GREENHOUSE PROPAGATION OF *VALLISNERIA AMERICANA*

A PRACTITIONER'S HANDBOOK



Taylor Breton & Katia Engelhardt

University of Maryland
CENTER FOR ENVIRONMENTAL SCIENCE
APPALACHIAN LABORATORY

Table of Contents

Authors and Collaborators.....1
Acknowledgments..... 2
Welcome.....3
Part I: Background and Key Findings..... 4
- **1.1** Introduction..... 5
- **1.2** *Vallisneria americana* Profile..... 9
- **1.3** Morphology and Key Features.....10
- **1.4** Life Cycle and Reproduction..... 12
- **1.5** Habitat Requirements.....16
- **1.6** Sampling and Genotyping..... 17
- **1.7** Cross-Factorial Experimental Design..... 20
- **1.8** Past Experimental Findings.....22

Part II: Greenhouse Operations..... 28
- **2.1** Specifications..... 29
- **2.2** Required Materials.....32
- **2.3** Planting Protocol..... 40
- **2.4** Planting Turions Protocol..... 43
- **2.5** Planting Seeds Protocol..... 44
- **2.6** Management and Monitoring..... 46
- **2.7** Data Collection..... 49
- **2.8** Pollination Protocol..... 50
- **2.9** Seed Pod Harvesting Protocol..... 51
- **2.10** Turion Harvesting Protocol..... 52
- **2.11** Clonal Division Protocol..... 55
- **2.12** Harvested Material Storage..... 57
- **2.13** Troubleshooting.....58

Table of Contents Cont.

Part III: Small-Scale Community Propagation..... 62

- **3.1** Small-Scale Propagation..... 63
- **3.2** Preparing for Propagation..... 64
- **3.3** Small-Scale Toolkit..... 65
- **3.4** Classroom Propagation..... 67
- **3.5** Indoor Propagation..... 69
- **3.6** Outdoor Propagation..... 70
- **3.7** Data Tracking..... 71
- **3.8** Collecting Propagated Plants..... 72

PART IV: Field Deployment..... 73

- **4.1** Restoration Planning..... 74
- **4.2** Site Selection Checklist..... 75
- **4.3** Genotype Selection Guidance..... 78
- **4.4** Transporting Plants..... 80
- **4.5** Field Deployment Strategies..... 81
- **4.6** Monitoring..... 84
- **4.7** Adaptive Management..... 86

References..... 88

How to Cite this Handbook

Breton, T., & Engelhardt, K. (2026). Greenhouse Propagation of *Vallisneria americana*: A Practitioner's Handbook. Technical Manual. University of Maryland Center for Environmental Science, Appalachian Laboratory.

Authors and Collaborators

LEAD AUTHORS



Taylor Breton, M.S.

As a recent UMCES Master's graduate and 2024–2026 NOAA Margaret A. Davidson fellow, Taylor's work is dedicated to bridging the gap between genetic diversity research and applied submerged aquatic vegetation (SAV) restoration to provide practitioners with management-ready guidance.



Katia Engelhardt, Ph.D.

Katia is a Research Associate Professor at the UMCES Appalachian Lab. She studies the effect of biodiversity on ecosystem functioning in various systems. She enjoys working at the interface between basic and applied science, which uses scientific principles to enhance management goals.

COLLABORATING ORGANIZATIONS

Hudson River National Estuarine Research Reserve (HRNERR) — Research collaboration and field support

New York State Department of Environmental Conservation (NYSDEC) — Research collaboration and field support

University of Maryland Center for Environmental Science (UMCES) Integration and Application Network (IAN) — Editorial support

University of Maryland, College Park — Genetic analysis of plant material

Acknowledgments

The creation of this handbook was a collaborative effort aimed at the propagation and restoration of *Vallisneria americana*. The protocols documented here were developed and tested through fieldwork in the Hudson River Estuary, as well as greenhouse propagation of Hudson River-native plants. We are grateful to those who contributed their expertise and labor to this project.

Thank you to our research collaborators at the Hudson River National Estuarine Research Reserve (HRNERR), Brian DeGasperis, Sarah Fernald, Daniel Miller, Christina Pacella, and Christopher Mitchell.

Thank you to Dr. Maile Neel at the University of Maryland, College Park, for sampling and conducting the genotype analysis of the UMCES Appalachian Lab genetic repository. Thank you to Jemima Gouala and Katie Kline for supporting the UMCES Appalachian Laboratory greenhouse operations. Thank you to the HRNERR volunteers and interns who assisted with fieldwork. Thank you to Rosh Nair-Gonzalez for your support with editing the handbook.

Special thanks to the Margaret A. Davidson Fellowship through the National Oceanic and Atmospheric Administration (NOAA), the Greenway Conservancy Grant through the New York State Department of Environmental Conservation (NYSDEC), and the Marine Estuarine Environmental Science program for providing financial support for this valuable work. Many thanks to the University of Maryland Center for Environmental Science and Hudson River National Estuarine Research Reserve for providing institutional support.

Welcome

Welcome to the Practitioner's Handbook for the greenhouse propagation and restoration of *Vallisneria americana*. This guide serves as a bridge between maintaining a genetic repository and strategically selecting genotypic stock to optimize restoration success. These protocols provide a scalable framework for practitioners across diverse estuarine systems.

Who are the intended users of this document?

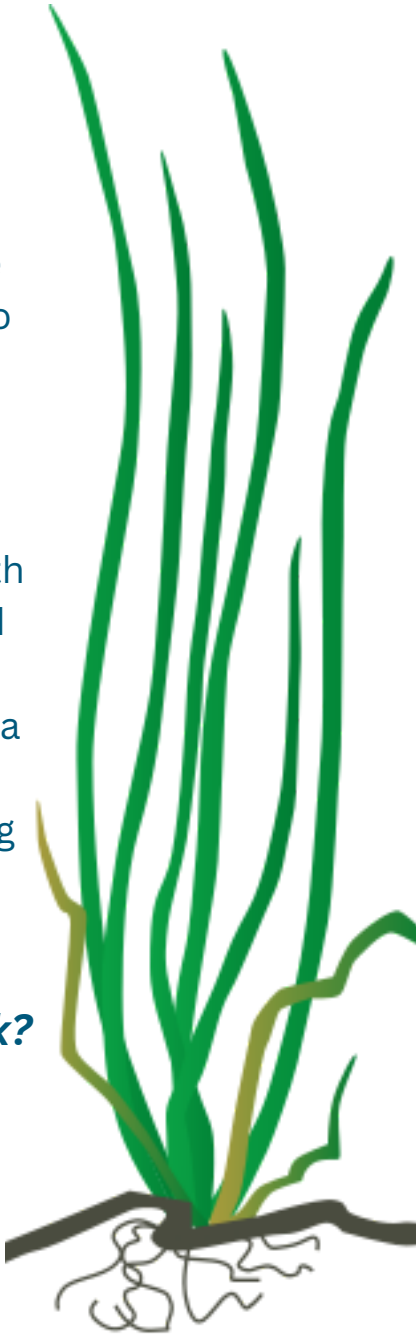
The primary audience for this handbook is submerged aquatic vegetation (SAV) managers and practitioners across aquatic systems who are interested in greenhouse rearing of *Vallisneria americana*. This document may also be of interest to citizen scientists pursuing small-scale propagation.

Why was this handbook developed?

Global declines of *Vallisneria americana* beds due to both environmental and anthropogenic stressors have created an urgent need for restoration efforts to re-establish resilient, genetically diverse populations. By maintaining a greenhouse genetic repository, managers can reduce the pressure of repeated donor bed harvesting while enabling strategic propagation of high-quality plant material for restoration projects.

How can this handbook be applied to future work?

By following the recommendations and protocols in this guide, practitioners and managers can ensure that the plants grown in the greenhouse are locally adapted and genetically suited for their target environment. Implementing these standardized methods directly increases the likelihood of long-term survival and enhances the overall resilience of restored populations.



Graphic Credit: Tracey Saxby, UMCES IAN Media Library

PART I: BACKGROUND AND KEY FINDINGS

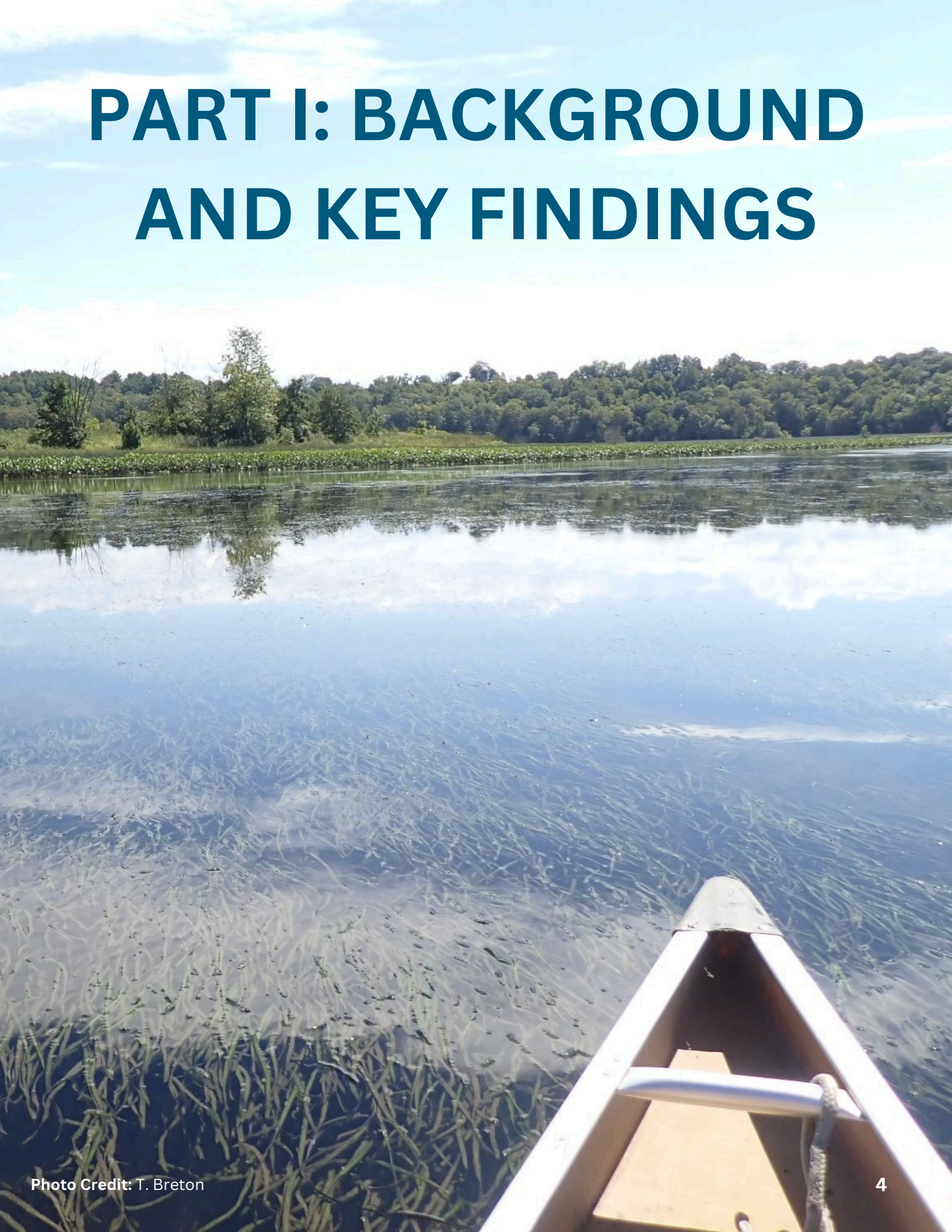


Photo Credit: T. Breton

1.1 *Introduction*

The presence of submerged aquatic vegetation (SAV) in shallow waters is often considered to be an indicator of a healthy water body (Scheffer et al., 1993; Scheffer et al., 2001; Scheffer & Carpenter, 2003; Folke et al., 2004) through its ability to immobilize pollutants, stabilize sediments, and provide habitat for diverse aquatic communities (Orth et al., 2006). Unfortunately, nutrient and sediment pollution combined with extreme weather events have reduced SAV extent in many tidal rivers and estuaries (Gurbisz et al., 2016; Orth et al., 2017; Lefcheck et al., 2018). Therefore, science-based restoration and active management are now key to reestablishing the structure and function of SAV beds.

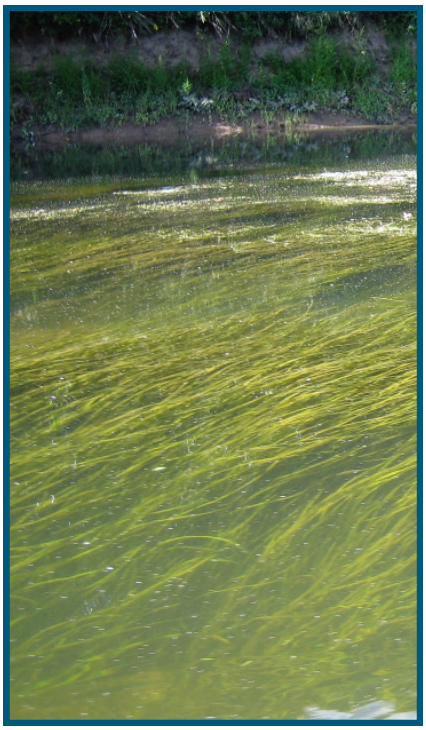


Image 1. *Vallisneria americana* bed with floating male flowers. Photo Credit: M. Neel

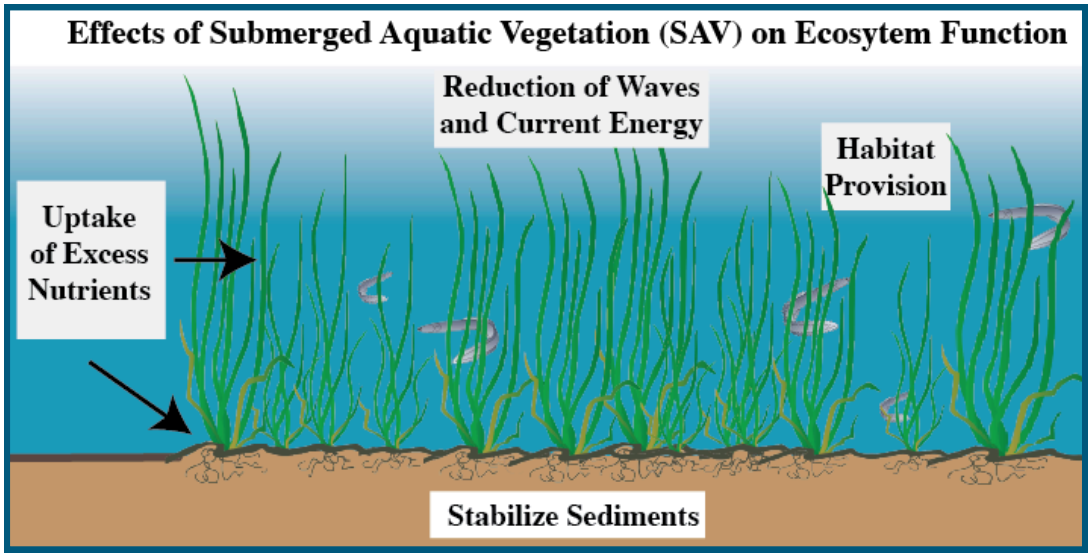


Figure 1. Ecosystem function and benefits provided by SAV.

One restoration approach is to jump-start the system (Hilderbrand et al., 2005) by introducing plant propagules that are able to establish rapidly, as well as provide sufficient species or genetic diversity for the restored SAV bed to be resistant or respond to change.

Genotypic identity and genetic diversity are known key drivers of ecosystem resilience (Reynolds et al., 2012; Hughes & Stachowicz, 2004), contributing to the long-term persistence of restored beds. Individual genotypes can differ in growth, reproductive output, and tolerance to stressors (Engelhardt et al., 2014), presenting the unique opportunity to both strategically select robust genotypes for restoration while also reintroducing multiple genotypes to bolster within-bed genetic diversity and trait variation.

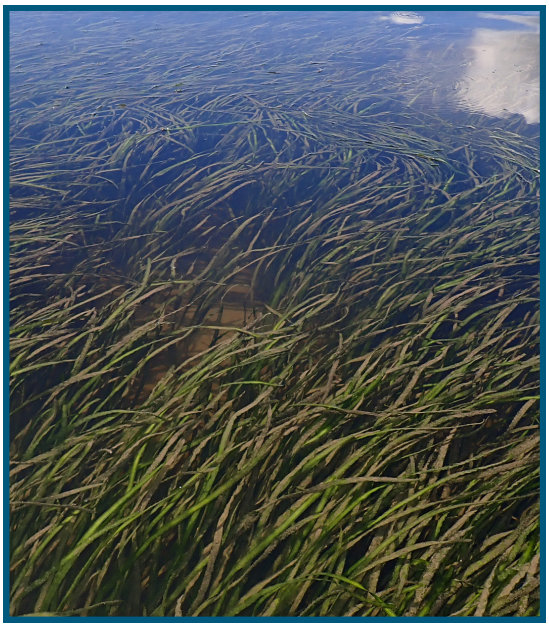


Image 2. Natural *Vallisneria americana* bed at Stockport Flats in Stockport, New York. Photo Credit: T. Breton

We focus here on *Vallisneria americana* Michx. because the species is a dominant SAV species in freshwater and oligohaline portions of the tidal and non-tidal Hudson River, New York (Findlay et al., 2006), and other tidal and non-tidal rivers in North America (Neel et al., 2025; Marsden et al., 2022; Rybicki & Carter, 1986).



Image 3. *Vallisneria americana* female flower.
Photo Credit: T. Breton

As with many aquatic species, *Vallisneria americana* is a dioecious (male and female flowers are on separate plants) submerged aquatic macrophyte that is capable of both clonal growth and sexual reproduction (Titus & Stephens, 1983; Lokker et al., 1997), with vegetative expansion often being the dominant form of propagation (Cook, 1987; Titus & Hoover, 1991).

The capacity of *Vallisneria americana* for both clonal growth and sexual reproduction makes it an ideal candidate for SAV restoration, providing rapid structural expansion of robust genotypes while allowing for long-term evolutionary change through genetic diversity.

Therefore, some SAV beds are composed of just one genotype where clonal spread is dominant, whereas others are highly diverse, illustrating the difficulty of harvesting plant material in the wild when its genetic diversity is unknown.



Image 4. *Vallisneria americana* is a clonal species that produces strings of ramets that are attached to each other through modified stems. When they are attached, each ramet along the string is genetically identical. Photo Credit: K. Engelhardt

Practitioners traditionally source restoration stock through two main avenues, both of which introduce critical ecological and operational trade-offs. Sourcing directly from local wild donor beds guarantees that the plant material is locally adapted to the target region, but risks over-harvesting fragile ecosystems with unknown genetic diversity. Sourcing bulk material from external commercial nurseries ensures adequate plant quantities, but it can introduce non-local plant material of unknown origin that may not be adapted or pre-adapted to regional conditions. Introducing plants from an unknown genetic source can also dilute the local gene pool, potentially causing outbreeding depression.

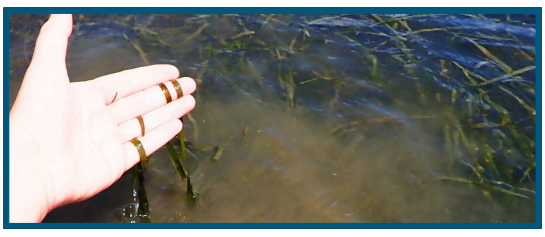


Image 5. Naturally occurring *Vallisneria americana* bed at Stockport Flats. Photo Credit: T. Breton

A dedicated, trackable greenhouse repository of genotypes bridges this gap by delivering bulk quantities of genetically known, locally adapted plant material without long-term impacts on ecosystems.

Greenhouse propagation requires access to restoration stock that is adapted to current local environmental conditions and has the capacity to adapt to future short- and long-term environmental change. Restoration stock of SAV can be in the form of plants, turions, or seeds. Greenhouse propagation serves as a good alternative to field harvesting while also serving as a genetic repository for SAV species that are at risk of being impacted by acute and chronic environmental stressors.



Image 6. *Vallisneria americana* propagation in plastic buckets at the UMCES Appalachian Lab greenhouse. Photo Credit: K. Engelhardt

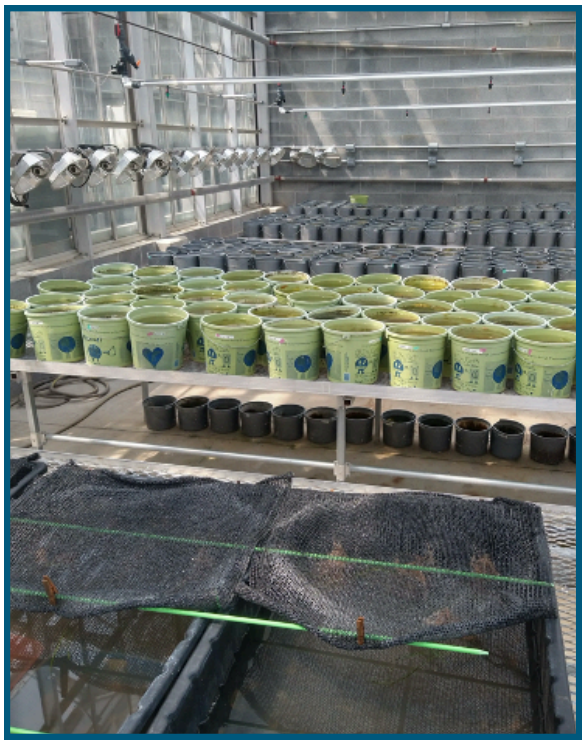


Image 7. Greenhouse cloning container setup. Gray containers are 5-quart, and green containers are 10-quart. Cement mixing trays in the foreground can support 4 small cat litter pans for seed germination or to store burlap bags containing turions. Photo Credit: K. Engelhardt

Creating a greenhouse genetic repository and propagating restoration stock for mass deployment in the field requires implementing propagation protocols that maximize vegetative reproduction through clonal spread, as well as the production of male and female flowers for seed production. Such turions and seeds can then be deployed in the field using vegetative mats (Boustany, 2003) or biodegradable mesh bags (Carew & Engelhardt, 2020). Using propagules and seeds reduces transplantation shock and enhances efficiency of deployment, thereby increasing the success of restoring sustainable SAV beds.

1.2 *Vallisneria americana* Profile

Overview

Vallisneria americana Michx., commonly known as wild celery, is a cosmopolitan perennial species found across freshwater and oligohaline systems within North America (Korschgen & Green, 1988). It is a member of the Hydrocharitaceae family of flowering plants, including tape-grass species. *V. americana* is a dioecious species, meaning male and female flowers are formed on separate plants. The species is capable of clonal expansion through "runners," which are plants connecting to the original plant by a horizontal stem. The plants can also expand through overwintering buds known as turions that form in the sediment.



Image 8. Close-up image of *Vallisneria americana* in a natural bed at Stockport Flats in Stockport, New York.
Photo Credit: T. Breton

Environmental Range

The presence of *Vallisneria americana* within a specific reach of a river or estuary is primarily determined by environmental drivers such as salinity, light availability, and substrate stability (Kemp et al., 2004). In estuarine systems, the "salt wedge" (the zone where freshwater meets saline tidal water) serves as a typical range limit (French & Moore, 2003). Practitioners should consult local submerged aquatic vegetation (SAV) inventories to identify existing persistent patches.



Figure 2. 2024 VIMS SAV Inventory Map of Accokeek, Maryland. Green regions represent SAV, including *Vallisneria americana*. Credit: Virginia Institute of Marine Science (VIMS)

1.3 *Morphology and Key Features*

Vallisneria americana is a distinctive SAV species, as it lacks any look-alike species that might cause misidentification.



Image 9. *V. americana* leaves.
Photo Credit: K. Engelhardt

Leaves

Long, basal, ribbon-like leaves that may reach up to several meters tall. Edges are toothless, with rounded tips, marked by a central line, and can range from reddish-brown to bright green in color. Leaves are all completely submerged.



Image 10. *V. americana* ramets.
Photo Credit: T. Breton

Ramets

Plants that are connected along stolons or rhizomes to form a clone of genetically identical plants.



Image 11. *V. americana* roots.
Photo Credit: K. Engelhardt

Roots

Strong, fibrous root system that grows from a stolon and anchors the plant into the sediment.

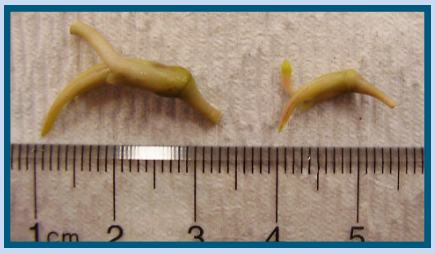


Image 12. *V. americana* turions.
Photo Credit: K. Engelhardt

Turions

White or gray overwintering buds form in the sediment at northern latitudes once the plant has gone dormant for the year. They range in size up to several centimeters.



Image 13. Female *V. americana* flower. Photo Credit: T. Breton

Female Flowers

The small white female flowers, ranging up to 3.5 mm, float on the water's surface and are connected to the base of the plant by a thin, coiled stalk (peduncle).



Image 14. Open spathe for *V. americana* male flowers. Photo Credit: T. Breton

Male Flowers

The male flowers are found at the base of the plant within a spathe structure that will burst. When mature, tiny flowers (<0.5 mm) detach and float to the water's surface to pollinate the female flowers.



Image 15. Seed pods in planting container. Photo Credit: K. Engelhardt

Seed Pods

Long, cylindrical fruit that suspends seeds inside a gelatinous substance. A single seed pod yields an average of 138 seeds (Marsden et al., 2013).

1.4 *Life Cycle and Reproduction*

Vallisneria americana is a persistent, perennial species that follows a life cycle consisting of vegetative and reproductive phases. Within the Mid-Atlantic and Northeastern regions of the United States, *V. americana* can propagate rapidly through asexual runner production, as well as seasonally through sexual reproduction and clonal persistence via dormant turions.

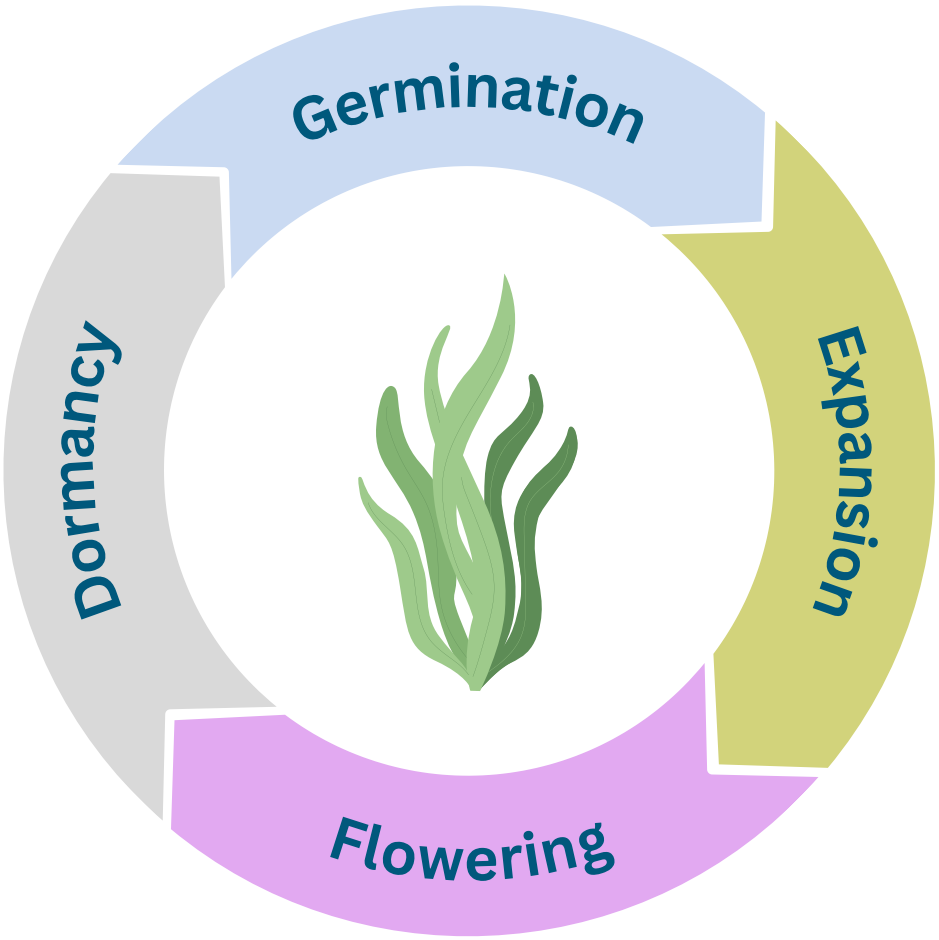


Figure 3. Life stages of *Vallisneria americana* in the Hudson River Estuary and other temperate environments.

Note on Regional Variability: These timelines are optimized for temperate regions, such as the Chesapeake Bay and Hudson River Estuary. *Vallisneria americana* in southern latitudes typically does not form turions and may become *V. tropicalis*.

I. Germination & Initial Growth

Late Spring to Early Summer (May to June)

As water temperatures rise and daylight increases, growth is initiated from seeds and turions.

II. Vegetative Expansion

Summer (June to September)

Leaves will elongate and the plants will send out ramets connected by a horizontal stem.

III. Flowering & Pollination

Mid to Late Summer (July to September)

Female flowers open at the water's surface to be pollinated. Male flowers and their pollen will attach to the female flowers. If pollination is successful, a seed pod will begin to form approximately 3–7 days later and mature by October.

IV. Senescence & Dormancy

Late Fall to Winter (November to January)

As the water temperatures begin to cool, the plants will begin to reallocate their energy from vegetative expansion and flowering to turion production, which is known as senescence. Plants will remain dormant until the spring or early summer.

Sexual Reproduction

Occurs via pollination of male and female plants

Pollination



Image 16. Male flowers released from spathe. Flowers are detached from the plant and on surface of the water.
Photo Credit: T. Breton

After the male flowers are released from the spathe and float to the water surface, they are trapped in a meniscus depression formed by the female flower.



Image 17. Pollination of a female flower.
Photo Credit: T. Breton

Pollination needs to occur within 1–2 days of the female flower opening into a small white flower and within 1–2 days of the release of the male flowers.

Seed Pod Production

Once pollinated, the female flower will mature into a seed pod that will naturally detach from the stalk or may be harvested. A single seed pod can yield an average of 138 seeds (Marsden et al., 2013). Seed pods are mature when they turn from green to brown in color.



Image 18. Attached seed pods floating at the water surface. Seed pods are often submerged as they mature.
Photo Credit: K. Engelhardt

Asexual Reproduction

Occurs via vegetative expansion of ramets or turion production



Image 19. Ramets connected via horizontal stem.
Photo Credit: K. Engelhardt

Vegetative expansion occurs when the original plant forms ramets, which are clonal plants that are connected by a horizontal stem.



Image 20. Partially senesced plant tissue.
Photo Credit: T. Breton

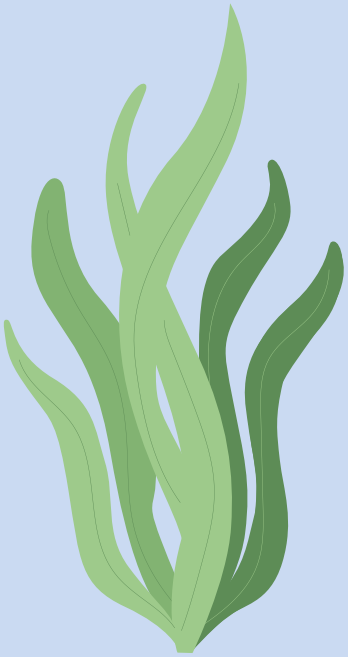
Turion production occurs during the winter months when the plants go through a dormancy phase known as senescence. This phase is identified by the plant's leaves turning translucent and detaching from the base of the plant. In southern latitudes, plants may not senesce and turions are not formed.



Image 21. *Vallisneria americana* turions.
Photo Credit: T. Breton

Once senescence occurs, turions can be harvested by washing the sediment through a sieve. When planted in the spring, turions will form new leaves and roots. The resulting plants are clones of the original plant.

1.5 *Habitat Requirements*



Salinity	*0–5 ppt (Jasinski et al., 2021)*
Light	>13% through water (Kemp et al., 2004)
Water Depth	General rule of thumb to plant in water less than 1 m deep at mean low water (MLW) (Jasinski et al., 2021)
Turbidity	Less than or equal to Secchi depth (Jasinski et al., 2021)
Dissolved Inorganic Nitrogen	NA (Kemp et al., 2004)
Dissolved Inorganic Phosphorus	<0.02 mg/L (Kemp et al., 2004)
pH	Neutral to slightly acidic conditions favor the availability of free carbon dioxide. However, V. *americana* can also utilize bicarbonate under alkaline conditions, which may increase the water column pH during photosynthesis (Pedersen et al., 2013).
Sediment Organic Matter	<6.5% (Hutchinson, 1975)
Sediment Grain Size	Fine sediments with more silt and clay and less coarse material (Rybicki & Carter, 1986)

1.6 *Sampling and Genotyping*

The collection and genotyping of the UMCES Appalachian Laboratory greenhouse genetic repository were conducted by Dr. Maile Neel at the University of Maryland, College Park. The repository contains genotypes originating from the Hudson River and the Chesapeake Bay and its tributaries, emphasizing the versatility of the outlined greenhouse protocols across aquatic systems.

Sampling Process

Thirty whole plants are typically collected from natural *Vallisneria americana* beds (Neel et al., 2025; Lloyd et al., 2012), unless populations are small and patchy. Individuals are collected along transects at regular spatial intervals of 5–10 meters, following established protocols (Neel et al., 2025).



Image 22. Restoration planting of *V. americana* in the Chesapeake Bay. Photo Credit: M. Neel

Whole plants of *Vallisneria americana* are sampled in the field by gently pulling green, leafy plants out of the sediments. When multiple ramets were attached to each other by a horizontal stem, they were left intact by gently pulling on the leaves near the base with one hand and digging below the root system with the other hand to loosen the roots from the sediments. Sampling a string of ramets increases the rate of establishment in the greenhouse, as each ramet is capable of producing multiple ramets. Intact ramets harvested in the field were placed immediately in water in plastic bags and transported on ice until planted within 48 hours in greenhouse monocultures.

Genotyping Process

To determine whether separate ramets are the same genotype, genetic analyses were completed by Dr. Maile Neel at the University of Maryland, College Park. Plant DNA was extracted from preserved leaf tissue using a DNeasy Plant kit and genotyped using polymorphic microsatellite markers developed specifically for *Vallisneria americana* (Burnett et al., 2009).



Image 23. Robert Burnett in molecular ecology lab at the University of Maryland, College Park. Photo Credit: M. Neel

This work can be conducted in-house if capabilities allow or the genotyping can be outsourced. By utilizing high-resolution microsatellite markers, practitioners can definitively identify unique individuals (genets) from clonal replicates (ramets). This distinction is critical for maintaining the genetic diversity of the repository.



Image 24. Turions labeled by genotype. Photo Credit: T. Breton

Maintaining the Genotype Repository

More than 150 clones from the Hudson River and the Chesapeake Bay are maintained and propagated at the University of Maryland Center for Environmental Science (UMCES) Appalachian Laboratory greenhouse under the direction of Dr. Katia Engelhardt.



Image 25. Monocultures of each genotype are maintained in a separate planting container. Photo Credit: T. Breton

Each whole plant that was sampled in the field has been grown in monoculture in 2.5-gallon containers filled to a depth of 5–10 cm with sterilized estuarine sediment and dechlorinated water (Engelhardt et al. 2014; Marsden et al., 2013). In the winter months, turions are harvested from these containers and propagated in the spring and summer in new planting containers to expand the genotypic stock.



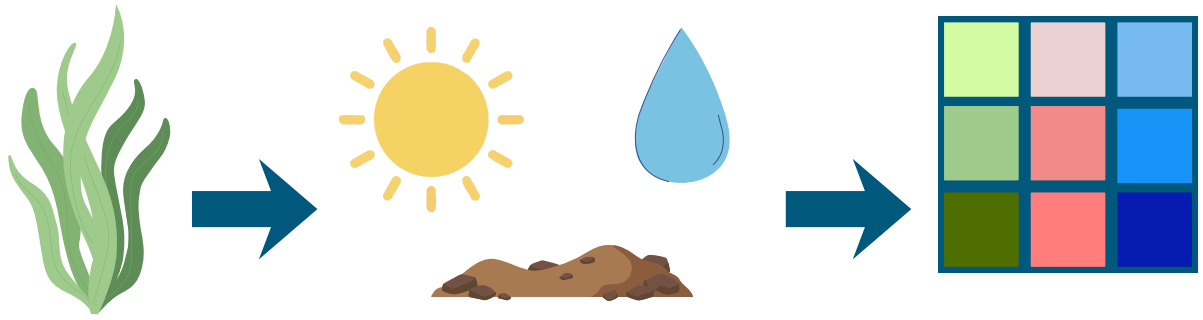
Image 26. Hudson River genotypes at the UMCES AL greenhouse. Genotypes are grown separately in containers and placed in water baths. Photo Credit: T. Breton

1.7 *Cross-Factorial Exp. Design*

Designing a cross-factorial experiment is a great method for isolating how specific variables interact. By testing multiple genotypes against varying environmental factors (such as light or nutrients), you can gain insights into how to enhance greenhouse propagation or which genotypes are best suited for field deployment. If genotypes are not available, these exact methodological steps can still be used to design a rigorous experiment for understanding performance across environmental gradients.

STEP 1: SELECTING VARIABLES

The first step is identifying which stressors or conditions you want to test against your genotypes. Common factors might include salinity, light availability, or substrate type. In a cross-factorial design, you pair these (e.g., 3 genotypes x 3 salinity levels = 9 unique treatments) to see how the plants react in different environments. You also want to make sure you have a minimum of three replicates per treatment, so that you can conduct a meaningful statistical analysis.



Select Genotypes ***Choose Factors*** ***Create Treatments***

of Genotypes **X** ***# of Levels of Factor*** **=** ***# of Unique Treatments***

Figure 4. Visual diagram of the steps to design a cross-factorial greenhouse experiment.

STEP 2: TREATMENT ALLOCATION

Once the variables are determined, organize your planting units (mesocosms) into “blocks” to account for microclimates in the greenhouse (like being closer to a cooling fan). Randomize the placement of treatments within these blocks to ensure the results are due to experimental factors and not just the mesocosms’ location. It can be helpful to draw a schematic of the greenhouse blocks and randomly assign the treatments within the sketch before planting.

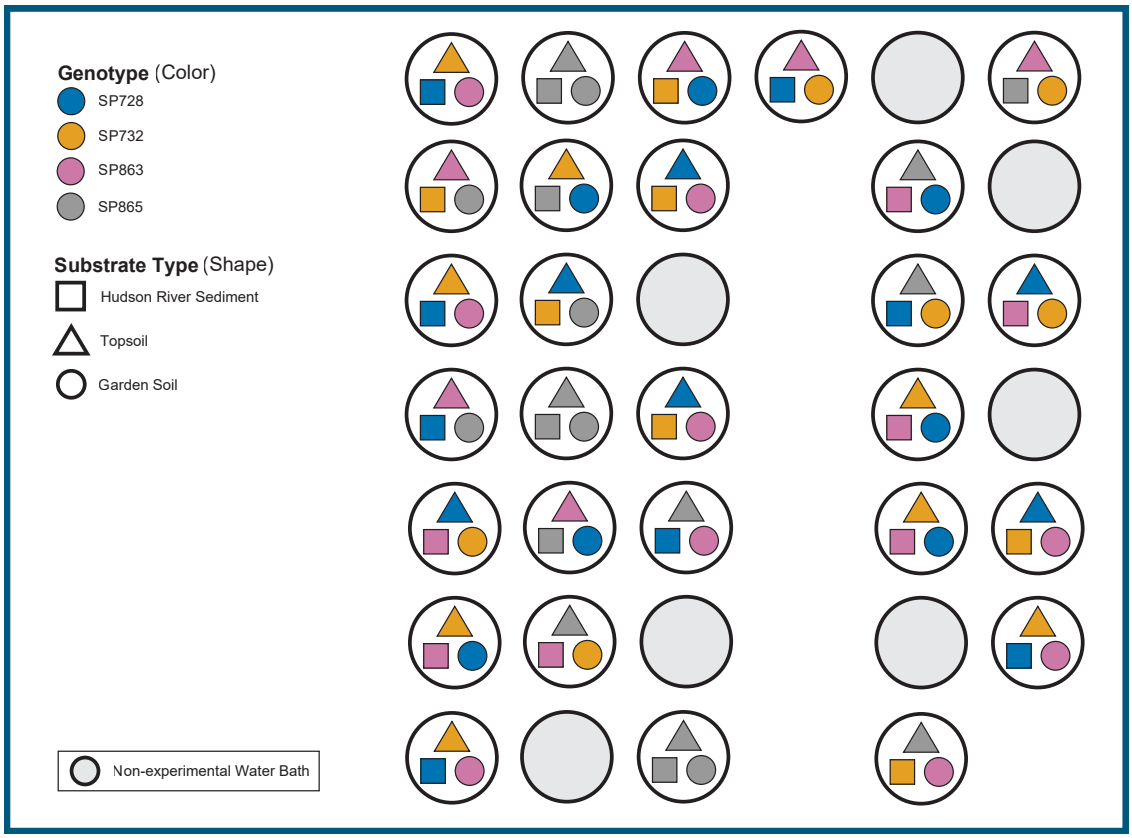


Figure 5. Schematic of genotype-sediment type cross-factorial experimental design block.

STEP 3: DATA COLLECTION

Define the data metrics that you want to collect early to ensure consistency across the study (See **Section 2.7**). Keep consistent data collection intervals, such as collecting data every six weeks starting in early July and ending in October.

1.8 *Past Experimental Findings*

Two experiments were conducted at the UMCES Appalachian Laboratory to assess the response of Hudson River-native genotypes to substrate type, additive fertilizer, and the presence of aeration. These experiments serve as a case study for evaluating genotype-specific responses under controlled conditions, as outlined in **Section 1.7**.

Genotype-Substrate Experiment

This experiment examined the effects of genotype, substrate type, and interaction effects on the establishment and productivity of *Vallisneria americana* under controlled conditions. Fixed experimental factors included four Hudson River genotypes (SP728, SP732, SP863, SP865) and three sediment types (garden soil, Hudson River sediment, and topsoil). Prior to planting, substrate samples were characterized to quantify properties relevant to plant growth, which included pH, organic matter content, and particle size analysis.

Table 1. Substrate characteristics (percent organic matter, pH, and grain size) of the three substrate types used in the genotype-substrate greenhouse experiment. Values are mean ± SD (n = 3).

Substrate Type	n	Organic Matter (%)	pH	Grain Size
Garden	3	4.26 ± 0.12	6.71 ± 0.0	32.95% gravel, 66.40% sand, 0.65% silt/clay
Hudson River	3	0.1 ± 0.04	6.61 ± 0.02	3.95% gravel, 88.27% sand, 7.78% silt/clay
Topsoil	3	0.69 ± 0.04	6.5 ± 0.01	25.74% gravel, 61.97% sand, 12.29% silt/clay



Figure 6. Cross-factorial design of the genotype-sediment type experiment.

In the spring of 2024, twenty-eight 25-gallon polyresin water baths were established in the greenhouse. Each water bath contained three 2.5-gallon plastic buckets that served as experimental units (mesocosms), with seven replicates per treatment combination, depending on turion availability at the time of planting. Each mesocosm was filled with approximately 10 cm of the assigned sediment and planted with five randomly selected turions from the designated genotype.



Image 27. Mesocosms in water baths. Photo Credit: T. Breton



Image 28. Ramets in Hudson River sediment.
Photo Credit: T. Breton

Data collection occurred in early July and mid-September to collect time series metrics on the number of ramets, length of the longest leaf, and the width of the longest leaf. Turions were harvested in the winter months and included in the data analysis. Data were analyzed using negative binomial mixed linear models to evaluate the fixed variables and interaction effects. Analysis of deviance was performed to evaluate the model and determine significance of main and interaction effects.

Genotype-Substrate Experiment Key Findings

Topsoil is the best substrate	Performance over time	Genotype-Specific Responses
Ramet production was significantly influenced by the type of substrate used. Across the board, commercial sifted topsoil vastly outperformed both garden soil and native Hudson River sediment.	**Mid-Summer (July):** Topsoil was the clear winner. Plants in garden soil and Hudson River sediment both performed equally poorly in comparison. **Late Summer (September):** Topsoil remained the highest producer. However, by this time garden soil began to marginally outperform the Hudson River sediment.	**SP728 & SP732:** Both genotypes strictly preferred topsoil, performing significantly better in it than either of the other two substrates. **SP863:** Performed significantly better in topsoil compared to Hudson River sediment, but there was no major difference in its performance between topsoil and garden soil. **SP865**: Also performed best in topsoil, with no significant differences in performance when comparing the Hudson River sediment to garden soil.

Genotype-Fertilizer-Aeration Experiment

This experiment was conducted to investigate how genotype, addition of fertilizer tablets, and water column aeration affect the establishment and productivity of *Vallisneria americana*. Fixed experimental factors included the four Hudson River genotypes (SP728, SP732, SP863, SP865), three levels of additive fertilizer (absent, moderate, high), and two levels of aeration (absent or present).

In spring 2025, thirty 25-gallon polyresin water baths were established in the greenhouse. Each water bath contained three mesocosms assigned a monoculture treatment consisting of one genotype, one aeration, and one fertilizer treatment, with three replicates per treatment combination. Each mesocosm was filled with approximately 10 cm of standard topsoil and planted with four randomly selected turions from the designated genotype, allowing approximately 12 weeks for plants to establish before treatment application.

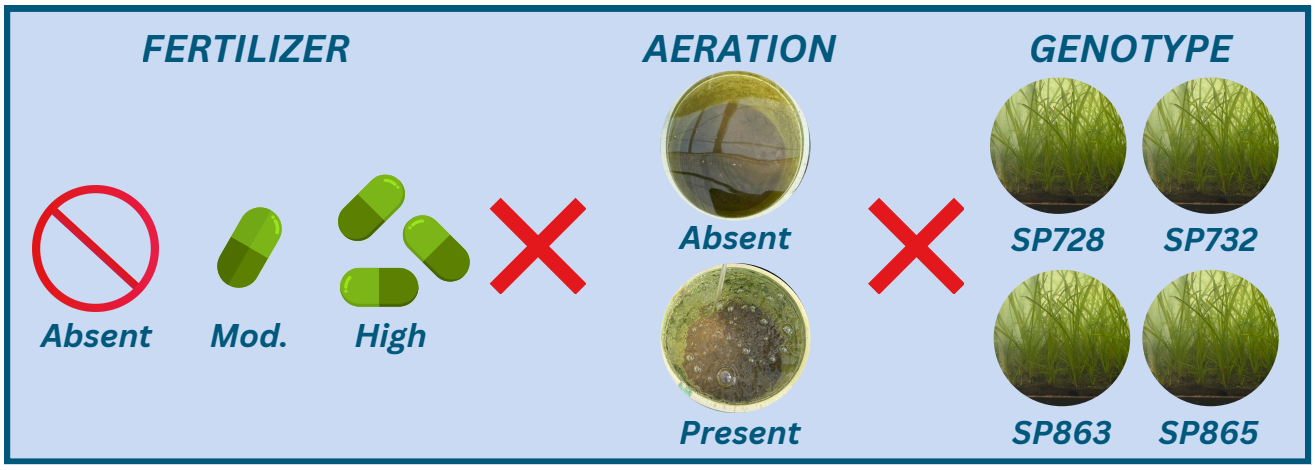


Figure 7. Cross-factorial design of the genotype-fertilizer-aeration experiment.

Aquatic plant slow-release fertilizer tablets (Thrive NilocG) were placed into the sediment of the “moderate” and “high” fertilizer treatments and applied twice during the experiment, approximately three months apart, with treatments receiving one or three tablets per application, respectively. Nutrient concentrations were calculated based on the manufacturer’s guaranteed analysis, which indicated that a full dose of three tablets had 2.57% total nitrogen and 1.69% available phosphate, along with other trace amounts of micronutrients. Tetra Whisper 10-gallon air pumps were used to provide aeration into each mesocosm. An airline splitter distributed airflow from a single pump into three mesocosms fitted with airstones.



Image 29. Aerated water bath with airline splitter into three separate mesocosms. Photo Credit: T. Breton



Image 30. Non-aerated water bath taken in September 2025.
Photo Credit: T. Breton

Data collection began in July 2025, at which time the total number of ramets, the length of the longest leaf, and the number of flowering events per mesocosm were recorded. These measurements were repeated in September and October 2025. Following plant senescence during the winter months, turions were harvested, and the total number produced per mesocosm was quantified. Data were analyzed using negative binomial mixed linear models to evaluate the fixed variables and interaction effects. Analysis of deviance was performed to evaluate the model and determine significance of main and interaction effects.

Genotype-Fertilizer-Aeration Experiment Key Findings

High Fertilizer Increased Flowering	Presence of Aeration Increased Ramet Count
Across the board, the full dosage of three fertilizer tablets produced higher rates of flowering compared to the moderate and absent treatments. High dosage treatments produced 3.2x more flowers than unfertilized plants and 2.3x more than the moderate dose.	Aerated treatments produced significantly more ramets compared to treatments with absent aeration. Aerated treatments produced 1.1x more ramets than non-aerated treatments.
Performance Over Time	**Genotype-Specific Responses**
For both vegetative growth (ramets) and sexual reproduction (flowering), October was the undisputed peak season. Production in October was significantly higher than in both July and September.	There were no significant genotype-specific responses.

Management Takeaways from Both Experiments

Greenhouse inputs should be actively managed based on goals.

Maximize Vegetative Biomass — Use standard topsoil and aeration without additive fertilizer.

Maximize Seed Production — Apply a full three-tablet dosage of Thrive NilocG tablets and select high-flowering genotypes.

Maximize Turion Production — Use standard topsoil.

PART II: GREENHOUSE OPERATIONS



Photo Credit: T. Breton

2.1 Specifications

Greenhouse Layout

Raised Tables

Raised tables are used in many greenhouses to enhance the ease of caring for plants as opposed to bending down to containers placed on the floor. Tables can therefore enhance accessibility; however, their typical height may not allow someone in a wheelchair to reach the mesocosm with ease. Tables constrain the size of the mesocosms (2.5-gallon maximum) placed on them, but they fit more mesocosms than water baths. Aisles should be maintained between tables to allow personnel and carts easy access. Tables should be made with heavy-duty material such as galvanized steel to disperse the weight of the mesocosms when they are filled with soil and water.

Floor Space

Having dedicated floor space alongside the raised tables allows for the use of large-scale (25-gallon) water baths, which are beneficial for maximizing propagule outputs. These floor-level baths support *Vallisneria americana* growth and expansion by providing the volume necessary for leaves to extend beyond their primary containers, increasing total photosynthetic surface area and facilitating natural pollination that is often restricted within smaller mesocosms.

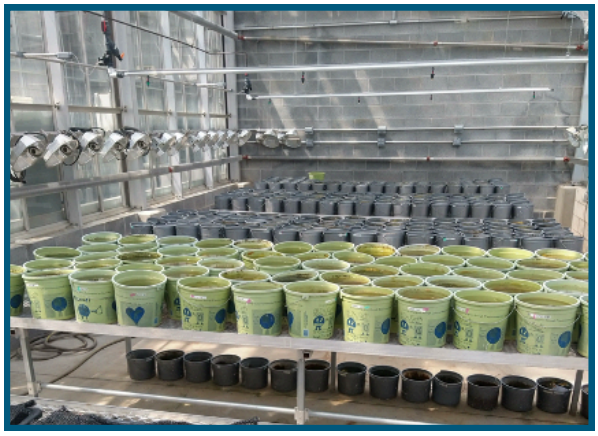


Image 31. Raised tables at the UMCES Appalachian Lab greenhouse.
Photo Credit: K. Engelhardt

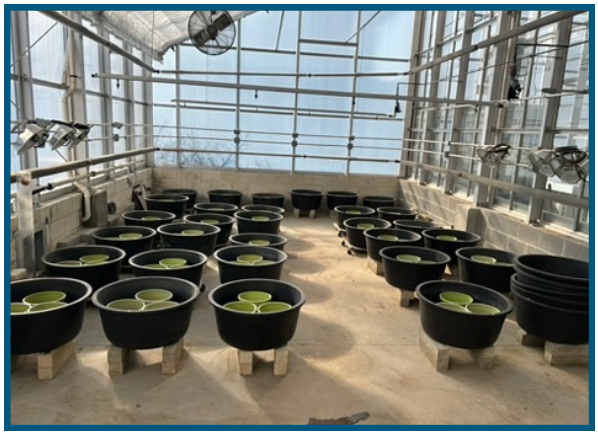


Image 32. Water baths utilizing floor space in the UMCES Appalachian Lab greenhouse.
Photo Credit: T. Breton

Lighting Requirements



Image 33. Shade cloth covering the UMCES Appalachian Lab greenhouse. Photo Credit: T. Breton

Light is a critical factor in supporting a productive greenhouse environment. However, too much direct light can bleach plant leaves and have just as many detrimental effects as insufficient light. Shade cloth is therefore important and also keeps temperatures down in the summer and decreases fan and cooling costs.

Black and reinforced shade cloth should preferably be fixed to cables inside the greenhouse to allow the shade cloth to open and close, depending on desired light levels.

Vallisneria americana is tolerant of low light levels and grows best at 15–19% incident light in greenhouse experiments. **We therefore recommend installing 70–80% shade cloth in temperate regions, such as the Northeastern and Mid-Atlantic United States.**

Climate Control and Air Circulation

Climate control and heating in the greenhouse can create a comfortable environment for *V. americana* establishment and vegetative expansion. However, warmer climates can inhibit plant dormancy and turion growth.

Swamp coolers can provide additional air circulation throughout the greenhouse and cool down the greenhouse interior throughout the summer months.

AL Greenhouse Climate Specs.

Target Range: 45 to 74°F

Humidity: 70%

Heating Set Points:
Day: 45°F (Heat on at 38°F, off at 46°F)
Night: 55°F (Heat on at 55°F, off at 56°F)

Cooling Set Points:
Day: 74°F **Night**: 80°F

Roof Vents (If no swamp coolers)**:**
Day: 83°F **Night**: 89°F

Shading (Cooling)**:**
Day: 73°F **Night**: 79°F

Water Supply

A non-chlorinated water supply (from a well or rain barrel) should be used for filling and maintaining *Vallisneria americana* mesocosms, if available. Tap water that is chlorinated is sufficient and does not damage plants. Reverse osmosis or distilled water should not be used because the water does not contain needed minerals, unless nutrients are added by hand for specific experimental treatments. A hose connected to a spigot is the most efficient way to fill water baths and mesocosms, and may be supplemented with overhead sprinklers to replenish evaporative water losses.



Image 34. Water spigot at the UMCES Appalachian Lab greenhouse.
Photo Credit: T. Breton

Overhead Sprinklers

Overhead water sprinklers are beneficial in reducing the likelihood of excess water loss and preventing plants from drying out during the hot summer months. To reduce water wastage, sprinklers do not need to be on more than once per week for 15 minutes.

However, it is still necessary to monitor the water levels of the buckets, especially during the summer months when water loss is higher, and either increase the time the sprinklers are on or water by hand as needed.



Image 35. Overhead sprinklers at the UMCES Appalachian Lab greenhouse.
Photo Credit: T. Breton

When water is added, the containers should be filled to overflowing so that water is flushed and a portion is replaced each time. Without this flushing, nutrients and salts will accumulate over time, which may harm the plants.

2.2 Required Materials

Substrate *(Necessary)*

Sifted topsoil is a highly recommended medium to support plant growth, as shown by prior greenhouse experiments. Sifting the topsoil removes any large rocks, sticks, or clumps that could impact *Vallisneria americana* growth. Sifted topsoil can be purchased from a local nursery. One ton typically lasts for one to three growing seasons, accommodating approximately 75–90 mesocosms per season. The topsoil can be stored loosely in the greenhouse (or outside in a sheltered place).



Image 36. Loose topsoil stored at the UMCES Appalachian lab.
Photo Credit: T. Breton

Each mesocosm should be filled to approximately 10 cm deep with substrate. For efficiency in filling multiple mesocosms, we calibrate the desired substrate depth by filling a small container that holds the desired amount, so the greenhouse technician can just scoop and dump. If, for some reason, topsoil is not available, practical recommendations are to select a substrate that has less than 5% organic matter and a 1:3 ratio of gravel to sand and fine sediments to best support plant growth.

Sand *(Necessary)*

A medium-grade silica sand should be placed on top of the sediment to prevent resuspension while the plants are still establishing.



Image 37. Silica sand.
Photo Credit: T. Breton

Planting Containers *(Necessary)*

The size of the container (mesocosm) directly impacts the expansion potential of *Vallisneria americana*. We find that 2.5-gallon plastic buckets provide enough room for the plants to expand clonally and produce a significant amount of turions. Using smaller plastic containers can also prove efficient for *V. americana* propagation; however, it may limit clonal expansion and turion yield.



Image 38. 2.5-gallon plastic container (mesocosm) typically used for planting.
Photo Credit: T. Breton

Labeling Tape *(Necessary)*

We use labeling tape as an alternative to a label maker. The writing on labeling tape fades in the sun, and the labels dissolve when submerged for a long time in water. We therefore only use this tape to keep track of daily pollination events or for labeling storage containers. Labeling tape is also great to have for a myriad of unexpected reasons.



Image 39. Printable labeling tape for mesocosms.
Photo Credit: T. Breton

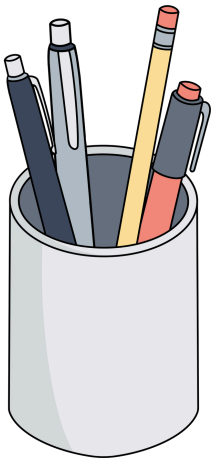
Label Maker *(Nice to Have)*

We use the Brother P-touch label maker and labeling tape to label all plastic buckets. The labels stick well to the surface, even when containers are submerged in water baths, and do not fade with exposure to UV light or water. We place the labels on the containers while they are completely dry.



Image 40. P-touch label maker.
Photo Credit: T. Breton

Pens and Permanent Markers *(Necessary)*



Pens and Sharpies are important for labeling purposes. We have found that permanent markers, such as Sharpies, are best. However, they do not write well when wet, so keeping multiple pens and markers on hand is important to avoid frustration.

Water Baths *(Nice to Have)*

Typical water baths consist of 25-gallon polyresin (rubber) tubs (29 x 29 x 13 inches) that are used to increase surface area, buffer plants from hot daytime or cold nighttime water temperatures, and promote vegetative and reproductive growth by allowing leaves, female flowers, and seed pods to expand beyond their individual containers. A 25-gallon round water bath can typically fit three 2.5-gallon mesocosms.

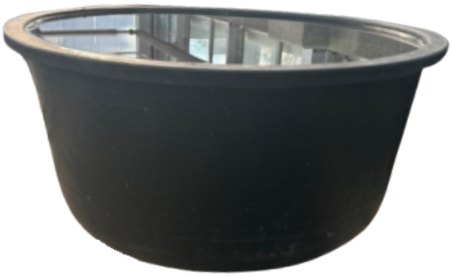


Image 41. 25-gallon rubber water bath. Photo Credit: T. Breton

While galvanized steel offers a smoother, easier-to-clean surface than rubber, its weight makes it difficult to maneuver. We recommend using smaller, hand-portable water baths elevated on cinder blocks or wooden risers to ensure accessibility and manual handling within tight greenhouse confines. Water baths can be fitted with plastic drains to facilitate drainage. Heavy baths should be placed on the greenhouse floor (or outside).

Mixing and Litter Tray System *(Nice to Have)*

As an alternative to deep water baths, 26-gallon UV-resistant mixing trays (36 x 24 x 8 inches) provide a successful growth system. These shallower trays can be placed on either raised tables or the floor, and may accommodate four standard litter trays for direct planting. This configuration is effective for managing smaller mesocosms while maintaining the benefits of shared water volume.



Image 42. Example mixing and litter tray system.
Photo Credit: T. Breton

Sponges *(Necessary)*

We use abrasive sponges and scour pads to clean algae and debris from the sides of the plastic containers.



Image 43. Abrasive sponges.
Photo Credit: T. Breton

Brushes *(Necessary)*

Large plastic and metal brushes can be used to clean out the water baths.



Image 44. Brushes used to clean water baths. Photo Credit: T. Breton

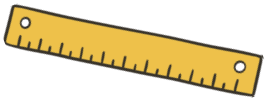
Scissors *(Necessary)*

Scissors have numerous and valuable uses in a greenhouse.



Plastic Rulers *(Necessary)*

Good for measuring out substrate and measuring leaf length, among other things.



Waterproof Notebooks *(Nice to Have)*

Good to keep on hand to communicate information about specific plants, pollination, collect data, etc.

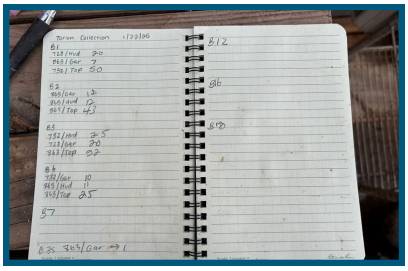


Image 45. Rite in the Rain notebook. Photo Credit: T. Breton

Twisted Cotton Rope *(Necessary)*

A 10-cm strand of cotton string can be tied to the stem of a female flower to indicate when a female flower has been pollinated by hand. This increases efficiency as plants will produce multiple flowers, and there is no need to pollinate the same flower multiple times. We use twisted cotton rope, which supplies three thin strings when the rope is pulled apart.



Image 46. Female flower that has been marked with a cotton string.
Photo Credit: T. Breton

The string is gently tied around the female flower stem and can stay attached for the entire growing season. Other types of string can be used; cotton is best as it is soft and not abrasive to the stem, and it is biodegradable.

Whirl-Paks *(Nice to Have)*

Small Whirl-Paks (non-sterile is sufficient) may be used to store seed pods that have been harvested. The small bags are cost-effective and pack well into storage containers for placement in a fridge. Bags with a white writing area are useful for labeling if the source of the seed pod needs to be tracked for research purposes. Ziploc bags are sufficient when the source of the seeds is unimportant, and all seed pods can be stored together in a bag.

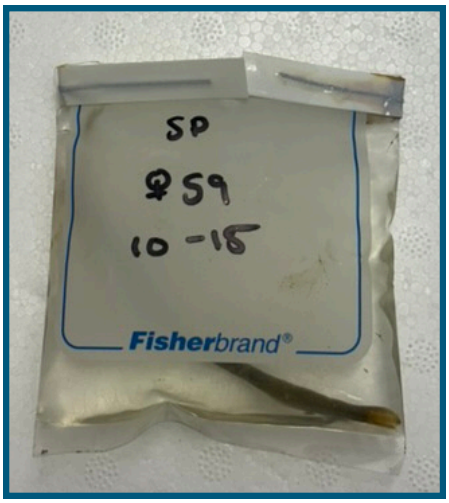


Image 47. Small Whirl-Pak holding a seed pod.
Photo Credit: T. Breton

Small Plastic Containers *(Necessary)*

Small plastic containers (120 ml or 4 fluid ounces) fitted with lids can be used for storing turions during the winter months after the turions have been harvested and washed from debris. Small containers are better to prevent storing too many turions together in the same containers, which can cause an entire batch of turions to degrade and lose viability.



Image 48. Small plastic containers holding turions.
Photo Credit: T. Breton

Labels can be placed on the containers to identify the containers' contents. The labels can be peeled off and the containers can be reused multiple times.

5-gallon Buckets *(Necessary)*

5-gallon buckets are used for harvesting turions. Using multiple buckets for turion harvest proves to be the most efficient method. One bucket is filled with clean water to sift turions from sediment, while a second bucket is used to dump sediment that has been cleared of turions. As sediment-collection buckets fill, they can be set aside to allow sediment to settle and dry before being discarded in a designated area. The 5-gallon buckets are also helpful stools for sitting in front of the sieving bucket.



Image 49. 5-gallon bucket.
Photo Credit: T. Breton

No. 10 Soil Sieve *(Necessary)*

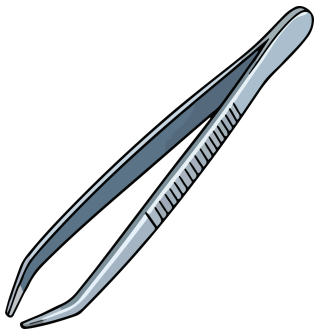
A soil sieve is necessary to sift the turions from sediments. A U.S. No. 10 (pore size = 2.00 mm) sieve allows most of the soil particles to pass through the sieve with relative ease without losing turions.



Image 50. US No. 10 soil sieve.
Photo Credit: T. Breton

Tweezers *(Nice to Have)*

Tweezers facilitate picking the turions from the soil sieve during harvest. Although not required or preferred by everyone, they may be used for planting.



Air Pumps *(Nice to Have)*

Ten-gallon air pumps provide aeration that helps support higher ramet counts and discourage algae from forming on the water's surface.



Image 51. 10-gallon Tetra air pump. Photo Credit: T. Breton

Fertilizer Tablets *(Nice to Have)*

Thrive NilocG fertilizer tablets slowly release a set of nutrients that are formulated for aquarium plant growth into the sediment. We have found that a three-tablet dose placed every 2–3 months induces higher rates of flowering.



Image 52. Thrive NilocG fertilizer tablets.
Photo Credit: T. Breton

2.3 *Planting Protocol*

Task	JAN	FEB	MAR	APR	MAY	JUN
Planting						
Monitoring						
Turion Harvest						

Task	JUL	AUG	SEP	OCT	NOV	DEC
Monitoring						
Assisted Pollination						
Seed Pod Collection						

Figure 8. Gantt chart showing when specific greenhouse tasks are typically performed.

Labeling & Preparation

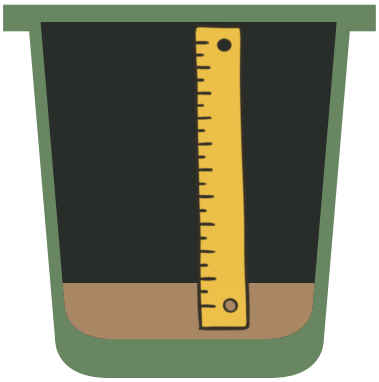
Before any planting begins, the mesocosms need to be labeled on the outside while completely dry. At the very least, the specific genotype should be labeled on the mesocosm. It helps to be consistent in where the labels are placed. We tend to place labels at the top on the outside of the mesocosm.



Image 53. Labeled mesocosm. Photo Credit: T. Breton

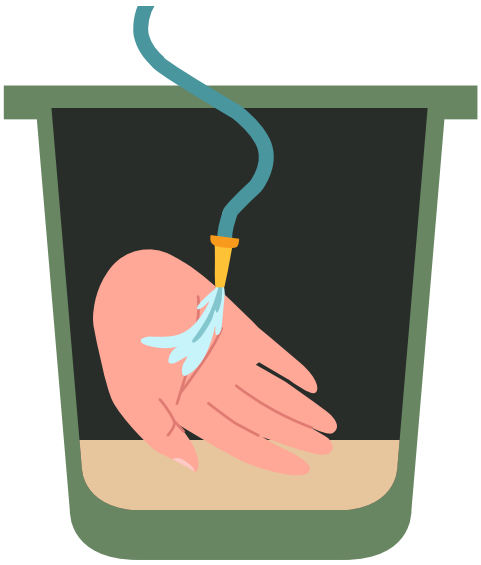
Substrate Preparation

Measure out approximately 10 centimeters of dry substrate per mesocosm. Lightly saturate the sediment before planting.



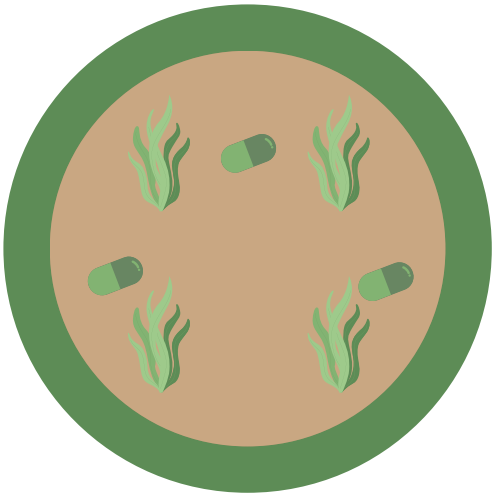
Filling with Water

Sediment resuspension is extremely common, especially when plants are not established. A good rule of thumb is to go low and slow when filling the water. We typically keep the water pressure low and place a hand above the sediment. We direct the hose right at the palm of the hand to evenly diffuse the water. As the water level rises, so does your hand until the container is completely full.



Fertilizer Additions

Once the ramets have begun to sprout within the mesocosms, typically in the summer months (July to September), fertilizer tablets can be added to the sediment to induce higher rates of flowering. A full dosage of 3 Thrive NilocG fertilizer tablets can be added to each mesocosm and refreshed every 2–3 months until the seed pods are harvested around October.



Aeration Additions

Aeration can enhance ramet count and can break up algae that accumulate at the water surface as a thin film. However, aeration is not mandatory, as *Vallisneria americana* and other SAV species are sufficiently productive without added aeration. If aeration is desired, air can be added to mesocosms using 10-gallon aeration pumps that pump air into attached airlines. Each aeration pump can pump enough air to supply three mesocosms, so an airline can be split three ways with an airstone attached to the end of each line. Splitting airlines reduces the number of airlines running through the greenhouse. Airstones can be placed into the middle of each bucket and will need to be periodically monitored to ensure they are not plugged. If they are plugged, the problem can often be solved by removing the airstones and reattaching.

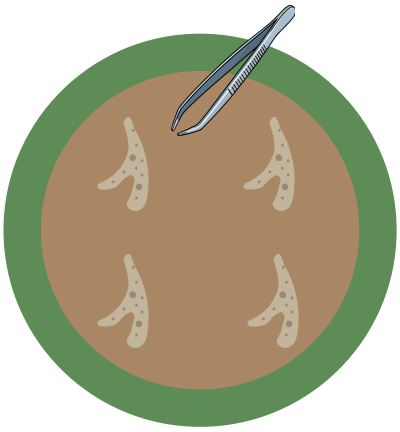


Image 54. Example airline, airstone, and three-way splitter. Photo Credit: T. Breton

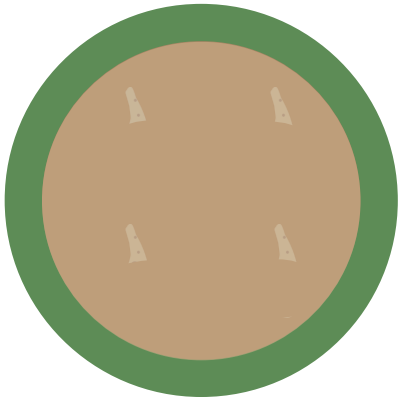
2.4 *Planting Turions Protocol*

Planting turions allows you to expand the genotypic repository stock by planting monocultures of a single genotype. This protocol ensures optimal spacing and proper orientation of the buds within the mesocosm, making it easy to track ramet emergence and quickly identify and replace non-viable turions.

Planting Turions Protocol



1. **Fill the Mesocosm:** To an empty 2.5-gallon bucket, add 10 cm of damp topsoil. It should be muddy, but not soupy in consistency.
2. **Determine the Geometric Planting Pattern:** To track growth and identify non-viable turions, always plant in a pattern based on your total turion count.
    - **3 Turions**: Triangle pattern
    - **4 Turions**: Square pattern
    - **5 Turions**: Pentagon or die-dot pattern

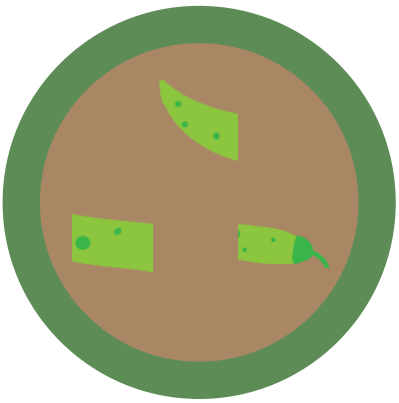


3. **Establish Spatial Buffers:** Always maintain 1–2 inches of space between individual turions.
4. **Insert the Turions**: Using tweezers or fingers, gently grip the turion and insert into the sediment with the growth tip pointing upwards. Embed the turion into the sediment, but allow a tiny bit of the growth tip to peek out.
5. **Apply the Sand Cap**: Gently add a layer of medium-grade silica sand (<0.25 inches) over the substrate.

2.5 *Planting Seeds Protocol*

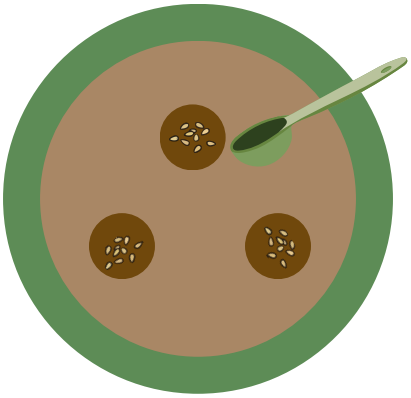
Planting seeds introduces genetic diversity by creating new, hybrid genotypes from parent crosses. While seeds are an excellent tool for restoration and scaling up *Vallisneria americana* stock, they do not produce genetic clones from the original plants like turions do.

Planting Seed Pods Protocol

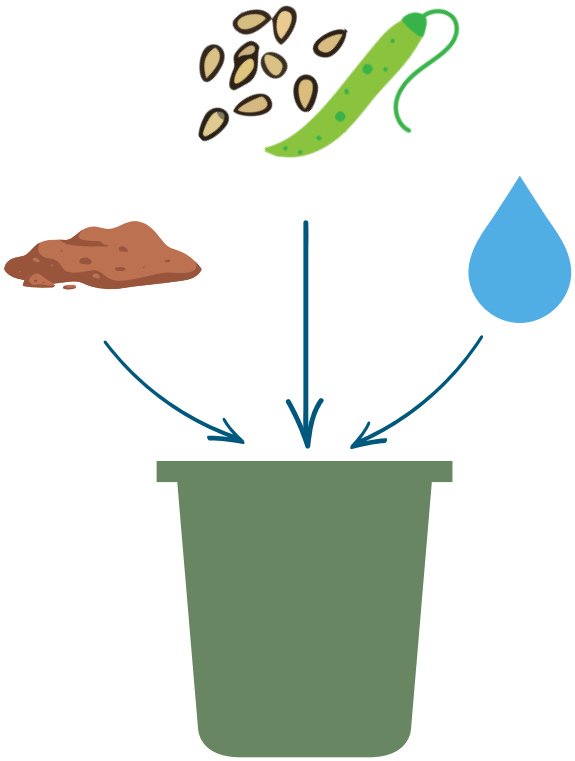


1. **Fill the Mesocosm:** To an empty 2.5-gallon bucket, add 10 cm of damp topsoil. It should be muddy, but not soupy in consistency.
2. **Cut the Seed Pod**: Cut the seed pods into 1–2 cm segments. We recommend planting no more than one seed pod per mesocosm to prevent crowding.
3. **Establish Spatial Buffers**: Always maintain 1–2 inches of space between segments. The seeds from the pod segments will form clusters, so there is no need to plant in a pattern like with turions.
4. **Place the Seed Pod Segments**: Using tweezers or fingers, place the pod segments on top of the substrate. Planting them deeper in the substrate will prevent the seeds from emerging.
5. **Apply the Sand Cap**: Gently add a layer of medium-grade silica sand (<0.25 inches) over top of the substrate.

Planting Loose Seeds Protocol



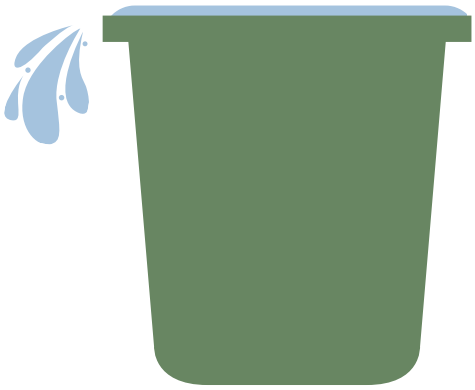
1. **Fill the Mesocosm:** To an empty 2.5-gallon bucket, add 10 cm of damp topsoil. It should be muddy, but not soupy in consistency.
2. **Create a Seed-Sediment Slurry**: In an empty bucket, make a slurry of topsoil and water; it should be muddy but not soupy in consistency. Add *Vallisneria americana* seeds (and any pod segments) directly into the slurry. Mix well.
3. **Plant the Seed Slurry**: Using a tablespoon, place seed slurry allotments approximately 1–2 inches apart in the mesocosm. No need to bury them.
4. **Apply the Sand Cap**: Gently add a layer of medium-grade silica sand (<0.25 inches) over top of the substrate.



2.6 Management and Monitoring

Water Level Maintenance

Water levels should be maintained at the top of the mesocosms. Any leaves that are not completely submerged in water will dry out. Water can be refilled using the palm method for even distribution. Filling the mesocosms until overflowing allows for additional water exchange within the mesocosms.



pH and Temperature Monitoring

We typically do not check the pH levels and water temperature of the individual mesocosms on a regular basis, relying on visual cues of plant health. In the past, we have monitored pH and temperature in the water baths, which range from 7.5–10, and 19–24°C, respectively.



Fertilizer Application

Fertilizer tablets should be applied after the ramets have begun to appear during the summer months, around June or July. They should be refreshed every 2–3 months until the seed pods are harvested around October.



Algae and Debris Management

Filamentous and surface algae often appear in the buckets, especially before ramets begin to establish. Filamentous algae can be gently pulled out using your hands, whereas surface algae can also be pulled out using a small metal sieve. The sides of the mesocosms can be gently scrubbed using an abrasive sponge to remove attached algae.



Image 55. Surface algae on a water bath. Photo Credit: T. Breton

Cleaning Buckets

When the buckets are no longer being used to propagate *V. americana*, it is best to give them a thorough cleaning to remove algae and debris. We never use soap, but instead use hot water and an abrasive sponge to scrub any visible residue. We let them air-dry and then place them into storage once completely dry and clean.



Cleaning Water Baths

When the water baths are no longer in use or algae overgrowth has occurred, we will drain the water baths to clean them. We use large metal and plastic brushes to remove debris from the walls of the bath and then spray them using a high-pressure setting on the hose to get any tough-to-reach spots.



Draining Buckets with Plants

If the algae inside a bucket have overgrown, draining the mesocosm may be necessary. You should gently but firmly place your hand over the sediment to prevent any spillage. Then slowly tip the bucket so the water drains.



Placing Buckets into a Water Bath

The best method to place the buckets into a water bath is to fill both the bath and the mesocosm separately. You can then float the mesocosm on top of the water in the bath; the weight of the water in the mesocosm should allow it to sink. You can also tip the side of the mesocosm and allow the water from the bath to very slowly enter.



Monitoring Air Pumps

It is best to keep air pumps out of areas where water can directly impact them. We keep the ones in our greenhouse up high, on ledges that are out of the way of the overhead sprinklers and accidental spillage. The pumps can be left running at all times, but it is a good idea to give them visual checks to make sure the airstones are still producing bubbles and are working properly.

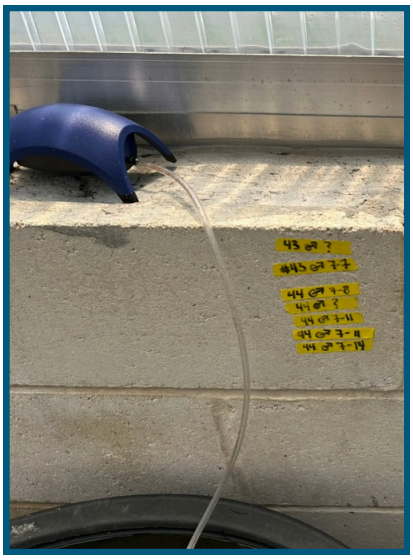


Image 56. Air pump on a ledge. Photo Credit: T. Breton

2.7 Data Collection

Vegetative Expansion

Common ways to quantify vegetative expansion include counting the number of individual plants (ramets) in each mesocosm. It is best to drain the mesocosm to provide better visualization of ramets. To keep track, select the first plant and count clockwise.



Image 57. Ramets in a mesocosm.
Photo Credit: T. Breton

Measuring the length and width of the longest leaf can also provide valuable insights into vegetative expansion. This can be achieved by gently lifting the plant leaves straight up and selecting the tallest shoot to measure.

Sexual Reproduction

Sexual reproduction can be measured by counting the number of male and female flowering events. The number of mature seed pods that are harvested may also be measured as a metric of sexual reproduction. To ensure the same flower is not counted multiple times, label the date of the flower using tape and tie a cotton string around the stalk.



Image 58. Mature seed pods in a mesocosm.
Photo Credit: K. Engelhardt

Turion Yields

Counting the number of turions harvested per mesocosm can be used as a metric of clonal reproduction. Sediments are washed through a sieve to separate turions.



Image 59. Harvested turions in a sieve.
Photo Credit: T. Breton

Data Logging

Data can be recorded in a Rite in the Rain notebook but should be immediately uploaded into a digital database.

2.8 *Pollination Protocol*

Natural Pollination

Natural pollination occurs when male and female plants share the same water bath, allowing the shared environment to facilitate the transfer of pollen. Mature female flowers rise to the water's surface, and their weight creates a slight surface depression in the water. The male flowers are released from the spathe at the base of the plant and float to the surface. The surface tension causes the male flowers to be drawn into the indent created by the female flower. This physical contact facilitates the transfer of pollen.



Image 60. Natural pollination of *Vallisneria americana*. Photo Credit: T. Breton



Image 61. Immature *Vallisneria americana* female flower. Photo Credit: T. Breton

Manual Pollination Protocol

Use this protocol when natural water movement is insufficient.

1. **Collection:** Scoop the free-floating male flowers from the surface of the water using a small plastic cup.
2. **Identification:** Locate the mature female flower. Gently tie a small piece of string around its stem to mark it for tracking.
3. **Application:**
    a. Gently grasp the base of the female flower
    b. Dip the flower directly into the collected male pollen
    c. Ensure that visible pollen grains have adhered to the female flower.
4. **Verification:** Return the flower to the water's surface. Confirm the pollen is attached and the flower is floating naturally. Make sure the stem of the female flower is completely submerged; if it dries out, the flower will not survive.

2.9 Seed Pod Harvesting Protocol

Identification of Mature Seed Pods

Seed pods typically mature by the fall (October) and can be identified by their length and green or brown coloring. Seed pods produced within a population can average around 9.5 cm in length (Marsden et al., 2013). If the pod appears translucent, it is time to harvest. Some seed pods will naturally sever from the stalk, while others can be manually cut from the stalk using a pair of scissors.



Image 62. Mature brown seed pod. Photo Credit: T. Breton



Image 63. Mature seed pods. Photo Credit: T. Breton

Seed Pod Storage

Once harvested, the seed pods can be placed in small Whirl-Paks with a small amount of water that fully covers the seed pod. Gently press the seed pod into the pack, but try not to bend or burst the pod. The packs should be labeled with the date harvested and information about the parent plants. They can be stored in the fridge until it is time to plant. The seeds may burst from the pod but will typically remain viable.

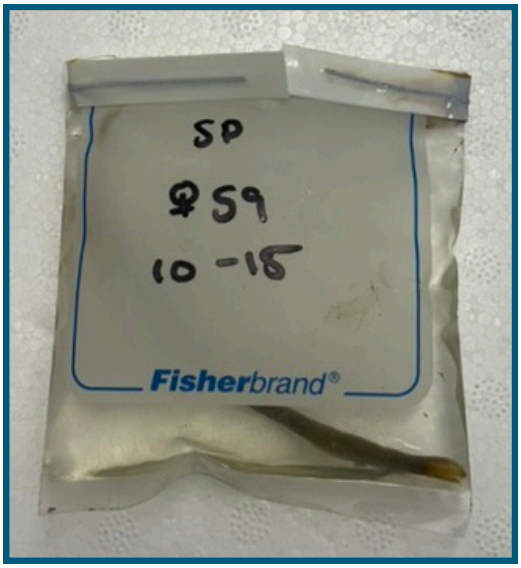


Image 64. Small Whirl-Pak holding a seed pod. Photo Credit: T. Breton

2.10 *Turion Harvesting Protocol*

When to Harvest

Typically, turions are harvested in the winter months of January to February; however, visual cues can help determine when exactly the turions should be harvested. The first cue to look for is whether the leaves are all translucent, suggesting that the plants have senesced. An absence of leaf tissue is also a good indicator that the plants are dormant.



Image 65. Partially senesced, translucent plant tissue. Photo Credit: T. Breton

Senescence is typically triggered by a drop in temperature, so it is possible that a climate-controlled greenhouse might not reach the desired temperatures to induce plant dormancy. In this instance, it is recommended to move the plants outdoors until the senescence cues are identified. However, the major caveat with this is that the mesocosms should not completely freeze over, as that will increase the likelihood that the turions will become non-viable.

Sieving Protocol

1. Remove the sediment from the mesocosm and place it into a U.S. No. 10 (2 mm) sieve. This might need to be done in batches so as to not overcrowd the sieve.
2. Rinse the sieve in the bucket filled with water, similar to panning for gold.
3. Once all the fine sediment has been removed, you can pick through the sieve with tweezers to pull out the turions and place them in a small plastic cup with water. Make sure there is enough water to cover the top of the turions.
4. Label the lid of the cup with the corresponding genotype and number of turions collected.
5. Put all of the leftover sediment from the sieve into an empty five-gallon bucket. Dump the sediment bucket every so often to avoid overflow.
6. Make sure to completely rinse out the sieve between mesocosms to avoid cross-contaminating turions originating from separate genotypes.



Image 66. Mesocosm ready to be harvested. Photo Credit: T. Breton



Image 67. Mesocosm sediment scooped into sieve.
Photo Credit: T. Breton



Image 68. Rinsed sediment with turions. Photo Credit: T. Breton

Identifying Viable Turions

Viable turions are firm and white in color. Sometimes multiple turions can be attached to one another. Turions that are not viable appear gray and mushy, often having a “marshy” odor. If you are unable to determine if a turion is viable or not, it is still worth a try to plant it; however, you should keep any turions that you suspect are non-viable separate from the healthy ones so as to not infect the others.



Image 69. Common types of viable turions.
Photo Credit: T. Breton

Turion Storage

Turions can be placed in small plastic containers with lids. They should be fully submerged in water. Turions can be stored in the fridge until planting in the spring.



Image 70. Turions in plastic containers.
Photo Credit: T. Breton

2.11 *Clonal Division Protocol*

This protocol may be used to guide practitioners in both harvesting mature shoots for field restoration and thinning out an overgrown mesocosm when space becomes limited.

Harvesting Protocol

1. Carefully slide your hand underneath the sediment in the mesocosm. Be careful not to damage the roots.
2. Gently scoop out the sediment with the adult shoots attached.
3. Brush off any excess sediment from the roots of the shoots.
4. Place the shoots in a tray filled with clean water to remove excess sediment from the roots and to keep them moist.



Image 71. Katia Engelhardt dividing clones into new planting containers. Photo Credit: T. Breton

Repotting Protocol

1. Fill a mesocosm with 10 cm of damp topsoil.
2. Gently press the roots of the harvested shoots into the sediment by gently pinching the bottom of the shoot and pushing into the sediment until the bottom of the container is found.
3. Place the shoots 1–2 inches apart to give them room to expand.
4. Gently add a layer of medium-grade silica sand (<0.25 inches) over the substrate.



Image 72. Clone that has been replanted in a new container. Photo Credit: T. Breton

Storing Mature Shoots

Unlike seed pods or turions that can tolerate extended cold storage, adult shoots are very vulnerable. The leaves are fragile, light-dependent, and highly susceptible to rot. It is best to plant them 24–48 hours after harvesting to avoid any complications. At most, they can remain in cold storage for 10 days before deteriorating. If shoots are stored for a few days, they can be placed in a gallon-sized Ziploc bag and filled with water. The leaves must be fully submerged. If the shoots are transported directly to the field, they can be wrapped in wet paper towels and kept in coolers until use or stored in shallow mixing trays filled with water.



Image 73. Shallow mixing tray holding harvested adult shoots. Photo Credit: T. Breton

2.12 *Harvested Material Storage*

Storage Containers

Turions may be stored in small plastic containers fitted with lids. Seed pods should be kept in small Whirl-Paks, and mature shoots can be kept in Ziploc bags with a small amount of water. All storage containers should be filled with just enough water to fully cover the propagule. Storage containers should be labeled with quantity, type of propagule, and date of harvest.

Temperature and Environment

We keep harvested propagules in a lab fridge (set to approximately 2.7–4.4°C). Lights in the fridge need to be kept off to reduce the likelihood of early sprouting.

Monitoring Stock

Stock should be monitored every 1–2 weeks. Check the water levels in the containers to make sure no leaks have occurred. Plant material should be checked for any signs of sprouting or deterioration. If the turions start to sprout, they need to be planted fairly soon. The seed pods may burst in storage but can still be planted as normal. Similarly to turions, seeds may not be viable if they are mushy and have an odor.



Image 74. Plant material being stored in the lab fridge. Photo Credit: T. Breton

2.13 *Troubleshooting*

Absence or Low Ramet Emergence



It is possible that shoots do not emerge from the planted turions because the turions are not viable, light is inadequate, algae is excessive, the turion was buried too deeply, or the water is too cold. Planting in a pattern is best, so turions that did not germinate can be identified and replaced. Wait 3–4 weeks after initial planting to allow ample time for the turion to sprout. First, you want to see if you can find the turion in the sediment to confirm that it is non-viable. If the turion is no longer viable, you can replace it with another one. If you find that the turion is still viable, investigate the conditions of the mesocosm and try to correct the issue.

Absence of Flowering

If flowering occurrence is low, a full, three-tablet dose of Thrive NilocG fertilizer tablets placed directly into the sediment can help induce higher rates of flowering.

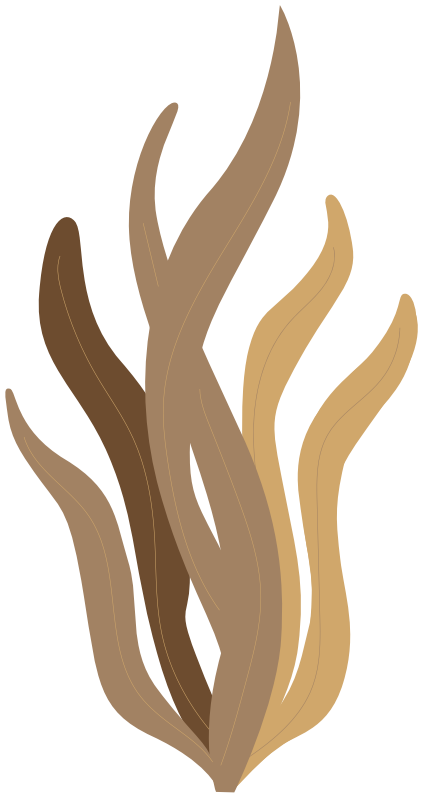
Turions or Seeds Sprouting Before Planting

Turions or seeds can sprout in the fridge before you are ready to plant them. If this occurs, you should begin planting as soon as possible. You can plant like normal but allow the sprout to emerge from the sediment.

Pest Outbreaks

Stagnant water provides a fantastic medium for pests to lay eggs, and it is possible the mesocosm can become overrun with aquatic insects. The best way to troubleshoot this issue is to cycle the water by filling more water into the mesocosms and allowing the pest-ridden water to overflow. Replace the water at a higher frequency.

Declining Plant Health



It is possible that plant health may decline throughout the growing season. Some visual cues that plant health is declining are yellowing or the development of spots on the leaves, unusual odors, algae overgrowth, or rotting at the base of the plant. If you notice the plants are declining, it can be beneficial to take water quality measurements such as temperature and pH. Adding fertilizer tablets or aeration may support the plant's needs. If you suspect the substrate is affecting plant health, remove the plants from the mesocosm and replant them with sifted topsoil and clean water.

Algae Overgrowth

When algae growth overtakes the mesocosms, first try scooping or hand-pulling any surface and filamentous algae from the water. You can use an abrasive sponge to scrub off any algae attached to the walls of the mesocosm. Adding aeration to the mesocosms can be used as a preventative measure to discourage surface algal growth.

Insufficient Substrate Volume

You may notice the base of some plants peeking out from the sediment, which is a clear indicator that the substrate depth is insufficient to accommodate the plant's expansive root system. This can be resolved by draining the water and adding more sediment to ensure a depth of at least 10 cm. Similarly, if "floaters" occur due to buoyant, sprouted turions becoming dislodged, simply reinsert them into the substrate; once they establish a firm root system, the plants will naturally remain anchored.

Abnormal Leaf Morphology

Sometimes *Vallisneria americana* can produce leaves that are not the standard bright green color, so it is important to know what variations are normal and which can indicate declining health.



Image 75. Healthy *Vallisneria americana* leaves.
Photo Credit: K. Engelhardt

- **The "Sunscreen" Tint (Normal):** A red or bronze tint is often normal. This occurs when the plant produces anthocyanins to act as a natural "sunscreen" and protect the leaves from intense light.
- **The "Accordion" Fold (Normal):** Leaves may appear folded like an accordion as they first emerge from the base. This is a common growth stage and not a sign of deformity.
- **The "Tiger Stripe" Pattern (Normal):** Leaves may develop small, dark rectangular "blocks" or dashed lines between the vertical veins. This is often a natural morphological feature of *Vallisneria americana*.
- **Yellowing or Browning (Warning):** It is not normal for the leaves to turn yellow or brown during the growing season. This could occur due to poor nutrients or water quality within the mesocosm.
- **Basal Rot (Warning):** If the base of the leaves begins to rot or brown, this can indicate that the base of the plant is buried too deep in the sediment or the substrate has become anaerobic.
- **Epiphytic "Fuzz" (Warning):** It can be common to see a brown or green "fuzzy" coating on the blades. This is actually algae; while a small amount is normal, a heavy coating can indicate too many nutrients in the water or too much light.
- **Translucent Leaves (Warning)**: Leaves that lose their green color during the growing season may be exposed to too much sunlight, bleaching the leaves. Use shade cloth to reduce incident light.

Poor Water Quality and Environmental Conditions

Water quality of the mesocosms may fluctuate. Relying on visual cues and in-house water quality testing, such as using a multimeter probe, can help you address any issues early on.

- **Turbidity:** If the water appears a milky brown color, light supply to leaf tissue is decreased. If this persists for more than a few hours, it is necessary to facilitate a partial water change.
- **High Light Intensity:** Excessive direct sunlight can increase water temperatures. If plants show signs of stress, like leaf bleaching or reduced vigor, reduce light exposure using shade cloth or move the mesocosms to a covered location.
- **Surface Scum:** Adding aeration can help break up accumulations of surface scum or algae.
- **Low Water Temperature:** If the temperature of the water is abnormally low (<15°C), it is possible dormancy might be triggered. Try moving the mesocosm to a spot in the greenhouse with direct light or add submersible heaters.
- **High Water Temperature:** If the water temperature is too high (>30°C), the plants might experience thermal stress, leading to melting or translucent leaves. Try increasing aeration to oxygenate the water, use shade cloth to block the sun, or move the mesocosm to a cooler area of the greenhouse.
- **pH Instability:** *Vallisneria americana* tolerates neutral to alkaline waters. If the pH drops below 7, try facilitating a partial or complete water change.



Image 76. Water baths in the UMCES Appalachian Lab greenhouse. Photo Credit: T. Breton

PART III: SMALL-SCALE COMMUNITY PROPAGATION



Photo Credit: T. Breton

3.1 *Small-Scale Propagation*

For some practitioners, maintaining a full-scale greenhouse operation is not feasible. However, limited institutional capacity should not prevent the preservation of *Vallisneria americana* genetic diversity. By distributing genotypes among schools, community members, and practitioners, a living genetic repository can be maintained to preserve genetic diversity while supporting education and future restoration efforts. This section outlines approaches for successfully propagating genotypes in classroom settings, indoor home stations, and backyard spaces, effectively turning the community into a decentralized nursery.

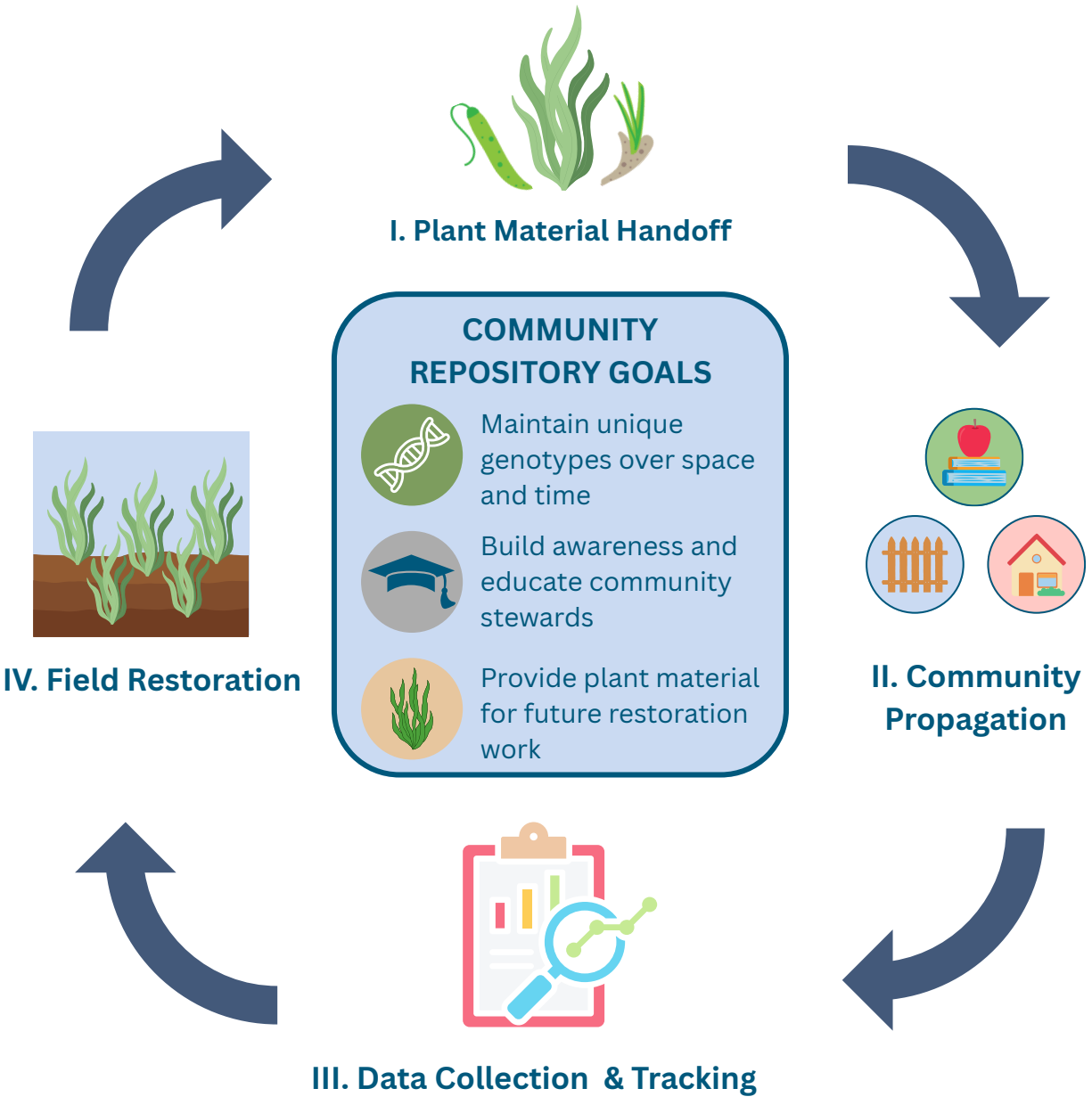


Figure 9. Workflow for maintaining a community-based genetic repository.

3.2 *Preparing for Propagation*

Employing community-based propagation and outsourcing the propagation work to citizen scientists requires seamless transitions and constant communication to ensure the repository remains healthy and robust. Making sure that citizen scientists are equipped with the correct genotypes and propagation material is critical to a successful nursery.

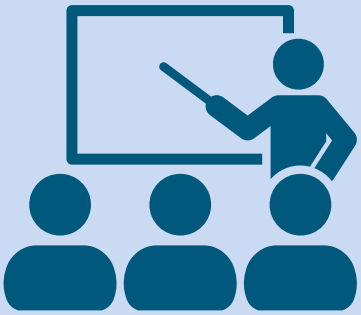
Source Genotypes and Assemble Toolkits

Lead practitioners manage the sourcing of specific plant genotypes and assemble standardized "toolkits" for community scientists (see **Section 3.3**) to ensure consistency.



Provide Standardized Trainings

Standardized trainings are the backbone of maintaining a community-based repository. They ensure every volunteer is fluent in specific propagation and data collection methods.



Handoff Materials

Direct handoff is more than a delivery; it is the launch of a new decentralized nursery site. Lead practitioners should provide materials directly to volunteers to eliminate barriers to entry.



With the handoff of materials complete, community scientists can begin small-scale propagation!

3.3 *Small-Scale Toolkit*

To successfully host a community-based genetic repository, you only need a few fundamental components that are outlined in this toolkit. The “Small-Scale Toolkit” is designed for portability and affordability, whether you are setting up a few plants indoors or a dozen tanks.

Planting Vessels

Choose the planting containers that best fit the size and location of where you want to propagate!



10+ Gallon Fish Tanks

Best for:

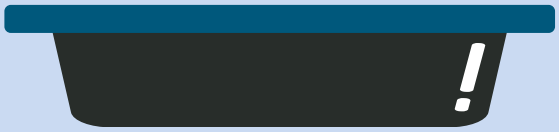
- Classrooms
- Indoors

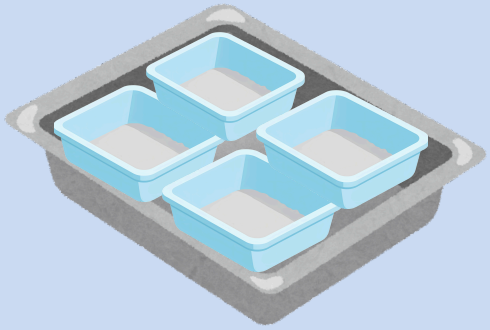


2.5-gallon buckets

Best for:

- Outdoors
- Indoors





Water Baths and Mixing Trays

Best for:

- Outdoors

Substrate



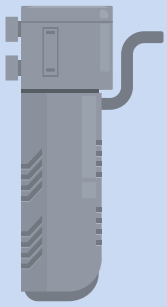
For all setups, use sifted topsoil. This can be purchased from a local nursery or garden store. Do not use bagged soil, as it often contains too much organic matter and is amended with nutrients.

Data Collection Gear



Datasheets and notebooks are great for keeping data organized. Plastic rulers can be used to collect measurement data. Multimeter probes can collect temperature and pH data.

Supplemental Gear



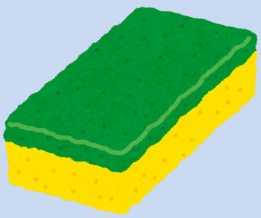
Tank Filter

Good for keeping water clear and preventing algae.



LED Grow Light

Provides plants additional light if natural light is insufficient.



Abrasive Sponge

Good for clearing algae out of planting containers.

3.4 *Classroom Propagation*

As students track plant growth and water quality, they witness firsthand the power of SAV and its ability to support broader ecosystem health. Programs such as **Grasses in Classes** (Maryland DNR) and **SAV in (and out of) the Classroom** (New York State DEC) have long set the standard, demonstrating that when students grow SAV, they aren't just completing a project but participating in a legacy of local restoration.

Classroom Tank Setup Options:

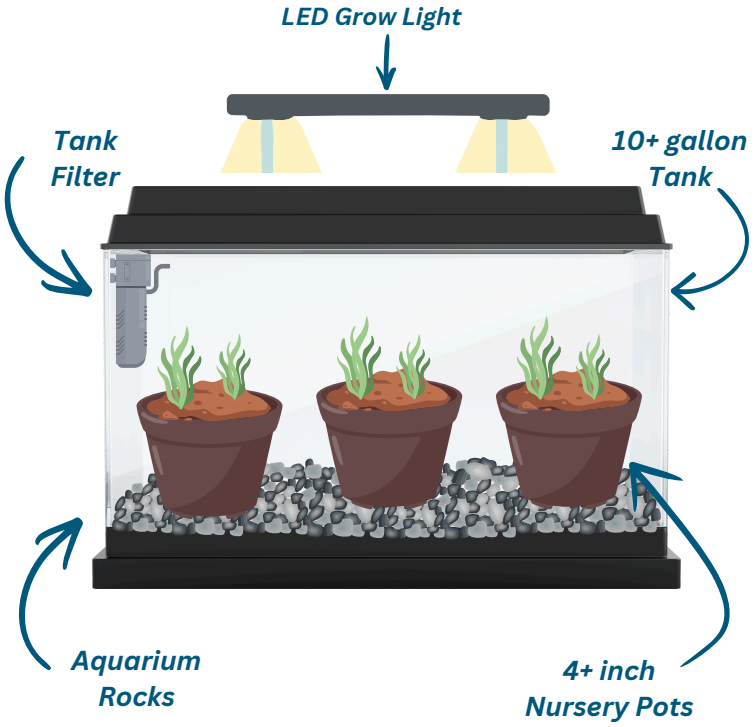
Fish Tank & Nursery Pots

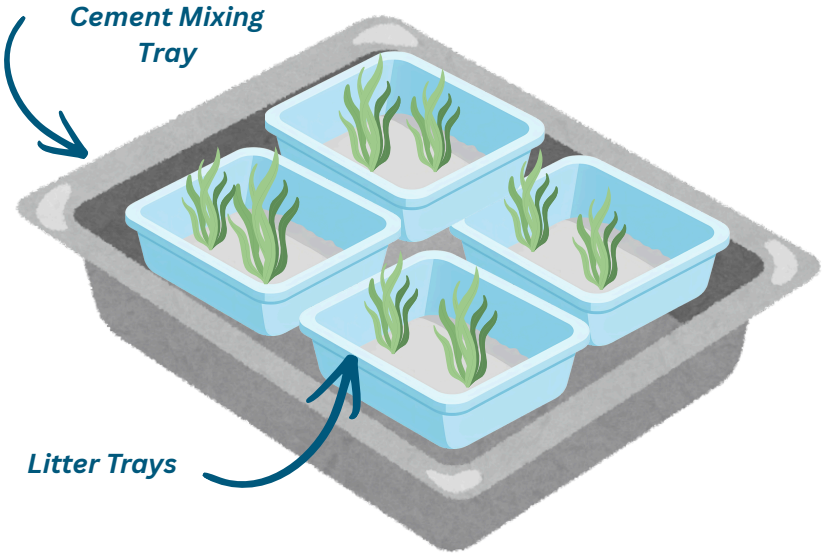
PRO:

- Each student can be responsible for monitoring their own potted plant.

CON:

- Pots might be too tiny to see significant plant expansion and growth.





Mixing Tray System

PRO:

- Trays allow larger surface area for plant expansion.

CON:

- Requires more classroom space than an aquarium setup.

Setup Instructions

1

Prepare Pots or Trays
Fill 4+ inch nursery pots or litter trays with 2–3 inches of topsoil. Add a genotype label on each container.



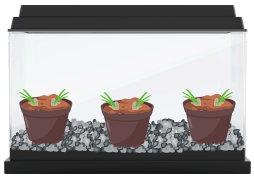
2

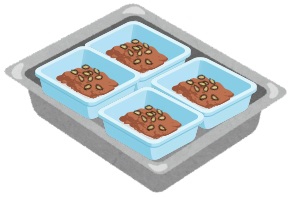
Plant the Containers
Add turions or seeds into the containers.



3

Arrange and Anchor
Place the pots in the empty tank. Fill the surrounding space with rocks. Or place four litter trays into a larger mixing tray.





4

Slow Fill
Use tap water to fill the tank or trays. Slowly diffuse the water over your hand to prevent disruption.



5

Optional: Power Up
Install a filter and LED grow light on the tanks. Keep the light on during the daytime.



3.5 *Indoor Propagation*

You don’t need a greenhouse to make a difference. Even single window sills can make a difference and serve as a vital link to a community-based genetic repository. Success in small spaces is all about optimization, such as maximizing light and ensuring the plants have room to expand.

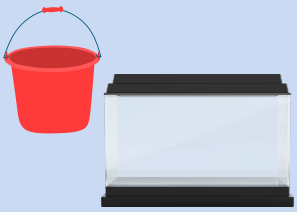
Setup Instructions

1. **Locate the Light**
Find a spot indoors with plenty of indirect light, such as a window. If you cannot find a spot with good lighting, an LED grow light can also work.

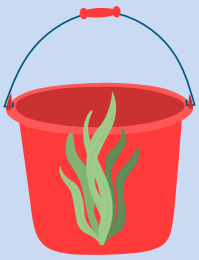
2. **Determine Planting Setup**
Depending on space, you can set the planting containers on your windowsill, raised tables & desks, or vertical shelving to maximize space.



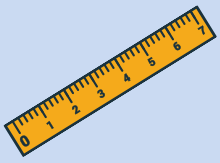
3. **Set Up Planting Containers**
Choose containers that optimize the planting space. You can select tanks or 2.5-gallon plastic buckets depending on what best fits in the space.



4. **Planting**
Fill the containers with 2–3 inches of sifted topsoil. Gently pour tap water into the containers, using your hand to diffuse the water.



5. **Monitoring and Data Collection**
Every week, make sure the water levels are replenished. Record leaf length and the number of plants per container.



3.6 Outdoor Propagation

Utilizing backyard space can be one of the best ways to expand your genetic repository when a greenhouse is not available. By moving outdoors, you harness the power of full natural cycles, allowing you to produce a higher volume of robust, “weather-hardened” plants ready for the field.

Pick an area in the yard that is semi-sheltered from weather exposure and wildlife. The ground should be stable enough to support the planting containers. Find a site with partial shade and plenty of light.

In the past, we have used 2.5-gallon buckets filled with 10 cm of sediment as planting vessels. However, you could also incorporate water baths or mixing trays filled with litter trays to optimize space. You can use a regular garden hose to fill the containers; make sure to diffuse the water with the palm of your hand.



Image 77. 2.5-gallon containers of *Vallisneria americana* in a backyard during a rainstorm.
Photo Credit: T. Breton



Image 78. Monoculture water baths of *Vallisneria americana* being stored outdoors.
Photo Credit: T. Breton

Monitor the planting containers once a week for unwanted pests, yard debris, and appropriate water levels. One trick is to lay mosquito netting over the tops of the containers to prevent pests and debris from falling in. The plants can tolerate most weather events, but if you experience extreme weather, you may need to take the containers inside.

Every 4–6 weeks, you can collect data on the plants, including leaf length and the number of plants per container.

3.7 Data Tracking

By tracking growth patterns, citizen scientists provide real-time insights needed to ensure the genetic stock remains healthy and ready for future restoration work.

Citizen Scientists Can Focus on 3 Primary Metrics:

1. **Visual Cues of Plant Health**
   Describe the color and overall appearance of the leaves. Measure the length of the tallest leaf.

2. **Number of Plants**
   Note how many individual ramets are in each container to track expansion.

3. **Water Quality Observation**
   - **Water Level:** Does the water need to be refilled?
   - **Clarity:** Is the water clear or are algae blooming?
   - **Temperature and pH (optional):** Is the water within optimal ranges for both metrics?

Submitting Data:

A common option for receiving data is for citizen scientists to complete data sheets. However, electronic options can include submitting the sheets to an online drive or filling out a Google Form to record data metrics.



3.8 Collecting Propagated Plants

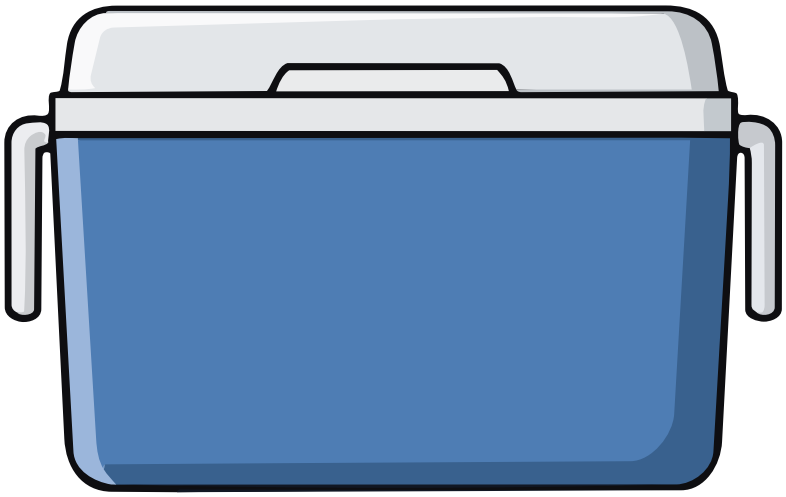
It should be up to the lead practitioners to facilitate the collection of propagated plant material. Lead practitioners can harvest the plant propagules, including turions, adult shoots, and seed pods, using protocols and guidance outlined in **Part 2** of this manual. Propagule storage techniques outlined in **Section 2.12** should be followed until needed for field restoration or future propagation.

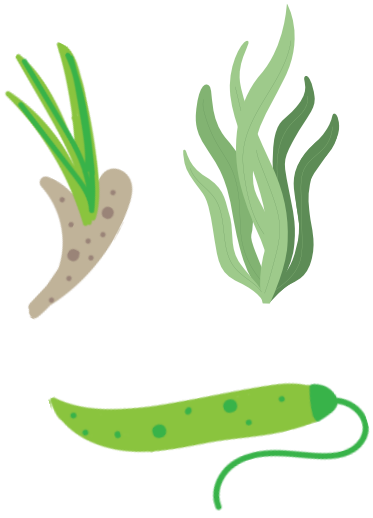
Collection Logistics

Climate Control
Propagules are sensitive to heat. Use insulated coolers with ice packs for transport from site to site. Do not leave propagules in a cooler overnight; they should be moved to a fridge as soon as possible.

Labeling
Every batch should be labeled with genotype ID, harvest date, and location.

Inventory Update
Keep a master repository database, such as an Excel spreadsheet, that details the genotype, collection date and location, as well as number of propagules.





PART IV:
FIELD DEPLOYMENT



4.1 *Restoration Planning*

Various methods exist for planning field restorations based on regional guidelines and best practices. The following techniques have been successfully used in dynamic estuarine systems, like the Hudson River Estuary, where large environmental gradients dictate site conditions.

Site Selection

Selecting ideal candidate sites requires evaluating both the historical presence of submerged aquatic vegetation (SAV) and current environmental conditions (see **Section 4.2**) to ensure that baseline thresholds are met to support bed establishment and long-term sustainability of their structure and function.

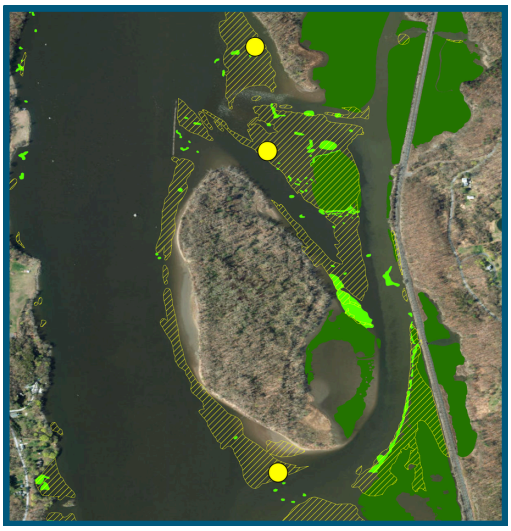


Figure 10. Map of candidate sites at Stockport Flats. Solid green represents presence of SAV in a 2022 NYSDEC SAV inventory. The dashed green area represents historical presence of SAV.

Permitting

After identifying candidate sites, determine which regional agencies hold jurisdiction. Allow a **minimum of 90 days** to submit and process applications before fieldwork begins. For example, at Stockport Flats, we secured a Temporary Revocable Permit (TRP) from NYSDEC and a recreation and historic preservation permit from the New York State Office of Parks (OPRHP).

Defining Goals and Outcomes

Beyond logistics, clearly define the project's goals and expected outcomes. Because no universal standards for SAV restoration success exist, establishing specific, measurable metrics, such as 70% plant survival by the end of the growing season, sets clear expectations for future site monitoring.

4.2 *Site Selection Checklist*

When selecting sites for restoration, sites need to meet the minimum suitability threshold for *Vallisneria americana* growth. You can leverage data from regional long-term water quality monitoring stations. While these stations provide excellent high-resolution data on parameters like dissolved oxygen, turbidity, and salinity to identify general trends, they may not capture localized discrepancies. Therefore, site-specific environmental data should be collected at each potential location before planting to account for these variations. **The following parameters and thresholds are based on our restoration work in the Hudson River and are intended to serve as a starting point for evaluating sites, rather than an exhaustive set of site suitability criteria.**

Elevation

Elevation can be used to identify areas that fall within a suitable depth range for SAV establishment. Because water depth varies with elevation across a site, regional data on elevations or depths where SAV has successfully established can help identify an appropriate target range for restoration. Variations in elevation can also influence light availability, water circulation, and sediment distribution, which may affect the establishment and growth of *V. americana*.



Image 79. Dan Miller (NYSDEC) using RTK to measure orthometric elevation. Photo Credit: T. Breton

In previous restoration work in the Hudson River Estuary, we used Real-time Kinematic (RTK) to identify an orthometric elevation within a successful depth for *V. americana*. A 30 x 30 m grid was then established, and RTK was used to measure orthometric elevation at 1 m intervals. These data were used to orient the planting grid within the target elevation range.

Exposure and Wave Energy

Exposure and wave energy can have strong impacts on the early establishment of propagules. This can easily be done by a visual survey of the site or via GIS software to assess the area for possible exposure to large channels, areas of high wave potential, etc. It is recommended to pick a location that does not experience chronic high wave energy or exposure from boat wakes or long fetches.

Sediment Characteristics

The **pH** of the sediment can affect the establishment of *V. americana*. Sediments outside of the optimal pH range could hinder the growth of founder colonies or lead to poor plant health. A pH meter can be used to measure the pH of the sediment by creating a 1:2.5 slurry of sediment and distilled water and measuring the pH promptly after preparation.

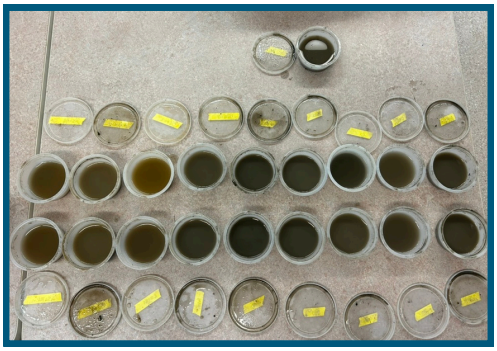


Image 80. Sediment slurry equilibrating to the environment. Photo Credit: T. Breton

Very coarse sediments hinder root establishment. Therefore, quantifying sediment grain size can help identify which sites *Vallisneria americana* is most likely to succeed. **Grain size** can be measured via sieving. The sample is dried in an oven to remove moisture, then weighed. After drying, the sediment is passed through a set of sieves with mesh sizes of 2 mm, 1000, 500, 250, 106, and 53 micrometers on a mechanical shaker. The sediment retained in each sieve is weighed to calculate the percentage of each size fraction. The Wentworth scale can be used to classify grain sizes.



Image 81. Sieve shaker at UMCES AL. Photo Credit: T. Breton

Organic matter (OM) plays an important role in nutrient cycling and availability, sediment structure, oxygen availability, and more, all of which affect the success of *Vallisneria americana*. Organic matter is measured using Loss on Ignition (LOI). The sediment is dried at 105°C to remove water, and the dried sample is weighed. The sediment is then placed in a crucible and ignited in a combustion furnace at 375°C for 4 hours. After cooling, the sample is weighed again, and organic matter is calculated based on the weight loss during ignition.



Image 82. Sediment in crucibles. Photo Credit: T. Breton

Pore Water Nutrients

Pore water is important to consider as it characterizes the nutrients in the sediment that are available for root uptake. Pore water can be collected using a pore water sampler referred to as a "peeper." These peepers are homemade using 1.0 µm polycarbonate filters placed through a hole in the cap of a 250 mL conical centrifuge tube. Vinyl and fiberglass mesh are secured on the inside and outside of the cap.



Image 83. Peepers to be deployed at Stockport Flats field sites. Photo Credit: T. Breton

The centrifuge tube is filled with DI water. Peepers should be placed into the sediment with about 1 inch of coverage and allowed to collect pore water for two weeks. Upon collection, they should be filtered using 25 mm, 0.45 µm PES syringe filters and 30 mL Luer-Lock syringes. Upon filtration, samples can be sent to a laboratory to be analyzed for oxidized inorganic nitrogen, ammonia/ammonium, and orthophosphate.

4.3 *Genotype Selection Guidance*

Strategically selecting genotypes to deploy in field restoration can be a beneficial strategy to enhance the long-term success and resilience of planted populations. One strategy is to select only high-performing genotypes. Another is to plant multiple genotypes to support genetic diversity within restored beds. Both strategies are important for facilitating establishment and long-term stability.

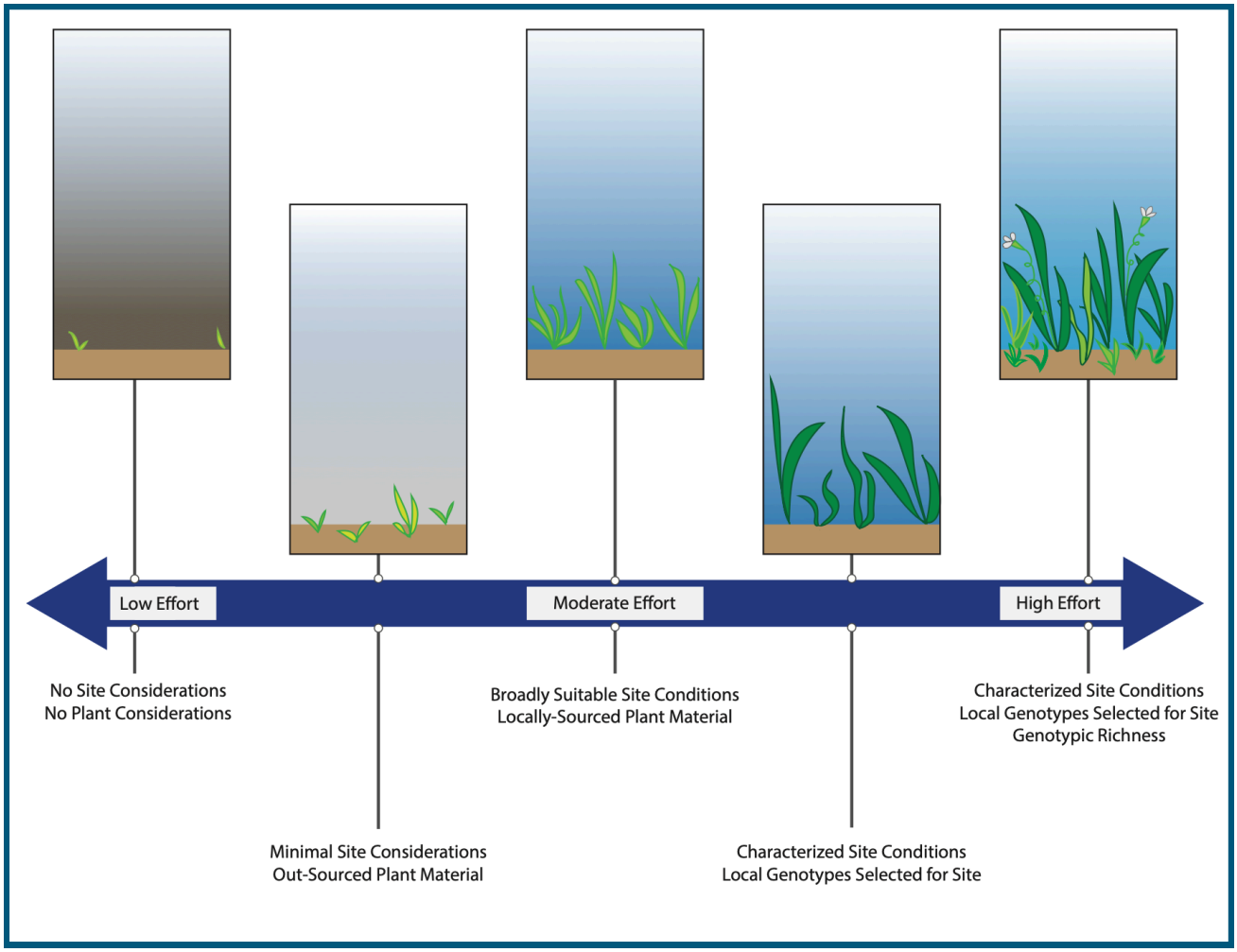


Figure 11. Graphic illustration of low, moderate, and high effort submerged aquatic vegetation (SAV) restoration techniques, highlighting the expected outcomes of each strategy.

Prioritizing Genotype Selection

Genotype selection is a targeted approach to maximize SAV restoration outcomes. If planting in a highly variable or stressful environment, strategically selecting genotypes may pose benefits to survival and early establishment. If the restoration site has a known environmental stressor, such as an unfavorable sediment type, planting a genotype that is known to be stress-tolerant from greenhouse experiments can likely improve restoration outcomes. Selecting a genotype that expands rapidly through clonal growth is beneficial for fast establishment.

Prioritizing Genetic Diversity

Broad genetic diversity should be selected for when the goal of the restoration is long-term resilience in planted populations. If the restoration sites are unpredictable, planting multiple genotypes allows for success even if some genotypes might fail under the environmental conditions. A wide pool of genetic material can support longevity in restored beds for years to come. Genetic diversity can be supported by planting multiple genotypes using turions and/or introducing seeds.



Image 84. Naturally occurring *Vallisneria americana* bed at Stockport Flats. Photo Credit: T. Breton

4.4 *Transporting Plants*

When transporting the propagules from the greenhouse, it is crucial to make sure proper measures are taken so the plants remain healthy during travel and have the best chance of survival in the field. It is best to move propagules from the greenhouse into the field within 24 hours.

All propagules can be transported in:

- Coolers filled with ice
- Shallow mixing trays filled with water

Transporting Seeds or Turions

If the seeds or turions have not sprouted, they can be transported in the same containers they were stored in the fridge. Make sure they have enough water to completely cover the propagules.

Transporting Leafy Material

Whether you are transporting adult shoots or burlap bags with leaf tissue, it is important to not let the leaf tissue dry out. The best option is to keep the leaf tissue submerged in water. If possible, shallow mixing trays filled with water are the best possible method of transportation. If that is not feasible, wrap the leaf tissue in wet paper towels and store in a cooler with ice.



Image 85. Burlap bags transported in gallon Ziploc bags and covered with wet paper towels. Photo Credit: D. Miller

4.5 *Field Deployment Strategies*

The following field deployment techniques are those that we have found to be successful in the Hudson River. This list is not exhaustive, and other techniques may be equally effective. Practitioners are encouraged to adapt these approaches or use alternative methods based on system needs.

Laying the Transects

Laying transects is one of the most efficient ways to plant plots (founder colonies) so that their location can be identified with ease for monitoring. Transects can be established prior to planting or on the day of planting. We have used a pre-marked 1-meter transect line strung between two snow poles to delineate plot locations. In past research (2025), we have marked snow poles every 3–5 meters. Snow poles are a good marker because they are flexible and will bend if a large object floats across them.



Image 86. Transects marked by snow poles at Stockport Flats. Photo Credit: T. Breton

Burlap Bag Method

Burlap bags provide an effective “safe site” to allow the plants to establish roots in the riverbed. They can be anchored into the sediment using 6-inch or 12-inch landscape staples to provide extra security. These bags are designed to biodegrade over time, so only the staples need to be removed from the water. We have found that the burlap material of the bags does not break down over the growing season (May–August).

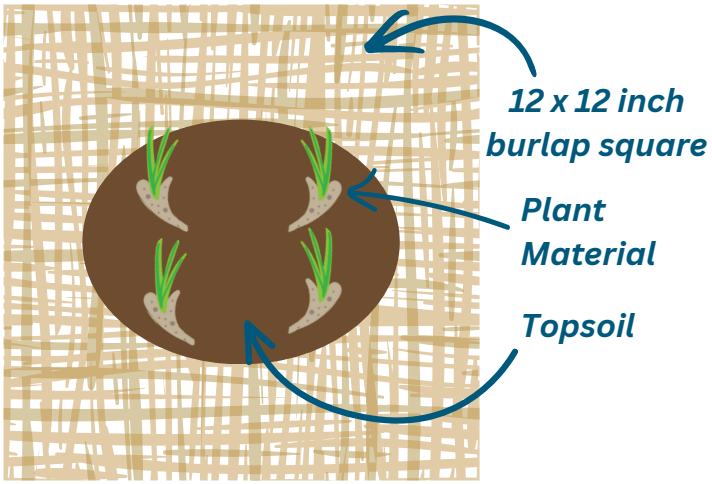


Figure 12. Graphic illustration of how the burlap bags are assembled.



Image 87. Burlap bag that supports *Vallisneria americana* leaf tissue. Photo Credit: K. Engelhardt

The bags are created using a 12 x 12-inch square of burlap with a “fist-sized” amount of wet topsoil in the center of the square. Turions or seeds can be placed directly into the sediment but not fully buried. Adult shoots may also be gently inserted through the openings of the burlap. Regardless of propagule type, be mindful not to place the propagules directly into the center of the sediment, as that is where the bag will get tied off. Gather the corners of the burlap square to the center of the bag and tie off with a string of burlap. Cut off the excess burlap, as it can attract algae. The bags can be grown in a greenhouse up to one month prior to planting to ensure there is leaf material present, or directly planted in the field as is. We recommend deploying burlap bags within one month, as the burlap weakens over time with exposure to water.

Field Installation

We have used a “founder colony” technique (Smart & Dick, 1998) for field restoration, which allows us to easily plant and monitor the plots with minimal disturbance to the growing plants. A founder colony refers to a 1-square-meter plot that contains a determined number of burlap bags.

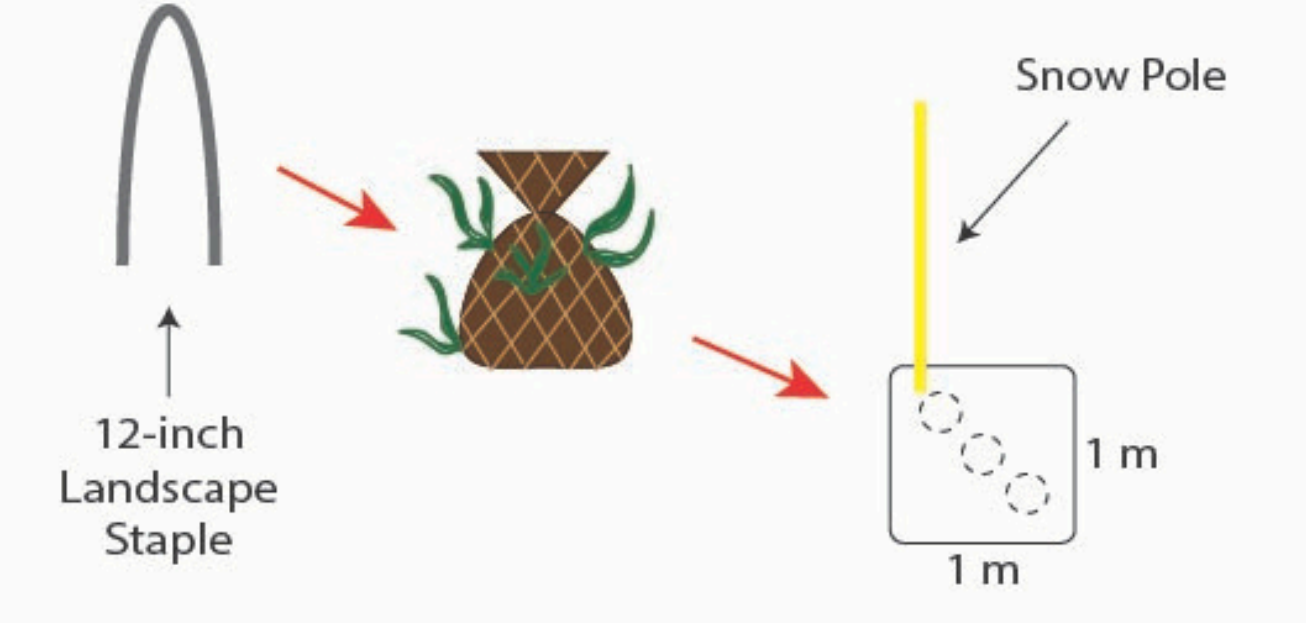


Figure 13. Graphic illustration of how the burlap bags are placed at the field sites.

To plant the founder colonies, we take a 1-square-meter PVC quadrat and orient it in the same direction over each snow pole marker. Placing the burlap bags in a specific pattern, such as a diagonal or square, is helpful for monitoring them later in the summer. Gently place a landscape staple directly over the top of the burlap bag, being careful not to clip any established leaf tissue. Place the bag and staple firmly so that the bottom of the bag is in good contact with the sediment. Remove the quadrat.

4.6 Monitoring

Field sites should be monitored beginning 4–6 weeks after planting.

Presence and Absence

Sites can be monitored for survivorship by determining the presence or absence of leaf tissue. In sediment-driven systems, this may have to be done completely by touch because turbidity reduces visibility of the sediment surface. You can gently feel for leaf tissue, which can take some practice and needs to be standardized among multiple observers.



Image 88. Leaf tissue found at restoration site on July 29, 2025. Photo Credit: T. Breton

Characterizing Leaf Tissue

If you feel a presence of leaf tissue, use a tactile 0–3 scale if you are interested in differentiating productivity among founder colonies. A score of 0 indicates the total absence of tissue, while a score of 3 represents a significant amount, approximately equivalent to a handful of *V. americana*. This standardized assessment allows for consistent documentation of material density across all samples.



Figure 14. Graphic illustration of a ranking scale for *Vallisneria americana* leaf tissue.

Expansion

Expansion can be monitored by counting the number of runners between ramets or by estimating percent cover using an established submerged aquatic vegetation monitoring protocol.



Image 89. Leaf tissue found at restoration site on August 26, 2025. Photo Credit: T. Breton

In past Hudson River Estuary work, we followed HRNERR monitoring protocols for estimating *V. americana* cover (Pacella, 2026). However, the most appropriate method may vary among systems and restoration objectives. When using percent cover, establish consistent cover classes and apply them consistently across monitoring periods. In some systems, selecting and measuring the length of the tallest leaf may provide information on the height of the canopy; however, in turbid systems like the Hudson River, selecting a representative leaf may provide a more practical approach.

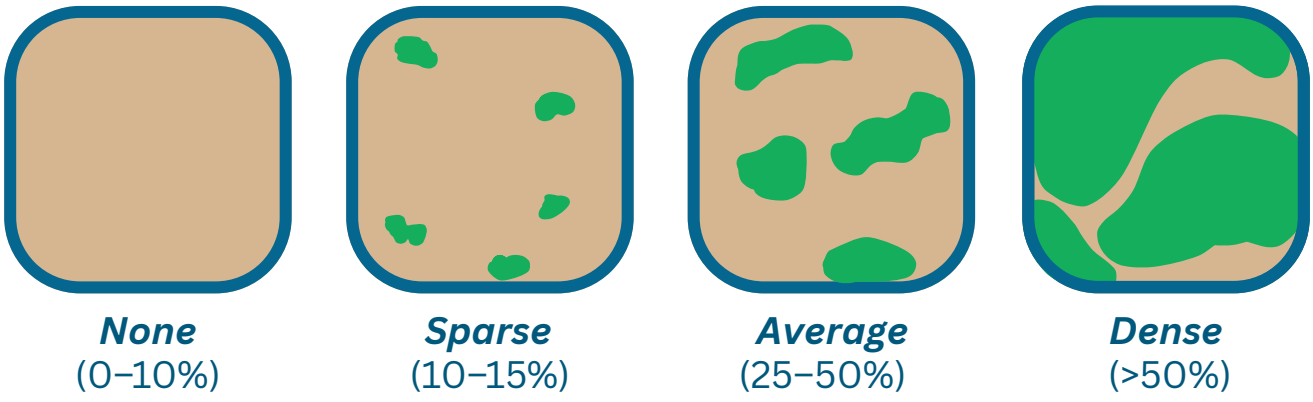


Figure 15. Graphic illustration of Hudson River National Estuarine Research Reserve (HRNERR) SAV percent coverage classes.

4.7 Adaptive Management

Restoration outcomes can vary among sites and over time, emphasizing the importance of regular monitoring. **Adaptive management** is an iterative approach to restoration in which monitoring results are used to evaluate outcomes and adjust management practices as needed. Adaptive management can help practitioners address challenges and improve restoration outcomes before the project is complete.

Common Adaptive Management Techniques Include:

Replace Lost Plant Material

In areas where plant material has been lost, replacing propagules can help maintain planting density and reduce gaps in developing SAV beds.



Image 90. Burlap bag with growing propagules.
Photo Credit: K. Engelhardt

Relocate Displaced Burlap Bags

If burlap bags have shifted or become dislodged, relocating and securing them with landscape staples or weights can help maintain proper placement and prevent further displacement.



Image 91. Taylor Breton securing bags with landscape staples with a quadrat.
Photo Credit: D. Miller

Adjust Planting Density

If establishment is consistently sparse or patchy, increasing the planting density in poorly established areas can help promote more continuous bed development.



Add Herbivory Fencing

If herbivory is suspected to be contributing to plant loss, herbivory cages can be installed during the growing season to reduce further losses. In the Hudson River Estuary, we constructed 5 x 5-ft cages from PVC pipe with a height of 2.5 ft. The cages were wrapped with plastic landscape fencing, including a lid, and anchored into the sediment using rebar stakes or posts at the corners.



Image 92. Herbivory cages deployed at Stockport Flats in Stockport, New York, at low tide. Photo Credit: D. Miller

Increase Monitoring

If plant loss or unexpected changes are observed, increasing monitoring frequency can help pinpoint issues and determine whether conditions are improving or if additional management actions are needed.

References

[1] Boustany, R. G. (2003). A pre-vegetated mat technique for the restoration of submersed aquatic vegetation. *Ecological Restoration*, 21(2), 87–94.

[2] Burnett Jr, R. K., Lloyd, M. W., Engelhardt, K. A. M., & Neel, M. C. (2009). Development of 11 polymorphic microsatellite markers in a macrophyte of conservation concern, *Vallisneria americana* Michaux (Hydrocharitaceae). *Molecular Ecology Resources*, 9(5), 1427–1429. https://doi.org/10.1111/j.1755-0998.2009.02578.x

[3] Carew, A. & Engelhardt, K. (2020). Factors Affecting the Growth and Establishment of *Vallisneria americana* in the Hudson River, NY. Section V: 1-23 pp. In D. J. Yozzo, S. H. Fernald, and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship, 2017. Hudson River Foundation.

[4] Cook, C. D. K. (1987). Dispersion in aquatic and amphibious vascular plants. In: Crawford, R. M. M. (ed.), Plant Life in Aquatic and Amphibious Habitats, pp. 179–190. Special Publication no. 5 of the British Ecological Society. Blackwell, Oxford.

[5] Findlay, S. E. G., Nieder, W. C., Blair, E. A., & Fischer, D. T. (2006). Multi-scale controls on water quality effects of submerged aquatic vegetation in the tidal freshwater Hudson River. *Ecosystems*, 9(1), 84–96. https://doi.org/10.1007/s10021-004-0053-7

[6] Engelhardt, K. A. M., Lloyd, M. W., & Neel, M. C. (2014). Effects of genetic diversity on conservation and restoration potential at individual, population, and regional scales. *Biological Conservation*, 179, 6–16. https://doi.org/10.1016/j.biocon.2014.08.011

[7] Folke, C., Carpenter, S., Walker, B., Scheffer, M., Elmqvist, T., Gunderson, L., & Holling, C. S. (2004). Regime shifts, resilience and biodiversity in ecosystem management. *Annual Review of Ecology, Evolution, and Systematics,* 35, 557–581. https://doi.org/10.1146/annurev.ecolsys.35.021103.105711

[8] French, G. T., & Moore, K. A. (2003). Interactive effects of light and salinity stress on the growth, reproduction, and photosynthetic capabilities of *Vallisneria americana* (Wild Celery). *Estuaries,* 26, no. 5: 1255–68. https://doi.org/10.1007/BF02803628.

[9] Gurbisz, C., Kemp, W., Sanford, L., & Orth, R. (2016). Mechanisms of storm-related loss and resilience in a large submersed plant bed. *Estuaries and Coasts*, 39. https://doi.org/10.1007/s12237-016-0074-4

[10] Hilderbrand, R. H., Watts, A. C., & Randle, A. M. (2005). The myths of restoration ecology. *Ecology and Society*, 10(1). https://www.jstor.org/stable/26267738

[11] Hughes, A. R., & Stachowicz, J. J. (2004). Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences of the United States of America*, 101(24), 8998–9002. https://doi.org/10.1073/pnas.0402642101

[12] Hutchinson, G. E. (1975). A treatise of limnology, limnological botany. John Wiley and Sons, New York.

[13] Integration and Application Network (2025). IAN Media Library: Pictures and icons [Media Library]. University of Maryland Center for Environmental Science. https://ian.umces.edu/media-library/

[14] Jasinski, D., Gurbisz, C., Huey, L., & Landry, B. (2021). Small-scale SAV restoration in Chesapeake Bay: A guide to the restoration of submerged aquatic vegetation (SAV) in Chesapeake Bay and its tidal tributaries. Approved by the CBP SAV Workgroup in October 2021. Available at chesapeakebay.net

[15] Kemp, M., Batleson, R., Bergstrom, P., Carter, V., Gallegos, C. L., Hunley, W., Karrh, L., Koch, E. W., Landwehr, J. M., Moore, K. A., Murray, L., Naylor, M., Rybicki, N. B., Court Stevenson, J., & Wilcox, D. J. (2004). Habitat requirements for submerged aquatic vegetation in Chesapeake Bay: Water quality, light regime, and physical-chemical factors. *Estuaries*, *27*(3), 363–377. https://doi.org/10.1007/BF02803529

[16] Korschgen, C. E., & Green, W. L. (1988). American wildcelery (*Vallisneria americana*): Ecological considerations for restoration. *U.S. Department of the Interior, Fish and Wildlife Service*.

[17] Lefcheck, J. S., Orth, R. J., Dennison, W. C., Wilcox, D. J., Murphy, R. R., Keisman, J., Gurbisz, C., Hannam, M., Landry, J. B., Moore, K. A., Patrick, C. J., Testa, J., Weller, D. E., & Batiuk, R. A. (2018). Long-term nutrient reductions lead to the unprecedented recovery of a temperate coastal region. *Proceedings of the National Academy of Sciences*, 115(14), 3658–3662. https://doi.org/10.1073/pnas.171579811

[18] Lloyd, M., Burnett, R., Engelhardt, K., & Neel, M. (2012). Does genetic diversity of restored sites differ from natural sites? A comparison of *Vallisneria americana* (Hydrocharitaceae) populations within the Chesapeake Bay. *Conservation Genetics*, 13, 1–13. https://doi.org/10.1007/s10592-012-0324-3

[19] Lokker, C., Lovett-Doust, L., & Lovett-Doust, J. (1997). Seed output and the seed bank in *Vallisneria americana* (*Hydrocharitaceae*). *American Journal of Botany*, 84(10), 1420–1428. https://doi.org/10.2307/2446140

[20] Marsden, B. W., Ngeve, M. N., Engelhardt, K. A. M., & Neel, M. C. (2022). Assessing the potential to extrapolate genetic-based restoration strategies between ecologically similar but geographically separate locations of the foundation species *Vallisneria americana* Michx. *Estuaries and Coasts*, 45(6), 1656–1673. https://doi.org/10.1007/s12237-021-01031-z

[21] Marsden, B. W., Engelhardt, K. A. M., & Neel, M. C. (2013). Genetic rescue versus outbreeding depression in *Vallisneria americana*: Implications for mixing seed sources for restoration. *Biological Conservation,* 167: 203–14. https://doi.org/10.1016/j.biocon.2013.08.012.

[22] Neel, M. C., Marsden, B. W., & Engelhardt, K. A. M. (2025). Going with the flow? Relative importance of riverine hydrologic connectivity versus tidal influence for spatial structure of genetic diversity and relatedness in a foundational submersed aquatic plant. *Ecology and Evolution*, 15(5), e71264. https://doi.org/10.1002/ece3.71264

[23] New York State Department of Environmental Conservation. (2025). Hudson Estuary Submerged Aquatic Vegetation (SAV 1997 and SAV 2022 layers) [Data set]. NYS GIS Clearinghouse. https://data.gis.ny.gov/datasets/nysdec::hudson-estuary-submerged-aquatic-vegetation

[24] Orth, R. J., Carruthers, T. J. B., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., Hughes, A. R., Kendrick, G. A., Kenworthy, W. J., Olyarnik, S., Short, F. T., Waycott, M., & Williams, S. L. (2006). A global crisis for seagrass ecosystems. BioScience, 56(12), 987–996. https://doi.org/10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2

[25] Orth, R., Dennison, W., Lefcheck, J., Gurbisz, C., Hannam, M., Keisman, J., Landry, J., Moore, K., Murphy, R., Patrick, C., Testa, J., Weller, D., & Wilcox, D. (2017). Submersed aquatic vegetation in Chesapeake Bay: sentinel species in a changing world. BioScience, 67, 698–712. https://doi.org/10.1093/biosci/bix058

[26] Pacella, C. M. (2026). *Sentinel sites monitoring at the Hudson River National Estuarine Research Reserve: Year 14 Report*. Hudson River National Estuarine Research Reserve. https://hrnerr.org/wp-content/uploads/2026/01/2024-BioMon-Report_final.pdf

[27] Pedersen, O., Colmer, T. D., & Sand-Jensen, K. (2013). Underwater photosynthesis of submerged plants – Recent advances and methods. *Frontiers in Plant Science,* 4:140. doi: 10.3389/fpls.2013.00140

[28] Reynolds, L. K., McGlathery, K. J., & Waycott, M. (2012). Genetic diversity enhances restoration success by augmenting ecosystem services. *PloS One*, 7(6), e38397. https://doi.org/10.1371/journal.pone.0038397

[29] Rybicki, N. B., & Carter, V. (1986). Effect of sediment depth and sediment type on the survival of *Vallisneria americana* Michx grown from tubers. *Aquatic Botany*, 24(3), 233–240. https://doi.org/10.1016/0304-3770(86)90059-8

[30] Scheffer, M., Carpenter, S., Foley, J. A., Folke, C., & Walker, B. (2001). Catastrophic shifts in ecosystems. *Nature*, 413(6856), 591–596. https://doi.org/10.1038/35098000

[31] Scheffer, M., & Carpenter, S. R. (2003). Catastrophic regime shifts in ecosystems: Linking theory to observation. *Trends in Ecology & Evolution,* 18(12), 648–656. https://doi.org/10.1016/j.tree.2003.09.002

[32] Scheffer, M., Hosper, S. H., Meijer, M. L., Moss, B., & Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends in Ecology & Evolution*, 8(8), 275–279. https://doi.org/10.1016/0169-5347(93)90254-M

[33] Smart, R., & Dick, G. (1998). Techniques for establishing native aquatic plants. *Aquatic Plant Management*, 36.

[34] Titus, J. E., & Hoover, D. T. (1991). Toward predicting reproductive success in submersed freshwater angiosperms. *Aquatic Botany, Ecology of Submersed Aquatic Macrophytes*, 41(1), 111–136. https://doi.org/10.1016/0304-3770(91)90041-3

[35] Titus, J. E., & Stephens, M. D. (1983). Neighbor influences and seasonal growth patterns for *Vallisneria americana* in a mesotrophic lake. *Oecologia*, 56(1), 23–29. https://doi.org/10.1007/BF0037821

[36] Virginia Institute of Marine Science (VIMS) SAV Monitoring and Restoration Program.
(2024). SAV Interactive Map. Retrieved from https://www.vims.edu/research/units/programs/sav/access/maps/

