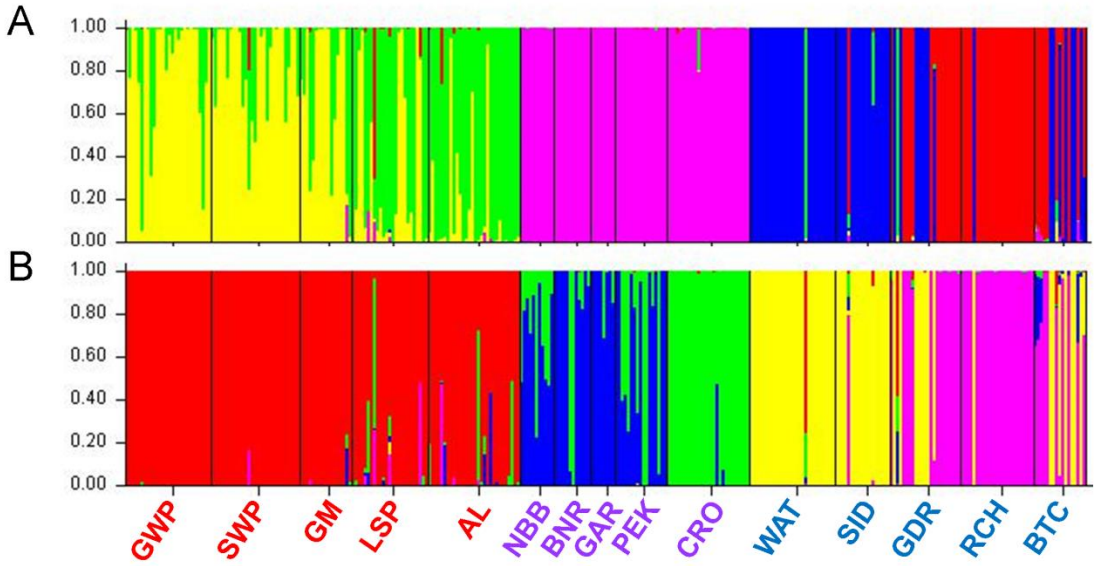

Figure 1.9: STRUCTURE (Pritchard et al. 2000) results partitioning *Vallisneria americana* multilocus genotypes (MLGs) collected from 15 sites spanning three geographically separated rivers, including the Potomac River (red labels), Hudson River (purple labels), and Kennebec River (blue labels), into five (K=5) Bayesian-modelled genetic clusters. STRUCTURE results grouped the five clusters into two different distributions, including (**A**) a distribution with two genetic populations in the Potomac River, one genetic population in the Hudson River, and two genetic populations in the Kennebec River (run 7 STRUCTURE results depicted; Table 1.7) and (**B**) a distribution with one genetic population in the Potomac River, two genetic populations in the Hudson River, and two genetic populations in the Kennebec River (run 9 STRUCTURE results depicted; Table 1.7). The distribution of genetic clusters depicted in the first panel (**A**) has the highest likelihood score (Table 1.7).

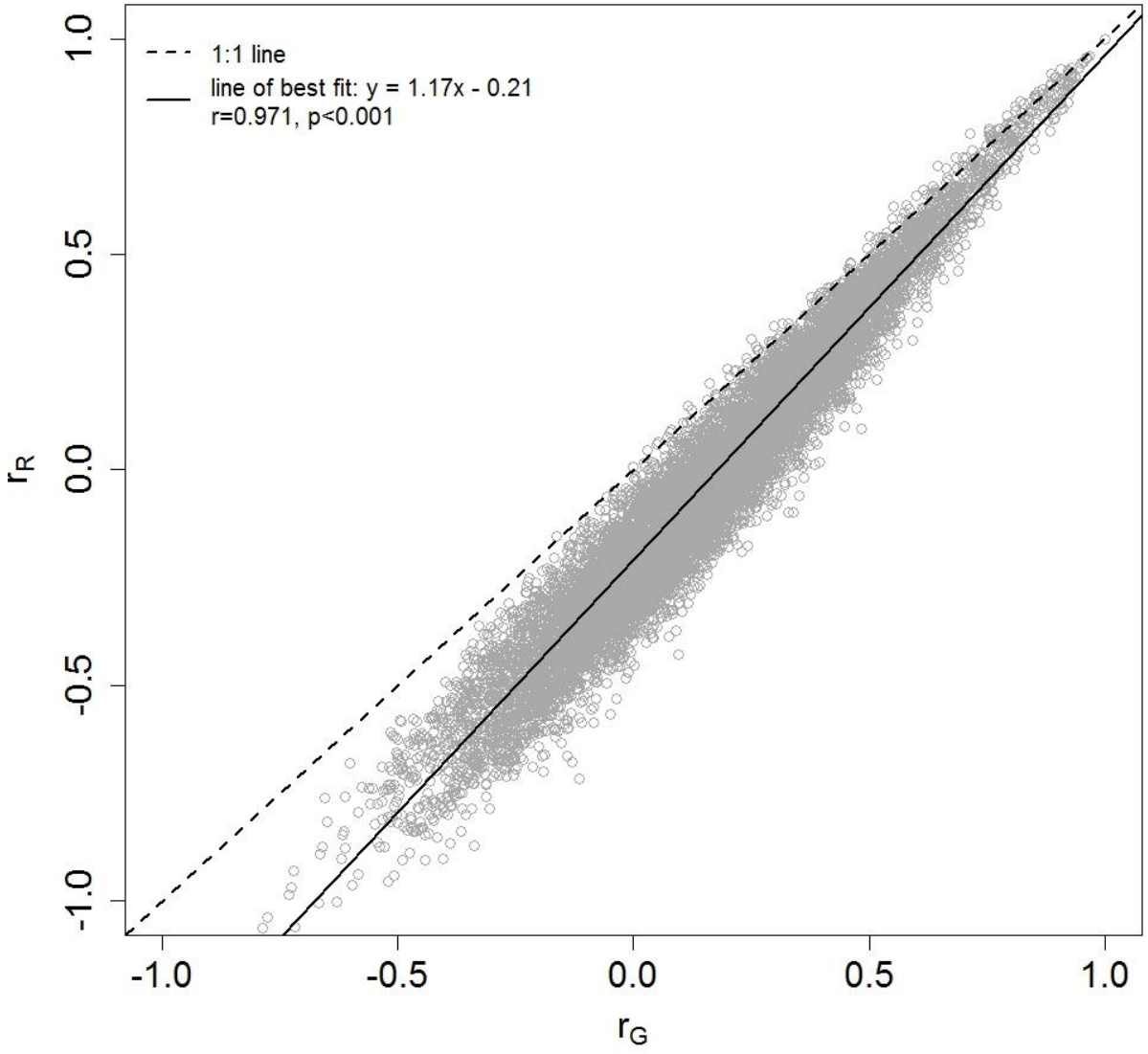

Figure 1.10: Scatterplot displaying the relationship between pairwise relatedness coefficients of *Vallisneria americana* calculated using region-specific allele frequencies (r_R) and global allele frequencies (r_G). Pearson correlation analysis revealed a strong, positive correlation between the two estimates of relatedness, but use of global allele frequencies consistently increased the relatedness coefficient estimate relative to estimates based on regional allele frequencies.

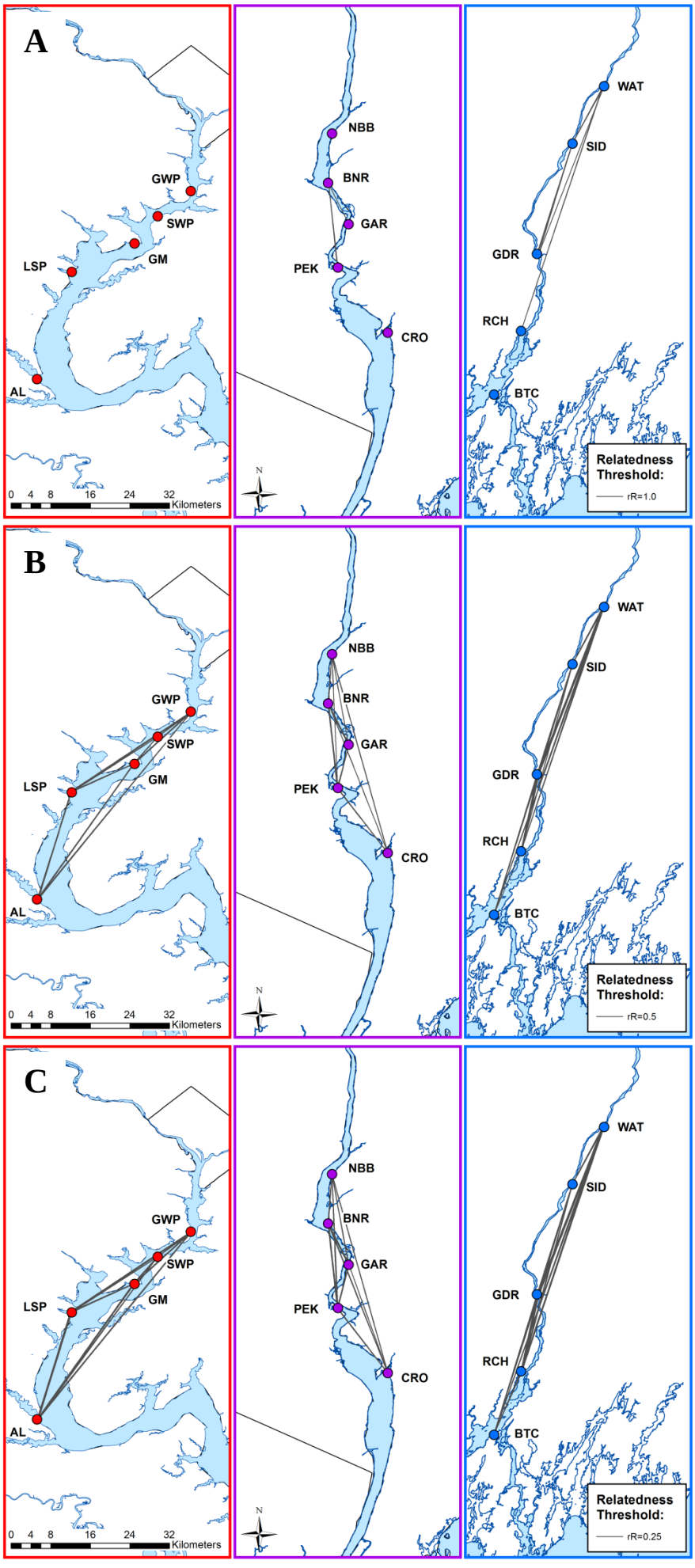

Figure 1.11: Spatially explicit individual-based networks of relatedness between *Vallisneria americana* samples collected from three major rivers spanning the northeast coast of North America, including the Potomac River in Maryland (red), the Hudson River in New York (purple), and the Kennebec River in Maine (blue). Networks depict the distribution of shoots within each region that are related to one another at thresholds of (**A**) $r_R = 1.0$, (**B**) $rR = 0.5$, and (**C**) $r_R = 0.25$, such that network nodes represent sampled shoots and edges represent connections between shoots at or above each threshold value. Pairwise relatedness coefficients between sampled shoots were calculated using the Wang (2002) estimator (implemented in COANCESTRY) based on region-specific allele frequencies (r_R). Site names are defined in Table 1.1.

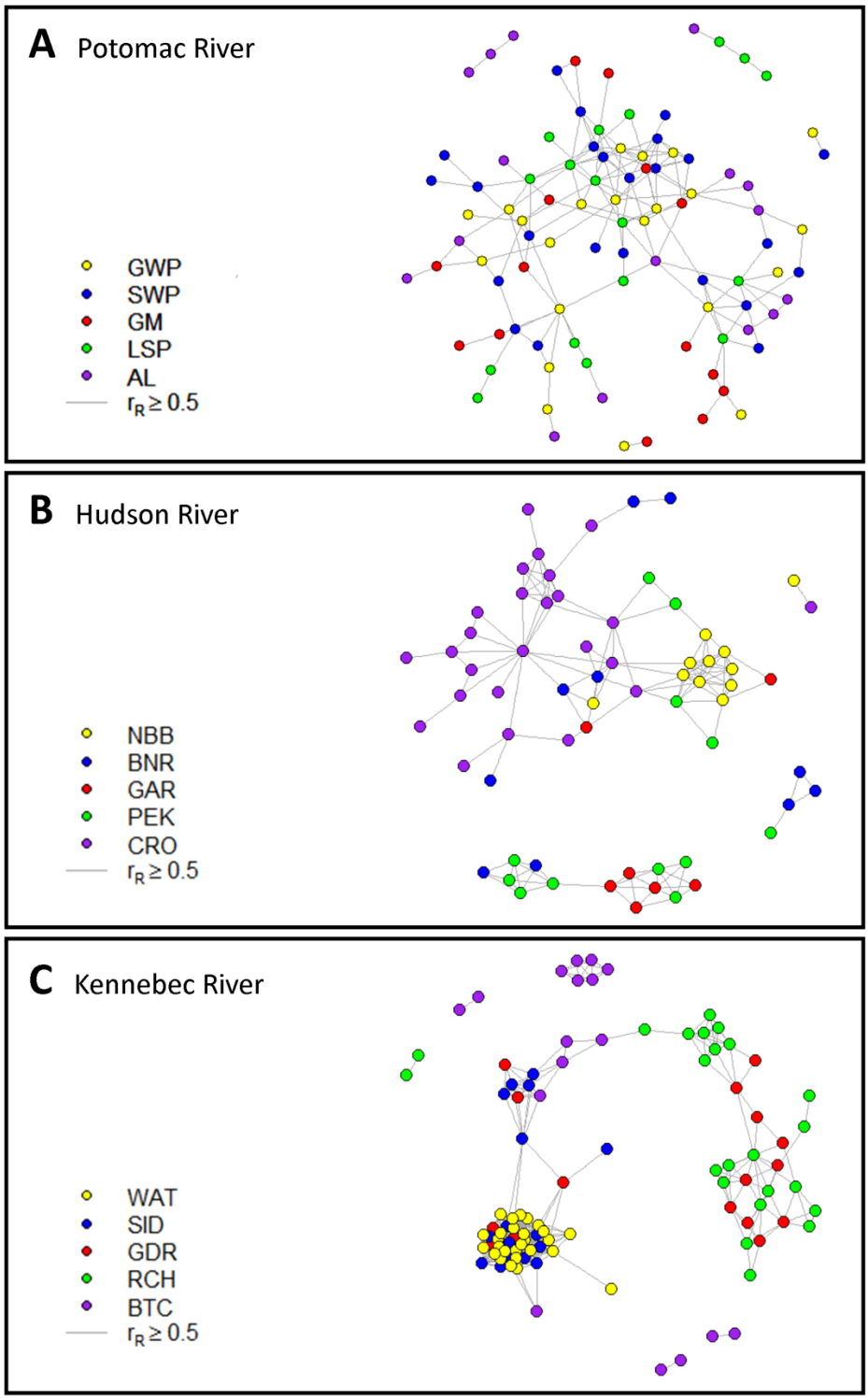

Figure 1.12: Spatially implicit individual-based networks of relatedness between *V. americana* multilocus genotypes (MLGs) from three rivers spanning the northeast coast of North America, including (**A**) the Potomac River, (**B**) the Hudson River, and (**C**) the Kennebec. Networks depict the degree of relatedness between MLGs within each region, such that the nodes represent MLGs and the edges represent MLGs related to one another at a level of $r_R \geq 0.5$. The edge length and distance between nodes is proportional to genetic distance (inverse of r_R). Pairwise relatedness between shoots were calculated using the Wang (2002) estimator based on region-specific allele frequencies (r_R). Networks were created using the *igraph* package (Csardi and Nepusz 2006) in R. Sample sites within each region are color coded and site names are defined in Table 1.1

Chapter 2: Quantifying the spatial distribution of genetic diversity & relatedness of *Vallisneria americana* along the Potomac River in Maryland: A comparison across tidal and non-tidal portions of a river

Abstract

Genetic diversity affects population persistence and resiliency through time in multiple ways, including increasing fitness, enhancing productivity, and enabling adaptation of populations. Knowledge of the spatial distributions of genetic diversity is an essential first step toward understanding not only what processes might promote or hinder spatial differentiation of genetic diversity, but also in identifying the scale over which dispersal, genetic drift, and selection might operate. Understanding how genetic diversity of submersed aquatic vegetation (SAV) varies and is structured along rivers is essential for the successful long-term maintenance and restoration of some of the most highly productive aquatic ecosystems. Therefore, I quantified the genetic diversity structure in 33 *Vallisneria americana* Michx. (Hydrocharitaceae) populations sampled along the species' entire distribution in the Potomac River, MD. Genotypic diversity ranged from 0.0-1.0 ($\bar{x} = 0.53$) while allelic diversity ranged from 1.5-5.4 ($\bar{x} = 3.1$) and observed heterozygosity ranged from 0.34-0.72 ($\bar{x} = 0.50$). Measures of population differentiation, STRUCTURE analysis, and network analysis on the relatedness between individuals revealed that there were

differences in the distribution of genetic diversity between tidal and non-tidal regions of the Potomac, such that the non-tidal Potomac was characterized by widespread connectivity while genetic diversity the tidal Potomac was more site-specific. Moreover, two widespread multilocus genotypes (MLGs) were discovered in 22 and 14 of the non-tidal Potomac River sites, spanning 239 and 159 river km, respectively. These two widespread MLGs had high levels of degree (ranked top 20%), closeness (rank top 15%) and eigenvector (ranked top 40%) centrality in networks of relatedness to other MLGs, suggesting that in addition to being highly expansive through asexual clonal growth, they are contributing disproportionately to the gene pool via sexual reproduction. The differences in *V. americana* genetic structure between the non-tidal and tidal Potomac River are likely driven by differences in environmental and hydrologic variables that impact local mating and dispersal mechanisms. We conclude by describing the different *V. americana* restoration strategies that may be suitable for either non-tidal or tidal sites of the Potomac River based on differences in genetic diversity.

Introduction

Spatial genetic structure within and among natural populations is largely dependent on interactions between life history traits, such as mating and dispersal mechanisms, and the biotic and abiotic factors that impact the expression of these traits. Understanding the impact of factors influencing the amount and structure of genetic diversity within natural populations is a key objective of ecological genetics and is a critical component for successful genetic management and restoration.

Genetic structuring in marine systems is often weak or has complex patterning that is described as random or 'chaotic' (e.g., Johnson and Black 1984, Becheler et al. 2010, Selkoe et al. 2010, Sinclair et al. 2014). Such patterning usually arises as a consequence of stochastic connectivity due to variable nearshore circulation patterns and alternating tidal flows that influence recruitment success (Siegel et al. 2008, Selkoe et al. 2010, Sinclair et al. 2014). Riverine systems, on the other hand, offer unique environments to aquatic species due to the linear arrangement of suitable habitats and continuous, unidirectional water flow (Pollux et al. 2007). The transition from non-tidal reaches of a river to tidal estuarine sections are particularly interesting because even though high gene flow is possible throughout the continuous connected network, the physical conditions between tidal and non-tidal areas are expected to yield different genetic structure. Although it is hypothesized that unidirectional gene flow will lead to erosion of genetic diversity in upstream river stretches and accumulation of genetic diversity in downstream stretches (Ritland 1989, Barrett et al. 1993), such associations have rarely been described (Gornall et al. 1998, Lundqvist and Andersson 2001, Liu et al. 2006, Pollux et al. 2007, Smith et al. 2015). Likewise, different abiotic factors such as wind speed and direction, wave action, tides, and regional circulation of water masses in shallow estuarine and coastal areas are likely to strongly influence local genetic structure in tidal rivers (Källström et al. 2008, van Dijk et al. 2009, Serra et al. 2010, Sinclair et al. 2014). Knowledge of the spatial distributions of genetic diversity is an essential first step toward understanding not only what processes might promote or hinder spatial differentiation of genetic

diversity, but also in identifying the scale over which dispersal, genetic drift, and selection might operate (Slatkin 1985, Heywood 1991, Ouborg et al. 1999).

For clonal plants, variation in abiotic environmental factors at local scales can have a major influence on spatial genetic structure by influencing levels of clonality, sexual reproduction, and recruitment (Sinclair et al. 2014). For example, studies have found that the relative proportion of sexual versus asexual reproduction in clonal plants varies with environmental parameters such that sexual reproduction is often suppressed in suboptimal conditions (Honnay and Bossuyt 2005). Sexual reproduction is necessary for gene flow among populations via pollen and seed dispersal; therefore, variation in sexual versus asexual reproduction directly affects overall genotypic diversity within populations and the spatial distribution of genetic variation among populations (Ellstrand and Roose 1987, Widen et al. 1994, Honnay and Bossuyt 2005). Many aquatic plants are characterized by the ability to reproduce both sexually and asexually (Barrett et al. 1993, Honnay and Jacquemyn 2008). To date, most studies on submersed aquatic vegetation (SAV) conclude that local environmental conditions are the main factors influencing the processes that determine genetic diversity and structure (e.g., Procaccini et al. 2001, Serra et al. 2010). Because these findings are so broad and inclusive, we propose first assessing current patterns in the structure of genetic diversity.

Understanding how SAV genetic diversity varies and is structured along environmental gradients is essential for the successful long-term maintenance and restoration of some of the most highly productive aquatic ecosystems (Costanza et al. 1997). SAV provide critical ecosystem services, including improving water quality

through sediment and nutrient sequestration (Brix and Schierup 1989, Takamura et al. 2003, Moore 2004, Gu 2008), physical sediment stabilization (Sand-Jensen 1998, Madsen et al. 2001), and erosion reduction (Fonseca and Cahalan 1992). Furthermore, both marine and freshwater aquatic plants promote healthy and diverse benthic communities (Orth et al. 2006) by providing shelter and nursery habitat to nearshore fish communities and acting as a primary source of food for waterfowl, fish, and invertebrates (Killgore et al. 1989, Perry and Deller 1996, Orth et al. 2006). Despite their importance, declines in SAV have been recorded in many parts of the world and restoration of SAV habitat following disturbance has become a priority (Orth et al. 2006, Waycott et al. 2009). Unfortunately, other than the generalization that local conditions are the main factors influencing genetic diversity and structure for SAV (Procaccini et al. 2001, Marsden CH1, Serra et al. 2010), we know very little about the patterns of population genetic structure in most SAV, let alone the processes that might be driving those patterns (Sinclair et al. 2014).

Because genetic information is often time consuming and expensive to obtain (summarized by Lloyd et al. 2011, Lloyd et al. 2012), assessments of genetic diversity are often not directly included in management and restoration plans. However, it is becoming increasingly important to understand the genetic factors that enable a population's persistence and resiliency through time because genetic diversity is fundamental to population resilience and has been associated with increased fitness (Williams 2001, Leimu et al. 2006), enhanced growth and productivity (Williams 2001, Reynolds et al. 2012a), improved population diversity (Booth and Grime 2003, Vellend 2006, Lankau and Strauss 2007), rapid response to

disturbances (Hughes and Stachowicz 2004, Reusch et al. 2005), and enhanced reproductive success and offspring fitness (Ellstrand and Elam 1993, Crnokrak and Roff 1999, Amos et al. 2001).

In this study, we examine the genotypic diversity within and genetic differentiation among 33 sites of *Vallisneria americana* Michx. (Hydrocharitaceae) spanning just over 400 river km in both tidal and non-tidal reaches of the Potomac River. Previous genetic analysis of *V. americana* across tidal reaches of three rivers in the northeastern United States found few consistent patterns in the spatial distribution of genetic diversity within rivers across latitude (Marsden CH1). More locally, genetic analysis of *V. americana* from the Chesapeake Bay found four genetically defined regions that corresponded with geographic location in the Bay (Lloyd et al. 2011). The objectives of this study were to understand how intrapopulation genotypic and genetic diversity and overall patterns of relatedness between individuals are spatially distributed across sites within the Potomac River and whether or not environmental differences within a single river, specifically differences between the tidal and non-tidal portions of the Potomac River, affect that distribution. We hypothesized that the high levels of interconnectivity and directional water flow that characterize riverine systems will facilitate the unidirectional exchange of genes among populations of *V. americana*, resulting in the downstream accumulation of genetic diversity. However, we also expected to find the structure of genetic diversity of *V. americana* in tidal portions of the Potomac to be more random and site-specific relative to *V. americana* in non-tidal portions of the river.

Methods

Study Species

The submersed aquatic plant *V. americana* is native to eastern North America and extends from southern Canada along the Atlantic coast to Florida and along the Gulf coast to Texas (McFarland and Shafer 2008). It is a perennial, dioecious, freshwater angiosperm and is capable of reproducing both sexually through the production of female flowers and male inflorescences as well as asexually through the vegetative expansion of clonal ramets and the production of overwintering turions (Wilder 1974, Titus and Hoover 1991).

Vallisneria americana is a dominant species in fresh to oligohaline waters across the eastern United States, but has declined in abundance and overall distribution (Brush and Hilgartner 2000, Shafer and Bergstrom 2010). It performs many of the functions widely documented for SAV, including producing oxygen and serving as habitat for fishes and invertebrates (e.g., Kemp et al. 2005, Findlay et al. 2006, Strayer and Malcom 2007, Findlay et al. 2014). Dramatic declines in *V. americana* cover and extent coupled with its important functions have led to targeted efforts to restore this species in historic but currently unoccupied areas (Rybicki et al. 2001, Schloesser and Manny 2007, Lloyd et al. 2012).

Study Area

The Potomac River originates at Fairfax Stone on the Allegheny Plateau of West Virginia and flows northeastward toward Cumberland, MD before turning southeast and ultimately discharging into the Chesapeake Bay at Point Lookout, MD. The Potomac River watershed drains just over 38,000 km^2 and the tidal influence

extends approximately 188 km from the mouth to Chain Bridge in Washington, DC (Mason and Flynn 1976, Carter and Rybicki 1986). The tidal portion of the Potomac River can be divided into three segments based upon salinity measured in parts-per-thousand (ppt); the upper freshwater (<0.5 ppt) reach ranges from Washington, DC to the Indian Head peninsula, the middle oligohaline (0.5-5 ppt) reach continues downstream to Morgantown, MD, and the lower mesohaline (5-20 ppt) reach stretches to Point Lookout, MD (Mason and Flynn 1976). The average annual flow in the Potomac River is 323 $m^3 s^{-1}$ and even though the net flow in the Potomac River is directed seaward at all depths (Carter and Rybicki 1986), tidal movement often exceeds river drainage 19 km south of Chain Bridge (Mason and Flynn 1976). The mean tidal range is approximately 0.88 m in the upper tidal region near Washington, DC and approximately 0.43 m near the Chesapeake Bay (Mason and Flynn 1976). The tidal portion of the river broadens from about 60 m near Washington, DC to about 10 km at its mouth (Mason and Flynn 1976) and is a relatively shallow estuary with an overall average depth of about 6 m (Carter and Rybicki 1986). Mean annual temperature in the Potomac River estuary is about 13°C (Mason and Flynn 1976) and the surface water temperature ranges from about 18.4°C in the spring to 25.9°C in the summer to 11.2°C in the fall (Carter and Rybicki 1986). The Potomac River is the second largest tributary of the Chesapeake Bay system and water quality is characterized by high nutrient and suspended sediment concentrations (Mason and Flynn 1976).

Coverage of submersed aquatic vegetation (SAV) has been highly variable in the tidal freshwater reach of the Potomac River. Historical records of the tidal

Potomac River describe shoals densely populated with *Potamogeton crispus*, *Ceratophyllum demersum*, and *Vallisneria americana* (Cumming et al. 1916). However, dramatic SAV declines commencing in the 1930s resulted from a combination of increased eutrophication and a series of major storm events in 1936 and 1937 (Rybicki et al. 2001). Natural resurgence of SAV in the upper tidal Potomac began in 1983 and was associated with decreased concentrations of phytoplankton, increased water clarity, and favorable flow and weather conditions (Carter and Rybicki 1986, Rybicki et al. 2001). Between 1983 and 1993 great variation in water clarity contributed to annual variation in SAV coverage and species composition (Carter and Rybicki 1994, Rybicki et al. 2001). More recent surveys indicate that from 1988 to 2005 SAV coverage has been fairly consistent in the middle tidal reach of the Potomac River and even steadily increasing in the lower tidal reach since 1992 (Karrh et al. 2007). Although reported species composition varies annually (Karrh et al. 2007, Rybicki et al. 2007), the most common SAV species include the native *Vallisneria americana* (wild celery) and *Zannichellia palustris* (horned pond weed), and the invasive *Hydrilla verticillata* and *Myriophyllum spicatum* (milfoil; Karrh et al. 2007). A third exotic, *Najas minor* (naiad), has been documented but has minimal coverage in the Potomac River (Rybicki et al. 2007). Some other SAV species that are commonly documented but are not dominant include *Najas quadalupensis* (southern naiad), *Elodea canadensis* (waterweed), and *Heteranthera dubia* (stargrass) as well as the alga *Chara vulg*aris (muskgrass; Rybicki et al. 2007).

Collections

Samples of *V. americana* were collected from 33 sites along tidal and non-tidal portions of the Potomac River, Maryland (Figure 2.1, Table 2.1). Samples from 13 sites were previously collected and described by Lloyd et al. (2011) in 2007 and 2008 (Table 2.1). Additional samples were collected from 15 sites in 2011 and five sites in 2013 (Table 2.1). In total, 845 samples of *V. americana* were collected. Distances between sampled sites ranged between 5 and 10 km and spanned a total of 400 km along the river. To be consistent with the sampling protocol of Lloyd et al. (2011), we aimed to collect ~30 shoots within each sampling site, each approximately 5–10 m apart. The actual number collected (range 5–33 shoots) depended on extent and density of the plants encountered. Shoots were collected along transects parallel with the river and distances among samples were kept as consistent as possible given the natural variation in densities within and between sites. Latitude and longitude coordinates were taken for each sampled shoot using a handheld GPS unit to allow us to not only quantify the genotypic and allelic diversity at each site but also to allow us to examine the spatial distribution of diversity within sites and along the course of the river. The approximate river mile for each sample site was estimated by projecting the GPS location to the midline of the river using ArcMap v10 (ESRI 2011), with river miles ranging from zero at the mouth of the river to 287 at the TC site (Table 2.1). Shoot (leaf) tissue was placed on ice within one hour of collection and after transport to the University of Maryland College Park, they were frozen at -20°C until DNA extraction.

DNA Extraction and Genotyping

DNA was isolated from shoots collected by Lloyd et al. (2011) using DNeasy Plant Mini Kit (QIAGEN). DNA from all newly collected shoots was first isolated using a modified Chelex Bead™ (Bio-Rad Laboratories) extraction method where a 1 cm^2 fragment of frozen leaf tissue was manually ground with a sterilized glass pestle in 200 µl of a 10% Chelex slurry. Samples were then boiled at 100°C for 10 minutes on an MJ Research PTC-200 Peltier Thermal Cycler. Supernatant containing DNA was removed and diluted 1:2 in sterilized deionized water for subsequent genotyping. Poor quality of some DNA extracts from this method led to downstream difficulty in assigning genotypes to approximately 1/3 of all newly collected samples. As a result, DNA was then also extracted from the leaf tissue of these samples using LGC sbeadx plant maxi DNA extraction kits (LGC) following the manufacturer's instructions.

Extracted DNA was amplified at ten polymorphic loci using primers previously developed for the species (Burnett et al. 2009). Polymerase chain reactions (PCR) were performed on an MJ Research PTC-200 Peltier Thermal Cycler using fluorescent labeled 500 LIZ™ forward primers (Applied Biosystems) and reagents in the TopTaq DNA Polymerase Kit (QIAGEN). Reaction conditions for all loci followed the protocols described by Burnett et al. (2009), with the modifications described by Lloyd et al. (2011). PCR products were separated and measured on an ABI 3730xl DNA Analyzer with GeneScan™-500 with the 500 LIZ™ Size Standard (Applied Biosystems). Peak data were then analyzed using GENEMAPPER v3.7 (Applied Biosystems) and all allele calls were visually inspected and made consistent

with the previously analyzed Potomac River data by following the standards set by Lloyd et al. (2011).

For quality control purposes, allele scoring in GENEMAPPER was done blind to sample number and site origin. All shoots that had DNA isolated from both the Chelex and LGC extraction protocols were genotyped and compared. Every ambiguous call was regenotyped up to three times, and if the call was still ambiguous after three attempts, the alleles were coded as missing. Our final data set contained 0.01% missing data, with 73 of 845 samples (8.6%) having missing allele information at one or more loci.

Genotypic Diversity

We assigned unique individual sampled shoots to multilocus genotypes (MLGs) using the program GENODIVE v2.0b17 (Meirmans and Van Tienderen 2004). To prevent underestimating genotypic diversity we required complete multilocus matches to assign individual shoots to the same MLG. However, this approach overestimated the number of MLGs because individual shoots with missing allele data were assigned to new MLGs. Therefore, we manually checked all shoots that had missing data and assigned them to unique MLGs only if their mutilocus genotype was unique despite missing loci (this occurred 55 times). If shoots with missing data were ambiguous we didn't assign them to any MLG and they were discarded from all subsequent analyses, even when they matched another MLG at all resolved loci (this occurred 18 times).

Within sites, the proportion of unique genotypes was calculated as (G - 1)/(N - 1), where G is the number of unique genotypes and N is the total number of shoots sampled and successfully genotyped (Arnaud-Haond et al. 2007).

Measures of Genetic Diversity

A suite of genetic diversity measurements were calculated for each sampled site using one representative of each MLG within each sampling site. The average number of alleles per locus (A), number of private alleles (A_p), percentage of polymorphic loci (P), and the mean observed (H_o) and expected (H_e) heterozygosity within each of the 33 sampled sites was calculated using GENALEX 6.5 (Peakall and Smouse 2006). Differences in measures of genetic diversity between tidal and non-tidal sites were assessed in R v3.0.1 (R Core Team 2013) using either independent two-way t-tests or Mann–Whitney tests when data didn't meet the assumption of normality under the Shapiro-Wilk test. Evidence of variation in levels of genetic diversity along the Potomac River was analyzed using spearman rank correlation analysis of each measure of genetic diversity against river mile using R.

Wright's F_{is} was calculated for the full dataset and for each sampling site using the estimator f (Weir and Cockerham 1984) in GDA (Lewis and Zaykin 2002) to test for site-level deviations from Hardy–Weinberg equilibrium. Significance of F_{is} for each locus was obtained using Exact tests in GDA with 3200 randomizations (Zaykin et al. 1995), and was assessed at the Bonferroni-adjusted $\alpha = 0.005$ ($n = 10$ comparisons). Significance of F_{is} for each sampled sited was tested by obtaining confidence limits around each estimate generated by 1000 bootstraps in GDA.

Significant departures from Hardy–Weinberg equilibrium can indicate a departure from random breeding.

To detect recent bottlenecks, we determined if expected heterozygosity exceeded levels expected at equilibrium using Wilcoxon's sign rank test in BOTTLENECK v1.2.02 (Cornuet and Luikart 1996). A two-phase mutation model (TPM) run for 1000 iterations was used because it provides results intermediate between an infinite allele model and a stepwise mutation model, which are considered to be most appropriate for microsatellites (Di Rienzo et al. 1994). Significance of the one-tailed Wilcoxon's sign rank test for heterozygosity excess was assessed at Bonferroni-adjusted $\alpha = 0.0015$ ($n = 33$ comparisons).

Estimation of Regional Genetic Structure and Differentiation

Analysis of molecular variance (AMOVA) was used to partition genetic variation within and among sampling sites and non-tidal versus tidal regions. AMOVA was conducted using GENALEX with population differentiation based on genotypic variance. This option produces an estimate of Φ_{pt}, an analogue of $F_{st.}$ The program interpolated missing locus information. Significance was assessed using 999 permutations.

Quantifying genetic differentiation between sites that may or may not be a part of a continuous, natural population is difficult and each method for assessing structure in genetic diversity has its limitations. Moreover, differences in the genetic make-up of different sites, especially in linear systems like rivers, may be more strongly driven by isolation-by-distance than any actual physical barrier to gene flow. Because of the limitations imposed by the use of any one way of assessing population

differentiation, we used a variety of methods including standard measures of genetic differentiation, spatial autocorrelation analysis, and STRUCTURE to gain multiple perspectives on the structure of *V. americana* genetic variation.

The distribution of diversity among non-tidal and tidal regions was analyzed using three measures, including Wright's F_{st} (Weir and Cockerham 1984), G'_{st} (Hedrick 2005), and D_{est} (Jost 2008). Even though genetic differentiation among populations is widely measured by calculating Wright's F_{st} statistic or its analogue for multiple alleles, G_{st} (Nei 1977), there are assumptions, including that population structure is based on the infinite island model, that complicate the interpretation of genetic divergence and gene flow among populations and these assumptions are almost always violated in natural systems (Bossart and Pashley Prowell 1998, Neigel 2002). Additionally, when individual populations have high allele richness and hypervariable microsatellite loci, F_{st} and G_{st} underestimate differentiation because they measure the amount of variation among populations relative to the total variation without taking into account the identity of the alleles (Hedrick 2005). One simple method to account for allelic richness and overcome the dependence of G_{st} on levels of heterozygosity is to scale G_{st} by the maximum G_{st} possible for the observed amount of heterozygosity (Hedrick 2005). The resulting statistic, $G'_{st,}$ varies from 0–1 in a way that better reflects the underlying patterns of genetic diversity, but remains fundamentally based on heterozygosity. To overcome issues associated with using heterozygosity as a means to describe genetic differentiation, we used Jost's (2008) statistic, D_{est}, based on effective numbers of alleles (Jost 2008, Meirmans and Hedrick

2011). All three measures of population differentiation were calculated using GENALEX and significance was assessed from running 1000 permutations.

Measures of pairwise differentiation were not calculated between sample sites because many sites, especially those in the upper Potomac River, had low sample sizes once replicate MLGs were removed from each site. Instead we performed spatial autocorrelation analysis on our MLGs to determine if the distribution of genotypes along the relatively continuous gradient of *V americana* habitat in the Potomac River was random or spatially structured. Because alleles in species with both clonal and sexual reproduction are more likely to cluster when ramets of the same genet are sampled multiple times at nearby locations (Reusch et al. 1999a), we performed a series of spatial autocorrelation analyses using GENALEX to determine if any detected spatial clustering was due to vegetative reproduction or the result of limited gene flow (isolation-by-distance). Spatial autocorrelation analysis was performed on 1) all collected samples, including duplicate MLGs, 2) one representative of each MLG from each sample site, and 3) one representative of each MLG from each sample site, excluding the two most extensive MLGs (MLG 199 and MLG 266). Geographic distance between all sampled shoots was calculated as the shortest distance over water using the ESRI Network Analyst Toolkit in ArcMap (ESRI 2011) and significance of the spatial autocorrelation analyses was assessed by running 1000 permutations. Furthermore, because population differentiation analysis between MLGs collected from non-tidal and tidal portions of the Potomac River showed significant levels of differentiation for all three measures (F_{st}, G'_{st}, and D_{est}),

the two tidal regions were treated as separate populations in the spatial autocorrelation analysis.

We used the program STRUCTURAMA v2.0 (Huelsenbeck and Andolfatto 2007) to identify theoretical a posteriori 'populations' from our Potomac River data based on minimal deviations from both Hardy–Weinberg and linkage equilibrium as described by Pritchard et al. (2000). STRUCTURAMA differs from the program STRUCTURE (Pritchard et al. 2000) in that the number of theoretical populations is included as a random variable in a Dirichlet process model (Pella and Masuda 2006) and is estimated from a posterior distribution for the probabilities of each number. Because Huelsenbeck and Andolfatto (2007) suggest that the estimation of the number of populations can suffer when the aggregation parameter of the Dirichlet process model (α) is mis-specified, we also let α act as a random variable represented by a gamma probability distribution with shape $\kappa=3$ and scale $\theta=2$. These parameter values allowed the Dirichlet process to estimate a variety of possible numbers of populations based on a range of α values from ~1 to 12. The sampler was run using four heated chains for 1,000,000 generations, and samples were taken every 25 generations for a total of 40,000 samples. Data were summarized after discarding 10,000 burn-in samples. We chose the mean partition value as the number of theoretical populations (K) containing the highest posterior probability.

Because STRUCTURAMA lacks clearly interpretable visualization of individual assignments we used STRUCTURE to assess distinctiveness of theoretical populations (Berryman 2002) by assigning individuals to the number of populations inferred by STRUCTURAMA. Following the recommendations of Onogi et al. (2011) for

unbalanced sample sizes, STRUCTURE was run assuming no prior admixture and no correlation of alleles, with 1,000,000 steps in the Markov Chain Monte Carlo sampler, using a burn-in of 50,000 steps. The analysis was run 10 times, and the best runs were selected based on the highest likelihood scores. One major limitation of STRUCTURE output is that when gradients of genetic variation are created by processes like neighbor mating, STRUCTURE tends to force continuous variation into genetic clusters (Schwartz and McKelvey 2009).

Estimates of Relatedness

We used the program COANCESTRY v1.0 (Wang 2011) to calculate the Wang (2002) estimator of pairwise relatedness among all collected individuals using allele frequencies calculated from the 33 Potomac River sampling sites and 12 additional Chesapeake Bay sites described by Lloyd et al. (2011). Calculating allele frequencies from a larger set of samples increases the accuracy in relatedness estimation (Bink et al. 2008). We chose Wang's estimator because previous Monte-Carlo simulations (Marsden et al. 2013) indicated it had the lowest variance and bias across various relationship categories (Van de Casteele et al. 2001).

We also sought to determine whether or not MLGs were genetically more related to other MLGs than expected from a randomly mating, panmictic population. Therefore, we compared the observed mean and variance of pairwise relatedness estimates against their expected distribution under the null hypothesis of panmixia using 1000 Monte Carlo permutations of the same number of alleles, as implemented in the program IDENTIX v1.1 (Belkhir et al. 2002). In addition to testing across the entire Potomac River, we also tested the null hypothesis of panmixia separately

within the non-tidal and tidal regions of the river. Due to limitations within IDENTIX, pairwise relatedness estimates were calculated with the Lynch and Ritland (1999) estimator for this analysis.

To understand the spatial distribution of relatedness within the Potomac River, we created a spatially explicit individual-based network of relatedness at thresholds of r =1.0, ≥0.5, and ≥0.25 in ArcMap v10 (ESRI 2011). Network nodes represent individual sampled shoots (including duplicate MLGs) in their geographic location, and edges represent connections between shoots that were at or above each relatedness threshold value.

Finally, we created an individual-based, spatially implicit network of individuals that were related at a threshold of $r \geq 0.5$ and visualized the pruned network using the *igraph* package in R (Csardi and Nepusz 2006). In contrast to the spatially explicit network, only one copy of each MLG was included in the spatially implicit network. To quantify connectivity between MLGs we calculated degree centrality (Freeman 1978, Wasserman and Faust 1994), closeness centrality (Freeman 1978), and eigenvector centrality (Bonacich 1987) for each MLG using the *igraph* package in R. Degree centrality is the number of adjacent edges of each node (MLG) in a network. MLGs with high degree centrality are directly related at $r \geq 0.5$ to many other MLGs within the river. Closeness centrality is a measure of how close a node (MLG) is to all other nodes in a network, measured as the reciprocal of the sum of the distances to all other nodes in a connected network. MLGs with high closeness centrality have more and shorter paths to all other MLGs within a network, and thus are more closely related to all MLGs. Finally, eigenvector centrality measures the

influence of a node in a network based on the influence of nodes to which it is connected. Therefore, MLGs that are closely related to many other MLGs that are in turn closely related to many other MLGs have higher scores. Evaluating centrality metrics will allowed us to determine if vegetatively expansive MLGs also contribute disproportionately to sexual reproduction. Differences between measures of centrality across the non-tidal and tidal region were assessed using non-parametric Mann–Whitney tests in R.

Results

Genotypic Diversity

We genotyped 828 of 845 sampled shoots, representing 413 unique MLGs. Missing data precluded the remaining 17 individuals from being unambiguously assigned to an MLG. Within each of the 33 collection sites, we sampled an average of 25.1 (*SD* = 6.6) shoots (Table 2.2). Genotypic diversity within sampling sites ranged from 0.00 to 1.00, with a mean of 0.54 (*SD* = 0.30; Table 2.2). Sample sites in the non-tidal region of the Potomac River had a broader range in genotypic diversity (spanning from 0.00 to 0.93; $\bar{x}$ = 0.39, *SD* = 0.22), than the genotypic diversity in the lower Potomac's tidal region, which ranged from 0.55 to 1.00 ($\bar{x}$ = 0.88, *SD* = 0.15). The non-tidal region of the Potomac had significantly lower genotypic diversity than the tidal region ($W = 9$; $p < 0.001$).

Thirty of the 413 MLGs identified in the Potomac River were found multiple times across the landscape and accounted for 442 (53.4%) of the 828 genotyped shoots. Eight of these MLGs were found across multiple sites in the non-tidal Potomac River (Figure 2.2; Table 2.3), whereas the other 22 MLGs were found

multiple times within single sites. Two of the MLGs found across multiple sites, MLG 199 and MLG 266, were far more widespread than other MLGs and they dominated within many sites (Table 2.3). MLG 199 was found 229 times in 22 different sites, comprising between 4-81% of the shoots genotyped within a site, whereas MLG 266 was found 134 times in 14 sites, comprising between 3-100% of the shoots genotyped within a site (Table 2.3). MLG 199 spanned 239 river km and MLG 266 spanned 159 river km. No MLGs were shared across sites in the tidal part of the river. MLGs that occurred multiple times within a single site were found two to five times, comprising between 6-40% of the shoots genotyped within a site. The spatial extent of MLGs found multiple times within a site ranged from 1.7 m in non-tidal sites to 2.2 km in tidal sites.

There was a negative correlation between genotypic diversity and river mile ($\rho = -0.74$, n = 33, $p < 0.001$), such that genotypic diversity tended to increase downstream (Figure 2.3). However, the negative correlation between genotypic diversity and river mile was not significant within just the tidal ($\rho = -0.57$, n = 10, $p = 0.09$) or non-tidal ($\rho = -0.39$, n = 23, $p = 0.07$) portions of the Potomac River (Figure 2.3).

Genetic Diversity

All 10 microsatellite loci were polymorphic. The proportion of polymorphic loci (*P*) within MLGs averaged across sites was $\bar{x} = 0.81$ (*SD* = 0.11). MLGs collected from non-tidal portions of the Potomac River had lower proportion of polymorphic loci ($\bar{x} = 0.78$; *SD* = 0.10) than MLGs collected from tidal portions of

the river ($\bar{x}$ = 0.87; *SD* = 0.09; *W* = 58; *p* = 0.020). In the full data set, seven loci departed significantly from Hardy–Weinberg equilibrium (Table 2.4).

The total number of alleles in the full data set was 75 (range 4-11 per locus) across all sampled MLGs and loci from all 33 sample sites. The average number of alleles per locus (*A*) within sites was 3.05 (*SD* = 0.92; Table 2.2). Non-tidal sites had lower allelic diversity ($\bar{x}$ = 2.60; *SD* = 0.55) than tidal sites ($\bar{x}$ = 4.08; *SD* = 0.76; $t_{13.246}$ = -5.56; *p* < 0.001). A strong, negative correlation between allelic diversity and river mile (ρ = -0.71, n = 33, *p* < 0.001) was primarily driven by these differences as there were no correlations within non-tidal (ρ = -0.42, n = 23, *p* = 0.05) or tidal (ρ = -0.05, n = 10, *p* = 0.89) regions of the river (Figure 2.3).

Across the 33 Potomac River sample sites, 13 private alleles occurred at frequencies of 0.02 to 0.10 (Tables 2.2 and 2.5). Six of the private alleles were found in six non-tidal sites and seven were found in five tidal sites (Table 2.2). Two tidal sites (AL and NC) had two private alleles (Table 2.2; 2.5), driving a negative correlation between the number of private alleles found at a site and river mile (ρ = -0.35, n = 33, *p* = 0.046). There were no correlations when the data was divided into non-tidal (ρ = -0.16, n = 23, *p* = 0.47) and tidal (ρ = -0.59, n = 10, *p* = 0.07) region of the river (Figure 2.3).

Average observed heterozygosity (H_o) of MLGs within all sample sites was 0.50 (*SD* = 0.09; Table 2.2). H_o in non-tidal sites ($\bar{x}$ = 0.53; *SD* = 0.09) was higher than H_o in tidal sites ($\bar{x}$ = 0.44; *SD* = 0.07; $t_{23.447}$ = 3.39; *p* = 0.002). The Potomac River had a strong, positive correlation between observed heterozygosity at a site and river mile (ρ = 0.64, n = 33, *p* < 0.001; Figure 2.4). Therefore, H_o was lower in

downstream locations. When the data were subdivided, the relationship remained significant into the non-tidal region ($\rho = 0.59$, n = 23, $p = 0.003$), but not the tidal region ($\rho = 0.12$, n = 10, $p = 0.75$; Figure 2.3).

Three sampled sites showed signs of heterozygote deficit (Table 2.2): GWP ($f = 0.184$; 95% CI 0.08 to 0.27), LSP ($f = 0.201$; 95% CI 0.12 to 0.30), and AL ($f = 0.210$; 95% CI 0.12 to 0.30). All three sites were located in the tidal Potomac. Nine non-tidal sites showed heterozygote excess (Table 2.2). Based on analysis with the program BOTTLENECK, none of the sites had evidence of a recent bottleneck when assessed at the Bonferroni-adjusted $\alpha = 0.0015$.

Estimation of Regional Genetic Structure and Differentiation

When AMOVA was used to partition the genetic variation within and among sampling sites and also among tidal and non-tidal regions, 78% of molecular variance was within sampling sites ($\Phi_{PT} = 0.221$; $p = 0.001$), 8% was among sampling sites ($\Phi_{PR} = 0.096$; $p = 0.001$), and 14% was among regions ($\Phi_{RT} = 0.138$; $p = 0.001$). *Fst* (0.045 p=0.001), *Gst* (0.125, p=0.002), and *Dest* (0.085, p=0.001) between all tidal versus all non-tidal samples were different from zero, signifying the regions were differentiated from one another.

We performed a series of spatial autocorrelation analyses to determine if the distribution of genotypes in non-tidal and tidal regions was random or was spatially structured due to either the vegetative propagation of clonal ramets or the result of limited gene flow (isolation-by-distance). When all collected samples were included in the analysis, allele frequencies in the non-tidal region were less similar at the three lowest distances classes (2.5, 7.5, and 12.5 km) than expected ($p < 0.05$; Figure

2.4A). The remaining distance classes showed signs of both positive and negative autocorrelation. By contrast, the four shortest distance classes were positively autocorrelated ($p < 0.05$) in the tidal region (Figure 2.4B). The transition from positive autocorrelation to negative autocorrelation in the tidal Potomac occurred around 19.9 km and reached a minimum near 72.5 km (Figure 2.4B).

Removing duplicate MLGs from each site did not alter the autocorrelation patterns observed in the tidal Potomac (Figure 2.4B). The first four distance classes still showed signs of positive autocorrelation ($p < 0.05$), and the lowest negative autocorrelation was still found around 72.5 km after transitioning from positive to negative autocorrelation at 20.2 km (Figure 2.4B). The first seven distance classes in the non-tidal correlogram of MLGs showed signs of positive autocorrelation ($p < 0.05$) as opposed to the negative autocorrelation observed when all sampled shoots were included in the analyses (Figure 2.4A). The transition from positive autocorrelation to negative autocorrelation in the non-tidal Potomac MLG correlogram occurred around 55.7 km and reached a minimum around 247.5 km (Figure 2.4B). Removing MLG 199 and MG 266, the two widespread MLGs in the non-tidal Potomac, did not alter this pattern.

Bayesian clustering analysis, as implemented by STRUCTURAMA, identified three genetic clusters from the 33 sites ($Pr[K = 3|X] = 1.00$). STRUCTURE was run assuming K = 2 (estimated ln probability of data = -8754.3) and K = 3 (estimated ln probability of data = -8379.1; Table 2.6) to visualize the individual genetic clusters (Figure 2.5). The groups recognized by STRUCTURE generally spatially clustered together (Figure 2.5). In both genetic partitions, there was a strong division between

genetic clusters at the tidal to non-tidal transition between the PL and GWP sample sites. In the K = 3 partition, a second genetic cluster in the non-tidal portion of the Potomac became prevalent at the ML site. The partitioning of *V. americana* into three genetic clusters based on minimal deviations from Hardy-Weinberg and linkage equilibrium may be driven by the low genotypic diversity and similar allele composition of MLGs in the upper reaches of the Potomac River. The second genetic cluster within the non-tidal Potomac coincided with the first occurrence of the widespread MLG 266 (Table 2.3). Three of the nine MLGs in the HCK2 site (originally described by Lloyd et al. 2011) clustered more closely with the non-tidal genetic cluster (Figure 2.5). Closer inspection of the raw allele data indicated that these assignments were not the result of either missing allele information or shared rare alleles between the HCK2 MLGs and the tidal Potomac MLGs. Rather, three HCK2 MLGs lacked alleles at some loci that were otherwise common combinations in the non-tidal region and instead had alleles that were either common in both the non-tidal and tidal regions or were rare in the whole data set. Finally, there were signs of admixture between the three genetic clusters as some MLGs sampled within one population clustered more closely with neighboring genetic populations (Figure 2.5).

Estimates of Relatedness

In the Potomac River, the average pairwise relatedness across all MLGs (r_A) was 0.115 (*SD* = 0.114; Table 2.2). Pairwise relatedness among samples from the non-tidal Potomac was higher ($\bar{x}$ = 0.267; *SD* = 0.294) than it was among samples from the tidal Potomac ($\bar{x}$ = -0.024; *SD* = 0.258; $t_{52709.24}$ = 120.83; $p < 0.001$). The average estimate of relatedness between MLGs from within the same sample site (r_W)

was 0.295 (*SD* = 0.262; Table 2.2) and differed between non-tidal and tidal portions of the Potomac River ($t_{3292.188}$ = 27.64; $p < 0.001$), such that r_W in the non-tidal Potomac was higher ($\bar{x}$ = 0.302; *SD* = 0.276) than r_W in the tidal Potomac ($\bar{x}$ = 0.061; *SD* = 0.270).

These differences resulted in a positive correlation between river mile and both r_A (ρ = 0.86; n = 33; $p < 0.001$) and r_W (ρ = 0.83; n = 33; $p < 0.001$; Figure 2.3). The correlation between r_A and river mile remained positive when the data were subdivided into non-tidal (ρ = 0.63; n = 23; $p = 0.001$) and tidal regions (ρ = 0.77; n = 10; $p = 0.009$; Figure 2.3). However, r_W was only positively correlated with river mile in the non-tidal portion of the Potomac River (ρ = 0.67; n = 23; $p < 0.001$; Figure 2.3).

According to permutation tests implemented in the program IDENTIX, MLGs were not, on average, more related to one another than expected from a null hypothesis of panmixia in the Potomac River, but they were approaching significance ($p = 0.067$). The variance in pairwise estimates of relatedness was higher than expected in the Potomac ($p = 0.001$), indicating that pairwise comparisons involved a combination of highly related and unrelated individuals (Belkhir et al. 2002). Within regions, MLGs on average were more related to one another than expected from panmixia in the non-tidal region ($p = 0.001$), but not in the tidal region ($p = 0.877$). The variance in pairwise estimates of relatedness was still higher than expected in both the non-tidal ($p = 0.001$) and the tidal ($p = 0.001$) regions.

Spatially explicit individual-based networks of Wang (2002) relatedness estimates show the distribution of relatedness across the Potomac River (Figure 2.6).

Edges in the network of r = 1.0 connect samples that were assigned to the same MLG, thus representing all clones that were sampled multiple times. Such clones accounted for 10.03% of pairwise comparisons among all sampled shoots (34,357 of 342,378; Figure 2.6A). They represented 20.44% (34,313 of 167,910) of the pairwise comparisons among non-tidal individuals, only 0.14% (44 of 30,628) of the pairwise comparisons among tidal individuals, and none of the 143,840 pairwise comparisons between non-tidal and tidal individuals (Figure 2.6A).

Connections between individual shoots that were related at the level of $r \geq 0.5$ (i.e., first degree relatives and higher), including r = 1, involved individuals from all 33 sites (Figure 2.6B). These edges accounted for 19.74% of all pairwise comparisons, 38.69% of non-tidal pairwise comparisons, 2.34% of tidal pairwise comparisons, and 1.32% of pairwise comparisons among individuals between tidal and non-tidal regions. Finally, at $r \geq 0.25$ (e.g., second degree relatives and higher), connections accounted for 35.01% of all Potomac River pairwise comparisons, 57.59% of non-tidal pairwise comparisons, 15.53% of tidal pairwise comparisons, and 12.81% of pairwise comparisons between tidal and non-tidal regions of the Potomac River.

Finally, the spatially implicit network of relatedness displayed how MLGs were related to one another within the Potomac River (Figure 2.7). The spatially implicit network at $r \geq 0.5$ comprised three components containing 390 of 413 MLGs connected by 3867 edges (Figure 2.7). Normalized graph-level degree centrality for the network was 0.168 ($\bar{x}$ = 19.83, range: 1 to 85); normalized graph-level closeness centrality for the network was 0.043 ($\bar{x}$ = 3.63×10^{-4}, range: 0.07×10^{-4} to 4.18×10^{-4});

and normalized graph-level eigenvector centrality for the network was 0.834 ($\bar{x}$ = 0.028, range: 0.00 to 0.167). MLGs from the non-tidal Potomac had higher values of degree centrality (W = 33352.5; $p < 0.001$; $\bar{x}$ = 31.63, SD = 19.12), closeness centrality (W =31837; $p < 0.001$; $\bar{x} = 3.83 \times 10^{-4}$, $SD = 0.16 \times 10^{-4)}$, and eigenvector centrality (W = 35290; $p < 0.001$ $\bar{x}$ = 0.054, SD = 0.047) than MLGs from the tidal Potomac (degree: $\bar{x}$ = 8.39, SD = 9.08; closeness: $\bar{x} = 3.43 \times 10^{-4}$, $SD = 0.58 \times 10^{-4}$; eigenvector: $\bar{x}$ = 0.004, SD = 0.010).

Six of the ten MLGs that had the highest degree centrality scores in the spatially implicit network also ranked in the top ten for eigenvector centrality (Table 2.7). Three other top ten MLGs for degree centrality also ranked in the top ten for closeness centrality (Table 2.7). All of the most highly ranked MLGs, with the exception of MLG 472 from PB and MLG 612 from NC, were found in the non-tidal region of the Potomac River (Table 2.7). Although not always ranked in the top ten, MLG 199 and MLG 266, the two most expansive MLGs, were highly ranked relative to the other 390 MLGs that were included in the spatially implicit network of relatedness (Table 2.8). This ranking indicates that these two MLGs are highly related to many other MLGs within the Potomac River and are thus likely contributing to sexual reproduction as well as dominating through vegetative reproduction (Figure 2.8).

Discussion

We found major differences in the range of genetic diversity, spatial distribution of genetic variation, and level of relatedness among *V. americana* samples collected from tidal versus non-tidal portions of the Potomac River. Clearly,

the environmental conditions and hydrological processes in the non-tidal part of the river have different effects on genetic structure of populations than in the tidal region. Because knowledge of the spatial distribution of genetic diversity is essential for understanding the scale at which dispersal, genetic drift, and selection might operate, natural resource managers can use this information to assist in managing populations so that they are resilient into the future. (Sgrò et al. 2011). Resilience refers both to the ability of populations to persist in their current state or to undergo evolutionary adaptation in response to changing environmental conditions (Sgrò et al. 2011). Differences in the structure and composition of *V. americana* genetic diversity in non-tidal versus tidal regions of the Potomac lead us to recommend different strategies for SAV restoration within each region. Briefly, the non-tidal Potomac River has evidence of widespread connectivity and we suggest that any site within the non-tidal Potomac River could be used as a source of restoration stock for another site within this region. However, accumulating evidence of local adaptation of *V. americana* in the tidal Potomac (Engelhardt et al. 2014b) leads us to suggest sourcing restoration stock from local, spatially adjacent SAV beds. Finally, a large gap in SAV coverage spanning approximately 54 km exists between the non-tidal and tidal Potomac which may be limiting connectivity between the two regions. High relatedness between non-tidal and tidal MLGs provides preliminary evidence of connectivity between the two regions, such that upstream non-tidal beds may act as sources for downstream beds. To facilitate a more resilient *V. americana* population in the long-term, natural resource managers may opt to focus on promoting natural re-establishment of *V. americana* beds between the non-tidal and tidal transition zone to

narrow the current gap. But, prior to these recommended actions, additional information is needed on the ecological role of the dominant and widespread MLGs discovered in the Potomac River.

Genotypic and Genetic Diversity in the Potomac River

We found a large range in the measures of genotypic and genetic diversity of *V. americana* across the species' distribution in the Potomac River (Table 2.2). Aquatic angiosperms have long been regarded as essentially clonal (Kendrick et al. 2005) and selfing SAV with strong clonal growth tend to have low levels of genotypic variation within populations (Procaccini et al. 2001) relative to outcrossing SAV species (Hamrick and Godt 1996, Wang et al. 2010). *V. americana* reproduces both clonally and sexually. Because *V. americana* is dioecious, (Wilder 1974), all offspring are the result of obligate outcrossing. Therefore, a broad range in genotypic variation is not unexpected and suggests that sites range from having either little detectable sexual reproduction to little detectable asexual reproduction. Genotypic diversity of *V. americana* in the Potomac River ranged from 0.0 to 1.0 (Table 2.2). Genotypic diversity was high ($\bar{x}$ = 0.53; Table 2.2) compared to the mean values observed for clonal terrestrial species ($\bar{x}$ = 0.17; Ellstrand and Roose 1987), but fell within the range of mean values observed for other clonal aquatic macrophytes ($\bar{x}$ = 0.20 to 0.71; Chen et al. 2007, Serra et al. 2010, Kamel et al. 2012, Sinclair et al. 2014). Although broad ranges in genotypic diversity is a common pattern for some outcrossing clonal aquatic species (Arnaud-Haond et al. 2010), the presence of dominant MLGs is a phenomenon that is usually associated with long-lived species characterized by sporadic sexual reproduction (Procaccini et al. 2001). The presence

of widespread and dominant MLGs like the two described in this study (Figure 2.2), has been observed in other *Vallisneria* species (e.g., Chen et al. 2007, Wang et al. 2010), including other *V. americana* populations (Lokker et al. 1994, Lokker 2000). One widespread *Vallisneria spinulosa* genotype was found in more than 75% of sampled sites that spanned approximately 900 km along the Yangtze River in China (Chen et al. 2007). Likewise, a single *V. americana* genotype dominated within six transects along the Detroit River, accounting for up 35-55% of the shoots sampled within each transect (Lokker et al. 1994).

Microsatellite allelic variation in *V. americana* ranged from 1.5 to 5.4 alleles/locus across the Potomac River (Table 2.2). The variation in allelic diversity falls within the range of *V. americana* allelic diversity previously reported for the Chesapeake Bay (1.5 to 5.8 alleles/locus; Lloyd et al. 2011), and more broadly within the range of microsatellite allelic variation for other clonal aquatic plants (1.3 to 6.09 alleles/locus; Reusch et al. 1999e, Pollux et al. 2007, Arnaud-Haond et al. 2010, Serra et al. 2010, Kamel et al. 2012, Reynolds et al. 2013). The site with the lowest allelic diversity, PL (A = 1.5), only supported one MLG (Table 2.2).

Although variation in factors that influence *V. americana* reproductive strategies among sites in the Potomac River may have a large effect on the observed variation and range of *V. americana* genotypic and genetic diversity, we believe that the high levels of interconnectivity and directional water flow that characterizes riverine systems also have an influence on the observed patterns. Hydrologic connectivity may facilitate the exchange of genes among populations of aquatic plants (Kudoh and Whigham 1997), but primarily unidirectional gene flow in rivers

results in the downstream accumulation of genetic diversity (Barrett et al. 1993). Consistent with this prediction, *V. americana* genotypic and allelic diversity was negatively correlated with river mile along the Potomac River (Figure 2.3). Unexpectedly, observed heterozygosity was positively correlated with river mile (Figure 2.3), a result driven by a few MLGs with higher than expected heterozygosity found in the upper reaches of the Potomac River (Table 2.2). This could be the result of some heterozygote advantage that enabled these MLGs to persist within the upper reaches of the Potomac. One recent study evaluating the ecological importance of different *V. americana* MLGs found that individuals with low levels of heterozygosity produced less turion biomass (Engelhardt et al. 2014b). Over time, increased turion production from MLGs with higher levels of observed heterozygosity could have major effects on the overall composition of MLGs within the upper Potomac if it leads to domination of a single MLG. Such decreased diversity could have negative consequences on the overall resilience of *V. americana* in the upper Potomac.

Ideally, the hydrologically connected sites in the Potomac could function as a single genetic metapopulation that acts as a reserve of genetic variation and reduces the effect of random genetic drift (Kudoh and Whigham 2001, Chen et al. 2007). However, there were clear divisions in patterns and structure of genetic diversity between the non-tidal and tidal portions of the river. In fact, most of the correlations between measures of genetic diversity and river mile were driven by the differences between the non-tidal and tidal segments (Figure 2.3). Genotypic diversity, allelic

diversity, and the total number of private alleles had no relationship once the tidal and non-tidal data were assessed independently (Figure 2.3).

Genetic Differentiation in the Potomac River

Using a variety of methods including standard measures of genetic differentiation, STRUCTURE analysis, relatedness networks, and spatial autocorrelation analysis to gain multiple perspectives on the structure of *V. americana* genetic variation, we found evidence of genetic differentiation between the *V. americana* collected from the non-tidal versus the tidal portions of the river and that the distribution of genetic diversity across sites is very different within each of these regions. Despite the limitations of F_{st}, we can use F_{st} measures from several previous studies on *Vallisneria* genetic differentiation for broader context for the observed levels of Potomac *V. americana* differentiation. The level of differentiation we observed among the non-tidal and tidal regions (F_{st} = 0.045) was similar to levels documented from other hydrologically connected sites, including *V. spinulosa* collected from the Yangtze River in China (F_{st} = 0.06; Chen et al. 2007) and *V. americana* collected from the Detroit River in the United States (F_{st} = 0.03; Lokker et al. 1994). Meanwhile, the observed F_{st} value is lower than that estimated for *V. natans* (F_{st} = 0.132) and *V. spinulosa* (F_{st} = 0.202) collected from hydrologically isolated lakes along the Yangtze River (Wang et al. 2010), as well as lower than that estimated for *V. americana* collected from different geographic regions of the Chesapeake Bay (F_{st} = 0.114; Lloyd et al. 2011). However, our estimate of D_{est} (D_{est} = 0.085) was similar to those found by Lloyd et al. (2011; D_{est} = 0.07) for *V. americana* collected from different Chesapeake Bay regions. There was lower genetic

differentiation between the non-tidal and tidal Potomac relative to hydrologically isolated *Vallisneria*, indicating some degree of connectivity between the two river regions. However, this differentiation was similar to levels found across broad regions of the Chesapeake Bay (Lloyd et al. 2011), indicating population subdivision between the non-tidal and tidal Potomac.

Population subdivision between *V. americana* from the non-tidal and tidal Potomac is further supported by the AMOVA, STRUCTURE, and relatedness results. The AMOVA results revealed a greater proportion of genetic diversity was within populations and the STRUCTURE results display a strong division occurring between the last non-tidal and the first tidal site when the *V. americana* MLGs are partitioned into two genetic clusters (Figure 2.5). Moreover, the MLGs from the non-tidal Potomac are significantly more related to all other Potomac River MLGs than MLGs from the tidal Potomac (Table 2.2), which is easily visualized by the tight clustering of the non-tidal MLGs in the spatially implicit relatedness network (Figure 2.7). Genetic differentiation between *V. americana* from non-tidal and tidal regions is also validated by the fact that a greater proportion of the connections in the spatially explicit relatedness networks occur within non-tidal and within tidal regions than among regions (Figure 2.6).

Beyond assessing genetic differentiation between *V. americana* between regions, we wanted to determine if there were similar patterns in the distribution and partitioning of alleles within regions. Therefore, tests of autocorrelation were used to determine if *V. americana* gene frequencies were randomly distributed within each of these regions. In plant populations, alleles often deviate from a random distribution

and reveal positive autocorrelation at short distances (Reusch et al. 1999a). Processes like gene flow and natural selection can make allele frequencies at nearby locations more similar than expected and frequencies at more distant locations less similar than expected. Processes like genetic drift and mutation might blur the patterns caused by gene flow and natural selection (Slatkin and Arter 1991). In addition, the contribution of clonal reproduction to genetic autocorrelation must be differentiated from the effects of gene flow (isolation-by-distance; Reusch et al. 1999a). For example, Reusch et al. (1999a) concluded that most of the significant genetic clustering observed in an SAV population of *Zostera marina* was due to clonal spread because there was no spatial autocorrelation once MLGs were identified and clustered into one representative clone for spatial autocorrelation analysis.

All sampled shoots in the non-tidal part of the river were negatively autocorrelated at short distances (Figure 2.4A), indicating that the alleles were less similar than expected at random. The extensive spread of MLG 199 and MLG 266 not only within sites but across multiple sites in the upper Potomac was driving the negative correlation because when only MLGs were analyzed we observed positive spatial autocorrelation at distances between 2.5 and 55.7 km (Figure 2.4A). A positive spatial autocorrelation was seen among tidal individuals located between 2.5 and 20 km apart, regardless of the inclusion or exclusion of multiple ramets from a single MLG (Figure 2.4B). Therefore, the nonrandom spatial structure of alleles in the tidal Potomac is likely due to limited gene flow among sites that are more than 20 km apart. The nonrandom spatial structure in the non-tidal Potomac is more complicated and likely the result of a combination of both extensive vegetative reproduction and

gene flow from pollen and seed. Gene flow in the non-tidal region is less restricted than the tidal region because there was significant positive autocorrelation across broader stretches of the Potomac. However, the inclusion of multiple ramets from each MLG dramatically altered the results of the spatial autocorrelation, demonstrating the importance of vegetative reproduction on patterns of genetic diversity in the non-tidal Potomac.

Potential Drivers of Differentiation and Variation in Genetic Structure

There are a wide variety of environmental and hydrological differences within and between the non-tidal and tidal regions of the Potomac that could be driving both the genetic differentiation between regions and the variation in the distribution of genetic diversity within regions. Three major factors that may influence genetic diversity and distribution of that diversity include rates of recruitment, trade-offs between vegetative growth and sexual reproduction, and levels of disturbance (Reusch 2006, Sinclair et al. 2014). A recent review of clonal plant reproduction describe two patterns of seedling recruitment that ultimately lead to populations that are either dominated by asexual, clonal growth or exhibit high levels of sexual reproduction and genetic diversity (Silvertown 2008). The first pattern, called the initial seedling recruitment (ISR) strategy (Eriksson 1993), describes a pattern where seeding recruitment is only able to occur at the initial establishment of the population and further development of the bed is largely restricted to asexual reproduction (Silvertown 2008). The alternative strategy, the repeated seedling recruitment (RSR), leads to higher genetic variation because seedlings are able to recruit regularly within stands of established SAV (Eriksson 1993, Alberto et al. 2005). ISR is common in

stable environments and RSR is typical in areas of frequent disturbance (Eriksson 1993, Becheler et al. 2014).

Populations of *V. spinulosa* in the Yangtze River are thought to have been founded primarily through fruit and seed dispersal (Piquot et al. 1998) and similar to ISR are subsequently maintained though clonal expansion. Long-distance dispersal of sexually derived propagules across hydrologically connected habitat was also used to predict why one widespread MLG was found in more than 75% of the sampled sites in the Yangtze River (Chen et al. 2007). The non-tidal region of the Potomac River can be classified as having continuous, directional water flow along relatively uninterrupted suitable habitat. If ISR is also characteristic of the non-tidal Potomac, then directional flow and widespread connectivity across sites with lower genetic diversity and more extensive vegetative growth creates an environment that enables the widespread expansion of MLG 199 and MLG 266.

Meanwhile, the tidal region lacks continuously suitable habitat due to deeper and wider channels, more turbidity, increased salinity, and changing water depth from tidal pulsing (Mason and Flynn 1976). The lack of continuously suitable habitat and more variable envrionmental conditions may limit gene flow between tidal sites of *V. americana*, ultimately impacting levels of genetic variation among sites. Other SAV studies have found that variation in local disturbance regimes have a strong influence on genetic diversity (e.g., Procaccini et al. 1999, Procaccini et al. 2001, Rhode and Duffy 2004, Sinclair et al. 2014). For example, meadows of the seagrass *Posidonia australis* had higher levels of genotypic and allelic diversity in open water sites with moderate levels of disturbance than they did at either highly exposed or highly

isolated sites (Sinclair et al. 2014). Restricted gene flow among sites coupled with moderate levels of disturbances across all tidal sites may lead to higher levels of genetic diversity and opportunities for local adaptation within the tidal Potomac if sites undergo RSR.

Despite evidence of strong differentiation between non-tidal and tidal sites in the Potomac, hydrochory (water dispersal) can result in long-distance seed or propagule dispersal events that effectively connect a discontinuous population (Waser et al. 1982, Kudoh and Whigham 1997, Chen et al. 2007). Even infrequent long-distance dispersal events can have dramatic effects on ecological and evolutionary processes (Levin et al. 2003, Levine and Murrell 2003, Nathan et al. 2008). With evidence that many *V. americana* individuals between non-tidal and tidal regions are related at $r \geq 0.5$ (Figures 2.6, 2.8), long-distance hydrochory is probable in the Potomac, even if infrequent. Future analyses on the rate of *V. americana* dispersal between sites may be able to confirm this. The establishment or re-establishment of meadows via long-distance dispersal events will be critical for the long-term sustainability and resilience of aquatic plant populations in the face of global declines (McMahon et al. 2014). Moreover, connectivity to established populations has been a primary driver of recovery of in many degraded SAV habitats (Duarte et al. 2013).

Implications for Restoration

Coastal aquatic ecosystems are among the most threatened in the world (Branch 1999, Kennish 2002). In addition to degradation brought on by current and historic anthropogenic land-use changes that cause increased nutrient and sediment runoff, nearshore aquatic communities will also be disproportionally affected by

changes in surface temperature and sea level rise (Kennish 2002). SAV species are in decline worldwide (Short and Wyllie-Echeverria 1996, Orth et al. 2006, Waycott et al. 2009), which is problematic because SAV provide crucial ecosystem services like sediment retention and erosion reduction that maintain water quality and provide valuable habitats for nearshore fisheries (Duffy 2006, Orth et al. 2006). In response to these global declines, marine protected areas that include SAV have increased and major SAV monitoring and restoration projects have been proposed and implemented worldwide (Orth et al. 2006). In fact, local resurgence of SAV in the Potomac River has been documented since 1983 and had been largely attributed to the increased water clarity associated with improved wastewater treatment that substantially reduced annual nutrient loading into the Potomac River (Carter and Rybicki 1986, Rybicki et al. 2001). More recent surveys indicate that SAV has been fairly consistent in the tidal reach of the Potomac River and even steadily increasing in some areas (Karrh et al. 2007). However, overall Chesapeake Bay nutrient reduction and SAV restoration goals have not been met (Ruhl and Rybicki 2010) and restoration of unvegetated sites are often unsuccessful (Tanner et al. 2010). Trends suggest that by 2025 estuaries will be significantly impacted by additional habitat loss from growing coastal populations, will suffer from increased incidences of hypoxia and anoxia from greater nutrient and sewage inputs, and will undergo ecological impacts associated with sea level rise, coastal subsidence and warmer temperatures (Kennish 2002). Many SAV populations are already negatively affected by these factors (e.g., Kemp et al. 1983, Orth and Moore 1983, 1984, Oviatt 2004, Tanner et al. 2010), so future

restoration will need to focus on creating SAV habitat that is resilient to and can mitigate against current and future environmental stressors.

The ultimate goal of ecological restoration is reestablishing self-sustaining ecosystems that will be resilient to future perturbation without ongoing human input (Procaccini and Piazzi 2001, Rice and Emery 2003, Ramp et al. 2006, Broadhurst et al. 2008, Liu et al. 2008). Genetic diversity is fundamental to resilience because it is associated with increased fitness (Williams 2001, Leimu et al. 2006), enhanced growth and productivity (Williams 2001, Reynolds et al. 2012a), rapid response to disturbances (Hughes and Stachowicz 2004, Reusch et al. 2005), and enhanced reproductive success and offspring fitness (Ellstrand and Elam 1993, Crnokrak and Roff 1999, Amos et al. 2001). Moreover, genetic diversity also increases chances for successful establishment and functioning of restored populations (Williams 2001, Reynolds et al. 2012a, Reynolds et al. 2012k). In fact, the recent success of large-scale *Zostera marina* restorations in Virginal coastal bays (Orth et al. 2012) could be driven by the fact that restoration using seeds collected from adjacent populations restored SAV beds to levels of genetic diversity that were higher than nearby SAV beds that were revegetated via natural recruitment (Reynolds et al. 2012k). Unfortunately, there has been very little research on the natural seed banks of *V. americana*, or more generally SAV. Most estuary seed bank studies focus on marsh plants (e.g., Leck and Simpson 1995, Leck 2003, Hilgartner and Brush 2006). However, one recent study on the seed banks in tidal estuaries of Louisiana found a lack of SAV seeds and concluded that reliance on seed banks for the restoration of SAV may prove unsuccessful (La Peyre et al. 2005).

Alternative approaches for selecting source material, seeds or shoots, for restoration tend to focus on either attempting to maintain current or known historic patterns of genetic diversity or attempting to augment current levels of genetic diversity. The range of restoration options fall along a continuum where restoration stock can be sourced from within local/adjacent sites, among several local sites within regions, or among regions. Selection of stock from local sites has the perceived benefit of reducing the risks of genetic dilution, maladaptation, and outbreeding depression (Montalvo and Ellstrand 2000, 2001, McKay et al. 2005), but risks decreasing fitness of individuals due to unmitigated inbreeding depression or inhibiting future adaptation should standing genetic diversity be too low (Fenster and Dudash 1994, Broadhurst et al. 2008, Hughes et al. 2008, Weeks et al. 2011). Therefore, we recommend the local selection of stock for restoration when populations have relatively high within site genetic diversity, no signs of inbreeding depression, and relatively high differentiation from other sites or regions. Selection of stock from multiple sites within a region has the perceived benefit of increasing genetic diversity, leading to genetic rescue (Fenster and Dudash 1994, Broadhurst et al. 2008, Hughes et al. 2008, Weeks et al. 2011). However, the risks of mixing stock from multiple sources include genetic dilution of locally adapted genotypes and potential outbreeding depression (McKay et al. 2005), which is why we recommend this strategy for populations that have relatively low within-site genetic diversity and evidence of inbreeding depression. At the extreme, selection of stock from different regions might be beneficial for long-term resilience of a population if it is able to match current local adaptations to the expected future environmental conditions of a

site (McLachlan et al. 2007). This strategy is most closely related to the concept of managed relocation (MR), which is emerging as a method to address biodiversity management in the face of climate change via the intentional movement of populations or appropriately adapted genotypes to locations where probability of future persistence is predicted to be higher (McLachlan et al. 2007, Richardson et al. 2009). MR might be considered when continued persistence is not possible due effects of climate change and natural dispersal distances preclude unaided establishment of populations. Choosing among source-selection strategies is a major decision in restoration, especially because the degree of differentiation among populations and inbreeding within populations vary independently, making the overall risks of inbreeding versus outbreeding depression site specific (Marsden et al. 2013). For example, several studies found no evidence of outbreeding depression in crosses between populations assigned to different genetic regions, and evidence of heterosis was not predicted by levels genetic dissimilarity among individuals (Marsden et al. 2013, Pickup et al. 2013).

Our results suggest that differences in the structure and degree of *V. americana* genetic diversity between the non-tidal and tidal regions of the Potomac River warrant different recommendations for future restoration activities in the two areas. In the non-tidal Potomac there is limited evidence of positive spatial autocorrelation due to prevalence of expansive MLGs. Even accounting for MLGs, spatial autocorrelation extended among sites. In addition, the overall degree of relatedness among MLGs from the non-tidal Potomac River is substantial and permutation tests revealed that individuals in the non-tidal Potomac were more

related to one another than expected in panmixia. Therefore, we feel that any site within the non-tidal Potomac River could be used to source stock for restoration of another non-tidal site within the river without posing much risk of genetic dilution or outbreeding depression. Wang et al. (2010) similarly suggested that any population of the *V. spinulosa* studied by Chen et al. (2007) could be used as stock for re-introduction in the Yangtze River because it had considerable genetic variation and low population genetic differentiation due to extensive hydrologic connectivity along the river. Even though sites in the non-tidal Potomac River had significantly lower levels of genotypic and allelic diversity relative to sites in the tidal Potomac (Table 2.2), there was no evidence of inbreeding depression in any sites. In fact, many sites actually had significant heterozygote excess.

However, high levels of clonal growth and relatedness among MLGs from the non-tidal Potomac are of concern because they limit the amount of standing genetic variation on which natural selection can act in the future. Although one strategy for increasing the standing genetic diversity in the non-tidal Potomac could involve supplementing non-tidal sites with individuals sourced from the tidal region of the Potomac River, we recommend first collecting additional information on the growth potential of the widespread MLGs. If MLG 199 and MLG 266 are widespread because of an ability to acclimate to many different conditions across many sites, then increasing the genetic diversity within the non-tidal Potomac River may not be necessary. In fact, bringing in outside variation may disrupt locally adapted gene complexes that are successful in this river region. On the other hand, if MLG 199 and MLG 266 are the result of an older bottleneck in the non-tidal region, then their

sexual reproductive success combined with their vegetative proficiency may lead to future risks of inbreeding depression within the non-tidal Potomac River.

In the tidal Potomac River there is evidence of spatial autocorrelation at distances up to about 20 km. In addition, although there is less overall relatedness between MLGs within the tidal Potomac, the high variance in pairwise estimates of relatedness indicated that there were groups of highly related MLGs and groups of unrelated MLGs (Belkhir et al. 2002). Therefore, we feel that sites within the tidal Potomac River should be evaluated independently and do not recommend sourcing material from just any site for restoration projects. For example, even though genotypic and allelic diversity was higher in the tidal sites relative to the non-tidal sites, three sites (GWP, LSP, and AL) had significant inbreeding coefficients that cause concern. Sourcing restoration stock from local, adjacent locations in the river seems practical for addressing cases of recolonization, inbreeding depression, or reduced genetic diversity. Previous studies on *V. americana* confirm that there is evidence of local adaptation within regions of the Chesapeake Bay (Engelhardt et al. 2014b) and that the success of mixing MLGs varies by individuals and sites and is not well predicted by levels of relatedness or differentiation (Marsden et al. 2013). Even though risk of outbreeding depression was found to be low for *V. americana* in the Bay (Marsden et al. 2013), most sites have sufficient genetic diversity that the potential cost of losing local adaptations outweighs the potential benefits of mixing multiple sources when attempting to increase coverage via restoration. Reynolds et al. (2012k) demonstrated that *Z. marina* seeds harvested from nearby beds can preserve genetic diversity in restored sites with no signs of inbreeding depression in either

donor or restored sites. Likewise, Lloyd et al. (2012) found that current *V. americana* restoration techniques that involve planting locally sourced material generally reflect levels of genotypic diversity, allelic diversity, and heterozygosity found in natural populations in the Chesapeake Bay, but not always the same composition of genotypes or alleles when restoration sources came from stock repositories.

Finally, we do not recommend sourcing material from the non-tidal region of the Potomac River to restore portions of the tidal Potomac without additional research. Given the accumulating evidence of *V. americana* local adaptation (Engelhardt et al. 2014b), there is great uncertainty about whether or not upstream MLGs could even survive in the different conditions associated with the tidal Potomac. Furthermore, without additional knowledge of the ecological role of the widespread MLGs from the non-tidal Potomac, we do not know if they have high acclimation potential (good for long-term resiliency) or just high vegetative growth capabilities that over time could come to dominate coverage within sites and limit the potential for future adaptation by lowering genetic diversity (bad for long-term resiliency).

That being said, pairwise relatedness estimates between all Potomac River MLGs were not different from the null hypothesis of panmixia and there were many highly related connections between MLGs from the non-tidal and tidal region (Figure 2.6). Combined with the correlations supporting the downstream accumulation of genotypic and allelic diversity (Figure 2.3), this connectivity between tidal regions is preliminary evidence that upstream *V. americana* beds act as sources for the re-establishment and maintenance of downstream *V. americana* beds. Despite heavy

field surveying, no SAV beds of *V. americana* were found in-between the PL and GWP sites in any of the sampling years. Therefore, restoration managers may opt to promote the natural re-establishment of *V. americana* beds between the non-tidal to tidal transition zone as a mechanisms to promote natural connectivity, gene flow, and future *V. americana* resiliency.

Conclusions

The differences in the *V. americana* genetic structure between the non-tidal and tidal Potomac River are likely driven by differences in environmental and hydrologic variables that impact local mating and dispersal mechanisms. These variables likely exist in other riverine habitats and may have similar impacts on the genetic diversity of similar SAV species. However, before suggesting that the results of this study can be used to make restoration decisions within other river systems or with other species, additional studies should investigate the generality of these findings. Pervious work, for example, found that the distribution and range of genetic diversity of *V. americana* within tidal regions of three major rivers in the Northeastern United States was region specific (Marsden CH1). Because genetic information is often time consuming and expensive to obtain (summarized by Lloyd et al. 2011, Lloyd et al. 2012) it is infrequently available prior to restoration. Therefore, it would be advantageous for restoration managers to know if there are general broad-scale patterns in the distribution of SAV genetic diversity across riverine systems. Thus far we can conclude that there are broad-scale differences in the distribution of SAV genetic diversity between non-tidal and tidal sites in the Potomac River that warrant different restoration strategies.

Table 2.1: Summary of *Vallisneria americana* collection details for 33 sites sampled from the Potomac River (MD).

Region	Site	Code	Old Code[a]	Latitude N	Longitude W	River Mile	Year Sampled
Non-Tidal	Town Creek	TC	-	39.5227	78.5399	287	2011
	Purslane Run	PR	-	39.5347	78.4640	282	2011
	Fifteenmile Creek Upriver	FMC1	-	39.6241	78.3848	259	2011
	Fifteenmile Creek Downriver	FMC2	TOUR1	39.6285	78.3833	259	2007/08
	Sideling Hill Creek	SHC	TOUR2	39.6340	78.3234	254	2007/08
	Sir John's Run	SJR	-	39.6516	78.2399	248	2011
	Hancock Upriver	HCK1	-	39.6971	78.1815	243	2011
	Hancock Downriver	HCK2	HCK	39.6974	78.1767	243	2007/08
	Licking Creek	LC	-	39.6506	78.0511	235	2011
	McCoy's Ferry	MF	-	39.6079	77.9688	228	2011
	Williamsport Upriver	WSP1	WSP	39.6053	77.8328	214	2007/08
	Williamsport Downriver	WSP2	-	39.6018	77.8294	214	2011
	Opequon Creek	OJ	-	39.5155	77.8606	205	2011
	Snyder's Landing	SL	-	39.4991	77.7682	191	2011
	Antietam Creek	AC	-	39.4200	77.7481	183	2011
	Brunswick Upriver	BWK1	BWK	39.3062	77.6164	168	2007/08
	Brunswick Downriver	BWK2	-	39.3063	77.6151	168	2011
	Point of Rocks Upriver	POR1	POR	39.2727	77.5416	163	2007/08
	Point of Rocks Downriver	POR2	-	39.2701	77.5307	163	2011
	White's Ferry Upriver	WF1	WF	39.1556	77.5195	150	2007/08
	White's Ferry Downriver	WF2	-	39.1550	77.5196	150	2011
	Edward's Ferry	EF	-	39.1030	77.4738	145	2011
	Pennyfield Lock	PL	PL	39.0533	77.2911	134	2007/08
Tidal	George Washington Parkway	GWP	GWP	38.7303	77.0416	101	2007/08
	Piscataway Park	SWP	SWP	38.6849	77.1019	97	2007/08
	Pohick Bay	PB	-	38.6770	77.1687	93	2013
	Gunston Manor	GM	GM	38.6353	77.1441	91	2007/08
	Belmont Bay	BB	-	38.6435	77.2001	88	2013
	Leeslvania State Park	LSP	LSP	38.5835	77.2583	86	2007/08
	Mattawoman Creek	MWC	-	38.5848	77.1613	84	2013
	Aquia Landing	AL	AL	38.3884	77.3213	71	2007/08
	Nanjemoy Creek	NC	-	38.4543	77.1497	59	2013
	Port Tobacco River	PTR	-	38.4814	77.0257	55	2013

a: Old code refers to site names of samples collected in 2007/08 and previously described in Lloyd et al. 2011

Table 2.2: Summary of multilocus genotypic and genetic diversity estimates for 33 *Vallisneria americana* sites sampled from the Potomac River (MD) based on 10 microsatellite loci.

	Site by Region	N	*G*	Genotypic Diversity	*A*	A_p	*P*	H_o	H_e	f	r_W	r_A
Tidal	TC	29	8	0.25	2.0	0	0.80	0.62	0.40	**-0.53**	0.652	0.193
	PR	21	5	0.20	2.3	0	0.90	0.70	0.41	**-0.63**	0.699	0.239
	FMC1	30	9	0.28	2.2	0	0.80	0.58	0.39	**-0.41**	0.544	0.200
	FMC2	15	4	0.21	2.1	0	0.70	0.60	0.38	**-0.48**	0.597	0.207
	SHC	14	3	0.15	1.9	0	0.70	0.67	0.37	**-0.72**	0.813	0.282
	SJR	27	8	0.27	2.5	1	0.80	0.62	0.41	**-0.47**	0.545	0.200
	HCK1	30	8	0.24	2.1	0	0.70	0.46	0.35	-0.23	0.510	0.199
	HCK2	25	9	0.33	3.1	0	0.70	0.50	0.43	-0.10	0.204	0.091
	LC	30	6	0.17	2.0	0	0.80	0.72	0.41	**-0.73**	0.778	0.209
	MF	30	18	0.59	2.9	1	0.90	0.47	0.41	-0.11	0.325	0.199
	WSP1	21	14	0.65	2.7	0	0.80	0.44	0.42	-0.01	0.243	0.147
	WSP2	31	20	0.63	3.6	1	0.90	0.48	0.46	-0.02	0.193	0.159
	OJ	15	5	0.29	2.5	0	0.70	0.50	0.39	**-0.16**	0.312	0.214
	SL	30	28	0.93	3.7	1	1.00	0.51	0.43	**-0.16**	0.331	0.190
	AC	18	10	0.53	2.9	0	0.80	0.55	0.45	-0.18	0.263	0.189
	BWK1	18	6	0.29	2.8	0	0.80	0.45	0.43	0.04	0.137	0.145
	BWK2	28	10	0.33	3.0	0	0.80	0.57	0.46	-0.19	0.241	0.191
	POR1	32	12	0.35	2.6	0	0.70	0.49	0.42	-0.13	0.306	0.177
	POR2	30	8	0.24	2.6	0	0.70	0.35	0.36	0.09	0.268	0.111
	WF1	20	11	0.53	3.0	1	0.80	0.51	0.42	-0.16	0.312	0.169
	WF2	28	14	0.48	2.7	1	0.80	0.46	0.42	-0.08	0.259	0.165
	EF	29	22	0.75	3.2	0	0.80	0.46	0.43	-0.03	0.216	0.161
	PL	29	1	0.00	1.5	0	0.50	0.50	0.25	N/A	N/A	0.143
Non-Tidal	GWP	30	28	0.93	4.1	1	1.00	0.36	0.43	**0.18**	0.098	-0.015
	SWP	30	29	0.97	4.1	0	0.80	0.41	0.44	0.08	0.130	-0.013
	PB	5	4	0.75	2.6	0	0.70	0.45	0.43	0.09	0.007	0.101
	GM	30	17	0.55	4.0	0	0.90	0.50	0.48	-0.02	0.074	0.018
	BB	30	29	0.97	4.4	0	0.90	0.55	0.53	-0.01	0.040	-0.077
	LSP	30	25	0.83	4.8	0	0.90	0.40	0.48	**0.20**	-0.039	-0.072
	MWC	23	19	0.82	4.3	1	0.80	0.48	0.48	0.02	0.043	-0.039
	AL	30	30	1.00	5.4	2	1.00	0.39	0.48	**0.21**	-0.055	-0.071
	NC	21	21	1.00	3.5	2	0.90	0.47	0.45	0.00	0.174	-0.131
	PTR	19	19	1.00	3.6	1	0.80	0.34	0.38	0.14	0.210	-0.090
	Average	*25.09*	*13.94*	*0.53*	3.05	*0.39*	0.81	0.50	0.42	*-0.12*	*0.29*	*0.11*
	SE	*1.15*	*1.53*	*0.05*	0.10	*0.11*	0.02	0.02	0.01	*0.05*	*0.04*	*0.02*

N number of genotyped shoots; *G* unique genets; Genotypic Diversity = (G - 1)/(N - 1); *A* average number of alleles per locus within a sampling site; A_p number of private alleles; *P* proportion of polymorphic loci; H_o observed heterozygosity; H_e expected heterozygosity; f Wright's inbreeding coefficient - the correlation of alleles within individuals within populations; r_W mean Wang (2002) coefficient of relatedness between multilocus genotypes (MLGs) from within sites; r_A mean Wang (2002) coefficient of relatedness between MLGs among all sites. f in bold type is significantly different from zero at $p < 0.05$.

Table 2.3: Number of *Vallisneria americana* shoots for each multilocus genotype (MLG) that is shared among sites along the Non-Tidal Potomac River, and the proportion (in italics) of the MLG within each sampling site.

MLG ID	TC	PR	FMC1	FMC2	SHC	SJR	HCK1	HCK2	LC	MF	WSP1	WSP2	OJ	SL	AC	BWK1	BWK2	POR1	POR2	WF1	WF2	EF	PL	Total # Shoots
199	22 *0.76*	17 *0.81*	21 *0.70*	10 *0.67*	11 *0.79*	19 *0.70*	23 *0.77*	13 *0.52*	22 *0.73*	12 *0.40*	2 *0.10*	10 *0.32*	11 *0.73*	2 *0.07*	8 *0.44*	6 *0.33*	1 *0.04*	2 *0.06*	2 *0.07*	5 *0.25*	6 *0.21*	4 *0.14*		229
205	1 *0.03*	1 *0.05*	2 *0.07*			2 *0.07*	1 *0.03*																	7
207	1 *0.03*								1 *0.03*			1 *0.03*										1 *0.03*		4
219				3 *0.20*																	1 *0.04*			4
230						1 *0.04*			4 *0.13*				1 *0.07*				1 *0.04*							7
266										1 *0.03*	6 *0.29*	3 *0.10*	1 *0.07*	2 *0.07*	2 *0.11*	8 *0.44*	19 *0.68*	20 *0.63*	22 *0.73*	6 *0.30*	10 *0.36*	5 *0.17*	29 *1.0*	134
278											1 *0.05*										1 *0.04*			2
346																1 *0.06*				1 *0.05*				2

Sites are ordered from upstream (left) to downstream.

Table 2.4: Genetic diversity of individual loci averaged over all *Vallisneria americana* sampled sites and the results of Exact tests for Hardy-Weinberg Equilibrium on each locus.

Locus	% Missing Data	A	H_e	H_o	f	*p*-value
atg002	0.48	3.85	0.60	0.76	-0.003	**<0.001**
aagx051	0.48	5.55	0.72	0.79	0.096	**<0.001**
aag002	0.48	3.09	0.55	0.73	-0.053	**0.001**
aagx012	0.60	1.73	0.09	0.10	0.086	0.062
m13	1.57	3.85	0.59	0.65	0.178	**<0.001**
m16	0.36	1.21	0.01	0.01	-0.004	1.000
aagx071	2.54	3.94	0.59	0.64	0.125	**<0.001**
m49	0.73	2.79	0.43	0.58	-0.022	**<0.001**
aag004	0.48	3.03	0.56	0.69	0.023	**<0.001**
aagx030	0.00	1.48	0.06	0.06	0.100	0.023
Average	*0.77*	*3.05*	*0.42*	*0.50*	*0.058*	
SE	*0.23*	*0.42*	*0.08*	*0.10*	*0.024*	

A = total number of alleles, H_o = observed heterozygosity, H_e = expected heterozygosity, f = the inbreeding coefficient. *p*-values in bold type are significantly different from zero at the Bonferoni adjusted $p < 0.005$.

Table 2.5: Frequency of 13 *Vallisneria americana* private alleles found in the Potomac River.

Site Code	Locus	Allele	Frequency
SJR	m13	253	0.063
MF	m16	180	0.028
WSP2	m49	159	0.050
SL	aag004	379	0.018
WF1	aag004	384	0.045
WF2	aag004	373	0.036
GWP	aagx012	214	0.036
MWC	atg002	144	0.026
AL	atg002	172	0.083
AL	aag004	400	0.017
NC	aagx012	205	0.100
NC	m13	283	0.025
PTR	aagx051	196	0.079

Sites are ordered from upstream (top) to downstream.

Table 2.6: Summary statistics for partitioning *Vallisneria americana* MLGs into three (K=3) Bayesian-modelled genetic clusters, as implemented in STRUCTURE (Pritchard et al. 2000). STRUCTURE was run assuming no prior admixture and no correlation of alleles, with 1,000,000 steps, using a burn-in of 50,000 steps.

Structure Run	Estimated Ln Probability of Data	Mean value of ln likelihood	Variance of ln likelihood
1	-8380.4	-8267.6	225.5
2	-8379.8	-8267.5	224.6
3	-8379.8	-8267.5	224.5
4	-8379.9	-8267.5	224.8
5	-8379.4	-8267.4	223.9
6	**-8379.1**	-8267.4	**223.4**
7	-8379.3	-8267.4	223.7
8	-8380.4	-8267.6	225.7
9	-8380.5	-8267.6	225.7
10	-8379.3	-8267.4	223.8

Values in bold represent the highest estimated probability score and the lowest variance from each of the STRUCTURE runs.

Table 2.7: The ten highest ranked *Vallisneria americana* multilocus genotypes (MLGs) for each graph measure calculated from the spatially implicit network of relatedness for the Potomac River. The networks was created using the *igraph* package (Csardi and Nepusz 2006) in R v3.0.1 (R Core Team 2013) at a relatedness threshold of r ≥ 0.5.

	Degree Centrality			Closeness Centrality			Eigenvector Centrality		
Rank	MLG	MLG Site (# Samples/Site)	Score	MLG	MLG Site (# Samples/Site)	Score	MLG	MLG Site (# Samples/Site)	Score
1	330	AC (1)	85	377	WF1 (1)	$4.18e^{-4}$	199	See Table 2.2	0.167
2	319	SL (1)	78	612	NC (1)	$4.17e^{-4}$	330	AC (1)	0.167
3	199	See Table 2.2	75	308	SL (1)	$4.14e^{-4}$	319	SL (1)	0.162
4	293	WSP2 (1)	73	296	WSP2 (1)	$4.13e^{-4}$	293	WSP2 (1)	0.161
5	223	SHC (1)	72	344	BWK2 (1)	$4.13e^{-4}$	243	HCK2 (3)	0.154
6	324	SL (1)	68	401	EF (1)	$4.12e^{-4}$	223	SHC (1)	0.153
7	308	SL (1)	68	472	PB (1)	$4.12e^{-4}$	265	MF (1)	0.152
8	377	WF1 (1)	67	402	EF (1)	$4.10e^{-4}$	200	TC (1)	0.151
9	243	HCK2 (3)	67	390	WF2 (1)	$4.10e^{-4}$	229	SJR (1)	0.148
10	305	SL (1)	64	305	SL (1)	$4.09e^{-4}$	210	PR (1)	0.147

Cells in white represent MLGs that only ranked in the top ten for one graph measure, cells in grey represent MLGs that ranked in the top ten for two graph measures. The MLG Site column describes the sites where each MLG was found as well as the number of times it occurred at that site (in parentheses).

Table 2.8: The rank of three calculated graph centrality measures for two widespread *Vallisneria americana* multilocus genotypes (MLGs) from the spatially implicit network of 390 Potomac River MLG's with relatedness r ≥ 0.5. The network was created using the *igraph* package (Csardi and Nepusz 2006) in R v3.0.1 (R Core Team 2013).

	MLG 199	MLG 266
Degree Centrality	#3 *(top 1%)*	#70 *(top 20%)*
Closeness Centrality	#43 *(top 15%)*	#49 *(top 15%)*
Eigenvector Centrality	#1 *(top 1%)*	#139 *(top 40%)*

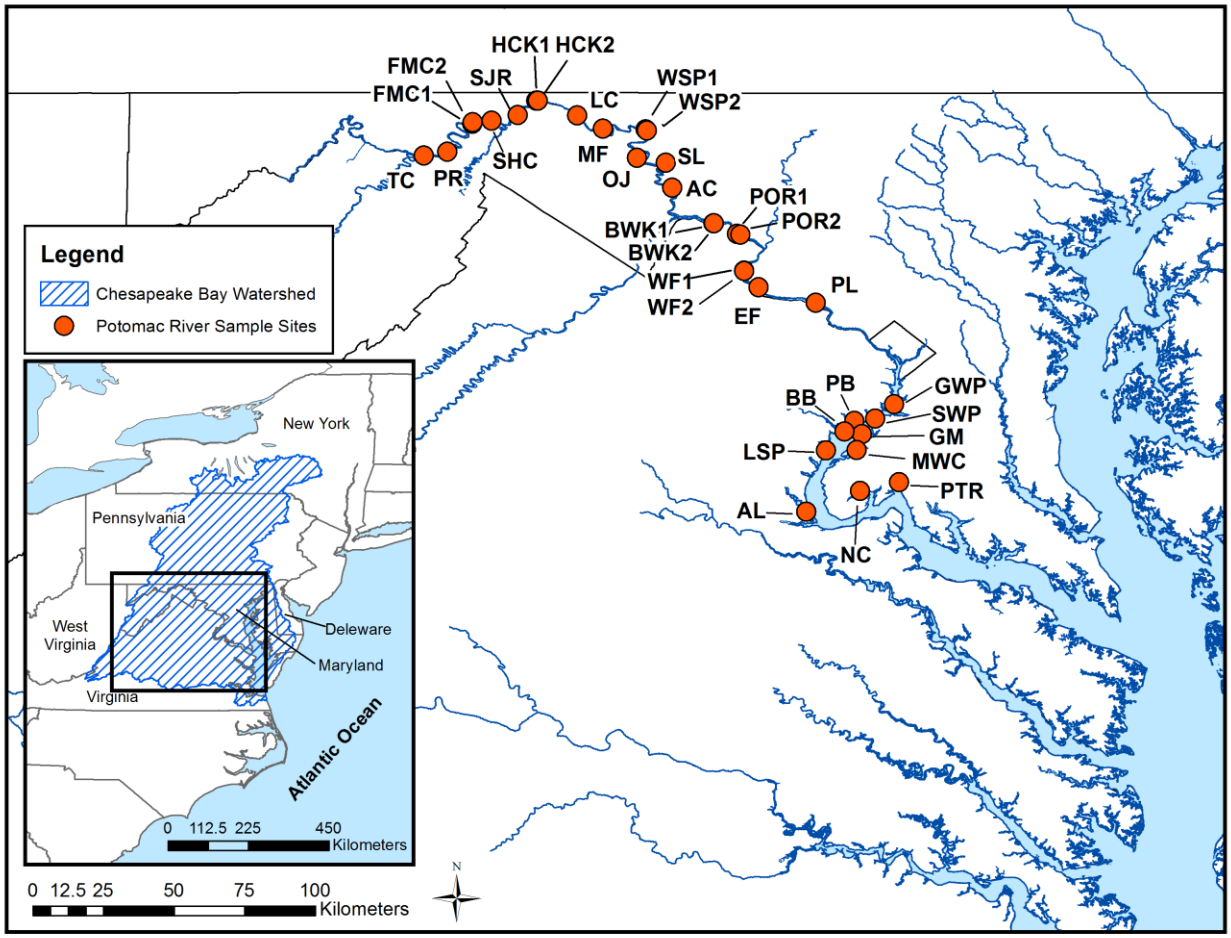

Figure 2.1: Map of the 33 *Vallisneria americana* sampling locations spanning the Potomac River. The Potomac River is located in the mid-Atlantic of North America and is part of the Chesapeake Bay watershed (hatched area). Site names and sample size are defined in Table 2.1.

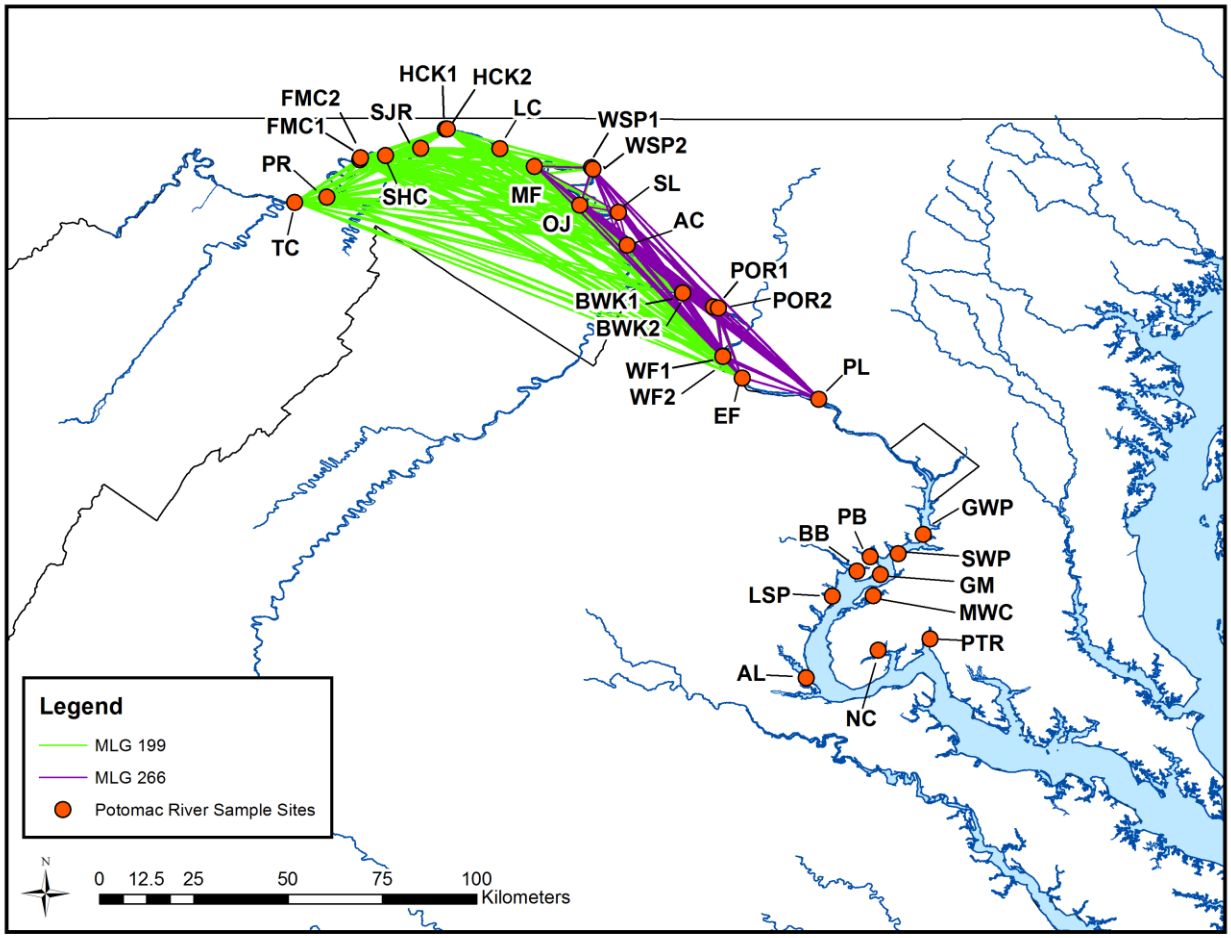

Figure 2.2: Map of the two *Vallisneria americana* multilocus genotypes (MLGs) that were found extensively within and across the 33 collection sites in the Potomac River. Lines connect individual samples of *V. americana* that are related to one another at r = 1.0. Pairwise relatedness coefficients between sampled shoots were calculated using the Wang (2002) estimator (implemented in COANCESTRY). Site names are defined in Table 2.1.

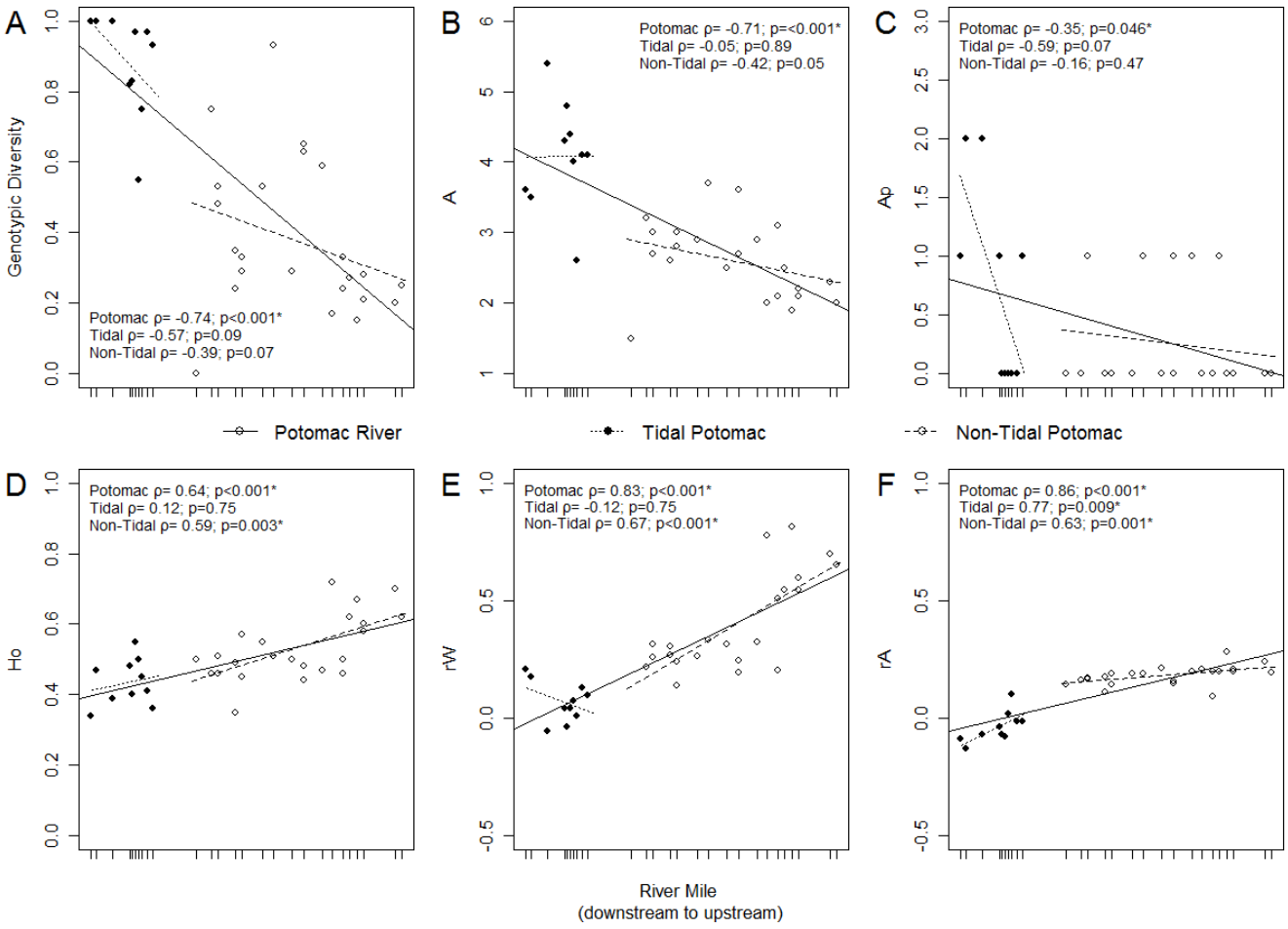

Figure 2.3: Scatterplots of measures of *Vallisneria americana* genotypic and genetic diversity along 33 sites in the tidal (filled circles) and non-tidal (open circles) Potomac River (MD). The x-axis represents the river mile location of each sample site, moving in a downstream to upstream direction. Results of nonparametric Spearman's rank correlation (ρ) analysis and corresponding *p*-values are provided on the plots for (**A**) genotypic diversity, (**B**) allelic diversity (*A*), (**C**) the number of private alleles found within each sampled site (A_p), (**D**) observed heterozygosity (H_o), (**E**) average relatedness of unique multilocus genotypes (MLGs) within each sampled site calculated using the Wang (2002) relatedness coefficient (r_W), and (**F**) average relatedness of MLGs among sampled sites calculated using the Wang (2002) relatedness coefficient (r_A). An * indicates significant rank correlations at $p < 0.05$.

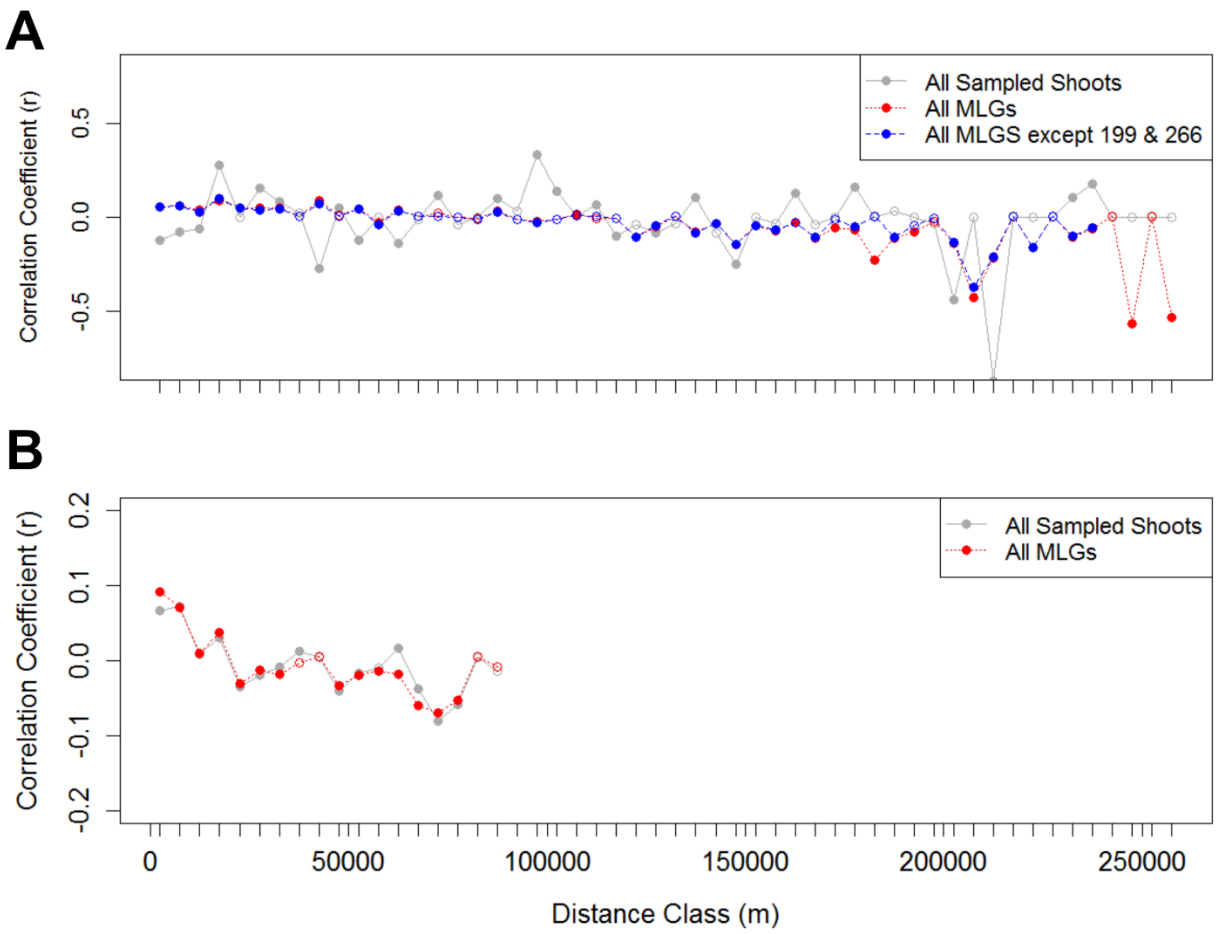

Figure 2.4: Correlograms of the spatial autocorrelation coefficient (r) for genetic distance calculated from 10 microsatellite loci for 52 distance classes (in river meters) covering the extent of the study area. Depicted are correlograms for all *Vallisneria americana* sampled shoots (grey), only multilocus genotypes (MLGs) within each site (red), and excluding the two expansive MLGs, MLG 199 and MLG 266 (blue), in both the (**A**) non-tidal and (**B**) tidal portions of the Potomac River. Open points are not significantly different from zero after 1000 permutations and filled points are significantly different from zero at $p < 0.05$. Note the change in the y-axis between (**A**) and (**B**).

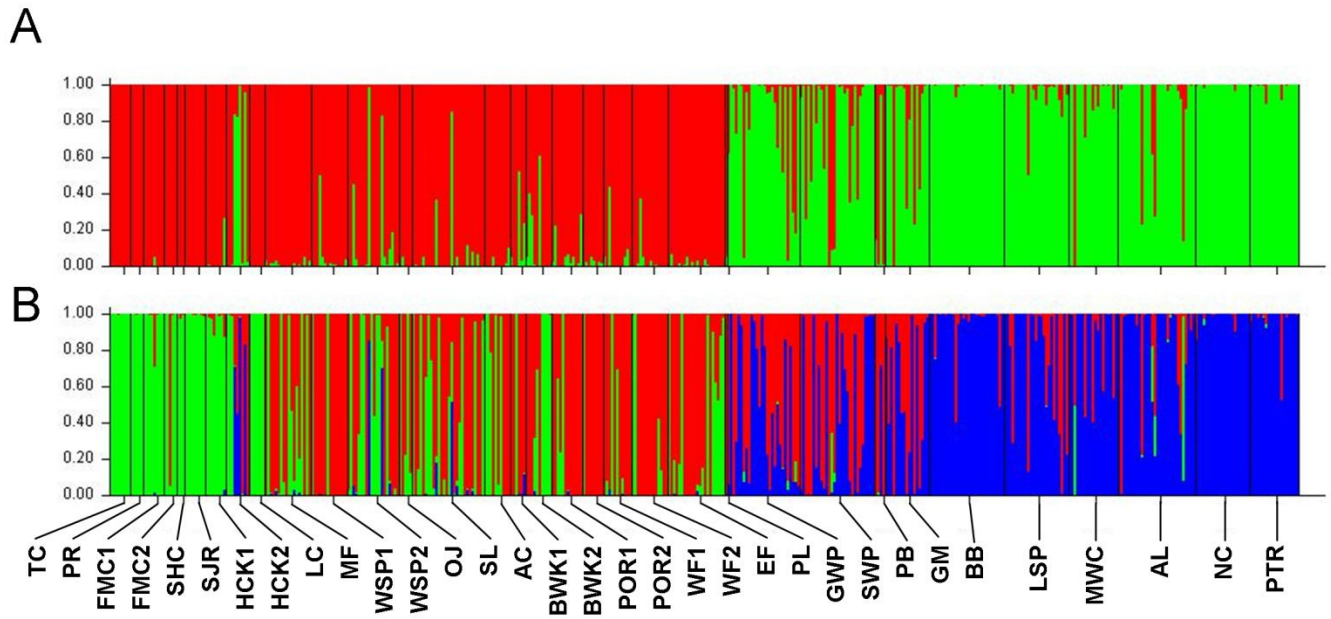

Figure 2.5: STRUCTURE (Pritchard et al. 2000) results partitioning *Vallisneria americana* multilocus genotypes (MLGs) collected from 33 sites spanning the Potomac River into (**A**) two (K=2) and (**B**) three (K=3) Bayesian-modelled genetic clusters. STRUCTURAMA (Huelsenbeck and Andolfatto 2007) results indicate that partitioning the MLGs into K=3 genetic clusters is the most probable based on minimal deviations from both Hardy–Weinberg and linkage equilibrium.

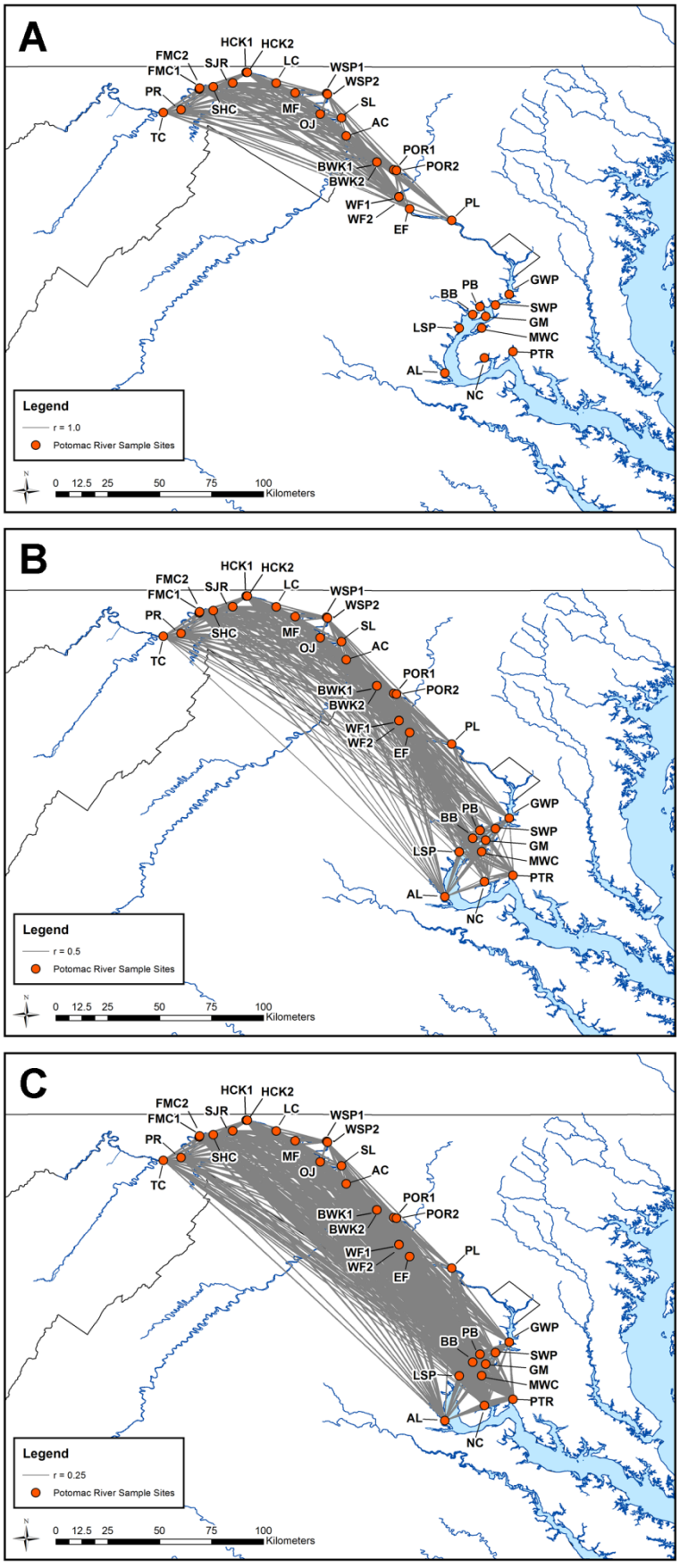

Figure 2.6: Spatially explicit individual-based networks of relatedness between *Vallisneria americana* samples collected from the Potomac River. Networks depict the distribution of shoots within and among sites that are related to one another at thresholds of (**A**) $r \geq 1.0$, (**B**) $r \geq 0.5$, and (**C**) $r \geq 0.25$, such that network nodes represent sampled shoots and edges represent connections between shoots at or above each threshold value. Pairwise relatedness coefficients between sampled shoots were calculated using the Wang (2002) estimator (implemented in COANCESTRY). Site names are defined in Table 2.1.

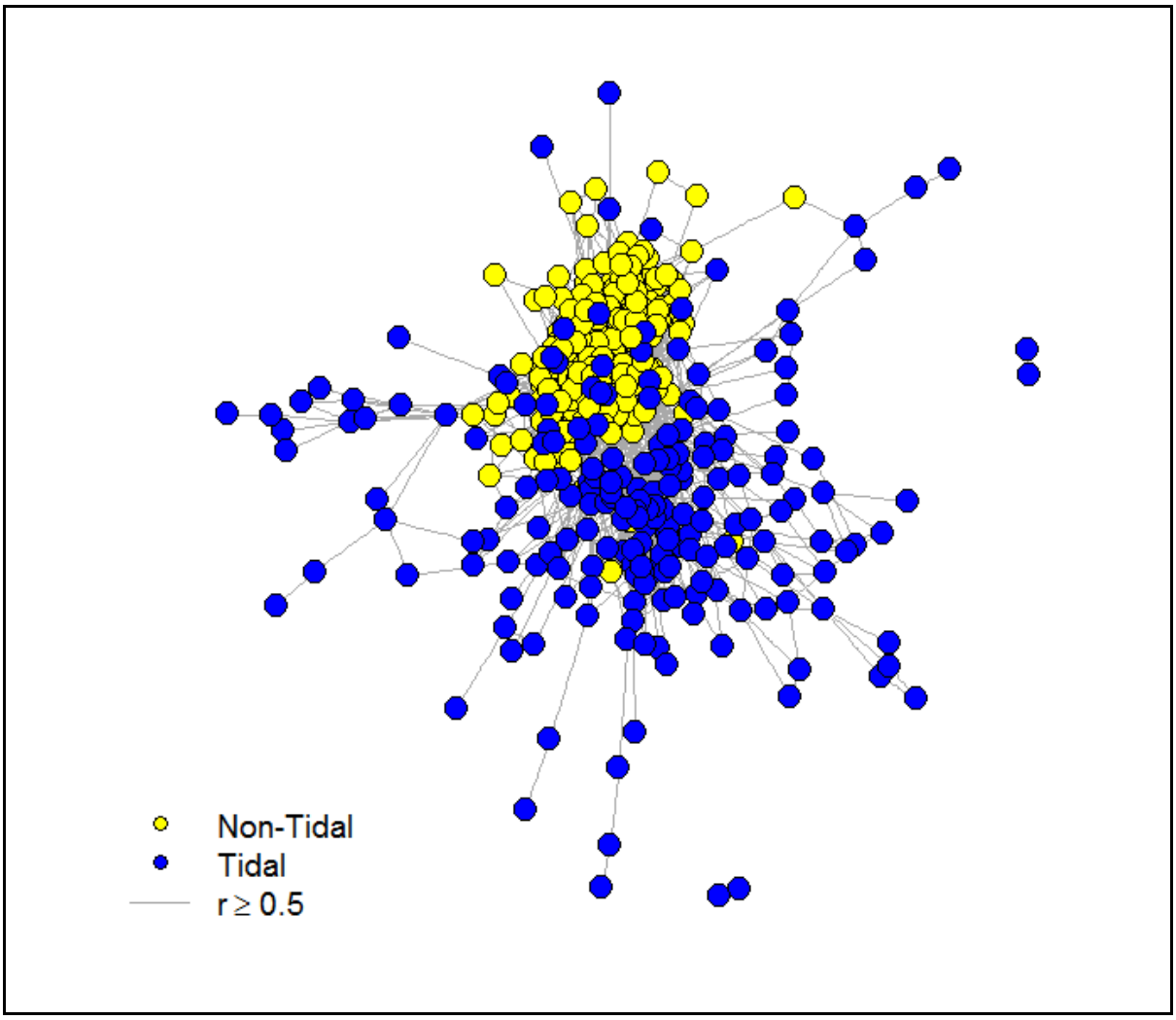

Figure 2.7: Spatially implicit individual-based network of relatedness between *Vallisneria americana* multilocus genotypes (MLGs) from the Potomac River. The network depicts the degree of relatedness between MLGs, such that the nodes represent MLGs and the edges represent MLGs related to one another at a level of $r \geq 0.5$. The edge length and distance between nodes is proportional to genetic distance (the inverse of r). MLGs collected from the tidal (blue) and non-tidal (yellow) regions of the Potomac River are color coded. Pairwise relatedness coefficients between MLGs were calculated using the Wang (2002) estimator (implemented in COANCESTRY). The network was created using the *igraph* package (Csardi and Nepusz 2006) in R v3.0.1 (R Core Team 2013).

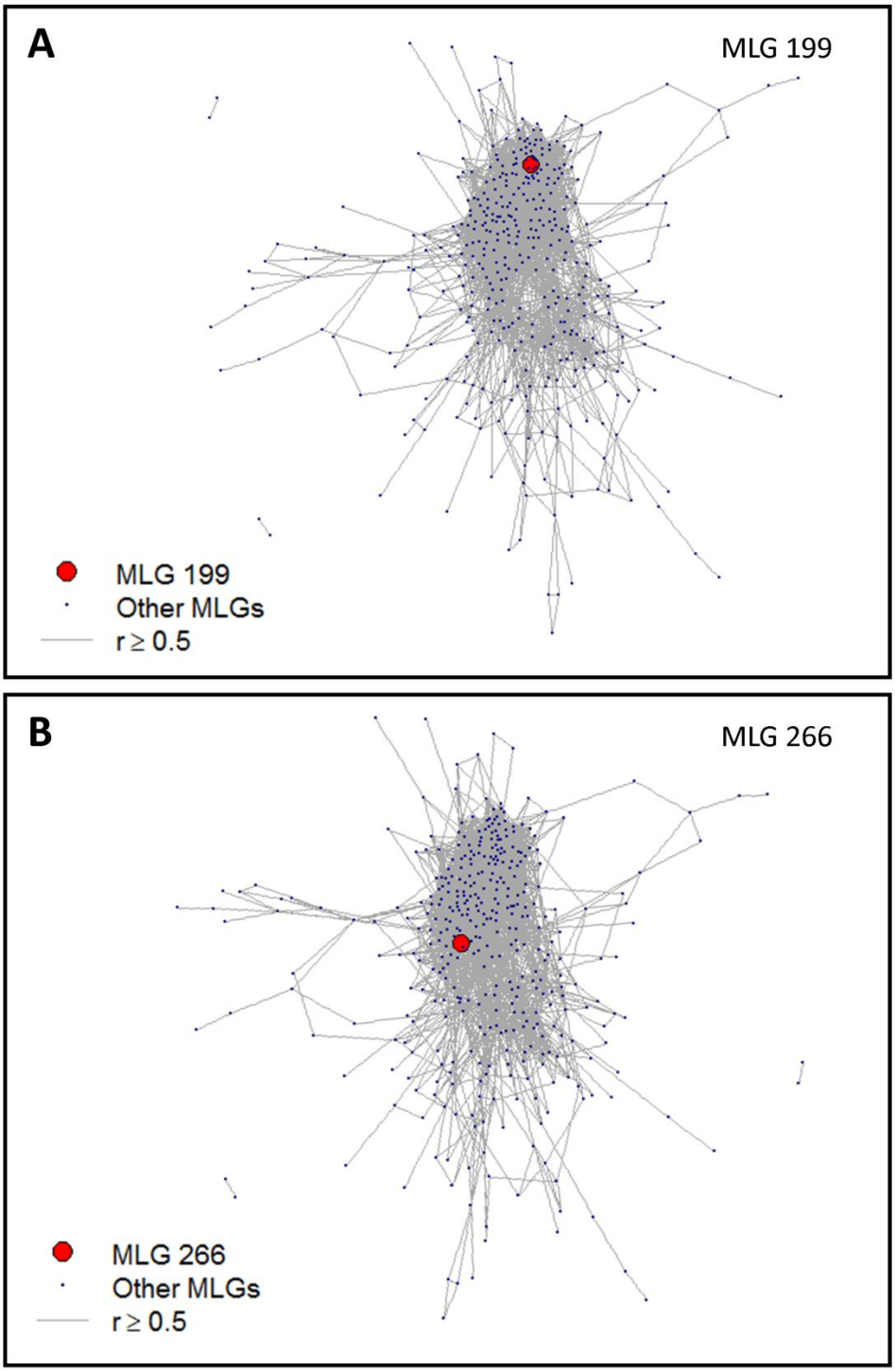

Figure 2.8: Spatially implicit individual-based networks of relatedness between *Vallisneria americana* multilocus genotypes (MLGs) from the Potomac River highlighting the location of the two most extensive MLGs, (**A**) MLG 199 and (**B**) MLG 266. Networks depict the degree of relatedness between MLGs, such that the nodes represent MLGs and the edges represent MLGs related to one another at a level of $r \geq 0.5$. The edge length and distance between nodes is proportional to genetic distance (the inverse of r). Pairwise relatedness coefficients between MLGs were calculated using the Wang (2002) estimator (implemented in COANCESTRY). The network was created using the *igraph* package (Csardi and Nepusz 2006) in R v3.0.1 (R Core Team 2013).

Chapter 3: Phenotypic responses of *Vallisneria americana* to temperature and photoperiod: Evaluating evidence of local adaptation and potential for acclimation

Abstract

Although local adaptation can be an important mechanism for maintaining genetic variation in a species, in the face of climate change local adaptation may limit the ability of populations to respond to emerging environmental conditions. Moreover, local adaptation may limit the success of restrict restoration and climate change mitigation strategies that translocate individuals or populations to regions where future conditions are projected to be more suitable. Common garden experiments were used to evaluate the local adaptation or acclimation potential of *Vallisneria americana* Michx. (Hydrocharitaceae), an ecologically important submersed aquatic plant, to different temperature and photoperiod conditions. Three temperature and photoperiod treatments simulated local conditions for *V. americana* collected from the Potomac River (MD; n=128), the Hudson River (NY; n=128), and the Kennebec River (ME; n=120). A fourth treatment simulated a future global warming scenario for the Potomac River. Morphological and life history traits of *V. americana* grown in each of these conditions indicated evidence of local adaptation only in plants from the Potomac River. Few overall differences in morphological and life history traits were observed between local and foreign sourced *V. americana*,

providing evidence of acclimation via phenotypic variation. However, *V. americana* grown under global warming conditions frequently had reduced growth and reproductive performance and we estimate that an optimal water temperature range for *V. americana* from all three regions is between 28-32°C. Overall, limited evidence of local adaptation and high acclimation to different conditions suggest that populations of *V. americana* in these rivers have high potential for resilience in the face of climate change, so long as temperatures do not exceed thermal tolerances, and may suffer few short-term negative consequences from climate change mitigation strategies that involve transplanting *V. americana*. However, long-term consequences of such restoration strategies remain unstudied.

Introduction

Mounting evidence regarding changing climate and increased climate variability highlight the importance of maintaining or restoring the resilience of natural populations and communities to ensure their future persistence. Climate projections reported by the Intergovernmental Panel on Climate Change (IPCC 2014) predict an increase from the current global mean surface temperature between 0.3°C to 0.7°C by 2035, with temperature increases likely to exceed 2°C by 2100. Recent analysis of global surface temperature by the World Meteorological Organization (2014) concluded that thirteen of the fourteen warmest years on records dating back to 1850 have all occurred in the twenty-first century. Each of the past three decades has been warmer than the last, culminating with 2001–2010 as the warmest decade on record. Likewise, sea level has risen globally by 19 cm since the start of the twentieth century, mostly because of the thermal expansion of the oceans and the melting of

glaciers and ice caps (World Meteorological Organization 2014). The current rate of global mean sea level rise is about 2.0 mm/year, but some projections estimate it will increase to a rate of 8-16 mm/year by 2100 (IPCC 2014). Extreme weather events are also expected become more intense and frequent (IPCC 2014, World Meteorological Organization 2014). As a result, ecosystems around the globe will face novel disturbance regimes that are different from historical conditions (Carpenter et al. 2011, Scheffer et al. 2012).

Given the documented effects of anthropogenic disturbances on natural ecosystems and accumulating evidence of perturbation from climate change, it is imperative to understand the factors that affect a population's resiliency through time, especially when considering management action (Pautasso et al. 2010). Resilience refers both to the ability of populations to persist in their current state and the ability to undergo evolutionary adaptation in response to changing environmental conditions (Sgrò et al. 2011). In other words, resilient populations have one or more of the following characteristics:1) phenotypically plastic individuals that can acclimate to changing environmental conditions, 2) heritable phenotypic and genetic variation across individuals that enables adaptation through natural selection, or 3) the ability to migrate through dispersal mechanisms to more suitable locations. In cases where there is insufficient phenotypic variation for acclimation, limited genetic variation for adaptation, and limited dispersal capability, extirpation is probable. The rapid environmental changes associated with climate change may make extirpation more likely for many taxa (McLachlan et al. 2007).

Rapid climate changes are affecting ecosystems and organisms, including the phenology, physiology, distribution, and ecosystem interactions of plant species (Walther et al. 2002, Root et al. 2003, Parmesan 2006, Parolo and Rossi 2008, Pautasso et al. 2010). Unlike terrestrial and intertidal fauna that counter the effects of warming climate by poleward expansion (Harley et al. 2006, Helmuth et al. 2006), flora are particularly sensitive to climate changes because of their limited ability to disperse to more suitable conditions (Abeli et al. 2012). Some terrestrial plant species have responded to warming through accelerated phenology, enhanced growth, and increased reproductive effort (Dormann and Woodin 2002). However, declines in reproductive success have also been observed as a response to thermal stress in plants when temperatures exceed the optimal range for metabolic and physiologic processes (Parsons 1990).

As conservationists continue to work to find approaches to manage biodiversity in the face of climate change, strategies like managed relocation (MR) are emerging (Richardson et al. 2009, Sgrò et al. 2011). These strategies involve the intentional movement of populations or appropriately adapted genotypes from currently occupied areas to locations where probability of future persistence is predicted to be higher (Richardson et al. 2009). Such translocations may be ineffective if individuals are poorly adapted to the new site and are not able to initially survive and establish populations. Additionally, long-term negative consequences may manifest if offspring from matings between local and foreign individuals have low fitness due to outbreeding depression (Montalvo and Ellstrand 2001). Greater understanding of the feasibility of strategies like MR and the risks

associated with them is critical. Specifically, understanding (1) the degree to which individuals within populations are locally adapted to their environment and (2) the potential for individuals to acclimate to new environments are necessary to evaluate both the current resiliency of populations in their current context given future climate change as well as the potential effectiveness of climate change mitigation strategies like MR.

Coastal aquatic ecosystems are already among the most threatened in the world due to the prevalence of stressors related to chemical and organic pollution, hydromorphological changes from land development, and invasive species (Branch 1999, Kennish 2002). The IPCC (2014) synthesis report notes that a large fraction of freshwater and marine species face additional extinction risk due to the current and future impacts of climate change. Nearshore aquatic communities will be greatly affected by changes in surface temperature (Kennish 2002) as many marine species exist at, or close to, their thermal tolerance limits (Helmuth et al. 2002). Coastal ecosystems will also be subject to additional effects of climate change, like rises in sea level and decreases in seawater pH from ocean acidification caused by increased levels of atmospheric carbon dioxide (Harley et al. 2006, Brierley and Kingsford 2009).

Submersed aquatic vegetation (SAV) is an important component of aquatic ecosystems that is already being negatively impacted by warmer water temperatures (Oviatt 2004), salt water intrusion from sea-level rise (Quammen and Onue 1993, French and Moore 2003), and large-scale disturbances (Kemp et al. 1983, Orth and Moore 1983, 1984, Fernald et al. 2012). Marine and freshwater rooted angiosperms

are keystone species in their communities. They provide critical ecosystem services, including improvement in water quality through sediment and nutrient sequestration (Brix and Schierup 1989, Takamura et al. 2003, Moore 2004, Gu 2008), physical sediment stabilization (Sand-Jensen 1998, Madsen et al. 2001), and erosion reduction (Fonseca and Cahalan 1992). SAV also promote healthy and diverse benthic communities (Orth et al. 2006) by providing shelter and nursery habitat to nearshore communities (Killgore et al. 1989, Orth et al. 2006) and acting as a primary source of food for waterfowl, fish, and invertebrates (Perry and Deller 1996, Strayer and Malcom 2007).

The function of SAV has been compromised by losses that have occurred worldwide over relatively short time periods and with increasing frequency (Walker and McComb 1992, Short and Wyllie-Echeverria 1996, Orth et al. 2006, Waycott et al. 2009). A recent review found that SAV area around the world is declining at a mean rate of 1.5% per year (Waycott et al. 2009). In response to these global declines, SAV monitoring and local restoration projects have been proposed and implemented worldwide (Orth et al. 2006). Restoration efforts have primarily operated at small, local scales (<10 ha), with only a few efforts being targeted at larger areas (>100 ha; Broadhurst et al. 2008). Many of these restoration efforts have had mixed or marginal success (Schenk and Rybicki 2006, Campanella et al. 2010g), which has stimulated the exploration of potential MR strategies that move plants across latitudinal regions to facilitate a populations' ability to respond to emerging environmental stressors (e.g., Campanella et al. 2010a, Campanella et al. 2010g).

The objective of this study was to quantify evidence of local adaptation and acclimation potential in the SAV species *Vallisneria americana* Michx. (Hydrocharitaceae) collected from three major rivers spanning a broad latitudinal gradient – the Potomac River in Maryland, the Hudson River in New York, and the Kennebec River in Maine. *V. americana* is a perennial, dioecious, freshwater angiosperm, capable of reproducing both sexually and vegetatively (Wilder 1974, Lloyd et al. 2011), and is broadly distributed along the eastern United States (McFarland and Shafer 2008). Understanding of the degree of regional *V. americana* local adaptation and acclimation potential will inform management decisions and restoration strategies intended to ensure long-term persistence and resilience of *V. americana* populations.

Studying Local Adaptation

Local adaptation of genotypes results in maximized growth and reproduction under local environmental conditions (Wright 1931). However, environments are variable and even local environments are not always optimal, resulting in fitness consequences for individuals due to some level of stress. Therefore, quantifying evidence of local adaptation by measuring maximized growth and reproduction of individuals is difficult because it requires some idea of the optimal habitat for an organism. Rather, by measuring the responses of organisms to different environments we can determine their relative fitness (Fischer et al. 2000).

Kawecki and Ebert (2004) identify two conditions that need to be met to demonstrate local adaptation in demes (local populations of one species that actively interbreed with one another and share a distinct gene pool) in experimental settings.

First, individuals from sites matching the experimental conditions need to have higher relative fitness than individuals from other conditions (the local vs. foreign criterion). Second, the relative fitness of individuals needs to be higher in their home condition than in alternative conditions (the home vs. away criterion). The local vs. foreign criterion provides evidence of divergent natural selection, the driving force of local adaptation. The home vs. away criterion is necessary to ensure that the differences in performance are not due solely to differences in habitat quality. For example, individuals from high quality home environment would have higher fitness in local than foreign conditions, but individuals from the poor-quality habitat could increase in relative fitness after transplant to high-quality habitat. Therefore, satisfying both the local vs. foreign and the home vs. away criteria provide the most robust evidence of local adaptation. On the other hand, the home vs. away criterion provides evidence of potential for acclimation to varying conditions if there are no differences between growth and reproductive patterns in home vs. away environments.

To distinguish local adaptation from other forms of deme × environment interactions, Kawecki and Ebert (2004) argue more than two environmental conditions need to be tested and replicate demes within each condition are necessary. If *a priori* knowledge generates a hypothesis regarding which environmental factors are relevant for divergent selection that might drive local adaptation, one would sample from several replicate demes from within each condition, and grow the samples from each deme in each habitat type (Kawecki and Ebert 2004). Because we hypothesize that temperature and photoperiod are driving forces in the local adaption of *V. americana* to different latitudes, samples for controlled growth chamber

experiments were selected from multiple sites within each of the three rivers and individuals from each site were grown in conditions reflecting each river.

Using these reciprocal common garden growth chamber experiments with photoperiod and temperature we evaluated (1) whether or not *V. americana* is locally adapted to regional climatic conditions within rivers and (2) whether or not regionally collected *V. americana* were able to acclimate to the conditions of another region or to conditions associated with global warming. We predicted that populations would be locally adapted and more productive in their local temperature and photoperiod conditions than foreign populations. Likewise, we predicted that populations would show limited signs of acclimation potential and be more productive in their home temperature and photoperiod conditions than in foreign temperature and photoperiod conditions.

Methods

Collection Locations and Greenhouse Propagation

In summer 2011, *V. americana* samples were collected from three major rivers in the Northeastern United States – the Potomac River, MD, the Hudson River, NY, and the Kennebec River, ME (Figure 3.1). We harvested up to 30 *V. americana* shoots with attached roots from each of four sites within each river. Because *V. americana* reproduces both sexually and vegetatively (Wilder 1974), populations may be composed of one clone or many different clones (Lloyd et al. 2011). To increase the sampling of different genotypes within demes we sampled every 5–10 meters along transects parallel with the river, following the protocol of Lloyd et al. (2011). Sampling distances were kept as consistent as possible given the natural variation in

densities within and among sites. Shoots and attached roots were immediately wrapped in wet paper towels and placed on ice within one hour of field collection. They were transported to the University of Maryland College Park, where each shoot was transferred into individual containers (diameter: 18 cm; height: 20 cm) filled 15 cm deep with steam-sterilized (Slack Associates, Inc. Model 1964) Chesapeake Bay sediment and capped with about 2 cm of washed and screened sand. Chesapeake Bay sediment harvested from Rocky Point, MD was selected because previous greenhouse experiments demonstrated that *V. americana* collected from multiple regions around the Chesapeake Bay grew well in this sediment (Engelhardt et al. 2014b). Steam sterilization killed any seed or turion propagules that could have been left in the sediments. Local microorganisms were reintroduced to propagation containers by planting the *V. americana* directly from the field.

To minimize environmental carryover from the field and reduce potential non-genetic effects from differences in handling, plasticity and maternal effects (Kawecki and Ebert 2004, Hughes and Stachowicz 2009), samples were maintained under common environmental conditions in the University of Maryland, College Park greenhouse for two to three seasons before use in experiments. Conditions in the greenhouse were kept as natural as possible, with no additional light or temperature control beyond normal seasonal variation. Because *V. americana* is diecious and plants were propagated in separate containers, male and female plants were not capable of cross pollinating during propagation.

Photoperiod and Temperature Experiment

Of the many environmental factors we could have manipulated to quantity evidence of local adaption (e.g. salinity, sediment, light intensity), we focused on temperature and photoperiod for two reasons. First, we hypothesize that temperature and photoperiod conditions are agents of divergent selection in *V. americana* across latitudinal regions. Second, we wanted to evaluate the potential for large-scale transplant of individuals across latitude. There is accumulating evidence that differences in temperate and photoperiod not only influence the distribution of species, but also restrict the ability of species to respond to rapid climate change (e.g., Harley et al. 2006, Helmuth et al. 2006, Cleland et al. 2007, Bradshaw and Holzapfel 2008, Abeli et al. 2012). Therefore, we grew *V. americana* in four growth chamber treatments, representing temperature and photoperiod profiles for the Potomac, Hudson, and Kennebec Rivers as well as a global warming profile for the Potomac River (Table 3.1).

During the first twelve weeks of the experiment, the temperature and photoperiod conditions within the four growth chambers were selected to simulate the three river regions during peak growing season and one global warming treatment (Table 3.1). Daytime temperatures were selected as the mean growing season temperature over the last 15-20 years for each location (USGS 2013). Temperature for the global warming treatment was always 4°C above the Potomac River (T1) temperature. This was selected based on modeled temperature predictions for mid-Atlantic estuaries at the end of the century under the A2 medium-high carbon dioxide emission scenario (Najjar et al. 2000, Najjar et al. 2009). Nighttime temperatures

were always set 2°C below daytime temperatures. Photoperiods also simulated regional conditions (data collected from U.S. Naval Observatory) and were changed weekly, steadily increasing to the photoperiod associated with the summer solstice of each location. To induce plant senescence, we began to decrease the photoperiod weekly after 12 weeks, moving toward the winter solstice of each location (Table 3.1). Temperatures were also decreased by 1°C every week until they reached either the thermal limit associated with their region or the lower limit of the growth chambers (8°C; Table 3.1). All other variables, including light intensity, humidity, and water regime were kept constant within each of the four growth chambers.

Each growth chamber could accommodate up to 48 *V. americana* containers, allowing replication of each plant source (Potomac River, Hudson River, Kennebec River) within each of the four climate treatments. In total, we planted 94 turions in each climate treatment across two trials – 8 from each of the four sites within the Potomac and Hudson Rivers (n=32 per river) and 6 or 8 from each of the four sites within the Kennebec River (n=30; Table 3.2; Figure 3.1). The first trial began in June 2013 and the second trial began in March 2014. The four treatments were randomly assigned to four growth chambers for each experimental trial. Turions for each trial were harvested from greenhouse cultures in January 2013 and 2014, respectively, and stored in 4°C water in the dark until subsequent planting. Turions were planted in the middle of individual containers (diameter: 18 cm; height: 20 cm) with 15 cm of steam-sterilized sediment capped with 2 cm of washed and screened sand. We measured turion length and width to account for initial turion area (length x width) in statistical tests. To account for any potential effects of microclimate within a growth

chamber, containers were randomly placed within growth chambers and were re-randomized every two weeks for the duration of the experiment. Water was added to each container every week to replace water lost through evaporation and any algae that accumulated were hand-scrapped or flushed from the container. After two weeks of initial establishment, stone aerators were added to each container to ensure continual mixing of water and to prevent algal mats from forming over the surface of the containers between flushing events.

Morphological and life history traits were measured for each planted *V. americana*. Morphological traits included the total number of ramets, total number of leaves, and longest leaf length and width. Morphological measurements were taken every week for the first six weeks, and then every two weeks until week 20, at which time *V. americana* within buckets began to die back. Life history traits included timing and frequency of male and female flower emergence, from which we calculated the length of flowering for each plant. After plant senescence in each trial in December 2013 and 2014, respectively, we harvested turions and measured their total abundance and wet weight biomass.

Genetic Variables

During the implementation of the experiment we genotyped *V. americana* leaf clippings from the greenhouse cultures using 10 microsatellite loci designed specifically for this species (Burnett et al. 2009), and assigned each *V. americana* sample to unique multilocus genotypes (MLGs; Marsden CH1, CH2). A total of 59 MLGs were used in the experiment, including 13 from the four Potomac River sites, 25 from the four Hudson River sites, and 21 from the four Kennebec River sites. Due

to limitations in availability, some MLGS were replicated within and across treatments, but many were not (Table 3.3). Thus, we could not analyze local adaptation and phenotypic plasticity of individual MLGs. Because variation in degree of genetic relatedness among individuals can be a source of uncontrolled variation, we calculated pairwise relatedness estimates among MLGs so that we could account for the effect of relatedness on the observed variation in morphological and life history traits measured in this experiment. We calculated the Wang (2002) estimator of pairwise relatedness among MLGs from each river using region-specific allele frequencies in the program COANCESTRY v1.0 (Wang 2011). We chose Wang's estimator because previous Monte-Carlo simulations (Marsden et al. 2013) indicated it had the lowest variance and minimal bias across various relationship categories (Van de Casteele et al. 2001). Mean pairwise relatedness was included as a continuous predictor variable in statistical analysis.

We also calculated the proportion of heterozygous loci for each MLG used in this experiment. Individual levels of heterozygosity can affect population performance and ecosystem function (Dudash 1990, Fenster and Dudash 1994). Reductions in heterozygosity, due to non-random mating, can influence probabilities of survival (Ellstrand and Elam 1993). Individual heterozygosity was also used as a continuous predictor variable in statistical analysis.

Data Analysis

All statistical analyses were carried out in R v3.0.1 (R Core Team 2013). To test for evidence of local adaptation we first compared the performance (number of leaves, ramets, turions and flowers, turion weight, leaf length, length of flowering,

and first flowering day) of 'local' plants to 'foreign' plants. This analysis assessed differences in performance (dependent variables) among the three river sources (independent variable) within each of the four climate treatments. Second, to determine if plants were able to acclimate to new conditions we compared the performance of plants in 'home' conditions relative to 'away' conditions. This analysis assessed differences in performance (dependent variables) among the four climate treatments within each of the three river sources.

None of the dependent variables we measured met the conditions of normality and homogeneity of variance, even after transformation. We therefore used generalized linear models (GLMs) for added flexibility through a link function for our analyses (Crawley 2012). GLMs with a Poisson distribution were used to analyze count data including maximum number of ramets, maximum number of leaves, total number of flowers produced, total flowering days, first flowering day, and total number of turions produced. GLMs with a gamma distribution were used to analyze two continuous dependent variables, including maximum leaf length and total turion biomass. GLMs for leaf length and turion biomass excluded data from plants that never grew and GLMs for flowering days also excluded data from plants that never flowered. Count data often showed signs of overdispersion. We compensated for overdispersion by fitting models using quasi-Poisson rather than Poisson error distributions (Crawley 2012). To test for the effects of river source or climate treatment on each dependent performance variable, we first created maximal models that included all of the following continuous predictor variables and factors: initial turion area (length × width), mean relatedness of each MLG, individual

heterozygosity, blocking by growth chamber, and the interaction of each of these continuous predictor variables with river source or climate treatment. Following the model simplification protocols outlined by Crawley (2012), we found the most parsimonious model for each of the measured dependent variables. The most parsimonious model only included the significant continuous predictors and interactions. During model simplification, the goodness of fit between successively simplified GLMs was compared using analysis of deviance with F-tests (Hastie and Pregibon 1992, Crawley 2012). Significant differences among main effects were subsequently evaluated with post-hoc Tukey-Kramer tests in R using the *multcomp* package (Hothorn et al. 2008).

To test differences in growth and life history traits through time, as opposed to simply testing differences between maximum performance measures, we used non-linear mixed effects models (NLMEs) to include repeated measures in the models. We used non-linear models for this analysis because the rate of change among weeks was variable, and often logistic in shape, as plants in individual containers reached their maximum growth. We built NLME models to test for differences in the number of ramets, total number of leaves, total number of flowers, and longest leaf length among 'local' and 'foreign' sourced plants. Time and river source were treated as fixed effects and repeated measures of plant performance was treated as a random effect. NLME models were also used to test for differences among plants in 'home' versus 'away' conditions, where time and climate treatment were fixed effects and repeated measures of plant performance was a random effect. We used the *nlme* package in R (Pinheiro et al. 2015) to run these models. Because time was always a

significant effect in these NLMEs (i.e., measured plant traits increased through time), interactions between river source or climate treatment and time were almost always significant. Therefore, significant differences among river sources or climate treatments were subsequently evaluated with post-hoc interaction analysis using the *phia* package in R (De Rosario-Martinez 2015).

Results

Overall, some morphological and life history traits differed by river source (Figure 3.2; Table 3.4). However, there were more differences in traits among *V. americana* from the same river but grown in different temperature and photoperiod conditions (Figure 3.2; Table 3.5). The same pattern was observed when differences in growth traits were assessed through time (Figure 3.4; Table 3.6).

Local versus Foreign Plants

In Potomac River conditions (T1), *V. americana* from the Potomac River produced more ramets, leaves, flowers, and turions than *V. americana* from the Kennebec River (Figure 3.2; Table 3.4; 3.6). *V. americana* from the Potomac River also produced more turions than *V. americana* from the Hudson River (Figure 3.2; Table 3.6). The area of the initial planted turion was the most common significant continuous predictor variable used in these GLMs, accounting for substantial variance in maximum leaf length, total number of flowers produced, and the number of days until the first flowering event (Table 3.4). Initial turion area and river source interacted to affect ramet production, maximum number of leaves, and total number

of turions produced (Table 3.4). The only other significant continuous predictor variable used in these GLMs was individual heterozygosity (Table 3.4).

Maximum leaf length was the only variable that differed by river source in the Hudson River conditions (T2), Kennebec River conditions (T3), and warm Potomac River conditions (T4; Table 3.4). Plants from the Hudson River had longer leaves than plants from the Kennebec River when grown in Hudson River conditions (Figure 3.2, Table 3.6). However, when grown in Kennebec River conditions, Hudson *V. americana* had longer leaves than both the foreign Potomac and local Kennebec *V. americana* (Figure 3.2, Table 3.6). Plants from the Kennebec were shorter than plants from either the Potomac or Hudson in the warm Potomac River conditions (Figure 3.2; Table 3.6). Common continuous predictor variables retained in GLMs for these three treatments included initial turion area, individual heterozygosity, and, occasionally, relatedness. The growth chamber blocking predictor factor was also retained in a few of the GLMs (Table 3.4).

Time was significant in most NLMEs for all measured variables. Within the Potomac River conditions, all measured traits differed by plant source (Table 3.7). Plants from the Potomac and Hudson Rivers produced more ramets, more leaves, and longer leaves when grown in Potomac River conditions than plants from the Kennebec River (Figure 3.3, Table 3.8). Potomac River *V. americana* produced more flowers than Hudson and Kennebec plants (Figure 3.3, Table 3.8). Leaf length was the only variable that differed by plant source in the Hudson, Kennebec, and warm Potomac conditions (Table 3.7). Hudson River *V. americana* produced longer leaves than Potomac River *V. americana* when grown in Hudson conditions (Figure 3.3;

Table 3.8). Hudson River *V. americana* also produced longer leaves than Potomac or Kennebec *V. americana* when grown in Kennebec conditions (Figure 3.3; Table 3.8).

Home versus Away Climate Conditions

For *V. americana* from the Potomac River, there were differences among climate treatments such that Potomac River plants produced more ramets, leaves, turions, and turion biomass when grown in Potomac River conditions than when grown in climate conditions from other rivers or in the warming condition (Figure 3.2; Table 3.5; 3.6). Common continuous predictor variables included in these GLMs were initial turion area, the interaction of initial turion area with treatment, growth chamber blocking effects, and individual heterozygosity (Table 3.5).

For *V. americana* from the Hudson River, there were differences among climate treatments such that Hudson plants grown in Hudson River conditions produced more leaves than when grown in Potomac River conditions and more turion biomass than when grown in Kennebec River conditions (Figure 3.2; Table 3.5; 3.6). Likewise, *V. americana* from the Hudson produced more ramets and leaves when grown in Hudson River conditions than warm Potomac River conditions. However, turion biomass was greater in plants from the Hudson River when grown in Potomac River conditions compared to Hudson River conditions (Figure 3.2; Table 3.6). Common continuous predictor variables retained in these GLMs were relatedness, growth chamber blocking effects, the interaction of growth chamber blocking effects with climate treatment, and initial turion area (Table 3.5).

For *V. americana* from the Kennebec River, there were differences among climate treatments such that *V. americana* from the Kennebec only produced more

ramets and leaves when grown in Kennebec River conditions than when grown in warm Potomac River conditions (Figure 3.2; Table 3.5; 3.6). In fact, plants from the Kennebec produced fewer leaves and less turion biomass when grown in Kennebec River conditions than when grown in Hudson River conditions, and they produced less turion biomass when grown in Kennebec River conditions than when grown in Potomac River conditions (Figure 3.2; Table 3.6). Continuous predictor variables retained in some of these GLMs included initial turion area, growth chamber blocking effects, relatedness, individual heterozygosity, and the interaction of initial turion area with treatment (Table 3.5).

Time was significant for all measured variables in NLMEs (Table 3.7). *V. americana* from the Potomac River differed across treatments in the number of ramets, number of leaves, and longest leaf length (Table 3.7). Potomac plants grown in Potomac River conditions outperformed plants grown in other treatments for number of ramets, leaves and longest leaf length over time (Figure 3.3, Table 3.8). Hudson River *V. americana* grown in Hudson River conditions outperformed plants grown in warm Potomac River conditions for longest leaf length through time (Figure 3.3, Table 3.8). Likewise, Kennebec River *V. americana* grown in Kennebec River conditions outperformed plants grown in warm Potomac River conditions for total number of ramets (Figure 3.3, Table 3.8).

Relatedness and Individual Heterozygosity

The pairwise relatedness among *V. americana* individuals within rivers differed across rivers (ANOVA; $F_{2,238} = 115.9$; $p < 0.001$). The mean relatedness of *V. americana* from the Potomac, Hudson, and Kennebec Rivers was 0.23 ($sd = 0.05$),

-0.04 (*sd* = 0.15), and -0.09 (*sd* = 0.20), respectively. *V. americana* from the Potomac River had higher relatedness than *V. americana* sourced from either the Hudson (Tukey HSD; $p < 0.001$) or Kennebec (Tukey HSD; $p < 0.001$) Rivers. Relatedness accounted for a significant portion of the variation in seven of the 56 morphological and life history trait GLMs (Table 3.4; 3.5). In all cases, there was a positive association between the relatedness and the morphological or life history trait. Individual heterozygosity of *V. americana* was also different across rivers (ANOVA; $F_{2,238} = 7.783$; $p < 0.001$). The mean individual heterozygosity of *V. americana* from the Potomac, Hudson, and Kennebec Rivers was 0.56 (*sd* = 0.16), 0.52 (*sd* = 0.12), and 0.47 (*sd* = 0.18), respectively. *V. americana* from the Potomac River had higher individual heterozygosity than *V. americana* from the Kennebec River (Tukey HSD; $p < 0.001$). Individual heterozygosity accounted for a significant portion of the variation in 12 of the 56 morphological and life history trait GLMs (Table 3.4; 3.5). In all cases, there was a positive association between observed heterozygosity and the morphological or life history trait.

Discussion

Local adaptation can be an important mechanism for maintaining genetic variation in species across populations. However, in the face of climate change, local adaptation of *V. americiana* to regional temperature and photoperiod conditions may limit a population's ability to respond to warming trends or affect the success of restorations that involve translocation of individuals or populations to regions where future temperature profiles are projected to be suitable. Overall, the common environment experiments provided little evidence of local adaption in *V. americana*

to temperature and photoperiod. Only Potomac River *V. americana* outperformed foreign plants in morphological and life history traits when grown in their local climate conditions (Figure 3.3; 3.2, Table 3.7; 3.8). On the other hand, *V. americana* collected from the Hudson or Kennebec Rivers often grew no differently in different climate conditions, providing some evidence of acclimation (Figure 3.3; 3.2, Table 3.7; 3.8). When there were differences in performance across climate treatments, *V. americana* collected from different rivers were often more productive in Potomac River conditions than their home conditions, but *V. americana* grown under the Potomac River warming conditions were frequently less productive (Figure 3.3; 3.2, Table 3.7; 3.8). Therefore, there may also be intrinsic differences in the overall growth and reproductive potential of *V. americana* to different temperatures and/or photoperiods.

Evidence of Local Adaption and Acclimation

Local adaptation is defined as the fine-tuning of populations to their local environment via natural selection (Sanford and Kelly 2011), and has been recognized as an important mechanism for maintaining genetic variation within species and across populations (Felsenstein 1976, Hedrick 1986, Kawecki and Ebert 2004). Local adaptation is promoted by low gene flow, strong directional selection coupled with moderate stabilizing selection, differences among habitats, and limited phenotypic plasticity (Kawecki and Ebert 2004). Persistent environmental gradients in heterogeneous environments may impose directional selection on a fitness advantage leading to evolution of differences in morphology, physiology, behavior, or life history traits that are most suited to local conditions.

Unfortunately, local adaptation in SAV is poorly studied. This is a major problem because many SAV populations are small, isolated, and facing rapidly changing environments to which they need to adapt. We expected temperature and photoperiod to greatly influence the overall fitness of *V. americana* by influencing the ultimate allocation of resources into either sexual (production of pollen and flowers) or asexual (production of turions) reproduction. We therefore expected *V. americana* collected from three different latitudinal regions to show signs of local adaption to temperature and photoperiod conditions. However, only plants sourced from the Potomac River showed evidence of local adaptation. The lack of significant differences in many of the local vs. foreign comparisons of plants sourced from the Hudson and Kennebec Rivers provide evidence of acclimation potential in *V. americana*. Moreover, the variation in morphological and life history trait responses of *V. americana* within each climate treatment demonstrates substantial phenotypic variation that may contribute to acclimation.

The results from this study conflict with emerging research on local adaptation in aquatic and marine environments. Local adaptation in aquatic and marine environments has been historically regarded as a rare phenomenon, restricted to a few species with low dispersal potential (Sanford and Kelly 2011). This expectation arose from the lack of apparent dispersal barriers in marine systems and the fact that many marine invertebrates and fish have planktonic larvae (Grosberg and Cunningham 2001). However, a growing body of research now suggests that many marine populations are less connected (reviewed by Palumbi 2004, Levin 2006) and exposed to more complex mosaics of abiotic and biotic conditions than previously

thought (Sanford and Kelly 2011). If this were the case, we would have expected to find greater evidence of local adaptation in *V. americana* because dispersal is limited across rivers and each river is characterized by difference local abiotic and biotic factors.

The downside of controlled environment experiments as opposed to *in situ* reciprocal transplants is that an experiment designed to mimic a specific environmental difference may neglect a key factor that is important for local adaptation (Kawecki and Ebert 2004). Populations of *V. americana* may not be locally adapted to temperature and photoperiod conditions, but rather may respond to more localized environmental variables (e.g. salinity, light, nutrients, sediment). It is possible that this experiment tested for local adaptation at the wrong scale for this species. Selective gradients in aquatic habitats can be very fine-grained, with strong differences in environmental conditions occurring over tens of meters (Sanford and Kelly 2011).

Another potential reason we did not detect stronger signals of local adaptation to temperature and photoperiod conditions in *V. americana* is because of the approach we used to measure plant performance. Because we were ultimately interested in the potential persistence and resiliency of *V. americana*, we wanted to assess the reproductive contribution of each plant to the next generation through either flower or turion production (e.g. life history traits). We also assessed performance in terms of morphological traits because in *V. americana*, plant biomass has been correlated with the onset of flowering (Titus and Hoover 1991). This experimental design assumes that such traits are monotonically related to fitness and are under directional selection.

However, fitness-related traits are often either under stabilizing selection or have trade-offs with other fitness components. Therefore, treating these morphological and life history traits as measures of performance may be misleading because intermediate trait values may be optimal for different conditions (Kawecki and Ebert 2004).

Effects of Genetic Diversity on Local Adaptation

The limited evidence of local adaption observed in our study suggests that *V. americana* populations are either connected across broad latitudinal gradients, have such reduced standing genetic variation that natural selection is restricted, or have such high levels phenotypic variation or individual plasticity that acclimation limits local adaption. If gene flow is high, the diversifying effects of selection can be counteracted by the homogenizing effects of gene flow, and local adaptation will tend to be counteracted (Sanford and Kelly 2011). Previous *V. americana* research shows population genetic sub-structuring at river and bay-wide scales (Lloyd et al. 2011; Marsden CH2). Therefore, prolonged gene flow across rivers located in three different latitudinal regions is highly unlikely, and ongoing gene flow is not limiting local adaption between these three rivers.

Local adaptation could also be constrained by reduced genetic variation (Antonovics 1976, Kawecki and Ebert 2004). Marsden (CH2) found that levels *V. americana* genetic diversity within the Potomac, Hudson, and Kennebec were relatively high and similar to other SAV species. Indeed, *V. americana* selected for analysis in this study had moderately high levels of individual heterozygosity. However, individuals selected from the Potomac River had high relatedness among

individual genets. Therefore, although individual heterozygosity was high in Potomac River individuals used in this experiment, overall levels of genetic diversity may actually be low because many of the individuals shared common alleles. The ability to adapt to new environments is often compromised in small populations because of reduced genetic diversity (Antonovics 1976, Stockwell et al. 2003, Pertoldi et al. 2007). Recent meta-analysis found that local adaptation was in fact very rare in small populations (e.g. <1000 flowering individuals; Leimu and Fischer 2008). There is also accumulating evidence that genetic diversity enables adaptation of individuals to local environments (Montalvo and Ellstrand 2000, Joshi et al. 2001, Montalvo and Ellstrand 2001, Hammerli and Reusch 2002, Hufford and Mazer 2003). Heritable genetic variation is the foundation of phenotypic variation, which controls the adaptive potential of populations (Eckert et al. 2008). Therefore, genetic diversity and its associated phenotypic variation is fundamental to long-term resilience because it enables adaptation and evolution to new conditions (Sgrò et al. 2011). The high levels of individual heterozygosity in Potomac River *V. americana* may have contributed to the fact that this population showed signs of local adaption to temperature and photoperiod. However, the high relatedness among individuals in the Potomac River may also mean that the potential for future adaption to new conditions is now limited.

Although subdivision of genetic diversity is not necessarily a precondition for adaptive differentiation (Sanford and Kelly 2011), genetic differentiation of *V. americana* across the Potomac, Hudson, and Kennebec Rivers has been documented with neutral microsatellite markers (Marsden CH1). Local adaptation is expected to

increase as distance among populations increases because reduced levels of gene flow will ultimately lead to increased levels of population differentiation (Becker et al. 2006, Leimu and Fischer 2008). These predictions are in stark contrast to the results of this study. Despite high levels of genetic differentiation among rivers, we found little overall evidence of local adaption.

Effects of Life History on Local Adaptation

Due to their effects on the degree and structure of genetic variation, plant traits such as mating system, clonality, and plasticity affect local adaption (Leimu and Fischer 2008). Evidence of stronger local adaptation has been found in short-lived and self-compatible species that are differentiated at smaller spatial scales as opposed to long-lived and/or outcrossing species (Linhart and Grant 1996, Leimu and Fischer 2008). Long-lived clonal plants might also be less adapted to local environments if genets are adapted to past conditions (Callaghan et al. 1996, Leimu and Fischer 2008). On the other hand, clonality may increase the potential for local adaptation due to restricted gene flow from reduced sexual reproduction (Van Kleunen and Fischer 2001, Knight and Miller 2004, Leimu and Fischer 2008).

In this study, lack of significant differences in many morphological and life history traits between local and foreign sourced *V. americana* provide limited evidence of local adaption. *V. americana* sourced from the Potomac River were the only plants that showed some evidence of local adaptation. The randomly harvested turions from the Potomac River that were used in this study had higher overall relatedness and more replicate clones than turions harvested from either the Hudson or Kennebec Rivers, which supports the above prediction that clonality increases the

potential for local adaptation. Obligate outcrossing in *V. americana* may have contributed to limited evidence of local adaptation in *V. americana* sourced from the Hudson and Kennebec Rivers, but probably not across the broad latitudinal scales encompassed in this study.

A pre-requisite for local adaptation is the failure of a population to evolve widespread phenotypic plasticity (Kawecki and Ebert 2004). Adaptive phenotypic plasticity leads to phenotypic differentiation without underlying genetic differentiation. Moreover, a genotype that is capable of producing the optimal phenotype in all locations will likely become fixed in a population (Kawecki and Ebert 2004). In the Potomac River, two multilocus genotypes (MLGs) were widespread within and across multiple sites (Marsden CH2). Therefore, when turions were randomly selected for inclusion in this experiment, MLG 199 and MLG 266 were overrepresented relative to the other MLGs (Table 3.3). It is possible that morphological phenotypic plasticity in these two MLGs has enabled them to grow optimally across widespread reaches of the Potomac River. Additional experiments at the genotype level will be needed to evaluate the potential phenotypic plasticity of *V. americana*. However, if these two Potomac River MLGs did have extensive phenotypic plasticity, then we would not have observed so many differences in morphological and life history traits in the home vs. away comparisons (Table 3.5; 3.7; Figure 3.2; 3.3).

Morphological phenotypic plasticity has been observed in many studies on clonal plants (Van Kleunen and Fischer 2001, Knight and Miller 2004). For example, plasticity in internode length and branching frequency of ramets in clonal plants with

spreading rhizomes allows clones to selectively place ramets in high quality patches and avoid adverse ones (Van Kleunen and Fischer 2001, Knight and Miller 2004). Plastic responses have also been observed in leaf length in many rosette plants (Hutchings and de Kroon 1994, Van Kleunen and Fischer 2001). Although environmental heterogeneity may increase local adaptation in non-clonal plants, it may favor the evolution of phenotypic plasticity in clonal plants, thus restricting the potential for local adaptation. In some clonal plants, plasticity itself is an adaptation to environmental heterogeneity (e.g., Van Kleunen and Fischer 2001). Plasticity in *V. americana* sourced from the Hudson and Kennebec Rivers is potentially a driving factor that enabled local and foreign sourced *V. americana* to perform similarly within treatments (Table 3.5; 3.7; Figure 3.2; 3.3). Additional experiments examining reaction norms at the genotypic levels is needed to fully investigate the potential phenotypic plasticity of different *V. americana* MLGs.

Physiological Responses to Temperature and Photoperiod

When there were differences in *V. americana* morphological and life history traits in home vs. away comparisons, *V. americana* grown in Potomac River conditions often outperformed plants grown in other conditions, regardless of original source (Tables 3.6, 3.8). Specifically, *V. americana* grown in Potomac River conditions had the highest mean maximum number of ramets, maximum number of flowers produced, total number of turions produced, and total turion biomass (Figure 3.2). Likewise, regardless of source, plants grown in warm Potomac River conditions often underperformed in morphological and life history trait measures relative to plants grown in other conditions (Tables 3.6, 3.8). Differences in morphological and

life history performance across environmental conditions, coupled with the lack of differences by source within treatments (best visualized in Figure 3.2), indicate fundamental differences in the overall growth and reproductive potential of *V. americana* to different temperatures and photoperiods.

Moreover, because photoperiod was consistent between the Potomac River treatment (T1) and warm Potomac River treatment, yet major differences in morphological and life history traits were found between these two treatments, there may be an optimal temperature range that affects the overall fitness of *V. americana*. Temperature responses in plant growth generally follow an optimum curve (Santamaía and van Vierssen 1997). Temperature is also plays a significant role in determining the distribution and productivity of plants. For example, morphology, especially the root to shoot ratio, is strongly affected by temperature in many plants and temperature often controls reproductive events, such as the induction of flowering and the germination of seeds (Santamaía and van Vierssen 1997).

A majority of SAV species show maximal photosynthesis at the relatively narrow temperature range of 25-35°C (Santamaía and van Vierssen 1997). Modeled temperature response curves of maximal *V. americana* photosynthesis estimated that the optimal temperature for V. americana was 32°C (Santamaía and van Vierssen 1997). This estimate was supported by a series of experiments that assessed the photosynthetic capability of *V. americana* collected from lakes in Madison, Wisconsin and found that the optimal temperature for photosynthesis was 32.6°C (Titus and Adams 1979). Field observations of *V. americana* in Nanjemoy Creek, Maryland revealed that clonal production of *V. americana* increased when water

temperatures rose above 25°C and laboratory experiments concluded that germination of *V. americana* seeds was most favorable at temperatures above 22°C (Jarvis and Moore 2008). Contrary to the above results, data from this experiment suggest that the optimal temperature range for *V. americana* collected from the northeast United States falls somewhere below 32°C and above 28°C.

Finally, it was also noteworthy that *V. americana* grown in Hudson River conditions produced, on average, more leaves of longer length than plants grown in other conditions, regardless of source (Figure 3.2). These results are the opposite of those found in photoperiod experiments on the aquatic angiosperm *Potamogeton pectinatus,* where a decrease in photoperiod resulted in an increase of leaf biomass (Pilon and Santamaria, 2002). However, the increased *V. americana* leaf production did not translate to subsequent increases in life history traits like flower or turion production.

Conclusions and Implications for Restoration

Potomac River *V. americana* demonstrated a pattern that was consistent with local adaptation in many of the measured morphological and life history traits. This pattern supports the conclusion that evolutionary processes like gene flow among the Potomac, Hudson, and Kennebec Rivers is limited and that Potomac River *V. americana* responded in the past to divergent selection to temperature and photoperiod. Even though Potomac River *V. americana* have been capable of responding to changes in local environmental conditions in the past, their ability to continue to respond to emerging conditions is questionable. Although *V. americana* from the Potomac River have high levels of individual heterozygosity, they also have

high levels of relatedness. Despite the fact that local adaptation can be an important mechanism for maintaining genetic variation across populations, adaptation to regional temperature and photoperiod may limit *V. americana* responses to warming trends and may affect the success of restoration practices like managed relocation.

On the other hand, *V. americana* populations from the Hudson and Kennebec Rivers fail to show signs of local adaptation in morphological and life history traits because of either limited divergent selection, too much gene flow, phenotypic plasticity or any combination of the above. The limited number of significant differences in measures of morphological and life history traits in local vs. foreign comparisons for *V. americana* sourced from the Hudson and Kennebec Rivers suggested that the populations have enough phenotypic variability to acclimate to novel temperatures and photoperiods. The phenotypic variation could derive from either underlying genetic variation or phenotypic plasticity. Phenotypic plasticity to temperatures and photoperiods would not only enable individuals to respond in situ to emerging conditions associated with climate change, but it would also facilitate the translocation of individuals to new regions. However, in the long-term phenotypic plasticity would limit the adaptation potential of *V. americana* if plastic phenotypes allow for phenotypic differentiation without underlying genetic differentiation. Additional experimental studies evaluating the response of individual geneotypes to varying environmental conditions is needed to determine if individual phenotypic plasticity is the source of the phenotypic variation that enabled *V. americana* sourced from different rivers to perform equally well within treatments.

Significant differences in plant performance in home vs. away conditions revealed that *V. americana* do not grow equally well in all conditions. Poor growth and reproductive success was documented for *V. americana* grown in the global warming scenario relative to plants from the same source grown in other temperature and photoperiod conditions. *V. americana* appear to perform optimally somewhere between 28-32°C, beyond which they appear to reach a thermal tolerace and suffer significant reductions in morphological growth and reproduction. Therefore, populations of *V. americana* at lower latitudes, like the Potomac River, may have reduced potential for long-term resilience in the face of climate change if temperatures exceed that thermal tolerance. This is especially a risk for *V. americana* populations, like the Potomac, that are already locally adapted and/or have reduced genetic diversity. Alternatively, the enhanced growth and reproductive success observed for *V. americana* grown in Potomac River conditions relative to plants grown in Hudson and Kennebec conditions indicates that slight increases in temperature in the more northern populations of *V. americana* will not reduce their potential for resilience.

Importantly, patterns of local adaptation tell us little about the underlying processes that drive the pattern (Kawecki and Ebert 2004). Future research studying the processes that foster, restrict, and interact with local adaptation would further help us to understand why local adaptation is apparent in some regions, like the Potomac, but not in others. A previous review found that local adaptation is actually less common in plant populations than generally assumed and when it is present, the degree of local adaptation in plants is independent of plant life history, spatial or

temporal habitat heterogeneity, and geographic scale (Leimu and Fischer 2008). Future research on local adaptation of *V. americana* should focus on testing such hypotheses. Specifically, additional experiments should evaluate temperature and photoperiod independently at the individual genotype level to accurately assess the driving force of local adaption in the Potomac River as well as whether or not phenotypic plasticity is limiting local adaption in the other rivers.

In this study, restricted evidence of local adaptation coupled with patterns of acclimation suggest that populations of *V. americana* in these rivers have high potential for resilience in the face of climate change and may suffer few short-term negative consequences from climate change mitigation strategies that involve managed relocation of *V. americana*. However, long-term consequences have yet to be evaluated and should not be overlooked. Although our results provide valuable insights, experiments were limited to fitness effects manifested during one growth *V. americana* growth season under benign greenhouse conditions. Long term fitness effects like outbreeding depression are often greater in later life stages (Holtsford and Ellstrand 1990, Husband and Schemske 1996), in subsequent generations (Edmands 2007, Broadhurst et al. 2008, Huff et al. 2011), and under stressful conditions (Carr and Dudash 1995, Keller 1998, Crnokrak and Roff 1999, Murren and Dudash 2012). Therefore, the success of reproduction crosses between *V. americana* sourced from different rivers should be evaluated prior to any managed relocation initiatives.

Table 3.1: Growth chamber temperature and photoperiod conditions for four climate treatments. Climate treatment T1, T2, and T3 simulate the natural conditions of the Potomac, Hudson, and Kennebec Rivers, respectively. Climate treatment T4 represents a global warming scenario for the Potomac River.

Climate Treatment	Simulated Region	Latitude (°N)	Maximum Daytime Temp (°C)	Minimum Daytime Temp (°C)	Initial Photoperiod at Week 0 (hours)	Maximum Photoperiod at Week 12 (hours)	Minimum Photoperiod (hours)	Change in Photoperiod (min/week)	Light Intensity (μmol $m^{-2}s^{-1}$)
T4	Warm Potomac River	n/a	32	15	12.50	14.70	9.45	11	200
T1	Potomac River	39.1-39.5	28	11	12.50	14.70	9.45	11	200
T2	Hudson River	41.2-41.5	25	8	12.50	15.00	9.15	12.5	200
T3	Kennebec River	44.0-44.5	21	8	12.50	15.30	8.85	14	200

Table 3.2: Source and total count of *Vallisneria americana* turions planted in each temperature and photoperiod treatment.

Source River	Source Site	Climate Treatment				**Site Total**
		T1: Potomac	T2: Hudson	T3: Kennebec	T4: Warm Potomac	
Potomac	LC	8	8	8	8	**32**
	OJ	8	8	8	8	**32**
	POR2	8	8	8	8	**32**
	EF	8	8	8	8	**32**
Potomac Total		**32**	**32**	**32**	**32**	
Hudson	NBB	8	8	8	8	**32**
	BNR	8	8	8	8	**32**
	PEK	8	8	8	8	**32**
	CRO	8	8	8	8	**32**
Hudson Total		**32**	**32**	**32**	**32**	
Kennebec	WAT	6	6	6	6	**32**
	GDR	8	8	8	8	**24**
	RCH	8	8	8	8	**32**
	BTC	8	8	8	8	**32**
Kennebec Total		**30**	**30**	**30**	**30**	
Grand Total		**94**	**94**	**94**	**94**	**376**

*Climate treatment conditions are defined in Table 3.1

Table 3.3: Count of *Vallisneria americana* multilocus genotypes (MLGs) from each river source planted in temperature and photoperiod treatments.

Source River	MLG	Climate Treatment			
		T1: Potomac	T2: Hudson	T3: Kennebec	T4: Warm Potomac
Potomac	199	11	12	11	13
	230	4	4	4	3
	266	9	7	9	7
	301	1		1	1
	364			1	
	365		1		
	369		1		1
	393	1	1		2
	394	1	1	2	1
	402	2	2	2	2
	406		1		
	407	1			
	411	2	2	2	2
Total # MLGs		**9**	**10**	**8**	**9**
Hudson	5	1	1	1	1
	7	4	4	4	4
	8			1	1
	13	3	3	2	2
	32	4	5	5	5
	33	1			
	34	2	2	2	2
	36	1	1	1	1
	40	2	2	2	2
	42	1		1	1
	44	2	2	2	2
	45	1	1	1	1
	48		1		
	50	2	2	2	2
	54		2	2	2
	55	2			
	59			1	
	62				2
	63			1	
	65		1		
	66	2	2	2	2
	69		1		
	70	2	2	2	2
	71	1			
	73	1			
Total # MLGs		**17**	**16**	**17**	**16**

Source River	MLG	Treatment			
		T1: Potomac	T2: Hudson	T3: Kennebec	T4: Warm Potomac
Kennebec	79	1	1		
	83	3	2	2	2
	96		1	2	2
	101	2	2	2	2
	103	2	2	2	2
	130	2	2	2	2
	139	2	2	2	2
	141	2	2	2	2
	153			1	1
	154	1	2	3	
	158	2			
	160				1
	162	2	2	2	2
	163				1
	164	1			
	166		2		
	168				1
	176	2	2	2	2
	181	2	1	1	2
	183	4	5	5	4
	na	2	2	2	2
Total # MLGs		**15**	**15**	**14**	**16**

*Climate treatment conditions are defined in Table 3.1; Potomac River MLGs are from Marsden CH2; Hudson and Kennebec River MLGs are from Marsden CH1

Table 3.4: Model results, including contribution of continuous predictor variables and factors, for the most parsimonious generalized linear models (GLMs) analyzing differences in *Vallisneria americana* morphological and life history traits by climate treatment to assess evidence of local adaptation via differences in 'local' versus 'foreign' sourced plants. Bolded numbers are significant at $\alpha < 0.05$.

	Morphological or Life History Variables																											
	Max Ramets				Max # Leaves				Max Leaf Length				Total # Flowers				# Flowering Days				First Flower Day				Total Turions				Total Turion Biomass			
	df	resid df	f stat	p-value	df	resid df	f stat	p-value	df	resid df	f stat	p-value	df	resid df	f stat	p-value	df	resid df	f stat	p-value	df	resid df	f stat	p-value	df	resid df	f stat	p-value	df	resid df	f stat	p-value
Treatment 1: Potomac River Conditions																												
Main Factor																												
Plant Source	2	91	8.58	**<0.001**	2	91	8.85	**<0.001**	2	62	1.15	0.324	2	91	6.73	**0.002**	2	22	0.93	0.411	2	22	1.15	0.335	2	91	10.7	**<0.001**	2	61	0.31	0.733
Continuous Predictor Variables/Factors																												
Initial Turion Area	1	90	0.22	0.639	1	90	0.84	0.361	1	61	10.82	**0.002**	1	90	5.82	**0.018**	-	-	-	-	1	21	7.47	**0.012**	1	90	0.0	0.9	1	60	1.91	0.172
Relatedness	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	59	14.16	**<0.001**
Ho	-	-	-	-	-	-	-	-	1	60	3.48	0.067	1	89	6.18	**0.015**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth Chamber	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Interactions																												
Plant Region:Initial Turion Area	2	88	3.40	**0.038**	2	88	3.13	**0.049**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	88	5.9	**0.004**	-	-	-	-
Plant Region:Relatedness	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plant Region:Ho	-	-	-	-	-	-	-	-	2	58	6.42	**0.003**	2	87	4.85	**0.010**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plant Region:Growth Chamber	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Model Error Structure	quasipoisson				quasipoisson				Gamma				quasipoisson				quasipoisson				quasipoisson				quasipoisson				Gamma			
Treatment 2: Hudson River Conditions																												
Main Factor																												
Plant Source	2	91	1.04	0.357	2	91	1.17	0.315	2	63	7.09	**0.002**	2	91	1.99	0.143	2	15	2.61	0.106	2	15	3.15	0.074	2	91	1.53	0.221	2	62	0.48	0.621
Continuous Predictor Variables/Factors																												
Initial Turion Area	1	90	3.83	0.530	1	90	5.55	**0.021**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	61	7.62	**0.008**
Relatedness	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	60	9.35	**0.003**
Ho	-	-	-	-	-	-	-	-	-	-	-	-	1	90	9.05	**0.003**	-	-	-	-	-	-	-	-	-	-	-	-	1	59	6.92	**0.011**
Growth Chamber	-	-	-	-	-	-	-	-	1	62	25.67	**<0.001**	1	89	7.06	**0.009**	-	-	-	-	1	14	8.34	**0.012**	1	90	4.15	**0.045**	-	-	-	-
Interactions																												
Plant Region:Initial Turion Area	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plant Region:Relatedness	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plant Region:Ho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plant Region:Growth Chamber	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Model Error Structure	quasipoisson				quasipoisson				Gamma				quasipoisson				quasipoisson				quasipoisson				quasipoisson				Gamma			
Treatment 3: Kennebec River Conditions																												
Main Factor																												
Plant Source	2	91	0.78	0.464	2	91	0.83	0.441	1	65	12.38	**<0.001**	1	91	0.31	0.735	2	16	0.75	0.491	2	16	0.50	0.615	2	91	2.36	0.100	2	63	1.07	0.351
Continuous Predictor Variables/Factors																												
Initial Turion Area	1	90	35.16	**<0.001**	1	90	32.20	**<0.001**	-	-	-	-	-	-	-	-	-	-	-	-	1	15	11.37	**0.004**	-	-	-	-	1	62	7.20	**0.009**
Relatedness	-	-	-	-	-	-	-	-	1	64	0.06	0.802	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ho	1	89	4.72	**0.033**	1	89	4.92	**0.029**	-	-	-	-	1	90	5.32	**0.023**	-	-	-	-	-	-	-	-	1	90	4.49	**0.037**	1	61	12.69	**0.001**
Growth Chamber	1	88	9.28	**0.003**	1	88	8.47	**0.005**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	89	13.28	**<0.001**	-	-	-	-
Interactions																												
Plant Region:Initial Turion Area	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plant Region:Relatedness	-	-	-	-	-	-	-	-	2	62	4.12	**0.021**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plant Region:Ho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plant Region:Growth Chamber	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Model Error Structure	quasipoisson				quasipoisson				Gamma				quasipoisson				quasipoisson				quasipoisson				quasipoisson				Gamma			
Treatment 4: Warm Potomac River Conditions																												
Main Factor																												
Plant Source	1	91	1.34	0.266	1	91	0.91	0.405	1	31	4.68	**0.017**	2	91	0.82	0.443	2	10	2.01	0.190	2	10	1.24	0.335	2	91	1.40	0.253	2	30	3.79	0.034
Continuous Predictor Variables/Factors																												
Initial Turion Area	-	-	-	-	-	-	-	-	1	30	5.85	**0.022**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Relatedness	-	-	-	-	-	-	-	-	-	-	-	-	1	90	3.03	0.085	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	9	8.08	**0.019**	-	-	-	-	-	-	-	-
Growth Chamber	1	90	7.96	**0.006**	1	90	10.11	**0.002**	-	-	-	-	1	89	13.53	**<0.001**	1	9	5.55	**0.043**	-	-	-	-	1	90	4.05	**0.047**	1	29	9.72	**0.004**
Interactions																												
Plant Region:Initial Turion Area	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plant Region:Relatedness	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plant Region:Ho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plant Region:Growth Chamber	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Model Error Structure	quasipoisson				quasipoisson				Gamma				quasipoisson				quasipoisson				quasipoisson				quasipoisson				Gamma			

Table 3.5: Model results, including contribution of continuous predictor variables and factors, for the most parsimonious generalized linear models (GLMs) analyzing differences in *Vallisneria americana* morphological and life history traits by river source to assess evidence of local adaptation via differences in plants grown in 'home' versus 'away' conditions. Bolded numbers are significant at $\alpha < 0.05$.

	Morphological or Life History Variables																											
	Max Ramets				Max # Leaves				Max Leaf Length				Total # Flowers				# Flowering Days				First Flower Day				Total Turions				Total Turion Biomass			
	df	resid df	f stat	p-value	df	resid df	f stat	p-value	df	resid df	f stat	p-value	df	resid df	f stat	p-value	df	resid df	f stat	p-value	df	resid df	f stat	p-value	df	resid df	f stat	p-value	df	resid df	f stat	p-value
Source River: Potomac River																												
Main Factor																												
Treatment	3	124	16.375	**<0.001**	3	124	14.699	**<0.001**	3	79	7.008	**<0.001**	3	124	2.736	**0.047**	3	32	1.754	0.176	3	32	1.861	0.157	3	124	27.435	**<0.001**	3	75	40.204	**<0.001**
Continuous Predictor Variables/Factors																												
Initial Turion Area	1	123	7.623	**0.007**	1	123	8.107	**0.005**	1	78	10.255	**0.002**	1	123	19.057	**<0.001**	-	-	-	-	1	31	13.42	**0.001**	-	-	-	-	1	74	5.71	**0.020**
Relatedness	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ho	-	-	-	-	-	-	-	-	-	-	-	-	1	122	36.44	**<0.001**	-	-	-	-	-	-	-	-	-	-	-	-	1	73	4.538	**0.037**
Growth Chamber	2	121	2.767	0.067	-	-	-	-	2	76	3.854	**0.026**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	71	2.823	0.067
Interactions																												
Treatment:Initial Turion Area	3	118	2.773	**0.045**	-	-	-	-	3	73	4.059	**0.010**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	68	0.815	0.490
Treatment:Relatedness	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Treatment:Ho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Treatment:Growth Chamber	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	67	7.254	**0.009**
Model Error Structure		quasipoisson				quasipoisson				Gamma				quasipoisson				quasipoisson				quasipoisson				quasipoisson				Gamma		
Source River: Hudson River																												
Main Factor																												
Treatment	3	124	7.992	**<0.001**	3	124	6.063	**0.001**	3	81	4.466	**0.006**	3	124	0.254	0.858	3	15	0.256	0.856	3	15	4.388	**0.021**	3	124	11.666	**<0.001**	3	80	33.752	**<0.001**
Continuous Predictor Variables/Factors																												
Initial Turion Area	-	-	-	-	-	-	-	-	1	80	8.749	**0.004**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Relatedness	1	123	11.662	**0.001**	1	123	9.287	**0.003**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	123	7.016	**0.009**	-	-	-	-
Ho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth Chamber	2	121	0.086	0.917	2	121	0.170	0.844	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	121	0.762	0.469	-	-	-	-
Interactions																												
Treatment:Initial Turion Area	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Treatment:Relatedness	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Treatment:Ho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Treatment:Growth Chamber	1	120	13.962	**<0.001**	1	120	15.870	**<0.001**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	120	4.899	**0.029**	-	-	-	-
Model Error Structure		quasipoisson				quasipoisson				Gamma				quasipoisson				quasipoisson				quasipoisson				quasipoisson				Gamma		
Source River: Kennebec River																												
Main Factor																												
Treatment	3	116	5.550	**0.001**	3	116	4.527	**0.005**	3	61	4.143	**0.010**	3	116	3.132	**0.028**	3	16	1.319	0.303	3	16	4.629	**0.017**	3	116	5.586	**0.001**	3	60	23.168	**<0.001**
Continuous Predictor Variables/Factors																												
Initial Turion Area	1	115	9.701	**0.002**	1	115	13.189	**<0.001**	1	60	4.928	**0.030**	1	115	9.771	**0.002**	-	-	-	-	1	15	15.39	**0.001**	1	115	9.687	**0.002**	-	-	-	-
Relatedness	-	-	-	-	-	-	-	-	-	-	-	-	1	114	29.888	**<0.001**	-	-	-	-	-	-	-	-	-	-	-	-	1	59	9.317	**0.003**
Ho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	114	7.128	**0.009**	-	-	-	-
Growth Chamber	-	-	-	-	-	-	-	-	2	58	5.597	**0.006**	2	112	9.702	**<0.001**	-	-	-	-	-	-	-	-	-	-	-	-	2	57	5.107	**0.009**
Interactions																												
Treatment:Initial Turion Area	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	111	2.883	**0.039**	-	-	-	-
Treatment:Relatedness	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Treatment:Ho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Treatment:Growth Chamber	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Model Error Structure		quasipoisson				quasipoisson				Gamma				quasipoisson				quasipoisson				quasipoisson				quasipoisson				Gamma		

Table 3.6: Post-hoc Tukey HSD analysis results for generalized linear models (GLMs) to assess differences in *Vallisneria americana* morphological and life history traits by climate treatment and river source. * denotes significance at $\alpha < 0.05$; ** denotes significance at $\alpha < 0.01$; *** denotes significance at $\alpha < 0.001$; · denotes no significance; - denotes traits without significant main effects. Original GLM results are on Tables 3.4 and 3.5.

	Morphological or Life History Variables							
	Max Ramets	Max # Leaves	Max Leaf Length	Total # Flowers	# Flowering Days	First Flower Day	Total Turions	Turion Biomass
Within Climate Treatments								
Treatment 1: Potomac River Conditions								
Potomac - Hudson	·	·	-	·	-	-	*	-
Potomac - Kennebec	***	***	-	*	-	-	***	-
Hudson - Kennebec	·	·	-	·	-	-	*	-
Treatment 2: Hudson River Conditions								
Potomac - Hudson	-	-	·	-	-	-	-	-
Potomac - Kennebec	-	-	·	-	-	-	-	-
Hudson - Kennebec	-	-	*	-	-	-	-	-
Treatment 3: Kennebec River Conditions								
Potomac - Hudson	-	-	**	-	-	-	-	-
Potomac - Kennebec	-	-	·	-	-	-	-	-
Hudson - Kennebec	-	-	***	-	-	-	-	-
Treatment 4: Warm Potomac Conditions								
Potomac - Hudson	-	-	·	-	-	-	-	-
Potomac - Kennebec	-	-	*	-	-	-	-	-
Hudson - Kennebec	-	-	*	-	-	-	-	-
Within River Sources								
Source: Potomac River								
T1: Potomac - T2: Hudson	*	·	·	·	-	-	*	***
T1: Potomac - T3: Kennebec	***	**	·	·	-	-	***	***
T1: Potomac - T4: Warm Potomac	***	***	·	·	-	-	·	**
T2: Hudson - T3: Kennebec	·	·	**	·	-	-	**	***
T2: Hudson - T4: Warm Potomac	***	***	·	·	-	-	·	·
T3: Kennebec - T4: Warm Potomac	*	*	*	·	-	-	*	***
Source: Hudson River								
T1: Potomac - T2: Hudson	·	·	**	-	-	·	·	**
T1: Potomac - T3: Kennebec	*	·	·	-	-	*	*	***
T1: Potomac - T4: Warm Potomac	***	**	·	-	-	·	·	**
T2: Hudson - T3: Kennebec	·	·	·	-	-	·	·	***
T2: Hudson - T4: Warm Potomac	**	*	·	-	-	·	·	·
T3: Kennebec - T4: Warm Potomac	·	·	·	-	-	*	·	***
Source: Kennebec River								
T1: Potomac - T2: Hudson	·	·	·	·	-	·	·	*
T1: Potomac - T3: Kennebec	·	·	·	·	-	·	·	***
T1: Potomac - T4: Warm Potomac	*	·	·	·	-	·	·	*
T2: Hudson - T3: Kennebec	·	·	*	·	-	·	·	**
T2: Hudson - T4: Warm Potomac	**	*	·	·	-	·	·	·
T3: Kennebec - T4: Warm Potomac	*	*	·	·	-	·	·	·

Table 3.7: Non-linear mixed effects model (NLME) results of *Vallisneria americana* morphological and life history traits (1) within climate treatments to assess evidence of local adaptation via differences in 'local' versus 'foreign' sourced plants and (2) within river sources to assess evidence of local adaptation via differences in plants grown in 'home' versus 'away' conditions.

Fixed Effects	Morphological or Life History Variables															
	# Ramets				# Leaves				Longest Leaf Length				# Flowers			
	df_n	df_d	f stat	p	df_n	df_d	f stat	p	df_n	df_d	f stat	p	df_n	df_d	f stat	p
Within Climate Treatments																
Treatment 1: Potomac																
Plant Source	2	91	8.57	**<0.001**	2	91	8.20	**<0.001**	2	91	5.36	**0.006**	2	91	6.06	**0.003**
Time (weeks)	1	1125	1411.6	**<0.001**	1	1125	1178.3	**<0.001**	1	1125	387.3	**<0.001**	1	1125	13.22	**<0.001**
Plant Source:Time	2	1125	88.66	**<0.001**	2	1125	82.17	**<0.001**	2	1125	20.13	**<0.001**	2	1125	4.29	**0.014**
Treatment 2: Hudson																
Plant Source	2	91	1.30	0.276	2	91	0.96	0.386	2	91	4.18	**0.018**	2	91	1.39	0.255
Time (weeks)	1	1125	1202.6	**<0.001**	1	1125	1194.3	**<0.001**	1	1125	477.3	**<0.001**	1	1125	34.47	**<0.001**
Plant Source:Time	2	1125	15.74	**<0.001**	2	1125	12.54	**<0.001**	2	1125	12.46	**<0.001**	2	1125	3.26	**0.039**
Treatment 3: Kennebec																
Plant Source	2	91	0.36	0.702	2	91	0.51	0.600	2	91	6.11	**0.003**	2	91	1.48	0.234
Time (weeks)	1	1125	1427.7	**<0.001**	1	1125	1327.4	**<0.001**	1	1125	495.5	**<0.001**	1	1125	48.29	**<0.001**
Plant Source:Time	2	1125	7.08	**0.001**	2	1125	6.29	**0.002**	2	1125	25.27	**<0.001**	2	1125	2.81	0.061
Treatment 4: Warm Potomac																
Plant Source	2	91	2.02	0.138	2	91	1.15	0.322	2	91	1.91	0.154	2	91	0.87	0.424
Time (weeks)	1	1125	384.3	**<0.001**	1	1125	313.7	**<0.001**	1	1125	159.0	**<0.001**	1	1125	6.93	**0.009**
Plant Source:Time	2	1125	18.41	**<0.001**	2	1125	9.02	**0.001**	2	1125	6.68	**0.001**	2	1125	1.22	0.296
Within River Sources																
Source: Potomac River																
Treatment	3	124	16.84	**<0.001**	3	124	15.47	**<0.001**	3	124	8.32	**<0.001**	3	124	2.367	0.074
Time (weeks)	1	1532	1968.5	**<0.001**	1	1532	1800.0	**<0.001**	1	1532	493.4	**<0.001**	1	1532	40.81	**<0.001**
Treatment:Time	3	1532	155.8	**<0.001**	3	1532	125.6	**<0.001**	3	1532	22.22	**<0.001**	3	1532	3.17	**0.024**
Source: Hudson River																
Treatment	3	124	5.71	**0.001**	3	124	5.24	**0.002**	3	124	2.96	**0.035**	3	124	0.09	0.964
Time (weeks)	1	1532	1470.7	**<0.001**	1	1532	1252.7	**<0.001**	1	1532	579.4	**<0.001**	1	1532	26.38	**<0.001**
Treatment:Time	3	1532	65.38	**<0.001**	3	1532	52.73	**<0.001**	3	1532	23.02	**<0.001**	3	1532	1.16	0.324
Source: Kennebec River																
Treatment	3	116	3.92	**0.011**	3	116	2.92	**0.037**	3	116	2.35	0.076	3	116	1.96	0.124
Time (weeks)	1	1436	851.39	**<0.001**	1	1436	813.3	**<0.001**	1	1436	442.9	**<0.001**	1	1436	27.99	**<0.001**
Treatment:Time	3	1436	43.39	**<0.001**	3	1436	39.63	**<0.001**	3	1436	27.91	**<0.001**	3	1436	5.57	**0.001**

Table 3.8: Post-hoc interaction analysis results for Non-linear mixed effects models (NLMEs) on *Vallisneria americana* morphological and life history traits (1) within each climate treatment to assess evidence of local adaptation via differences in 'local' versus 'foreign' sourced plants and (2) within each river source to assess evidence of local adaptation via differences in plants grown in 'home' versus 'away' conditions. Traits without significant main effects are designated by -. Bolded numbers are significant at $\alpha < 0.05$. Original NMLE results are from Table 3.7.

	Morphological or Life History Variables											
	# Ramets			# Leaves			Longest Leaf Length			# Flowers		
	df	X^2	p	df	X^2	p	df	X^2	p	df	X^2	p
Interactions Within Climate Treatments												
Treatment 1: Potomac River Conditions												
Potomac - Hudson	1	3.01	0.083	1	3.17	0.075	1	0.49	0.484	1	8.25	**0.008**
Potomac - Kennebec	1	17.03	**<0.001**	1	16.35	**<0.001**	1	9.80	**0.005**	1	9.77	**0.005**
Hudson - Kennebec	1	5.85	**0.031**	1	5.25	**0.044**	1	5.96	**0.029**	1	0.09	0.764
Treatment 2: Hudson River Conditions												
Potomac - Hudson	-	-	-	-	-	-	1	3.53	**0.120**	-	-	-
Potomac - Kennebec	-	-	-	-	-	-	1	0.97	0.324	-	-	-
Hudson - Kennebec	-	-	-	-	-	-	1	8.04	**0.014**	-	-	-
Treatment 3: Kennebec River Conditions												
Potomac - Hudson	-	-	-	-	-	-	1	10.24	**0.004**	-	-	-
Potomac - Kennebec	-	-	-	-	-	-	1	0.12	0.725	-	-	-
Hudson - Kennebec	-	-	-	-	-	-	1	7.82	**0.010**	-	-	-
Treatment 4: Warm Potomac Conditions												
Potomac - Hudson	-	-	-	-	-	-	-	-	-	-	-	-
Potomac - Kennebec	-	-	-	-	-	-	-	-	-	-	-	-
Hudson - Kennebec	-	-	-	-	-	-	-	-	-	-	-	-
Interactions Within River Sources												
Source: Potomac River												
T1 - T2	1	12.29	**0.002**	1	12.64	**0.002**	1	1.63	0.404	-	-	-
T1 - T3	1	22.18	**<0.001**	1	21.14	**<0.001**	1	14.83	**<0.001**	-	-	-
T1 - T4	1	48.25	**<0.001**	1	44.20	**<0.001**	1	17.91	**<0.001**	-	-	-
T2 - T3	1	1.45	0.229	1	1.09	0.297	1	6.64	**0.030**	-	-	-
T2 - T4	1	11.84	**0.002**	1	9.57	**0.006**	1	8.74	**0.012**	-	-	-
T3 - T4	1	5.00	0.051	1	4.21	0.081	1	0.14	0.703	-	-	-
Source: Hudson River												
T1 - T2	1	1.62	0.405	1	2.16	0.341	1	2.11	0.534	-	-	-
T1 - T3	1	9.27	**0.012**	1	9.30	**0.011**	1	0.11	0.740	-	-	-
T1 - T4	1	13.81	**0.001**	1	12.76	**0.002**	1	2.25	0.534	-	-	-
T2 - T3	1	3.13	0.230	1	2.50	0.341	1	3.18	0.373	-	-	-
T2 - T4	1	5.96	0.059	1	4.42	0.142	1	8.72	**0.019**	-	-	-
T3 - T4	1	0.45	0.502	1	0.27	0.602	1	1.37	0.534	-	-	-
Source: Kennebec River												
T1 - T2	1	0.06	1.000	1	0.08	1.000	-	-	-	-	-	-
T1 - T3	1	0.02	1.000	1	0.06	1.000	-	-	-	-	-	-
T1 - T4	1	7.10	**0.031**	1	4.99	0.102	-	-	-	-	-	-
T2 - T3	1	0.01	1.000	1	0.00	1.000	-	-	-	-	-	-
T2 - T4	1	8.45	**0.022**	1	6.31	0.072	-	-	-	-	-	-
T3 - T4	1	7.86	**0.025**	1	6.09	0.072	-	-	-	-	-	-

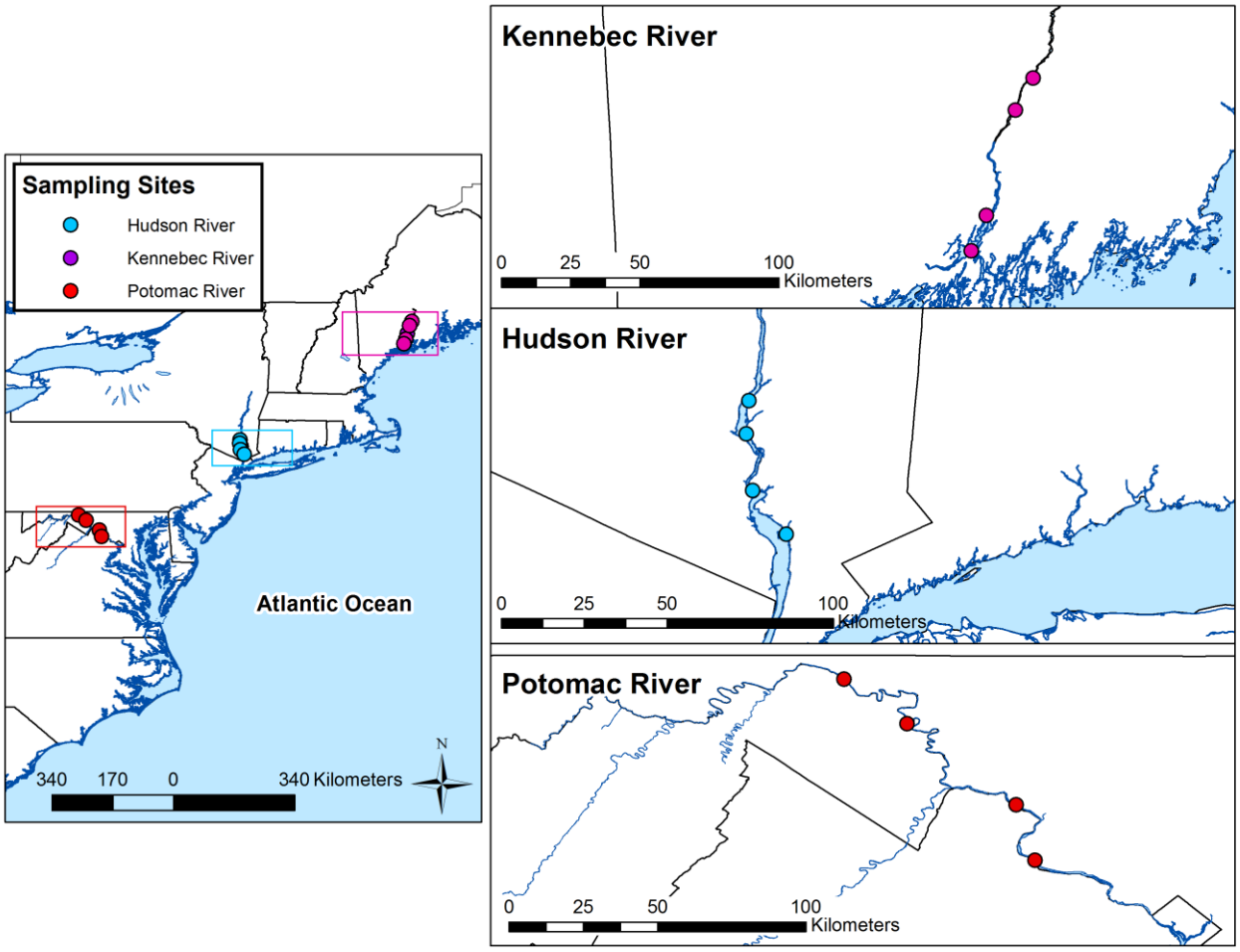

Figure 3.1: Map of 2011 *Vallisneria americana* collection locations in the Kennebec River (ME), Hudson River (NY), and Potomac River (MD). Collected shoots were propagated in the University of Maryland Greenhouse. Turions were harvested from four randomly selected sites within each river (pink circles) in January 2013 and 2014 for use in temperature and photoperiod experiments.

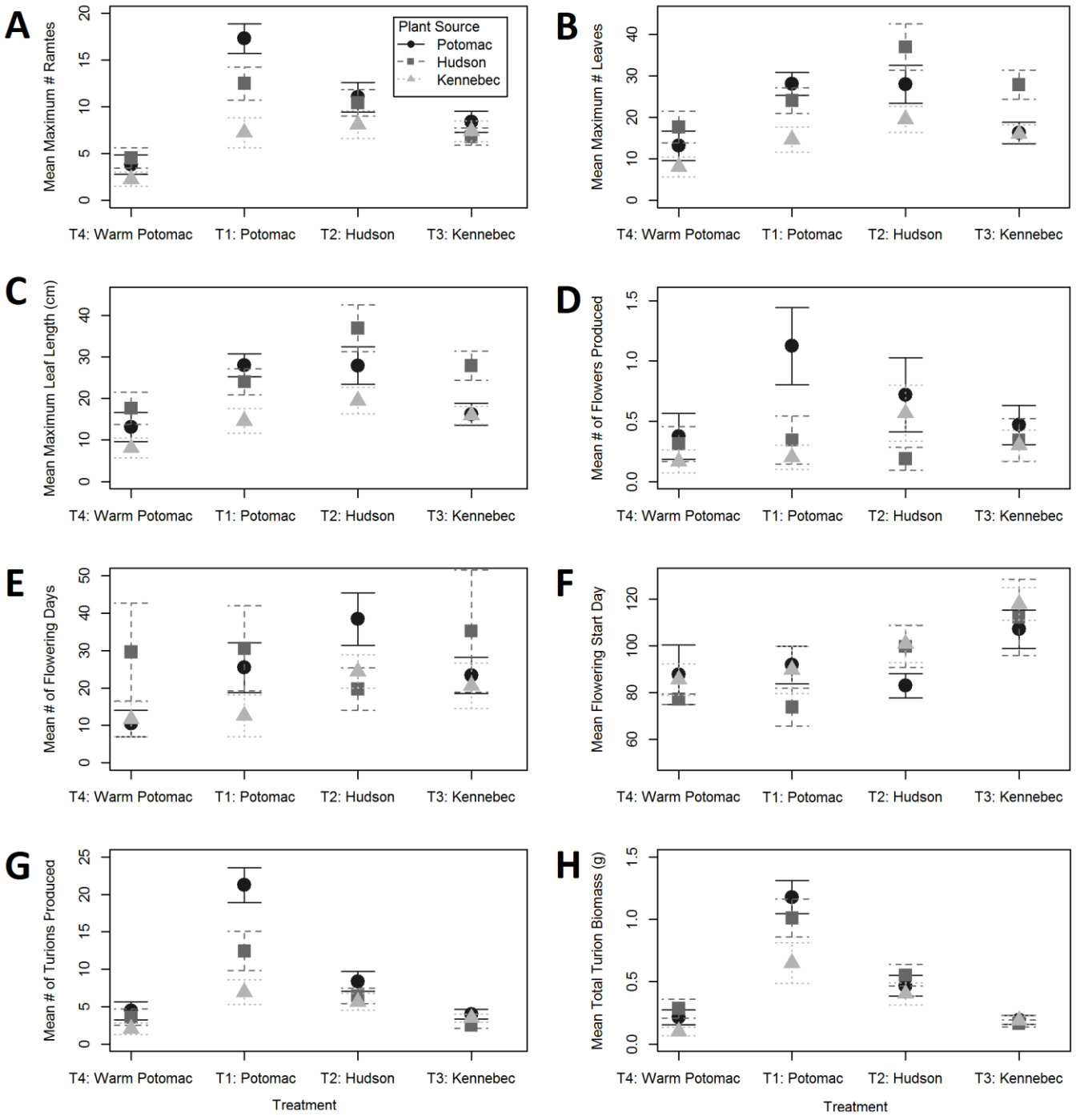

Figure 3.2: Mean and standard error of *Vallisneria americana* morphological and life history traits (**A**-**H**) measured across four temperature and photoperiod growth chamber treatments. Treatment conditions, defined in Table 3.1, simulate source regions of collected plants. Plants were sourced from the Potomac River (black circles), the Hudson River (dark grey squares), and the Kennebec River (light grey triangles).

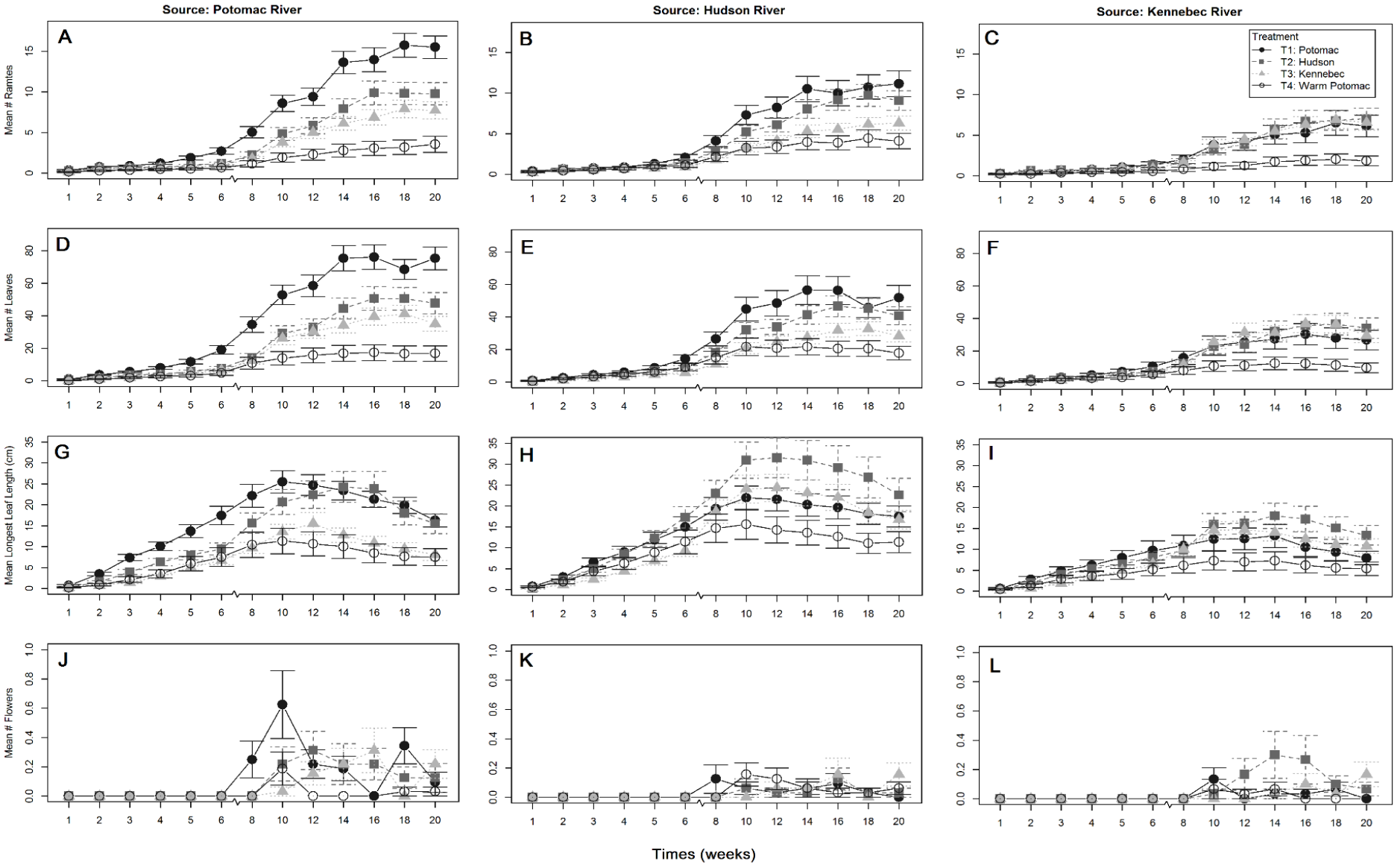

Figure 3.3: Mean and standard error of *Vallisneria americana* morphological and life history traits (rows 1–4) measured though time on plants sourced from either the Potomac River (**A**, **D**, **G**, **J**), Hudson River (**B**, **E**, **H**, **K**), or Kennebec River (**C**, **F**, **I**, **L**) and grown in four temperature and photoperiod growth chamber treatments. Treatment conditions are defined in Table 3.1.

Chapter 4: Genetic rescue and outbreeding depression in controlled crosses of *Vallisneria americana*: Implications for mixing seed sources for submersed aquatic vegetation restoration*

*Reprinted from *Biological Conservation*, 167, **B. W. Marsden**, K. A.M. Engelhardt, and M. C. Neel, Genetic rescue versus outbreeding depression in *Vallisneria americana*: Implications for mixing seed sources for restoration, pp. 203-214, Copyright (2013), with permission from Elsevier (Appendix B).

Abstract

Selection of seed stock for restoration remains a complex issue. Using local stock reduces the chances of outbreeding depression or genetic dilution, whereas mixing sources may increase diversity and counteract inbreeding depression. Evaluation of these opposing approaches remains difficult when planning a restoration project but is needed to increase chances of long-term population persistence. We evaluated seed production and germination success of seeds from controlled reproductive crosses of the submersed aquatic plant *Vallisneria americana* (wild celery) collected from populations throughout the Chesapeake Bay. We assessed differences in seeds, capsules, and germination success in three types of crosses: 1) individuals within-populations, 2) among-populations but within-genetically differentiated regions, and 3) among-regions. We observed population level differences in within-population and among-region crosses. Levels of genetic relatedness among individuals, genetic

diversity within populations, or differentiation across populations did not predict reproductive success. Our data show that mixing sources from different populations and regions have both benefits and drawbacks. Thus, minimizing the risks of outbreeding and inbreeding depression, presented as a mostly dichotomous issue in the restoration literature, is not an either-or issue in *V. americana*.

Introduction

Two contradictory paradigms for selecting source materials create a major tension in restoration ecology. One approach argues for maintaining purity of local genetic stock by using propagules from one or a few sites in close proximity to a restoration site. The underlying hypothesis is that local stock is well adapted to environmental conditions of a site and will successfully establish with no risk of outbreeding depression from gene flow of non-local alleles (Montalvo and Ellstrand 2000, 2001, McKay et al. 2005). Risk of restoration failure, however, can be high when source populations are small, have been isolated and drastically reduced in size, or have low diversity or low fitness due to inbreeding depression (Broadhurst et al. 2008, Weeks et al. 2011).

The alternative approach is to increase diversity and counteract local inbreeding by introducing genotypes from foreign source populations or by mixing genotypes from multiple populations (Broadhurst et al. 2008). Proponents argue that stock from multiple sources promotes persistence if associated phenotypes are adapted to a broader range of environmental conditions than individuals from any single population and mating among them following restoration results in heterosis (Fenster and Dudash 1994, Broadhurst et al. 2008, Hughes et al. 2008, Weeks et al.

2011). Immediate negative consequences of such plantings arise if phenotypes are poorly adapted to local conditions and cannot survive and establish. Long-term consequences arise if reproduction between local and foreign stock is not possible or fitness of their offspring is compromised. Advocates of mixing propagules from many populations argue that benefits of increased diversity outweigh any potential negative consequences of outbreeding depression (Broadhurst et al. 2008) and argue that risks of outbreeding depression are overstated and unsubstantiated (Frankham et al. 2011, Weeks et al. 2011).

Inbreeding and outbreeding depression are increasingly presented as extreme dichotomous conditions. We argue here that degrees of differentiation among populations and inbreeding within populations are continuous gradients that vary independently. Managing the risks of using local or disparate sources of restoration stock, therefore, needs to account for the genetic context of natural source populations. In general, it appears that mixing slightly differentiated, inbred populations can lead to increased fitness whereas mixing extremely differentiated, locally adapted populations can result in outbreeding depression (Waser 1993, Hereford 2009, Forrest et al. 2011, Hufford et al. 2012, Pickup et al. 2013). For example, recent studies by Forrest et al. (2011) and Hufford et al. (2012) found that plants crossed at intermediate-distances outperform within-population crosses in terms of germination success and survival while long-distance hybrids show signs of outbreeding depression. These studies furthermore concluded that spatial autocorrelation and genetic differentiation can be used to determine the optimal distances in which seeds can be mixed for restoration purposes (Forrest et al. 2011,

Hufford et al. 2012). In another study, Pickup and colleagues (2013) found no evidence of outbreeding depression in crosses between pairs of populations across multiple generations, but they did detect evidence of heterosis. In contrast to previous studies, Pickup at colleagues (2013) found that heterosis was not limited to crosses between populations assigned to different genetic regions based on genetic dissimilarity. Therefore, empirical evidence for where natural populations lie along continua of genetic diversity and differentiation, and how that translates into risks for inbreeding or outbreeding depression, is essential to make informed decisions on what restoration stock to use to maximize fitness and long-term population persistence.

To assess relative risks and benefits of these two restoration approaches, we evaluated reproductive success in terms of fruit size, seed number, seed size, and germination in controlled-environment crosses of individuals from within versus among 11 populations of the submersed aquatic plant species *Vallisneria americana* Michx. (wild celery; Family Hydrocharitaceae) in the Chesapeake Bay of eastern North America. These metrics were selected because seed supply is an important driver of initial establishment in restorations (Broadhurst et al. 2008) and they represent long-term potential for persistence and maintenance of genetic diversity via successful sexual reproduction. *Vallisneria americana* has characteristics and a history that would indicate potential risk of both inbreeding depression and outbreeding depression. Once a dominant species influencing ecosystem function in freshwater and oligohaline portions of the Bay (e.g. Kemp et al. 2005), *V. americana* has greatly declined in abundance and distribution (Brush and Hilgartner 2000) such

that populations are a small fraction of their historical size (Orth and Moore 1983). As a dioecious species, small populations have an elevated risk of lacking compatible mates and may suffer increased effects from mating among relatives. Genotypic diversity in 26 Chesapeake Bay populations varies greatly, ranging from 0 (populations consisting of one single clone) to 1 (populations made up of completely unique genotypes; Lloyd et al. 2011), a phenomenon also seen for other clonal aquatic species (Arnaud-Haond et al. 2010). This means that sites ranged from having no detectable sexual reproduction to no detectable asexual reproduction. Variation in genotypic diversity within populations is mirrored by microsatellite allelic variation, which ranges from 1.5-5.8 alleles/locus. Heterozygosity ranges from moderate heterozygote deficit (F_{IS}=0.193), indicating potential risk of inbreeding, to large excess (F_{IS}=-0.667), indicating either recent bottlenecks or the presence of a heterozygote advantage. At the same time, evidence of genetic differentiation (Lloyd et al. 2011) and local adaptation (Engelhardt et al. 2014b) is accumulating. Assignment tests indicate four genetic regions in the Bay (Figure 4.1), suggesting long-term limitations to gene flow among some populations and connections among others (Lloyd et al. 2011). Common garden experiments have demonstrated population level differences in growth rates and allocation of resources to leaf extension versus ramet production that are also mediated by the environment (Engelhardt et al. 2014b).

We predicted that if local adaptation is strong, crosses within populations would produce more, higher quality seeds that germinate than crosses among populations within genetic regions, which, likewise, would be more successful than

crosses among regions. Alternatively, we expected that crosses between individuals from different populations would yield higher trait values if inbreeding in populations is relieved. To move beyond simple dichotomous comparisons of within versus among population crosses, we explicitly tested if reproductive success was affected by degree of relatedness among individuals, amount of genetic diversity within populations, or differentiation among populations.

Methods

Collection Locations and Protocol

We sampled *V. americana* in summer 2007 from tidal and non-tidal reaches of Chesapeake Bay tributaries (Lloyd et al. 2011), collecting ~30 shoots, 5-10m apart, from 11 populations. Individuals from the populations were propagated in estuarine sediment at the University of Maryland Center for Environmental Science Appalachian Laboratory greenhouse. Shoots had previously been genotyped at 10 microsatellite loci (Burnett et al. 2009, Lloyd et al. 2011) and grouped into four regions based on minimal deviations from both Hardy-Weinberg and linkage equilibrium (Figure 4.1). Regions were designated as the North-Chesapeake (including CP, EN, FB, and SASS), Mid-Chesapeake (DC, HWC, MP, and SFP), Potomac River (MATTA and SWP), and York River (HL).

In order to produce replicates of genotypes that had little field condition legacy we cloned all collected plants (n≈330) over multiple seasons in a common environment (Kawecki and Ebert 2004). Genotype sex was determined by production of staminate versus pistillate flowers. To clone the samples we harvested turions after senescence in fall 2007, stored them in 4˚C water in the dark, and planted multiple

turions of each genotype in 2008. Turions were again harvested at the end of the growing season.

Reproductive Crosses

In 2009, we planted turions from 2008 in separate containers. We planted ~6 replicates for each unique female and male genotype. Maternal turion size (length and width) was measured for a subset of the planted genotypes (n=15). Reproductive crosses were designed to include males and females 1) from within the same population, 2) from different populations within the same genetic region, and 3) from different populations from different regions. Replication of crosses was limited by timing and quantity of male and female flowers. *Vallisneria americana* pollen is only viable for a few days (McFarland and Shafer 2008), and we found that female flowers were only receptive for ~24 hours. These limitations precluded a full factorial design of within-versus-among population crosses. Therefore, we emphasized within-population crosses (n=158) as well as crosses that included females from each population pollinated by males representing two distinct populations and genetic regions – HWC from the Mid-Chesapeake Region (n=113) and SWP from the Potomac River region (n=94; Table 4.1). In sum, 300 crosses were produced that involved the use of 71 unique female and 50 unique male *V. americana* genotypes.

As plants bloomed, female flowers were hand pollinated using pollen from one male genotype per female replicate to ensure unambiguous attribution of paternity. Even though plants produce multiple flowers per reproductive event, just one was pollinated per replicate bucket. Various fathers were used to pollinate

different replicates of the same female genotype. Successful pollination led to the production of a single fruit per cross. We harvested mature fruits in October and measured fruit and seed traits. Fruits are cylindrical capsules that contain hundreds of small, dark seeds embedded in a clear gelatinous matrix. We measured capsule length and width to calculate capsule area. We counted the number of seeds in every capsule and calculated average length per cross from 10 randomly chosen seeds. Seeds were stored in tap water in the dark at 4˚C until germination trials.

In January 2010, we assessed germinability of 10 randomly selected seeds from each harvested fruit by planting seeds in Petri dishes. To remove orientation effects on germination, we stabilized the seeds in a horizontal orientation in 0.2% agar covered with a thin layer of dechlorinated tap water (Baskin and Baskin 1998). We randomly placed Petri dishes in a growth chamber at 30˚C with a 12 hour light-dark cycle at ~200 μmol $m^{-2}s^{-1}$ of fluorescent light, conditions found to be optimal for *V. americana* germination in previous research (Jarvis and Moore 2008). Water was added daily to compensate for evaporation and the locations of petri dishes were rerandomized weekly. We monitored germination, defined as emergence of the radicle at least 1mm from the seed coat (Jarvis and Moore 2008), daily for 30 days and calculated percent of successful germination events per cross.

Estimating Relatedness

Variation in degree of genetic relatedness among crossed individuals can be a source of uncontrolled variation, especially in species with large ranges in genotypic diversity and broad distribution of a few clones (Lloyd et al. 2011). Because full diallel crosses were not possible we wanted to account for the effect that relatedness

might have on seed production and germination between any two crossed individuals. Randomly crossing more or less related individuals within or among populations or regions may bias our results. In absence of known pedigree information, estimated relatedness can be used to understand the genetic component of phenotypic similarity (see Appendix A). To account for effects of this variation on reproductive success we used multilocus genotypes (Lloyd et al. 2011) to calculate Wang's (2002) estimator of pairwise relatedness between crossed individuals. We chose Wang's estimator because Monte-Carlo simulations (Table A1) indicated it had the lowest variance and minimal bias across various relationship categories (Van de Casteele et al. 2001). Relatedness ranges from 0 (unrelated) to 1 (identical clones). Sometimes Wang's relatedness estimates are negative, which is also interpreted as unrelated (Wang 2002). Pairwise relatedness was included as a random factor in all subsequent data analysis.

Statistical Analysis

Statistical analyses on reproductive fitness were performed using The SAS® System for Windows (SAS Institute, Inc.). We used nested one-way ANOVAs with the Satterthwaite approximation to account for unequal sample variances to determine if capsule area, seed count, or seed length differed between regions in the within-population crosses. Population source was treated as a random effect nested within region. Pairwise relatedness was included as a random effect. Likewise, one-way ANOVA was used to test for differences among populations in the within-population crosses, followed by post-hoc Tukey-Kramer tests. Differences in germination by region and population were examined with chi-square tests of independence.

In the within population crosses we used Spearman rank correlation (R Core Team 2011) to quantify relationships between variation in seed trait variables with one another as well as with genetic diversity and differentiation metrics. Specifically, capsule area, seed count, seed length, and germination success were compared with the genotypic diversity (the proportion of unique genotypes found in a population), average number of alleles, number of private alleles, observed and expected heterozygosities of each population, the average population relatedness of all individuals originally sampled from each population in the Chesapeake Bay, and the average relatedness among only the crossed individuals. We estimated relatedness among populations by averaging relatedness estimates for pairwise comparisons of genotypes collected from different populations (Table 4.2). Average among population relatedness was compared to Hedrick's heterozygosity-corrected measure of population divergence (G'_{ST}; Hedrick 2005) as calculated from the program SMOGD (Table 4.2; Crawford 2010). Hedrick's G'_{ST} is a derivative of Wright's F_{ST} that is more appropriate for comparisons of loci that have different mutation rates, like microsatellites. To conserve family-wise error rates among multiple correlation comparisons, Bonferroni corrections were applied.

To quantify effects of mixing sources on capsule area, seed count, or seed length, we performed a suite of statistical analyses on crosses that used only HWC or SWP pollen. First, we used one-way ANOVAs and Tukey-Kramer tests to test for differences in fruit and seed traits in crosses classified as either within-population (e.g., HWC x HWC), among-population within the same region (e.g., SFP x HWC), or among-region (SASS x HWC). Using the same analyses, we also tested for

differences in fruit and seed traits across all pairwise population combinations. We then used two-way ANOVAs on data from HWC- and SWP-pollinated crosses to determine whether interactions between maternal and paternal population sources could be observed.

The effects of different pollen sources on fruit and seed production were assessed using one-way ANOVA on mothers crossed with pollen from either within their population, from HWC, or from SWP. Contrasts within mothers were compared using F-tests to determine whether differences in seed or capsule production by pollen source exist. Differences in germination in the among-population crosses were examined with Chi Square tests of independence.

Maternal turion size was only collected for 15 of the 71 maternal genotypes used in crosses, spanning five Chesapeake Bay populations (DC, HWC, SFP, SWP, and MP). One-way ANOVA on this subset of the data found that maternal turion length was not significantly different among populations (ANOVA; $F_{4,10}$=1.71; p=0.224) or regions (ANOVA; $F_{1,13}$=2.61; p=0.130). Overall, 78 of the 300 crosses used flowers from these maternal genotypes, so we also used Spearman rank correlation (R Project v2.12.2, 2011) to determine if there were significant relationships between average maternal turion length and the capsule area, seed count, seed length, and percent germination resulting from crosses using these individuals. There were no significant correlations. Therefore, maternal turion size was not used as a covariate in the analyses.

Results

Within-Population Crosses

Of the 300 capsules produced, within-population crosses yielded 138 capsules, with an average length of 9.5±0.2cm (2.0-17.9cm) and width of 3.0±0.1mm (1.3-5.2mm). On average these capsules produced 137.7±6.5 seeds (0-385 seeds), with lengths averaging 2.6±0.02mm (1.91-3.20mm).

We observed no seed trait differences in within-population crosses among the four genetic regions. Despite lack of regional differences, individual populations differed from one another in capsule area (ANOVA; $F_{10,63.8}$=2.29; p=0.023) and seed count ($F_{10,63.4}$=2.51; p=0.013; Figure 4.2). The SFP and HL within-population crosses exhibited the lowest values in multiple traits (Figure 4.2). Germination also varied by population ($X^2_{10,1530}$=74.44; p<0.001), but not by region (Figure 4.3). At the extremes, seeds from crosses within SFP (3%), SASS (10%), and SWP (14%) germinated poorly whereas DC and MATTA had the highest germination success (43% and 38%, respectively).

Even after correcting for multiple comparisons with the Bonferroni correction, there are significant positive correlations between capsule area and seed count (r_s = 0.79, p<0.001), capsule area and percent germination (r_s = 0.29, p<0.001), and seed count and percent germination (r_s = 0.28, p<0.001). The average relatedness estimate for each population was positively correlated with the average relatedness of individuals used in the crosses (Table 4.3), indicating that crossed individuals represented their source populations. Without correcting for multiple comparisons, both estimates of population relatedness were negatively correlated with some genetic

diversity metrics (Table 4.3), however, genotypic diversity, average number of alleles per population, and the observed or expected heterozygosity of each population were not correlated with reproductive traits (Table 4.3). Average population relatedness was negatively correlated only with seed count (Table 4.3). However, after controlling for family-wise error rates among the multiple comparisons, these correlations are no longer significant. Thus, we observed no consistent association between reproductive variables and relatedness values for sampled Chesapeake Bay *V. americana*.

Among-Population Crosses

The among-population crosses produced 138 capsules with average lengths of 8.7±0.2cm (2.0-16.8cm) and widths of 2.9±0.04mm (1.3-5.0mm). On average, capsules produced 111.3±4.9 seeds (0-307), with lengths averaging 2.60±0.02mm (1.91-3.46mm).

HWC-pollinated crosses differed in seed count whereas SWP-pollinated crosses differed in seed length (Figure 4.1, 4.5). At the regional level, maternal sources from the North-Chesapeake region pollinated by HWC (from the Mid-Chesapeake region) produced more seeds than the other among-region crosses (ANOVA; $F_{4,63.9}$=4.55; p=0.003; Figure 4.4). Likewise, maternal sources from the Mid-Chesapeake pollinated by SWP (from the Potomac River) produced longer seeds than York-Potomac crosses (ANOVA; $F_{4,51}$=4.13; p=0.006; Figure 4.4). Region-level ANOVAs masked subtler differences in seed count and seed length between specific population combinations (Figure 4.5). However, no one cross type consistently outperformed the others (Figure 4.5).

Although certain combinations of regions or populations differed in capsule and seed production, no interactions between maternal and paternal population source on capsule area, seed count, and seed length were observed. Maternal population source accounted for some variation observed in seed count ($F_{10,75.6}$=3.43; p=0.001) and seed length ($F_{10,109}$=2.69; p=0.006). Regardless of pollen source, crosses involving mothers from populations in the North-Chesapeake typically produced many large seeds whereas crosses involving mothers from MATTA and HL consistently produced fewer, shorter seeds. However, comparison of fruit and seed production from crosses from a single maternal source and three different pollen sources (within-population, HWC, or SWP) revealed significant paternal effects in seed count (ANOVA; $F_{30,276}$=12.22; p<0.001) and seed length ($F_{30,271}$=4.49; p<0.001; Figure 4.6). Some populations crossed with SWP pollen were outperformed by the within-population or HWC-pollinated crosses, while other populations did better with SWP pollen (Figure 4.6). Thus, capsule and seed production tended to be population specific and differences were not consistent enough to produce an overall paternal-maternal interaction.

Germination success among crosses was also population specific. For example, SFP mothers crossed with either HWC (within-region) or SWP pollen (among-region) had 2.5% and 0% germination success, respectively, whereas offspring of CP mothers had high germination rates regardless of paternal source. Germination was higher overall for SWP-pollinated crosses than for HWC-pollinated crosses ($X^2_{1,2120}$=24.13; p<0.001; Figure 4.3), but this was largely driven by a few specific cases (Figure 4.3). Germination success did not differ between crosses that

occurred within-versus-among genetically defined regions. In contrast, within (32% germination) versus among (39% germination) population crosses differed ($X^2_{1,3000}$=5.17; p=0.023).

Not surprisingly, among population relatedness was negatively correlated with levels of population differentiation (r_s = -0.461, p<0.001). Individuals from populations in the North-Chesapeake or the Potomac River regions had the highest levels of relatedness to one another and these regions had the lowest differentiation among populations (Table 4.2). Despite similar levels of relatedness and differentiation, populations from the North-Chesapeake tended to produce many large seeds whereas Potomac River populations had less robust seed production (Figures 4.2, 4.4, 4.5).

Discussion

Risk of Inbreeding and Outbreeding Depression

Most restoration practitioners would agree that benefits and risks of genetic rescue (alleviation of inbreeding and recovery of genetic diversity; Frankham 2010, Frankham et al. 2011) versus outbreeding depression (McKay et al. 2005, Broadhurst et al. 2008, Frankham et al. 2011) must both be considered. Differences in opinions arise regarding which risks are higher and more pervasive. Increasingly, advocates of restoration strategies that involve mixing sources suggest that risks of outbreeding depression are overemphasized and poorly supported (Frankham et al. 2011, Weeks et al. 2011). In contrast to the simplistic dichotomous framework for dominance of one risk over the other, we find that neither has overwhelmingly strong or consistent effects in *V. americana* from the Chesapeake Bay. The fact that many within-

population crosses were more successful than among-population crosses provides evidence for local adaptation and concerns over outbreeding depression. In contrast, more and larger seed production with higher rates of germination in some among-population crosses indicate potential genetic rescue. It is disconcerting that none of the easily measured aspects of genetic diversity were useful in predicting which populations might need genetic rescue and which would be at risk of outbreeding depression. Rather, we see a complicated picture in which reproductive success varies independently of measured genetic diversity and relatedness.

Frankham et al. (2011) suggest that risk of outbreeding depression in crosses among populations is heightened when populations have fixed chromosomal differences, have had limited gene flow during the past 500 years, or inhabit different environments. Information from previous studies on *V. americana* in the Chesapeake Bay shows differentiation among populations. The four genetic regions in the Bay (Figure 4.1) suggest long-term limitation to gene flow between populations assigned to different regions and connections among populations within regions (Lloyd et al. 2011). Populations exist in a variety of environmental conditions (e.g. salinity, turbidity, sediment composition) and there is mounting evidence of local adaptation to specific habitats (Engelhardt et al. 2014b). At the same time, populations have been reduced to a fraction of their historical size and now occupy isolated patches in the Bay, a situation that is known to increase risk of reducing genetic diversity and experiencing inbreeding depression (Ellstrand and Elam 1993, Aguilar et al. 2008, Frankham et al. 2011). For such populations, genetic rescue by reestablishing gene

flow or by supplementing individuals from more genetically diverse populations (Frankham 2010) is often suggested.

Weeks et al. (2011) proposes that when population divergence is low, translocation of individuals among populations can occur without the need to go through a risk-assessment for outbreeding depression. However, they do not define 'low divergence' and instead offer a few case studies of species with very different life histories. In our experiments, populations with lower measures of divergence (e.g. G'_{ST}=0.09 between CP and SWP and G'_{ST}<0.01 between MATTA and SWP) produced significantly fewer seeds than when crossed within-populations (Figure 4.6). Likewise, when we control for family-wise error rate, none of the population genetic diversity metrics were correlated with reproductive output traits (Table 4.3). Thus, low divergence and genetic structure of populations may not be the best predictor of successful population mixing.

Correlation between levels of genetic diversity and fitness may be weak if the genetic markers used to estimate genetic diversity are neutral, genetic variation is nonaddative, or there is differential selection on the measured traits (Reed and Frankham 2001, Reed and Frankham 2003). Despite these theoretical limitations, a large body of literature suggests that genetic diversity estimates from neutral markers like allozymes and microsatellites, are good proxies for population fitness and adaptive potential (Merilä and Crnokrak 2001, Reed and Frankham 2003, Reynolds et al. 2012a). For example, despite differences in magnitude between quantitative traits and measures of genetic differentiation, Merilä and Crnokrak (2001) found the measures were positively correlated, suggesting that divergence in neutral markers

may be indicative of the degree of genetic differentiation in quantitative traits. Likewise, Hufford and colleagues (2012) were able to use molecular marker data to predict the scale of outbreeding depression while other studies have found measures of genetic diversity, like level of inbreeding and number of alleles, were consistent predictors of heterosis when mixing individuals from different populations (Pickup et al. 2013). Studies like these have led to the creation of plant restoration guidelines for the translocation of individuals that rely primarily on levels of genetic diversity and differentiation (e.g. Weeks et al. 2011). However, the results presented here as well as in other studies (reviewed in Reed and Frankham 2001) find low correlation between molecular markers and measured traits, suggesting that molecular markers alone cannot be used to predict population fitness and potential for population persistence.

Because among-population and among-region crosses did not consistently outperform within-population or within-region crosses in seed production (Figure 4.4, 4.5) there is no strong evidence of genetic rescue benefits. Specific population combinations, however, had reduced or enhanced reproductive output. Unfortunately, seed production was not predicted by any genetic metrics that sometimes indicate outbreeding or inbreeding depression risk. For *V. americana*, therefore, common-garden or field based experiments that cross individuals among populations are needed to assess potential outbreeding depression and rescue effects prior to restoration.

Although our results provide valuable insights, experiments were limited to fitness effects manifested early in the *V. americana* life cycle under benign

greenhouse conditions. Our results were further limited to seed production traits, but there may be differences in correlations between genetic differentiation metrics and morphological versus life history traits (e.g. Merilä and Crnokrak 2001). While we found no correlation between seed production and measures of genetic diversity and differentiation, it is possible that the morphological traits that have already demonstrated population level differences in growth rates and allocation of resources to leaf extension versus ramet production (Engelhardt et al. 2014b) may be better correlated with genetic diversity. Furthermore, fitness effects of both inbreeding and outbreeding are often greater in later life stages (Holtsford and Ellstrand 1990, Husband and Schemske 1996) and in subsequent generations (Edmands 2007, Broadhurst et al. 2008, Huff et al. 2011) as well as under stressful conditions (Carr and Dudash 1995, Keller 1998, Crnokrak and Roff 1999, Murren and Dudash 2012). This research focused specifically on sexual reproductive fitness because of its importance in establishing diverse populations post restoration. Research on other macrophytes has demonstrated that genetically diverse assemblages do better in terms of plant productivity in both stressed and non-stressed environments (e.g. Reusch et al. 2005, Reynolds et al. 2012a).

Additional Factors Affecting Reproductive Output

Vallisneria americana reproduces vegetatively and sexually (McFarland and Shafer 2008), and we see evidence that suggests a tradeoff between seed production and allocation to vegetative expansion or turion production. For example, Lloyd et al. (2011) found that in most populations >70% of samples were unique genotypes, but some populations consisted of a single clone. Only 29% of the sampled genotypes

from the SFP population were unique, but relatedness estimates were low (Figure 4.2) indicating high *in situ* vegetative reproduction and low inbreeding during sexual reproduction. Despite no indication of inbreeding, this population had poor seed production (Figure 4.2). It did, however, rank high relative to the other populations in turion production (K. Engelhardt, 2011, UMCES Appalachian Laboratory, Frostburg, MD, unpublished data), producing a mean of 18 turions per replicate clone (n=6) within one growing season. In contrast, 89% of genotypes in the CP population were unique (Lloyd et al. 2011). Crosses involving CP mothers had higher seed production than average (Figure 4.2), yet the mean number of turions per clonal replicate within one growing season was <7 (K. Engelhardt, 2011, UMCES Appalachian Laboratory, Frostburg, MD, unpublished data). These observations suggest an inverse relationship between vegetative and sexual reproductive fitness, irrespective of the degree of relatedness among crossed individuals. Furthermore, in other aquatic plants there is evidence of tradeoffs between sexual and asexual reproduction that are mediated by the environment (e.g. Prati and Schmid 2000, Xie and Yu 2011). The presence of stressful environments or increased competition may lead to an increase in sexual allocation of resources relative to asexual reproduction (e.g.Prati and Schmid 2000). Alternatively, the submersed macrophyte *Potamogeton crispus* produces turions of greater mass in nutrient-poor sediment compared with plants grown in nutrient-rich sediment (Xie and Yu 2011). If our populations are genetically adapted to reproduce dominantly by either sexual or asexual reproduction under low stress conditions, then our seed production data may be biased since all plants were grown in a stress-free greenhouse environment. How these reproductive tradeoffs

interact with and influence genetic diversity and population persistence over time are key future research topics.

Implications for Restoration

Our objective was to evaluate relative risks and benefits of using local versus non-local plantings in restoration as indicated by *V. americana* seed production and germination success. Restoration of aquatic species in the Chesapeake Bay typically involves planting locally sourced material, including whole individuals harvested from beds in the same tributary, individuals reared from seeds harvested from nearby beds, or individuals from repositories that were initially established from local populations (Lloyd et al. 2012). Reynolds et al. (2012k) demonstrated that *Zostera marina* seeds harvested from multiple parents from nearby beds can preserve genetic diversity in restored sites with no signs of inbreeding depression in either donor or restored sites. Lloyd et al. (2012) found that current *V. americana* restoration techniques generally reflect the genetic diversity found in natural populations in the Chesapeake Bay. We see no strong argument against local sourcing in this case because most populations are not inbred based on microsatellite markers, and population level differences in seed production (Figure 4.2) and germination (Figure 4.3) suggest potential for local adaptations or differences in compatibility among populations. Similarities in seed production and germination between crosses that occurred within-regions (Figure 4.4) indicate that movement within-regions does not substantially affect local adaptation if it exists. Additionally, very few of the among-population crosses were substantially better than within-population crosses, indicating little benefit from genetic rescue. Some specific populations were consistently weak

(e.g. HL, SFP) or had low replication (e.g. DC, SFP) and thus warrant further investigation.

In summary, the accumulating evidence for *V. americana* in the Chesapeake Bay is that most remnant populations are diverse in terms of the number of genotypes and alleles and do not suffer from heterozygote deficiencies (Lloyd et al. 2011). Although we do see evidence of population level differences in morphology and reproductive success, we do not see systematic patterns that indicate widespread inbreeding or outbreeding depression. Increasing submersed aquatic grass coverage worldwide is a major restoration goal because of the vital ecosystem services they provide the Chesapeake Bay (Orth and Moore 1983). Even though risk of outbreeding depression is low for *V. americana* in the Bay, most populations have sufficient genetic diversity and the potential cost of losing local adaptations outweighs the potential benefits of mixing multiple sources when attempting to increase coverage. The most disconcerting finding was that the performance of populations and crosses was not consistently explained by easily quantified genetic diversity, differentiation, and relatedness metrics suggested for assessing risk of inbreeding versus outbreeding depression. The degrees of differentiation among populations and inbreeding within populations fall along continuous gradients that vary independently. This finding highlights the need of identifying better metrics or methods to help conservation practitioners efficiently select restoration stock that best balances the risks of inbreeding/outbreeding depression, which are not as dichotomous as previously suggested, while providing the most benefit in terms of genetic rescue and long-term persistence.

Table 4.1: Replication (rep.) numbers of controlled *Vallisneria americana* reproductive crosses by maternal (rows) and paternal (columns) population sources nested within four genetic regions of the Chesapeake Bay.

			Paternal Source											
	Region		North-Chesapeake Bay				Mid-Chesapeake Bay				Potomac River		York River	Total Rep.
		Population	CP	EN	FB	SASS	DC	HWC	MP	SFP	MATTA	SWP	HL	
Maternal Source	North-Chesapeake Bay	CP	5	-	-	-	-	2	-	-	-	4	-	11
		EN	-	13	-	-	-	3	-	-	-	3	-	19
		FB	-	-	12	-	-	7	-	-	-	8	-	27
		SASS	-	-	-	26	-	14	-	-	-	7	-	47
	Mid-Chesapeake Bay	DC	-	-	-	-	3	3	-	-	-	4	-	10
		HWC	-	-	-	-	-	56	-	-	-	42	-	98
		MP	-	-	-	-	-	9	19	-	-	5	-	33
		SFP	-	-	-	-	-	4	-	3	-	2	-	9
	Potomac River	MATTA	-	-	-	-	-	5	-	-	8	5	-	18
		SWP	-	-	-	-	-	5	-	-	-	9	-	14
	York River	HL	-	-	-	-	-	5	-	-	-	5	4	14
	Total Rep.		5	13	12	26	3	113	19	3	8	94	4	300

Table 4.2: Genetic relatedness among *Vallisneria americana* individuals within or among populations and differentiation of population within and among four genetically defined regions of the Chesapeake Bay. Hedrick's heterozygosity-corrected measure of divergence (G'_{ST}) is above the diagonal (white), the average Wang pairwise relatedness measure for individuals in populations is on the diagonal (dark grey) and the mean relatedness of all pairs of individuals among the specified populations is below the diagonal (light grey). Relatedness estimates above zero are in bold. Population abbreviations are defined in Figure 4.1

Region		North-Chesapeake Bay				Mid-Chesapeake Bay				Potomac River		York River
	Pop	CP	EN	FB	SASS	DC	HWC	MP	SFP	MATTA	SWP	HL
North-	CP	**0.03**	0.03	<0.01	0.02	0.08	0.07	0.09	0.13	0.05	0.09	0.21
Chesapeake	EN	<-0.01	**0.11**	0.04	0.02	0.07	0.05	0.12	0.12	0.03	0.07	0.22
Bay	FB	**0.05**	**0.02**	**0.08**	0.04	0.06	0.03	0.09	0.10	0.02	0.09	0.19
	SASS	-0.04	-0.02	-0.03	-0.03	0.10	0.08	0.09	0.12	<0.01	0.08	0.19
Mid-	DC	-0.16	-0.16	-0.11	-0.19	-0.05	0.015	0.02	0.05	0.06	0.12	0.22
Chesapeake	HWC	-0.16	-0.13	-0.12	-0.17	-0.13	**0.05**	0.02	0.07	0.02	0.13	0.15
Bay	MP	-0.16	-0.20	-0.12	-0.18	-0.09	-0.10	-0.01	0.10	0.10	0.19	0.20
	SFP	-0.18	-0.12	-0.10	-0.14	-0.06	-0.19	-0.12	**0.14**	0.09	0.15	0.19
Potomac	MATTA	-0.03	**0.02**	-0.05	-0.02	-0.10	-0.16	-0.19	-0.02	**0.29**	<0.01	0.02
River	SWP	-0.10	-0.02	-0.07	-0.05	-0.16	-0.19	-0.12	-0.11	**0.16**	**0.18**	0.16
York River	HL	-0.12	-0.12	-0.11	-0.09	-0.17	-0.05	-0.10	-0.07	**0.11**	-0.04	**0.51**

Table 4.3: Spearman Rank Correlation coefficients among measures of relatedness for crossed individuals, average population relatedness, population genetic diversity metrics (from Lloyd et al. 2011), and average seed trait variables from within each *Vallisneria americana* population from the Chesapeake Bay. Correlation coefficients significant at the 0.05 level without correction for multiple comparisons are designated boldface. Superscripts denote changes to correlation coefficients after correcting for family-wise error rates.

	Crossed Individual's R	Average Population R	Genotypic Diversity	A	A_p	H_o	H_e
Crossed Individual's R	--	**0.65**	-0.35	-0.61	-0.21	-0.12	**-0.72** [A]
Average Population R	**0.65**	--	**-0.69** [A]	**-0.78** [A]	-0.40	0.05	-0.49
Capsule Area	-0.27	-0.58	0.23	0.47	0.08	0.08	-0.13
Seed Count	-0.26	**-0.74**[B]	0.40	0.50	0.16	-0.05	-0.07
Seed Length	-0.29	-0.31	0.08	0.43	-0.28	0.52	0.50
% Germination	-0.03	-0.20	0.16	0.17	-0.08	-0.02	-0.24

R = Wang's (2002) estimator of relatedness, A = average number of alleles, A_p = number of private alleles, H_o = observed heterozygosity, H_e = expected heterozygosity. Genotypic diversity = $(G - 1)/(N - 1)$

[A]: These correlation coefficients are no longer significant after controlling for family-wise error rate with the Bonferroni correction across the 10 comparisons between the 5 genetic metrics and the 2 estimated relatedness metrics.

[B]: This correlation coefficient is no longer significant after controlling for family-wise error rate with the Bonferroni correction across the 28 comparisons between seed traits variables and genetic metrics.

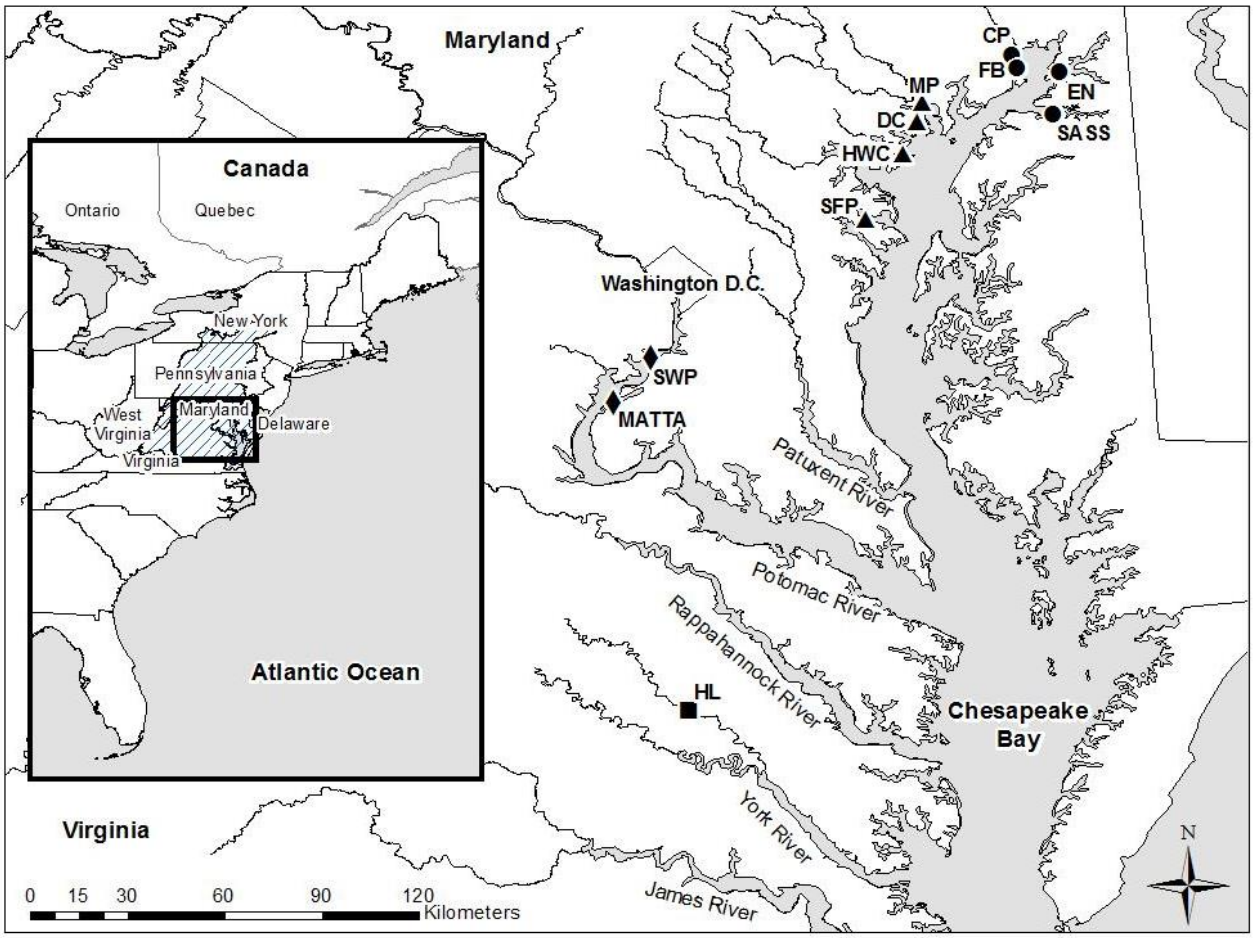

Figure 4.1: *Vallisneria americana* collection locations in the Chesapeake Bay. Population abbreviations are as follows: Concord Point, Susquehanna Flats, MD (CP), Elk Neck, Elk River, MD (EN), Fishing Battery, Susquehanna Flats, MD (FB), Sassafras River, MD (SASS), Dundee Creek, Gunpowder River, MD (DC), Rocky Point Hawks Cove, Back River, MD (HWC), Mariner Point, Gunpowder River, MD (MP), South Ferry Point, Magothy River, MD (SFP), Mattawoman Creek, Potomac River, MD (MATTA), Piscataway Park, Potomac River, MD (SWP), and Horse Landing, Mattaponi River, VA (HL). Regional assignments to the North-Chesapeake (circle), Mid-Chesapeake (triangle), Potomac River (diamond), and York River (square) were based on previous population genetic analysis (Lloyd et al. 2011).

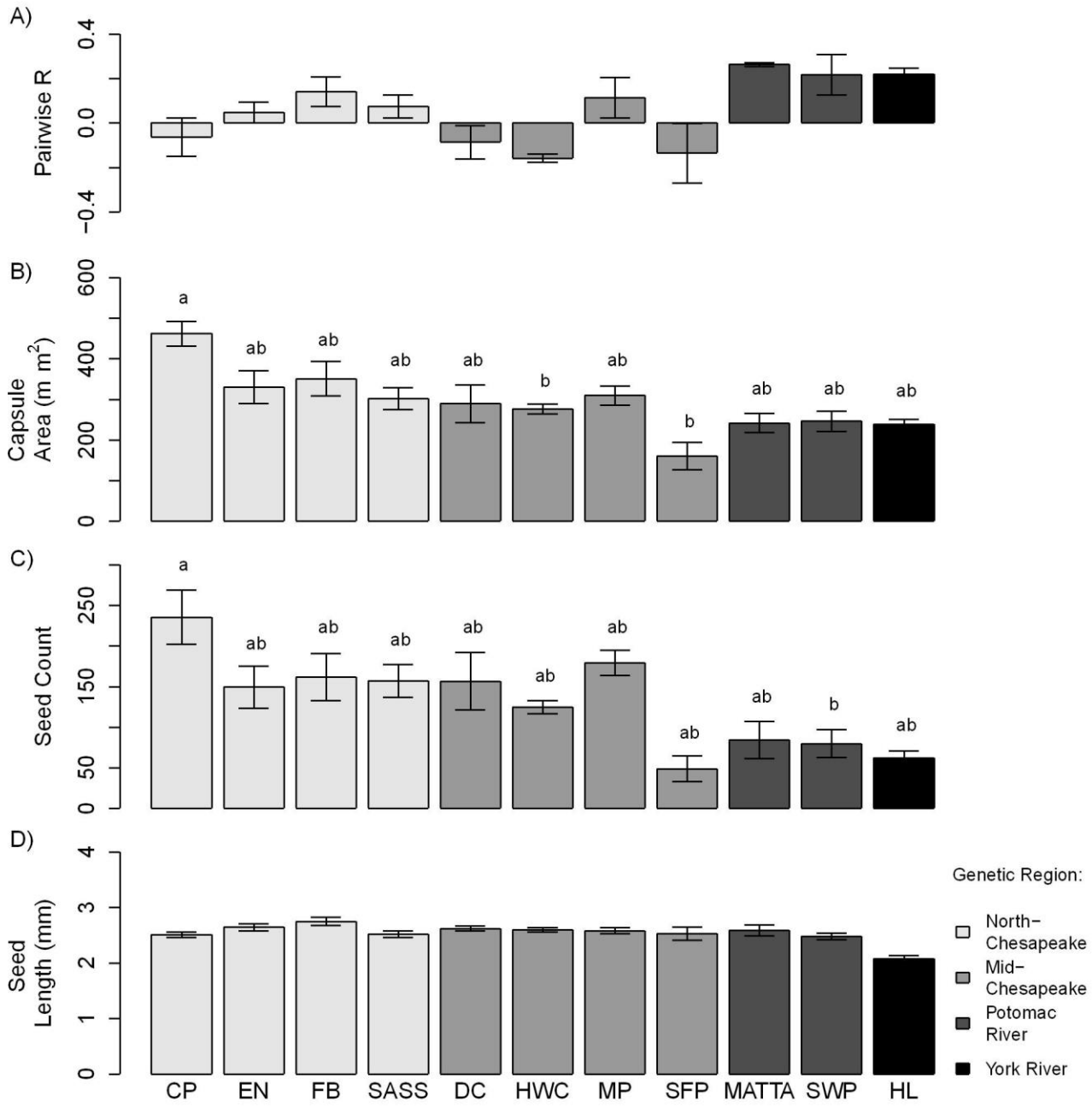

Figure 4.2: Population means and standard errors of a) pairwise relatedness between crossed individuals, b) capsule area, c) seed count, and d) seed length from Chesapeake Bay within-population *V. americana* crosses. Different letters in panels b and c denote significant differences between pairs of means at the 0.05 level based on ANOVAs with the Satterthwaite approximation to account for unequal sample variances and posthoc Tukey-Kramer tests. ANOVAs were not used to assess differences in relatedness. Light gray indicates populations from the North-Chesapeake Region, gray indicates populations from the Mid-Chesapeake Region, dark gray indicates populations from the Potomac River, and black indicates populations from the York River.

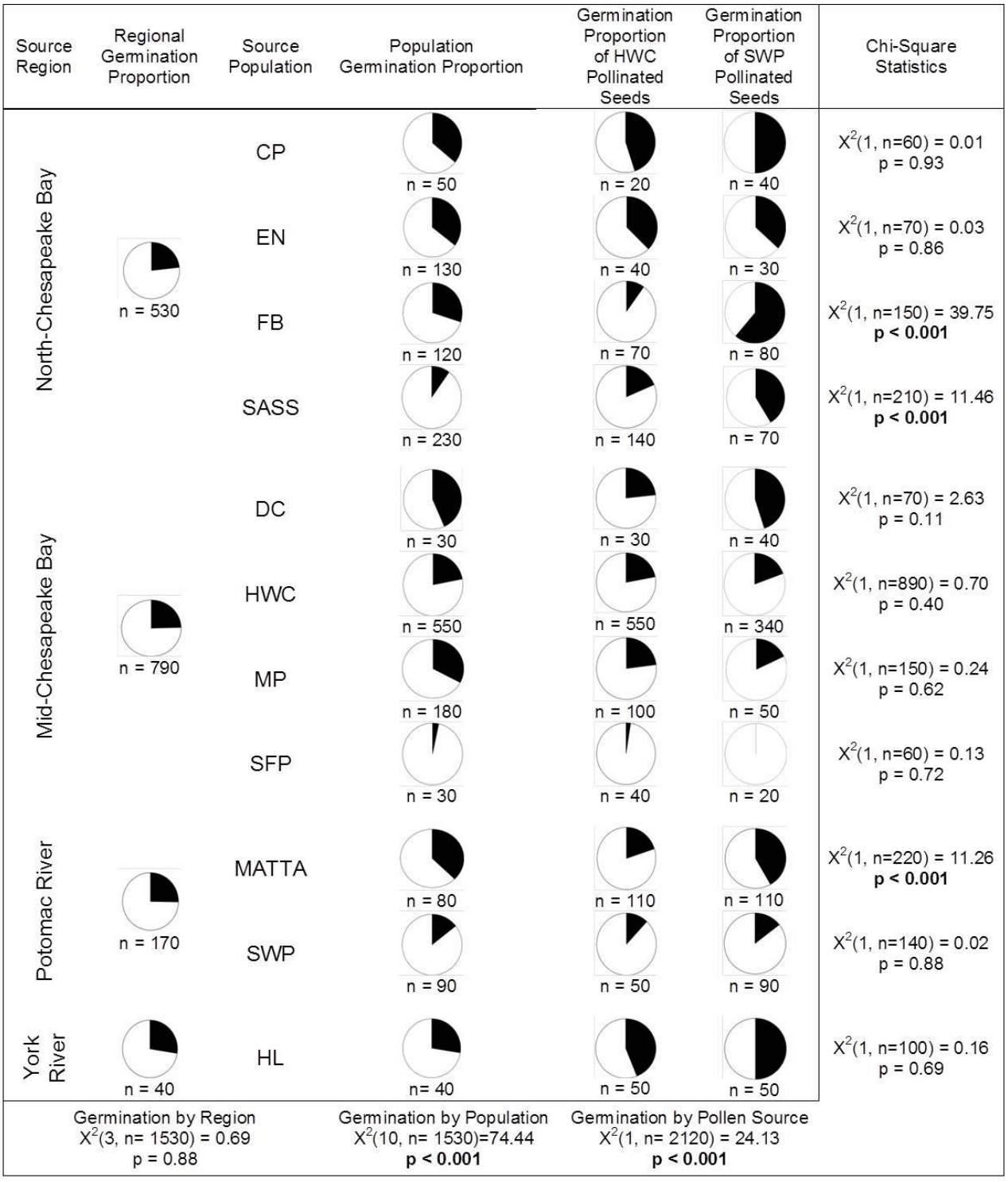

Figure 4.3: Proportion of successfully germinated *V. americana* (10 seeds per cross) pollinated within each region and population as well as from either HWC or SWP sources. Chi Square tests of independence were used to determine if germination count varied significantly by region, population, or by HWC versus SWP pollen source. Black designates successful germination and white designates unsuccessful germination.

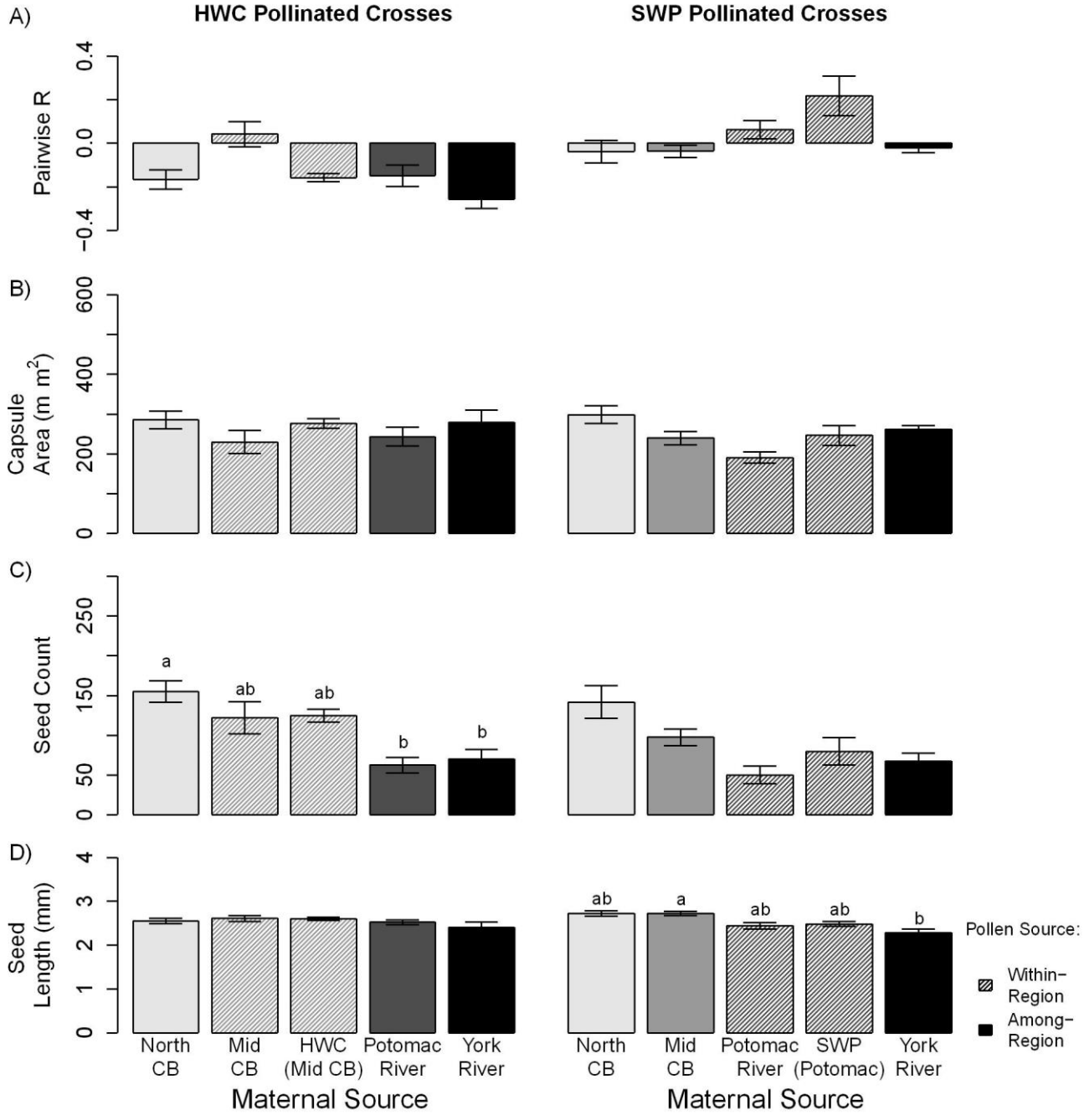

Figure 4.4: Means and standard errors of a) pairwise relatedness between crossed individuals, b) capsule area, c) seed count, and d) seed length from Chesapeake Bay (CB) *V. americana* crosses pollinated by either HWC or SWP pollen, grouped by maternal region. Different letters in panels c and d denote significant differences between pairs of means at the 0.05 level based on ANOVAs with the Satterthwaite approximation to account for unequal sample variances and posthoc Tukey-Kramer tests. Lack of letters denotes no observed significant differences. ANOVAs were not used to assess differences in relatedness.

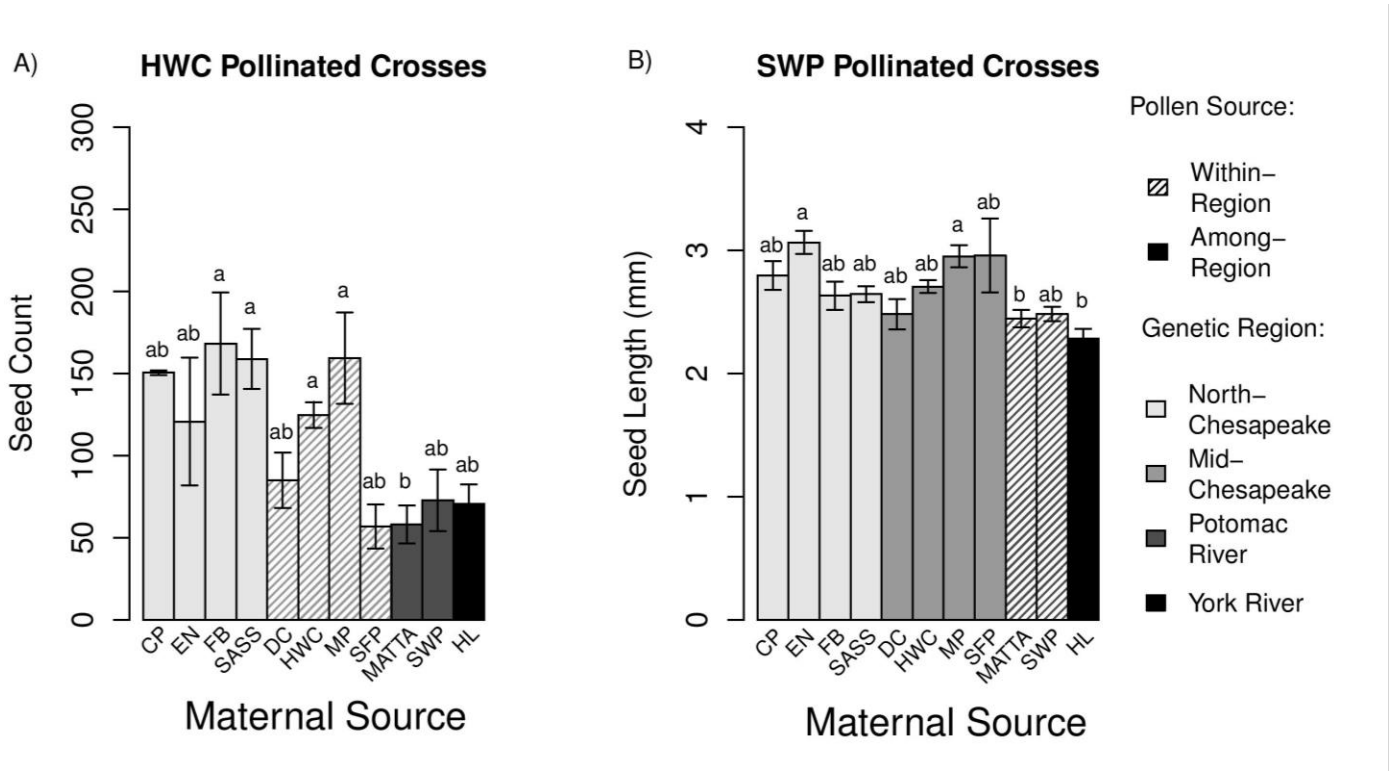

Figure 4.5: Means and standard errors of a) seed count from Chesapeake Bay *V. americana* crosses pollinated by HWCpollen and b) seed length from Chesapeake Bay *V. americana* crosses pollinated by SWP pollen. Results are grouped by maternal population. Different letters denote significant differences between pairs of means at the 0.05 level based on ANOVAs with White's heteroscedasticity correction and posthoc Tukey-Kramer tests.

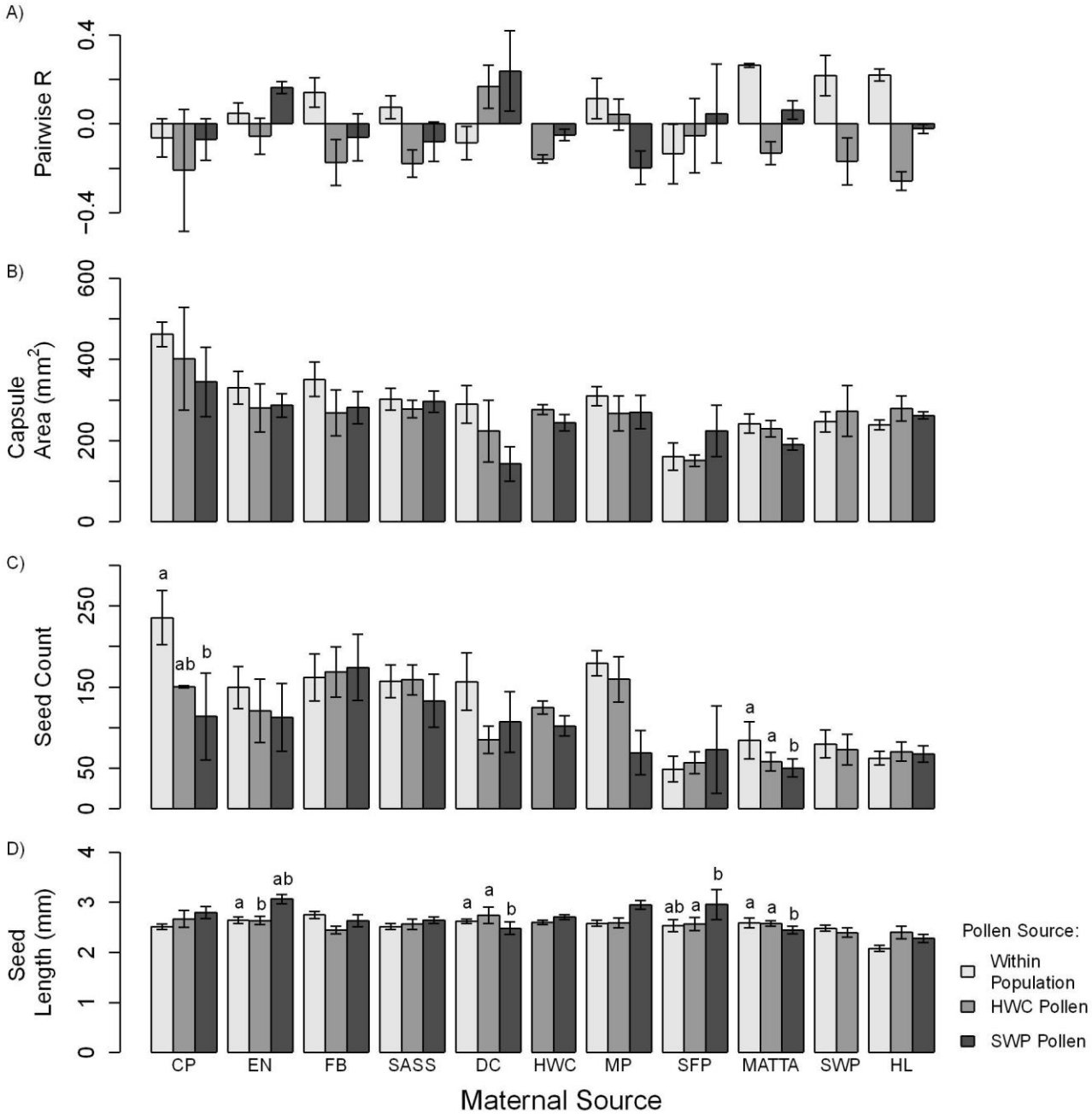

Figure 4.6: Means and standard errors of a) pairwise relatedness between crossed individuals, b) capsule area, c) seed count, and d) seed length from Chesapeake Bay *V. americana* crosses pollinated within-populations or with HWC or SWP pollen, grouped by maternal population. Different letters in panels c and d denote significant differences between pairs of means within a maternal population at the 0.05 level based on ANOVAs with White's heteroscedasticity correction and posthoc F- tests with comparison-wise error rates. Lack of letters denotes no observed significant differences. ANOVAs were not used to assess differences in relatedness.

Chapter 5: Conclusions and future directions of *Vallisneria americana* management, restoration, and research

A Growing Cause for Concern

Climate change and its effects on the potential persistence of natural populations is an increasing issue. Ecosystems around the globe will face novel disturbance regimes with increasingly greater differences from historical conditions (Carpenter et al. 2011, Scheffer et al. 2012). Coastal aquatic ecosystems, in particular, are already among the most threatened in the world and will be disproportionally affected by changes in sea surface temperature and sea level rise (Kennish 2002). Mounting evidence regarding changing climate and increased climate variability highlight the importance of maintaining or restoring resiliency to ensure the future persistence of natural populations and communities. Persistence of any population is ultimately a function of phenotypic diversity and plasticity (i.e. acclimation potential) and standing genetic and phenotypic variance (i.e. adaptation potential).

Submersed aquatic vegetation (SAV) is an important component of aquatic ecosystems that is already being negatively impacted by warmer water temperatures (e.g. Oviatt 2004), sea-level rise and salt water intrusion (Quammen and Onue 1993, French and Moore 2003), and large-scale disturbances (Kemp et al. 1983, Orth and Moore 1983, 1984, Fernald et al. 2012). *Vallisneria americana* Michx. (Hydrocharitaceae) was once a broadly distributed SAV species that dominated

freshwater to oligohaline environments in eastern North America. *V. americana* has declined dramatically and is the target of many conservation and restoration initiatives. Dispersal of *V. americana* across rivers separated by broad spatial scales is limited, thereby restricting the ability of *V. americana* to expand to locations that might be more suitable in the context of climate change. The ultimate objective of this research was to evaluate the potential for future persistence of *V. americana* through either acclimation or adaptation. Specifically, I assessed *V. americana* by 1) quantifying the structure of genetic diversity at multiple spatial scales, including a broad-scale assessment across tidal regions of three rivers in the Northeast United States (Marsden CH1) and a fine-sale analysis evaluating the structure of genetic diversity within the Potomac River (Marsden CH2), 2) evaluating evidence of either local adaptation or acclimation potential for V. *americana* sourced from the three rivers (Marsden CH3), and 3) assessing the scales at which sources of restoration stock can be mixed to reduce chances of outbreeding depression without exacerbating local inbreeding depression (Marsden CH4).

The results of this dissertation found that levels of *V. americana* genotypic and genetic diversity, local adaption, acclimation potential, and inbreeding versus outbreeding risk are site specific. Genotypic and genetic diversity varied greatly within the Potomac River and across the Potomac, Hudson, and Kennebec Rivers. Moreover, the distribution of genetic diversity had very different patterns of structure both within the Potomac River, primarily separated by divisions between the tidal and non-tidal regions, and across the three northeastern rivers. Plants sourced from the Potomac, Hudson, and Kennebec performed differently in controlled environment

experiments, with only plants from the Potomac River showing evidence of local adaptation to regional temperature and photoperiod. A lack of differences in the morphological and life history responses between *V. americana* sourced from different rivers provides evidence of acclimation potential to temperature and photoperiod. Finally, controlled reproductive crosses between individuals sourced from different spatial scales across the Chesapeake Bay, including within sites, across sites, and across genetically pre-defined regions show site dependent results with no overall patterns in the risks of inbreeding depression within sites or outbreeding depression when mixing sources across sites. Unfortunately, the site specific interactions found in this research preclude restoration managers from using information learned about genetic diversity, local adaption, acclimation, or risks of inbreeding versus outbreeding in one system to inform practice at another.

Restoration Practice: Past and Future

In response to global declines in SAV (Waycott et al. 2009), marine protected areas that include SAV have increased and major monitoring and restoration projects have been proposed and implemented throughout the world (Orth et al. 2006). To date, restoration efforts have primarily been implemented at small, local scales (Broadhurst et al. 2008) and used information on SAV sensitivity to water quality and light availability in order to guide selection of appropriate revegetation sites (Kemp et al. 2005). Unfortunately, many of these restoration efforts have been met with mixed or marginal success, with only a slight increases in SAV populations since the 1980s (e.g. Reid et al. 1993, Moore et al. 2000, Bologna and Sinnema 2005, 2006, Schenk and Rybicki 2006).

Assessments of genetic diversity are often not included in restoration planning and management decisions because this information is typically absent and it is expensive to obtain (summarized by Lloyd et al. 2011, Lloyd et al. 2012). Further, issues arising from low levels of genetic diversity are often seen as being secondary threats to more immediate concerns. However, the need to include processes that maintain genetic diversity and adaptive potential in restoration planning has been advocated for some time (Pressey et al. 2007, Mace and Purvis 2008) and some managers are starting to consider the potential benefits of accounting for genetic diversity in restoration planning (e.g. Campanella et al. 2010a, Campanella et al. 2010g, Reynolds et al. 2012a, Reynolds et al. 2012k). Genetic diversity affects population persistence in dynamic environments (e.g. Lande and Shannon 1996) and increases the chances for successful establishment and functioning of restored populations (Williams 2001, Reynolds et al. 2012a, Reynolds et al. 2012k).

This research provides evidence that most remnant populations of *V. americana* in the Potomac, Hudson, and Kennebec Rivers are diverse in terms of the number of genotypes and alleles and do not suffer from heterozygote deficiencies. These conclusions are consistent with previous findings by Lloyd et al. (2011) for *V. americana* in the Chesapeake Bay. There was also evidence of site level differences in the distribution of genetic variation, morphology, and reproductive success. Evidence of local adaptation to temperature and photoperiod conditions, for example, was restricted to Potomac River sites. However, limited evidence of local adaption in Hudson and Kennebec Rivers *V. americana* does not indicate that local adaption isn't present, only that plants from these sites are not locally adapted to these two factors.

Due to the heterogeneous nature of aquatic environments (Sanford and Kelly 2011), directional selection on *V. americana* populations may be driven by responses to more localized environmental variables like salinity, light, nutrients, or sediment. Therefore, even though risk of outbreeding depression is low for *V. americana* in the Chesapeake Bay and there is some evidence of acclimation potential, the potential cost of losing local adaptations outweighs the potential benefits of mixing multiple sources when attempting to increase SAV coverage via restoration.

As the effects of climate change continue to threaten SAV communities across the globe, restoration strategies are emerging to address management of natural populations when persistence, adaptation, and dispersal are not possible. Such strategies include managed relocation (MR; Richardson et al. 2009) and genetic translocation (Sgrò et al. 2011), which involve the intentional movement of populations or appropriately adapted genotypes from currently occupied areas to locations where probability of future persistence is predicted to be higher (Richardson et al. 2009). The importance of MR as a restoration strategy is likely to grow as changes in climate become more pronounced (Richardson et al. 2009). In fact, in their work studying the genetic diversity of the SAV species *Zostera marina*, Campanella et al. (2010a, 2010g) suggest that *Z. marina* from the regions of the Chesapeake Bay or northern Maine would serve as good donor sites to source restoration stock for planting in Barnegat Bay, NJ. However, because they only assessed one site within each studied region they were not able to examine the variability or distribution of genetic diversity within each latitudinal region. My data on *V. americana* spans a similar latitudinal gradient and shows signs of strong genetic

differentiation and dissimilarity across these scales. Therefore, mixing individuals or populations from different latitudes may not be successful and could even be detrimental if individuals are maladapted to the new region and offspring from mating between local and foreign individuals result in low fitness due to outbreeding depression (e.g. Montalvo and Ellstrand 2001).

Additional research is needed to evaluate the consequences of mixing individuals of *V. americana* across such genetically distinct populations. Although no signs of outbreeding depression were observed in controlled reproductive crosses within the Chesapeake Bay, it is possible that they would arise in reproductive crosses between individuals from different latitudes.

Summary of Pilot Projects

Initially this dissertation aimed to quantify the genetic diversity and phenotypic variation in *V. americana* collected across the species' entire latitudinal range, from Florida to Maine (Figure 5.1). In addition to the *V. americana* samples collected from the Potomac, Hudson, and Kennebec Rivers in 2011, samples were also collected from the Caloosahatchee River (n = 22), the Loxahatchee River (n = 30), and the St. John's River in Florida (n = 137) as well as the Santee River in South Carolina (n = 110). *V. americana* has genotypically based variation in growth characteristics observed at both regional levels (e.g. Engelhardt et al. 2014b) and geographic levels (e.g. Les et al. 2008). However, I have found preliminary evidence of phenotypic and genetic differences between northern and southern *V. americana* that may warrant reclassification of these two groups into either ecotypes or even different species, including lack of turion production in southern sourced *V.*

americana and limited reproductive success between northern and southern *V. americana* crosses.

Turion Production

In temperate climates *V. americana* populations overwinter as dormant winter buds (turions) buried in the sediment (Titus and Hoover 1991), while southern populations grow year round and never completely dies back in winter (Dawes and Lawrence 1989). Even though there are accumulating descriptions from natural resource managers that southern *V. americana* are non-turion producing, no studies have explicitly tested whether or not southern *V. americana* are even capable of producing turions when grown in conditions that lead to senescence.

Therefore, in January 2013 I tested turion production in 11 northern sourced *V. americana* from the Potomac, Hudson, and Kennebec Rivers and 11 southern sourced *V. americana* from the Loxahatchee and St. John's River. Containers that had been propagating in the University of Maryland greenhouse since 2011 were divided into four equal quadrants and replanted in new containers. One container from each *V. americana* sample was randomly placed into four growth chambers. Two growth chambers were set to Hudson River December solstice conditions (Temperature: 8°C; Photoperiod: 549 min) and two were set to St. John's River December solstice conditions (Temperature: 16°C; Photoperiod: 611 min; USGS, Observatory). By March 2013 all *V. americana* in lower temperature and photoperiod had gone through senescence as well as several of the northern sourced *V. americana* in the higher temperature and photoperiod treatment. All containers were harvested and turion production was only found in for the northern sourced *V.*

americana. No turions were produced in any of the containers of southern sourced *V. americana*.

Reproductive Success

Although we found evidence of site level differences in morphology and reproductive success between controlled crosses of *V. americiana* within the Chesapeake Bay, we did not find systematic patterns that indicate either widespread inbreeding or outbreeding depression from crosses within sites, among sites, or among genetically defined regions. To begin evaluating whether or not outbreeding depression would be a greater risk in more genetically distinct and geographically separated populations, I performed a pilot study crossing southern and northern sourced *V. americana*.

In July and August 2011 I crossed southern sourced *V. americana* from the Caloosahtchee River with northern sourced *V. americana* from the Chesapeake Bay (Figure 5.2). These plants had been propagating in the University of Maryland greenhouse since collection in 2010 and 2007, respectively. I performed three types of crosses: males and females from the Caloosahatchee River (n = 8); males and females from the Chesapeake Bay (n = 9); and males and females from different regions (n = 27). Fertilization success, fruit production, and seed germination from all crosses were assessed. Fertilization was successful for all reproductive cross types and there were no significant differences in measures of fruit or seed size. However, germination success was significantly higher in crosses that occurred within regions compared to between region crosses ($X^2_{1,26}$=1.440; $p<0.001$). Moreover, in the crosses between northern and southern *V. americana*, none of the seeds produced by southern

females germinated, indicating reduced viability and fitness of reproductive crosses between northern and southern sourced *V. americana*.

Future Research Directions

Vallisneria americana in the United States is largely described and managed as one species (except see Les et al. 2008). Papers make casual mention of different growth morphologies in *V. americana* between tropical and temperate regions (Dawes and Lawrence 1989, McFarland and Shafer 2008), but the above pilot studies demonstrate the need to reassess the genetic relationship between northern and southern *V. americana*. Moreover, if such major differences in life history traits like turion production and reproductive viability exist between *V. americana* sourced from the Chesapeake Bay and Florida, then it is probably that similar differences exist between *V. americana* populations separated by similar latitudes (e.g. between Potomac River and Kennebec River *V. americana*). Such differences would have major implications for restoration strategies like MR.

To expand upon initial work by Les et al. (2008) to examine phylogenetic differences in species of *Vallisneria*, I Illumina sequenced eight *V. americana* genotypes collected from across the latitudinal range of the species. *V. americana* genotypes were sourced from the Loxahatchee River (FL), the St. John's River (FL), the Santee River (SC), the Potomac River (MD), the Susquehanna flats in the Chesapeake Bay (MD), the Hudson River (NY), the Kennebec River (ME), and a repository in Wisconsin (described in Lloyd et al. 2012). A *Vallisneria neotropicalis* genotype provided by Dr. Donald Les was sequenced for comparison. *De novo* genome assemblies were completed in August 2014 using ABySS (Simpson et al.

2009). Identification of single nucleotide polymorphisms (SNPs) in highly variable regions across all nine genomes will allow us to use targeted resequencing protocols like restriction-site associated DNA (RAD) sequencing (Davey and Blaxter 2010) to genotype all *Vallisneria* samples. Moreover, Microsatellite markers are selectively neutral and thus follow Mendelian inheritance, which allows them to be used as a tool for detecting demographic patterns (Selkoe and Toonen 2006). In assessing genetic variation among populations, neutral marker variation is limited to assessing genetic drift of random mutations in populations (Nielsen 2005). Alternatively, non-neutral genetic markers can be used to assess variation in genetic diversity due to natural selection. Therefore, RAD-Seq will enable comparisons of neutral and non-neutral SNP markers to investigate the effects of genetic drift versus selection on variability within and among populations of *V. americana*. This will allow even better assessment of the potential resiliency of populations of *V. americana* in the face of climate change.

Figure 5.1: Map of the 55 *Vallisneria americana* sampling locations in seven major rivers along the eastern coast of North America. The sampled rivers include the Caloosahatchee River, Loxahatchee River, and St. John's River in Florida (red circles), Santee River in South Carolina (orange circles), Potomac River in Maryland (green circles), Hudson River in New York (blue circles), and Kennebec River in Maine (purple circles).

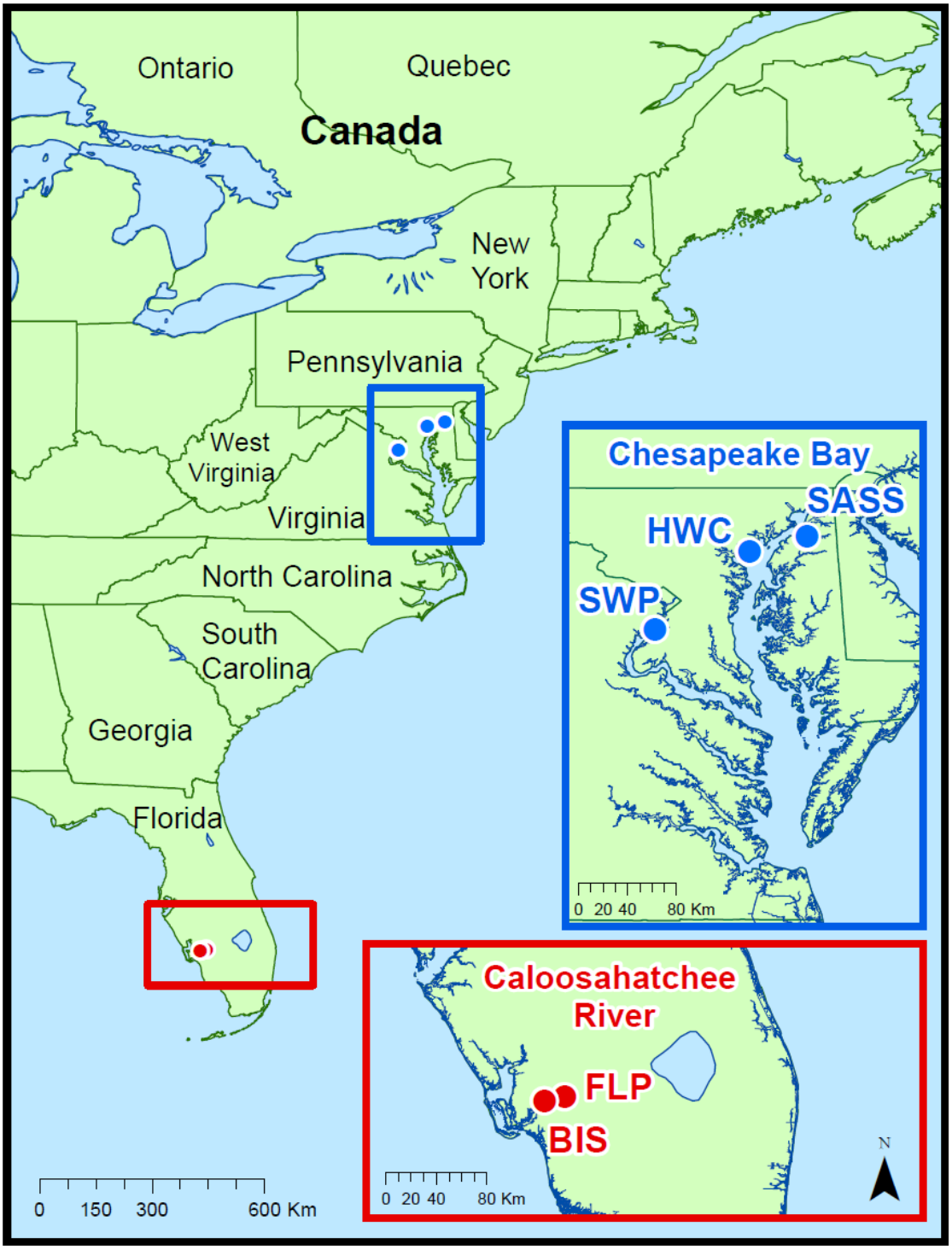

Figure 5.2: Locations of northern sourced *Vallisneria americana* from the Chesapeake Bay, MD and southern sourced *V. americana* from the Caloosahatchee River, FL used in reproductive cross experiments assessing fertilization, fruit and seed production, and germination success in crosses within and between regions.

Appendices

Appendix A: Evaluation of pairwise relatedness estimates

A relatedness estimator quantifies the degree to which individuals share alleles and estimates the probability that the genes are identical by descent based on population level allele frequencies. Higher estimates indicate a greater degree of relatedness such that first degree relatives (e.g., parent-offspring, full-sibs) average a relatedness coefficient of 0.5, second degree relatives (e.g., half-sibs) 0.25, third degree relatives (e.g., first cousins) 0.125, and unrelated individuals average a relatedness coefficient of 0. The properties of any relatedness estimator (bias and variance) depend upon the distribution of gene frequencies in the studied population. For this reason, we followed the recommendations of Van de Casteele et al. (2001) and ran Monte-Carlo simulations to determine which relatedness estimator was best suited given our data (Table A1). We used the program COANCESTRY v1.0 (Wang 2011) to generate four data sets of 999 pairs of unrelated, half-sib, full-sib, or parent-offspring genotypes using the allele frequencies for the 10 *V. americana* microsatellite loci as estimated from Lloyd et al.'s (2011) collections throughout the Chesapeake Bay (n=680 individuals). Allele frequencies were calculated from a larger set of samples than those that were used in the reproductive crosses to increase the accuracy of the allele frequencies used in relatedness estimation (e.g. Bink et al. 2008). Method-of-moment relatedness estimators, including the Queller and Goodnight (1989), Ritland (1996), Lynch and Ritland (1999), and Wang (2002) estimators, as well as two maximum-likelihood relatedness estimators were calculated

for each simulated pair of genotypes. Bias of the estimators was determined using two-sample T-tests to test the significance of difference between the estimated relatedness and the simulated relatedness (Table A.1). The Wang (2002) estimator had the lowest variance and minimal bias across various relationship categories, and thus most accurately estimates relatedness.

Table A.1: Mean relatedness ± variance for simulated populations consisting of 999 pairs of unrelated, half-sib, full-sib, or parent-offspring pairs with allele frequencies from 10 loci of Vallisneria americana (Lloyd et al. 2011). In parentheses are two-tailed P-values of t-tests that test for a significant difference from expected relatedness value. Significance (*) was calculated following sequential Bonferroni correction (Pcrit=0.013) for four tests (one for each relationship category). A significant difference indicates bias. The smallest sampling variances per relationship category of estimators that did not show significant bias are in bold. MOM = method-of-moments, ML = maximum likelihood.

		Relationship Category			
Estimator Type	Estimator	Unrelated	Half-Sib	Full-Sib	Parent-Offspring
Expected relatedness		0.00	0.25	0.50	0.50
MOM	Queller & Goodnight	-0.00002 ± 0.04193	0.24515 ± 0.04104	0.4987 ± 0.03578	0.49446 ± 0.01914
		(p=0.998)	(p=0.449)	(p=0.828)	(p=0.206)
	Ritland	-0.00083 ± 0.02455	0.21084 ± 0.16902	0.53096 ± 1.83053	0.50155 ± 1.57881
		(p=0.867)	(p= 0.003*)	(p= 0.470)	(p=0.969)
	Lynch & Ritland	**-0.00285 ± 0.01913**	0.2479 ± 0.04749	0.50852 ± 0.05166	0.51138 ± 0.03795
		(p=0.515)	(p=0.761)	(p=0.237)	(p=0.065)
	Wang	-0.00269 ± 0.04604	**0.25514 ± 0.03935**	**0.50925 ± 0.03224**	**0.50032 ± 0.01159**
		(p=0.692)	(p=0.413)	(p=0.104)	(p=0.925)
ML	Dyadic Likelihood	0.08132 ± 0.01441	0.28389 ± 0.03075	0.51711 ± 0.02658	0.53285 ± 0.00521
		(p<0.001*)	(p<0.001*)	(p<0.001*)	(p<0.001*)
	Triadic Likelihood	0.09166 ± 0.01627	0.29601 ± 0.03204	0.53245 ± 0.02563	0.5359 ± 0.00609
		(p<0.001*)	(p<0.001*)	(p<0.001*)	(p<0.001*)

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