Small-scale SAV Restoration in Chesapeake Bay

A Guide to the Restoration of Submerged Aquatic Vegetation (SAV) in Chesapeake Bay and its Tidal Tributaries

Cover photo credit: Zack Kelleher, Sassafras RiverKeeper

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photo credit: Maryland Department of Natural Resources

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Acknowledgements

The Chesapeake Bay Program's SAV Workgroup and Green Fin Studio would like to sincerely thank everyone who contributed to the development of this SAV Restoration guidance document and the restoration techniques described within. The information and guidance shared here has been compiled from experts and a thorough review of relevant literature, though we acknowledge that methods will continue to evolve as practitioners learn more and refine their methods.

Of critical importance to the development of this guide were the folks who took the time out of their busy schedules to speak with us and share their extensive knowledge about SAV and its restoration in Chesapeake Bay. Their efforts in research and restoration over the past few decades are the source of much of the guidance we detail in this document. We would especially like to thank Michael Norman and Steve Ailstock at Anne Arundel Community College, Mark Lewandowski, Becky Golden, and Mike Naylor at Maryland Department of Natural Resources, Chris Patrick and Bob Orth at the Virginia Institute of Marine Science, Gabby Troutman and Chris Moore at the Chesapeake Bay Foundation, Jorge Bogantes Montero at Anacostia Watershed Society, Bowdoin Lusk at The Nature Conservancy, Peter Bergstrom at NOAA (retired), and Elle Bassett, Miles-Wye Riverkeeper, for their expertise in the field of SAV Restoration and for sharing their SAV restoration methods for public use. It will take all of us working together to fully restore SAV in Chesapeake Bay.

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Welcome

Welcome to *Small-scale SAV Restoration in Chesapeake Bay: A Guide to the Restoration of Submerged Aquatic Vegetation (SAV) in Chesapeake Bay and its Tidal Tributaries*. This document is a comprehensive resource for those interested in the process of directly restoring SAV in the tidal waters of the Chesapeake Bay. Its development was sponsored by the Chesapeake Bay Program's SAV Workgroup.

Who is this document for?

As conceived, the intended audiences for this document are Riverkeeper and watershed organizations, local jurisdictions, and federal and state agencies. However, anyone with an interest in the Chesapeake Bay and its living resources will likely find something of value here.

Why was this document developed?

SAV is a vital habitat of the Chesapeake Bay, and achieving and sustaining historical abundance and distribution is an important restoration goal of the Chesapeake Bay Watershed Agreement. To accelerate the recovery of SAV in the Chesapeake Bay and its tidal tributaries, it is necessary to supplement natural recovery with direct restoration efforts in which seeds or mature plants are planted in areas where water quality is sufficient for growth and expansion, but where an existing SAV bed, and therefore a source of seeds, is not present. You can also restore a species that is not present within an existing SAV bed to increase diversity and the health of the system.

The goal of this document is to share proven restoration methods for SAV species common in Chesapeake Bay to advance progress toward the Chesapeake Bay Program's 185,000-acre Bay-wide SAV restoration target. The guidance within is intended to help readers directly restore SAV in appropriate areas of tributaries and waterways while also simultaneously providing outreach and educational opportunities for watershed group constituents and volunteers. Increasing the frequency and effectiveness of direct SAV restoration efforts using the best available methods will accelerate the timeline in which the Chesapeake Bay Program's SAV restoration target is met compared to relying solely on improvements in water quality to passively promote recovery.

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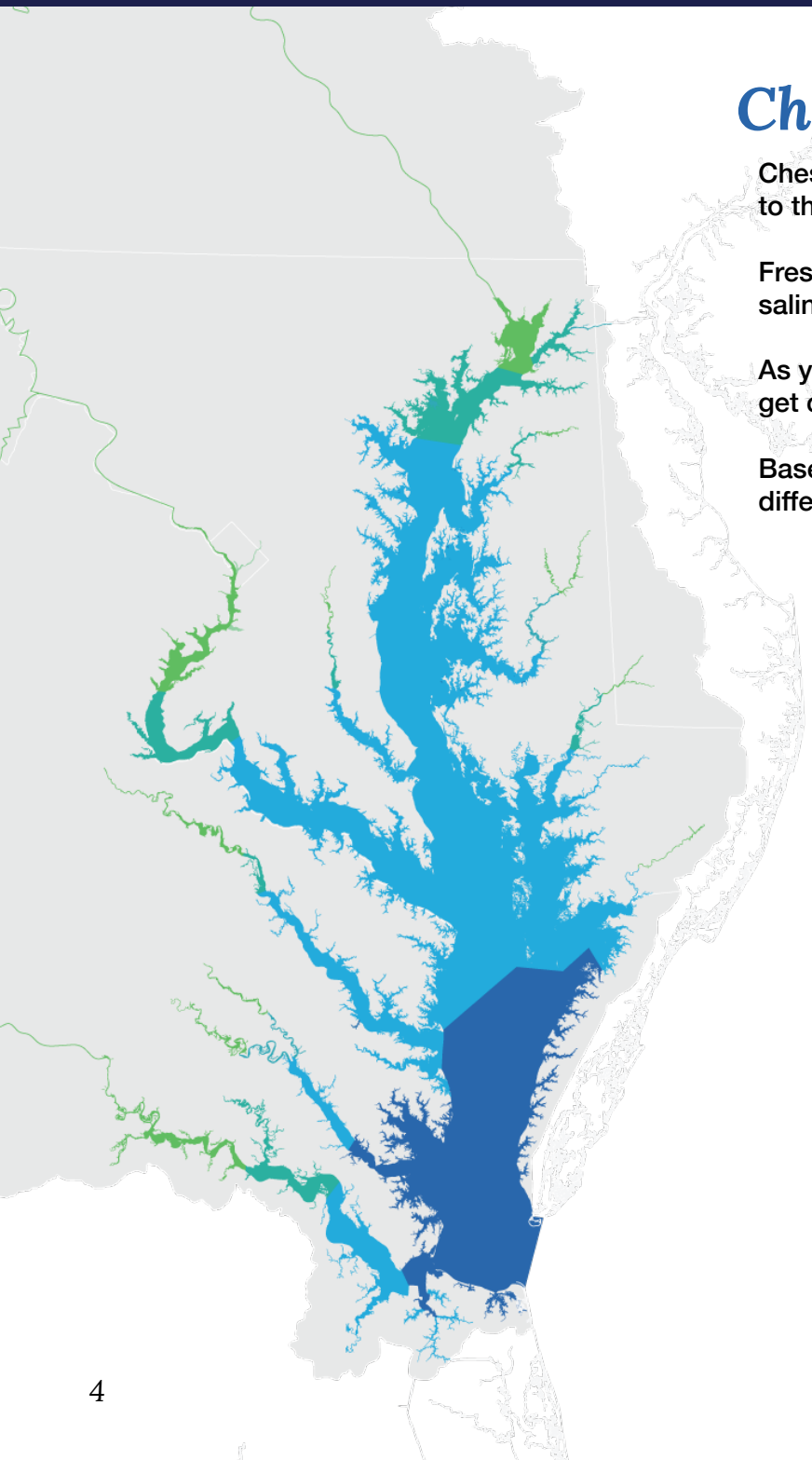
Introduction

Submerged aquatic vegetation (SAV), also known as "bay grass" in the Chesapeake Bay, is a group of flowering plants which live completely underwater (with the exception of seeds and flowers in some species). In the marine environment, submerged flowering plants are often called "seagrasses." However, we use the term "SAV" in the Chesapeake Bay because these underwater plants inhabit the full range of estuarine salinities, from tidal fresh (< 0.5 parts per thousand, or ppt) and low salinity (oligohaline, 0.5-5 ppt) to brackish (mesohaline, 5-18 ppt) and saltwater (polyhaline, >18 ppt).

SAV is different from macroalgae or seaweed because it has a vascular system which transports nutrients between the sediment, roots, and leaves – just like terrestrial plants. SAV also reproduces through flowering, pollination, and seeds, whereas most macroalgae reproduce via spores. Some SAV species can also reproduce asexually through adult plants spreading to create "baby" clones, called ramets, or plant fragments sprouting into new plants.

photo credit: Maryland Department of Natural Resources



Chesapeake Bay Salinity

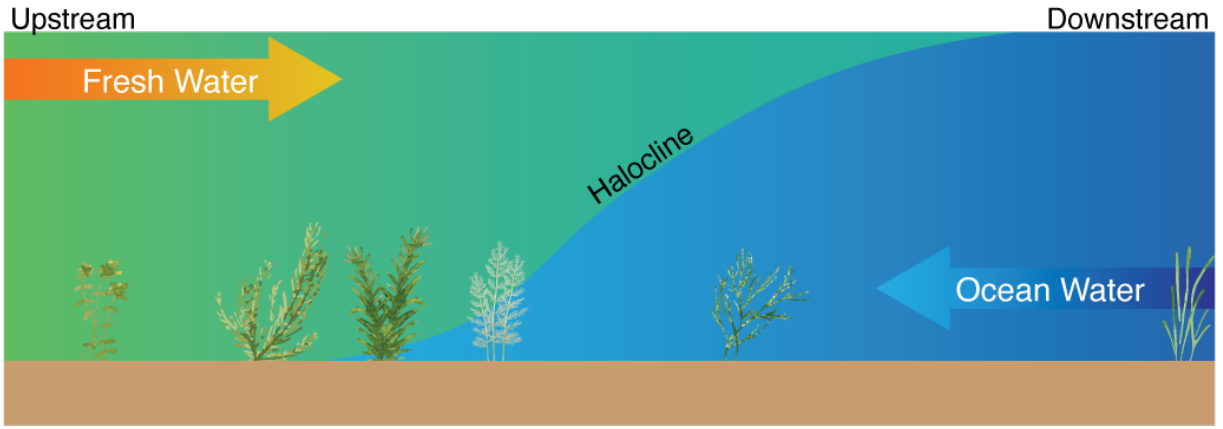
Chesapeake Bay is an estuary, or a body of water that connects rivers and streams to the ocean.

Fresh, low salinity water flows into the Bay from rivers where it mixes with high salinity water from the ocean.

As you move down each river and the Bay, salinity will gradually increase as you get closer to the ocean.

Based on the range of salinity with distance from the ocean, the bay is divided into different salinity zones:

- *Tidal Fresh*
- *Oligohaline*
- *Mesohaline*
- *Polyhaline*



Because the fresh river water is less dense than the colder, saltier ocean water, the freshwater floats on top of the saltwater as it flows downstream. Because of this, in deeper portions of rivers and the Bay, salinity is lower at the surface than at the bottom. The point in the water where the fresher surface water meets the saltier water it is floating on is called the halocline.

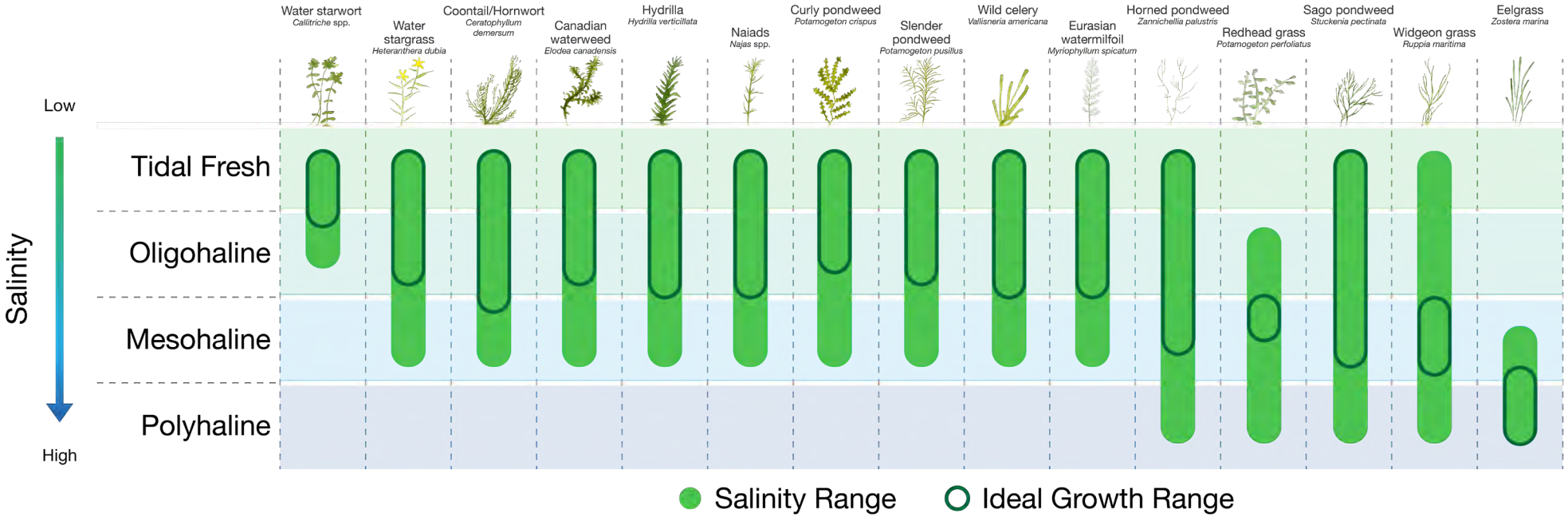


Figure 1 - Shown are the common SAV species found in Chesapeake Bay and the salinity zones in which they grow. (Source: Bergstrom et al. 2006)

About seventeen SAV species are found in the Chesapeake Bay and its tidal tributaries. The most common species include wild celery (*Vallisneria americana*), redhead grass (*Potamogeton perfoliatus*), sago pondweed (*Stuckenia pectinata*), widgeon grass (*Ruppia maritima*), and eelgrass (*Zostera marina*). Other species of SAV include multiple pondweeds (*Zannichellia palustris* and Potamogeton spp.), water stargrass (*Heteranthera dubia*), Canadian waterweed (*Elodea canadensis*), coontail (*Ceratophyllum demersum*), water starwort (Callitriche spp.), several naiads (Najas spp.), hydrilla (*Hydrilla verticillata*) and a few milfoil species (Myriophyllum spp.). Their distribution is based primarily on their preferred salinity (Figure 1).

As you move from the lower salinity to higher salinity regions of the Bay, SAV species diversity decreases. In the tidal fresh and oligohaline portions of the Bay, it is possible to see a dozen or more species in one area. As you move into the mesohaline areas, diversity decreases to include only four to five more salt-tolerant species, and in the polyhaline waters of the lower bay, only eelgrass and widgeon grass are abundant.

SAV is an important component of the Chesapeake Bay ecosystem. As a foundation species, SAV provides food, nursery, refuge, and habitat for diverse assemblages of organisms. These include economically important species such as the blue crab. SAV can also improve water quality by storing and processing nutrients and trapping suspended sediment. In addition, large, dense SAV beds or meadows can dampen waves and currents, which protects nearby shorelines. SAV also helps mitigate climate impacts by taking up carbon dioxide and buffering ocean acidification. Humans benefit from all of these ecosystem services provided by SAV (Figure 2).

Historically, SAV may have blanketed more than 200,000 acres of the shallow Bay bottom. A number of interacting factors, however, have contributed to the overall loss of SAV in Chesapeake Bay (Figure 3). A primary culprit is water running off the land and carrying nitrogen (N) and phosphorus (P) into the creeks, streams and rivers that flow into Chesapeake

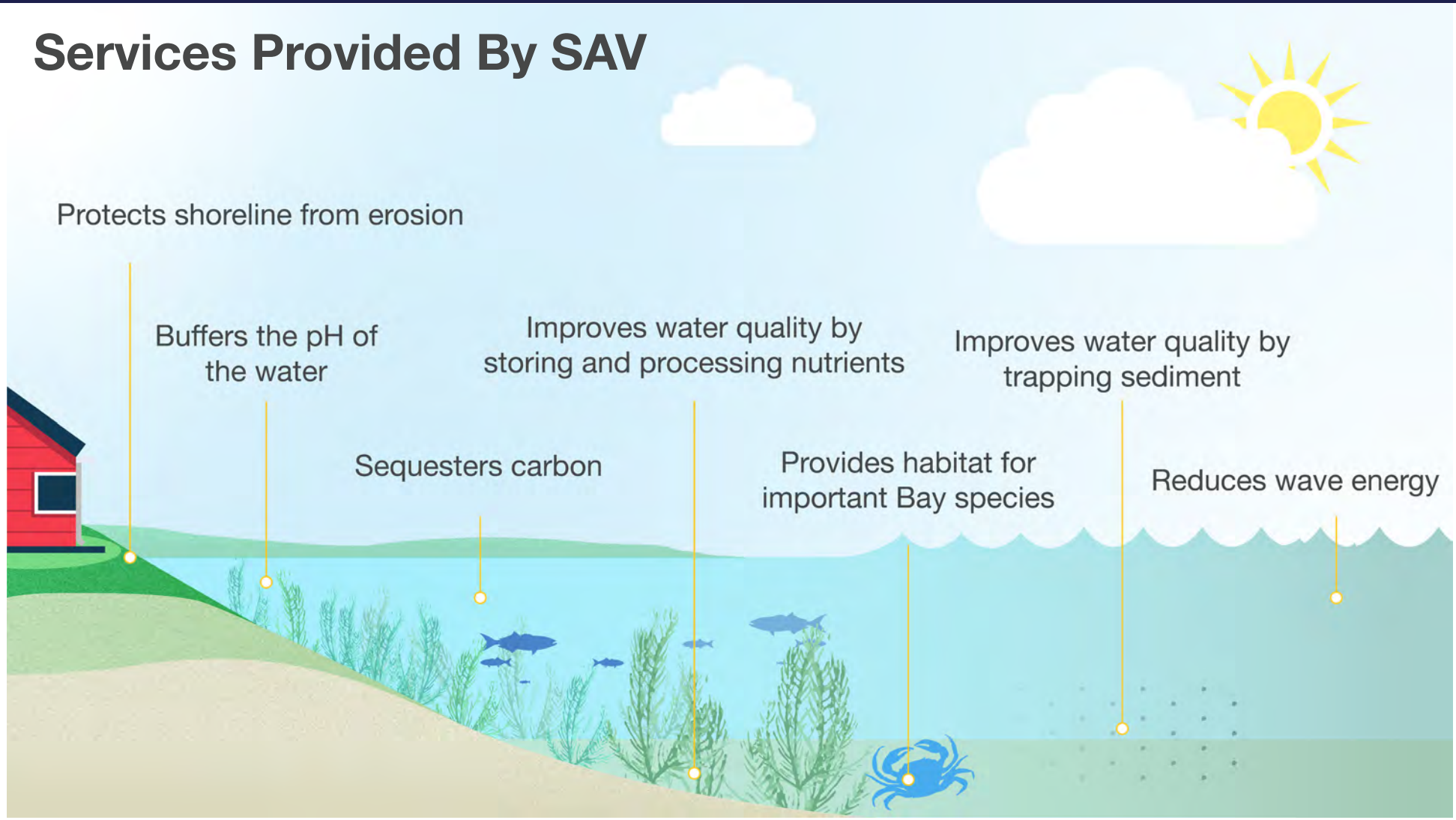


Figure 2 – Depicted are the many ecosystem services provided by healthy SAV beds in Chesapeake Bay.

Bay. Primary sources of N and P include both residential and agricultural fertilizer, stormwater, and sewage treatment plants. This nutrient pollution can cause blooms of free-floating microscopic algae, known as phytoplankton, as well as the growth of epiphytic algae attached to SAV leaf surfaces (epi- means "upon" or "on" and -phyte means "plant"). Both types of algal blooms prevent sunlight from reaching SAV leaves, which means their capacity to photosynthesize decreases and they weaken or die. SAV is also sensitive to storms and floods, which increase the amount of sediment suspended in the water by churning the bottom or washing sediment off the land and into the Bay. Suspended sediment, like algal blooms, decreases water clarity, meaning that sunlight cannot shine through the water to reach the leaves of SAV below the surface. When waves and floods scour the shallow areas where SAV lives, they can dislodge SAV from the sediment. One of the projected

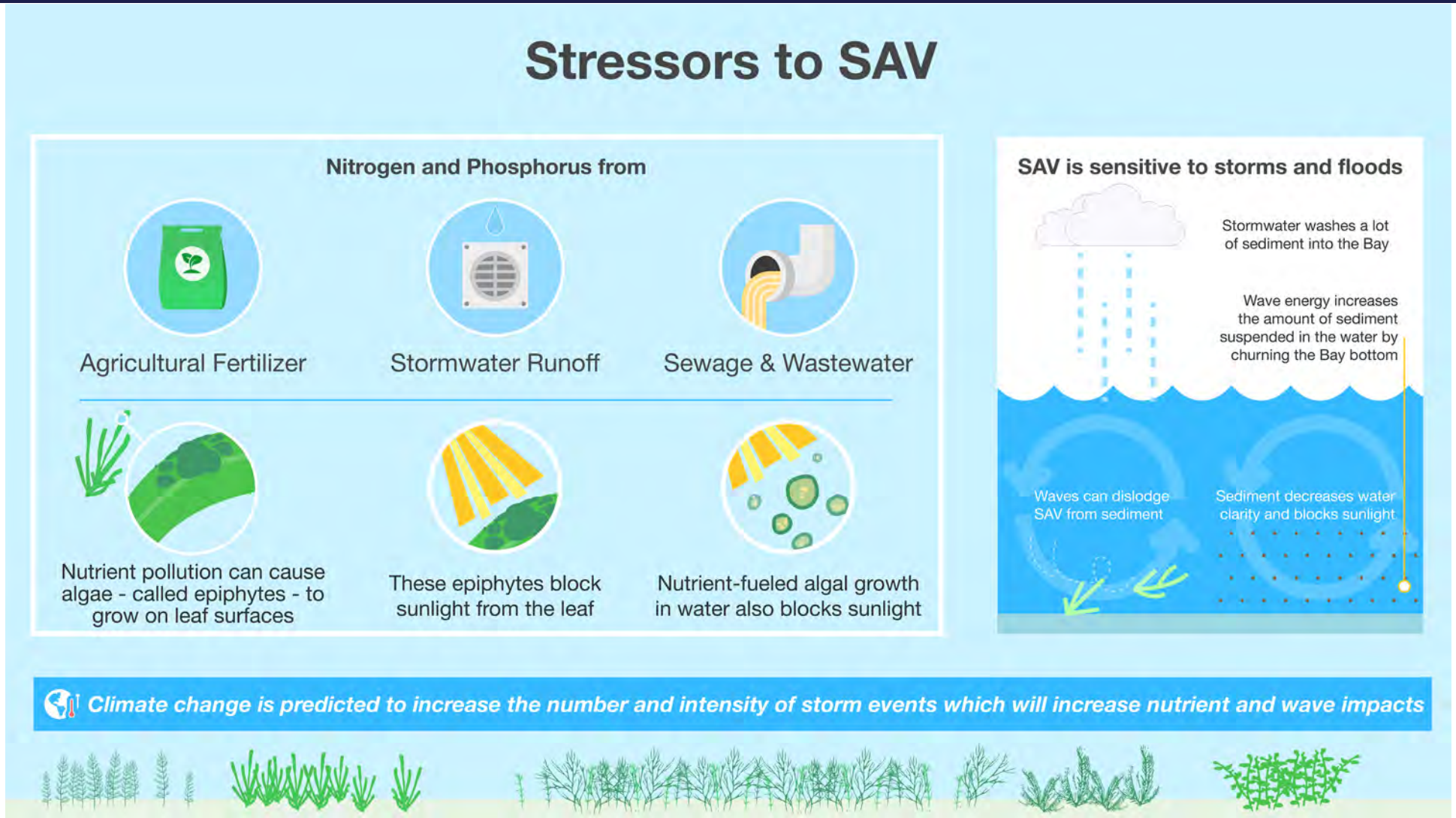


Figure 3 - Nutrient pollution, erosion, and climate change are important factors impacting SAV survival in Chesapeake Bay.

impacts of climate change to the Chesapeake region is an increase in the frequency and intensity of storm events which could have negative effects on SAV in the Bay.

Impacts to SAV became particularly evident in the early to mid-20th century. As agriculture intensified and urban areas expanded throughout the Chesapeake Bay watershed, N and P inputs increased, triggering gradual, widespread SAV declines. Then in 1972, Tropical Storm Agnes caused one of the largest floods on record, which destroyed most of the already stressed Chesapeake Bay SAV populations.

Several additional factors have further contributed to regional SAV losses. A slime mold that causes "wasting disease" resulted in massive eelgrass die-offs throughout the North Atlantic region in the 1930s, including the lower Chesapeake

SAV Area Over Time

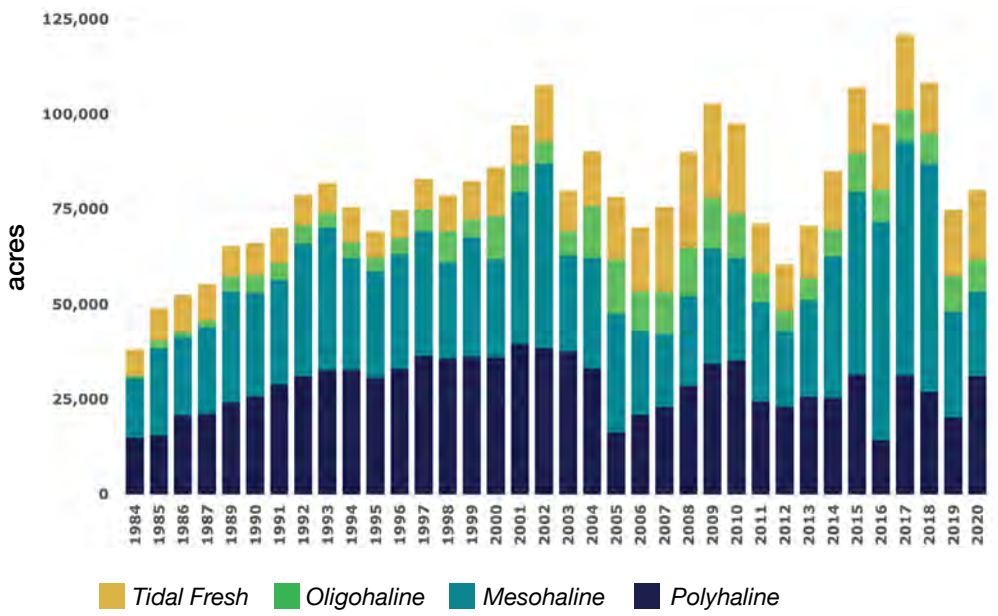


Figure 4 - Area of SAV by salinity zone over time in Chesapeake Bay. (Source: Virginia Institute of Marine Science)

Bay. Although eelgrass largely recovered from the wasting disease, it is now threatened due to its sensitivity to poor water quality and increasing temperatures and heat events associated with climate change.

Given the important ecological functions that SAV beds carry out, SAV restoration has become an integral component of Chesapeake Bay management initiatives. SAV can be restored by planting new SAV beds and by alleviating the underlying stressors that prevent their natural recovery - namely nutrient and sediment loads. Reducing nutrient and sediment loads to the Bay and its tributaries from the watershed is currently the primary method by which the U.S. Environmental Protection Agency's Chesapeake Bay Program and its partners are working to restore SAV. Through policies and investments that are part of the Bay's pollution diet (the Chesapeake Bay Total Maximum Daily Load, or TMDL), we are indeed seeing natural SAV recovery, particularly in lower-salinity regions of the Bay and its tributaries (Figure 4). Some sections of the Bay have even reached their restored SAV acreage goals. However, as water quality continues to improve, restoration via planting is now a viable approach to kick-start SAV recovery in areas where natural recovery has been slow to take off.

The Role of Regional Watershed Groups

Regional watershed groups such as Riverkeepers and River Associations play a vital role in environmental advocacy, conservation, and restoration, including SAV restoration. SAV restoration projects are becoming a popular outreach and engagement activity for these organizations. Some groups use plantings to engage with their target audience about the Chesapeake ecosystem. Other organizations aim to restore viable SAV beds to sites that have historically supported them. The guidance within this document will ensure that watershed groups follow the best available protocols to increase the likelihood of success. SAV restoration projects may also serve as a means for watershed groups to work with waterfront property owners who are interested in contributing to the recovery of the Chesapeake Bay.

Although methods will continue to improve as more practitioners engage in SAV restoration activities, this guide lays out the latest available, science-based protocols to guide small-scale (<1-acre) SAV planting projects in the tidal waters of Chesapeake Bay and its tributaries. There are essentially six major steps in the process:

- ☑ Understanding the regulations and obtaining the permits
- ☑ Selecting your restoration site
- ☑ Collecting your seeds and/or plants
- ☑ Processing and storing seeds
- ☑ Planting your SAV bed
- ☑ Monitoring and assessing your SAV bed

Obviously, within each of these steps there a several important smaller steps with options and variables to consider. This document aims to walk you through each step to make the process of SAV restoration understandable and efficient, if not necessarily simple.

SAV restoration can be cheap and quick or expensive and labor-intensive. For example, you might spread some seeds and hope for the best, or you may decide to follow rigorous site-selection, seed processing, and monitoring procedures. Either approach can potentially result in a restored SAV bed. However, using a more scientifically rigorous, systematic approach increases the probability of success and decreases the chance of wasting a valuable resource – SAV seeds. In this manual, we provide a "menu" of protocols that include simple and inexpensive but, perhaps, less rigorous options as well as state-of-the-art, but often more expensive and time-consuming, options. You should choose a combination of menu items that optimizes your organization's goals and available resources.

Restoration Species

The protocols described here focus on SAV restoration practices for only the most common species in the Bay for two reasons. First, those species have been the most heavily studied and more information is available regarding their successful restoration techniques. Second, it is important to restore SAV beds with species that are known to thrive and have abundant seed sources in neighboring areas. Additional supporting information can be found in a companion publication, Chesapeake Bay SAV Restoration Methods: Literature review[1].

We will be focusing on five species throughout this guide. They are wild celery (*Vallisneria americana*), sago pondweed (*Stuckenia pectinata)*, redhead grass (*Potamogeton perfoliatus)*, widgeon grass (*Ruppia maritima)*, and eelgrass (*Zostera marina*). Wild celery is used in freshwater restoration projects. Sago pondweed and redhead grass are used in mesohaline projects. Widgeon grass can be used in mesohaline and/or polyhaline projects, while eelgrass is used in polyhaline projects. Each plant is described in detail on the following pages. More information can be found in this SAV Identification Key[2] (see Appendix A for website URLs if you are viewing a print version of this guide).

Wild celery (Vallisneria americana)

Figure 5 - Wild celery

Wild celery is found throughout the tidal fresh and oligohaline regions of Chesapeake Bay and its tributaries. It prefers coarse silty to sandy soils, and is fairly tolerant of murky waters and high nutrient loading. The plant has long, ribbon-like leaf blades with bluntly rounded tips and a visible, bright green stripe that runs down the center of each leaf. Leaves grow out from the base of the plant and can be 1.5 m or more in length, but are generally only about 1 cm wide.

Sexual and asexual reproduction are both common in wild celery. Asexual reproduction occurs when winter buds form at the base of the plants in late summer. These winter buds elongate in spring, sending a horizontal stem along the sediment surface from which a new plant emerges. During the growing season, each plant also sends out underground

stems from which new plants emerge. Sexual reproduction occurs from late July to September. Wild celery is dioecious, meaning individual plants are either male or female. Individual female flowers occur in a tubular sheath that grows to the water surface at the end of a long stalk called a peduncle. Male flowers are crowded into an ovoid sheath on a short stalk near the base of the plant. Eventually the stalk of male flowers breaks free and floats to the surface where it releases pollen. Fertilization occurs when pollen floats into contact with female flowers. When fertilization is complete, the stalk of the female flower coils up and fruit develops underwater.

Fertilization produces a long cylindrical pod containing small, dark seeds. This seed pod is what is collected and used for wild celery restoration efforts.

Wild celery is particularly valuable as a food source for waterfowl. Canvasback and other diving ducks such as scaups, scoters, and redheads rely on the winter buds and rootstock of wild celery during migration and in their wintering habitats.

Sago pondweed (Stuckenia pectinata)

Sago pondweed was once common in oligohaline regions of the Bay, but today is more widely distributed in the Bay's mesohaline regions. It can tolerate high alkalinity and it grows in silty-muddy sediments. It also tolerates strong currents and wave action better than many bay grasses because of its long rhizomes and runners under the sediment.
Sago pondweed has slender stems that are abundantly branched, giving it a moderately bushy appearance, with leaf clusters that fan out at the surface. It has thread-like leaves that are 3 to 10 cm long and 0.5 to 2 mm wide, and taper to a point. Its basal sheaths sometimes have a pointed tip that aids in identification when plants are not in flower.

Reproduction is by sexual and asexual means. Sexual reproduction occurs during early summer with the formation of a spike of flowers that have both male and female reproductive organs and appear like beads on the plant's slender stalk.

Figure 6 - Sago pondweed

Flowers release pollen that floats on the water surface, resulting in fertilization. Developing seeds look somewhat like grape bunches and form terminal clusters that remain on the stem until autumn when they are dispersed into the water. Asexual reproduction is achieved through vegetative growth as well as the formation of tubers (underground structure that bears buds) and turions (buds that can grow whole plants) which grow into plants the following spring.

Sago pondweed is widespread throughout the United States and is considered one of the most valuable food sources for waterfowl in North America. Its nutrient-filled seeds and tubers, as well as leaves, stems and roots, are consumed by many species of ducks, geese, swans and shorebirds. Sago pondweed beds are also excellent fish habitat.

Redhead grass (Potamogeton perfoliatus)

Redhead grass is typically found in the moderately brackish, mesohaline waters of Chesapeake Bay. It grows best in firm, muddy soils and in quiet water with slow-moving currents. The

Figure 7 - Redhead grass

paddle-like leaves of redhead grass make it one of the most easily recognizable species of SAV in the Bay. They are short, flat, and oval-shaped and grow in an alternating arrangement directly from the stem. Branching is more developed in the upper portion of the plant growing near the surface. At its base, redhead grass has an extensive root and rhizome system that securely anchors the plant.

Asexual reproduction occurs by vegetative growth and fragmentation, and by the formation of over-wintering tubers that grow at the ends of rhizomes. Sexual reproduction regularly occurs in early to mid-summer. Spikes of tiny flowers emerge from where leaves meet the upper stem. The flower spikes extend above the water surface and the pollen is wind-carried. As fruits mature, they sink below the surface where they release seeds.

Redhead grass probably got its common name from the redhead ducks often found feeding on it. Redhead grass is considered an excellent food source for waterfowl.

Widgeon grass (Ruppia maritima)

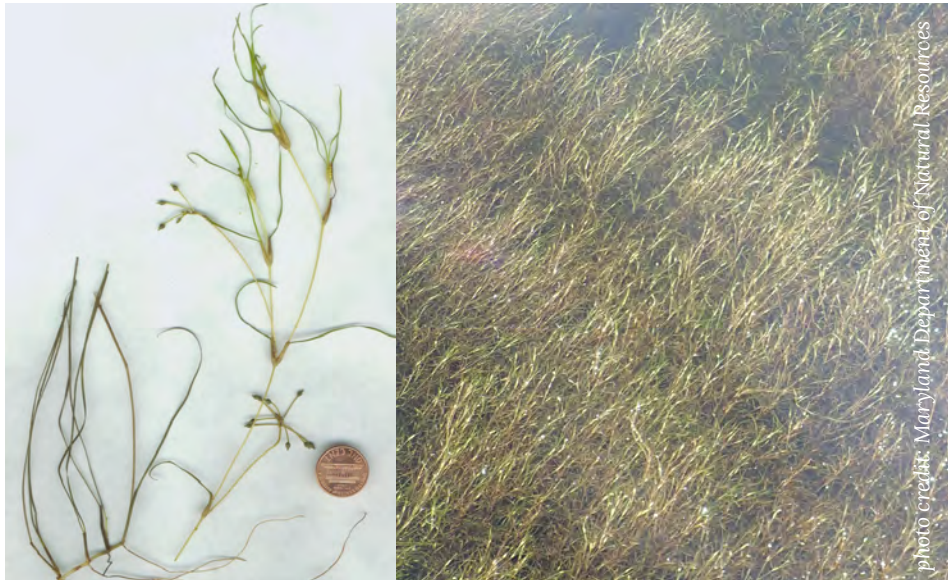


Figure 8 - Widgeon grass

Widgeon grass is one of the most abundant species of SAV in Chesapeake Bay thanks to its relatively broad salinity tolerance. Although it tolerates lower salinities, in Chesapeake Bay it is most commonly and abundantly observed in mesohaline and polyhaline regions with sandy substrates. It is also frequently observed growing in soft, muddy sediments and it does not thrive in high-energy environments.

Widgeon grass has linear, thread-like leaves that are 3 to 10 cm long and approximately 0.5 mm wide. These leaves are arranged alternately along slender, branching stems and have rounded tips that may appear slightly pointed. Widgeon grass has an extensive root system of branched, creeping rhizomes that lack tubers. There are two growth forms of widgeon grass in Chesapeake Bay: an upright, highly branched form present during flowering (summer); and a creeping growth form with the leaves appearing at the base of the plant from early spring through late fall.

Widgeon grass reproduces both asexually and sexually. Asexual reproduction occurs by emergence of new stems from the root-rhizome system. Sexual reproduction takes place in late-summer. During sexual reproduction, the plant produces a flower head with two flowers containing male and female reproductive organs. The flowers are enclosed in a basal sheath of leaves and extend towards the water's surface on a flower stalk. Pollen floats in bubbles on the water's surface until contacting the female reproductive organ. Fertilized flowers produce four black, oval-shaped fruits with pointed tips. Individual fruits extend on stalks, which occur in clusters. The fruits are dispersed in the water by currents but also through consumption by fish and waterfowl.

Widgeon grass is one of the more valuable food sources for waterfowl and all parts of the plant have excellent nutritional value. It is also an important and easily-accessible habitat for many micro and macro invertebrates and some small fish.

Eelgrass (Zostera marina)

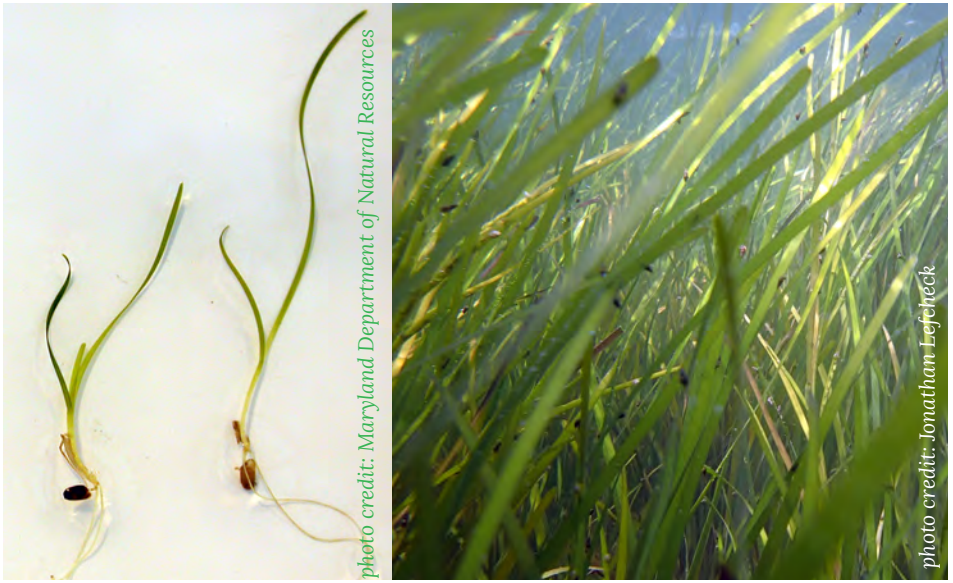


Figure 9 - Eelgrass

Eelgrass is the only true seagrass in Chesapeake Bay. Once dominant throughout the polyhaline region of the Bay, it has declined in abundance in recent years as a result of increasing water temperatures and poor water quality. Its current distribution is from Tangier Sound south to the mouth of the Bay and in the lower reaches of high salinity tidal rivers.

Eelgrass has a thick creeping rhizome with abundant roots and leaf nodes spaced 1 to 3.5 cm apart. Ribbon-like leaves with rounded tips arise from these nodes and grow to 1.2 m long and 2 to 12 mm wide. Eelgrass leaves are relatively small and narrow where the plants grow on shallow, sandy, physically exposed substrates, but longer, wider leaves occur on plants growing in deeper, muddy areas.

Eelgrass reproduces both asexually and sexually. Asexual reproduction occurs through growth and elongation of the rhizome from which new stems arise, and by formation of turions. Sexual reproduction occurs through seed formation, and begins with flowering in May and June. An eelgrass plant has both male and female flowers and fertilization occurs by drifting pollen. Male and female flowers mature at different times on the same plant to prevent self-fertilization. Once fertilized, the flowers develop into seed-bearing generative shoots that eventually break off and float to the surface, releasing their seeds as they drift.

Unlike other Chesapeake Bay SAV species, eelgrass is most productive during the cooler months of early spring and again in the fall after the summer heat subsides. During the summer months, it mostly dies back. Eelgrass is an important habitat for blue crabs that use the beds as nursery grounds and for protective cover during mating and shedding. It is also an important habitat for seahorses, pipefish and speckled sea trout, and important food source for brant geese. Canada geese, widgeon, redhead ducks, black ducks, and green sea turtles also feed on eelgrass.

4

Regulations and Permitting

Regulations for harvesting and planting SAV differ by jurisdiction as do the processes for obtaining permits. While the process may vary for each jurisdiction, it cannot be stressed enough how important it is to obtain a permit prior to harvesting SAV plants or seeds. Following all permitting guidelines is essential to avoid harvesting from recently recovered SAV beds and to avoid potential over-harvesting of donor beds. Following years when poor water quality has reduced the overall distribution and abundance of SAV in the Bay, permits may not be granted in order to protect recovering beds. Information for Maryland, Virginia, and Washington, D.C. waters are provided on the following page. Additionally, the Chesapeake Legal Alliance has put together a comprehensive summary of statutes and regulations affecting SAV titled "Existing Chesapeake Bay Watershed Statutes and Regulations Affecting Submerged Aquatic Vegetation." This document is available here[3].

photo credit: Chesapeake Bay Program

Maryland

Permits are required for harvesting SAV or collecting seeds for any reason in Maryland tidal waters. Approval of permits comes from the Department of Natural Resources under Natural Resources Article 4-213[4]. Interested parties should email Becky Golden (rebecca.golden@maryland.gov) with the following information to obtain an SAV collection permit:

- contact information (for person and organization)
- project information (the purpose of collection - research, education, etc.)
- harvesting method
- approximate time frame
- location of donor bed or beds (latitude and longitude required) and verification that they meet the density and stability requirements to serve as donor beds (the bed must be at least 5 years old and have a density of 70-100% as mapped by VIMS)
- the extent of SAV to be removed (for example, the number of shoots or seeds, volume of reproductive material, or square meters)
- for transplants, transplant techniques that will be used
- the location the seeds or plants will be dispersed or planted (latitude and longitude required)
- a follow-up monitoring plan

A report will be required after project completion and must include info on where the seeds were dispersed, how many were dispersed, and if the project was successful as evidenced by the growth of an SAV bed.

Virginia

The Title 4 Virginia Administrative Code 20-337-30[5] outlines detailed regulations for SAV harvesting and planting. Any request to remove SAV from or plant SAV upon state bottom requires completion of a Joint Permit Application submitted to the Virginia Marine Resources Commission (VMRC). According to the regulation, applications should provide information that will enable the VMRC to "evaluate the probabilities of transplantation success while minimizing impacts to established donor bed populations [and] track the progress of various projects and learn from previous projects which methods and sites are promising for further restoration or enhancement efforts." This information includes:

- Site coordinates and evidence that planting site will likely support restored SAV beds
- Transplant source(s), technique, and timing
- Monitoring plan

Details for each of these components are available in 4VAC20-337-30[5], but they are also, in large part, consistent with the recommended protocols provided in this manual.

You can contact VMRC at (757) 247-2200.

Washington, D.C.

Washington D.C. Municipal Regulations Title 21, § 1401 states, "every person desiring to harvest, cut or otherwise remove or eradicate submerged aquatic vegetation from any land under the tidal waters of the District below the elevation of mean high tide, shall obtain plan approval from the Director" via a Wetland and Stream Permit and Water Quality Certification. The D.C. Department of Energy & Environment (DOEE) website[6] includes detailed instructions and guidance. The process will involve creating an account on the Department of Energy and the Environment's Surface and Groundwater System. This is where you will submit your permit application. A U.S. Army Corps Section 404 nationwide permit from the Baltimore Regulatory District may also be required depending on the scope of work. Details are available on the Baltimore District Website[7].

You can contact DDOEE at (202) 535-2600.

5

Site Selection

Site selection is one of the most imporant, if not *the* most important, elements to consider to facilitate SAV survival and expansion (Fonseca 2011). A fundamental rule of thumb is to plant in similar conditions from which the seeds or plants were harvested (Fonseca et al. 1998; Fonseca 2011). The planting site should also be one where SAV has grown previously or where nearby SAV beds are present. These basic guidelines help to ensure that conditions at the planting site can support SAV growth.

The next several sections identify parameters that can help determine if your site is suitable for SAV. These parameters are grouped according to whether they are high priority or low priority. High-priority parameters are known to strongly constrain SAV growth and should, therefore, be measured whenever possible. Low-priority parameters can also constrain SAV growth, but measuring them is not absolutely necessary. Rather, low-priority data serve to paint a more complete picture of why a particular site is or is not suitable for SAV growth.

photo credit: Chesapeake Bay Program

For each parameter, we provide detailed information on how to measure it. In many cases, multiple options exist for making these measurements. Often, the most precise and accurate measurements are also the most time-consuming and expensive. We describe these so that if you have the resources, you can follow a more rigorous approach. However, we also suggest alternative, less resource-intensive but, perhaps, less accurate and precise, methods which have nonetheless been shown to be sufficient in most cases. We use a series of icons that indicate for each parameter and method described, the time it takes to measure it, how important it is to measure it, the cost to measure it, and precision and accuracy of the method described. These icons are shown in figure 10.

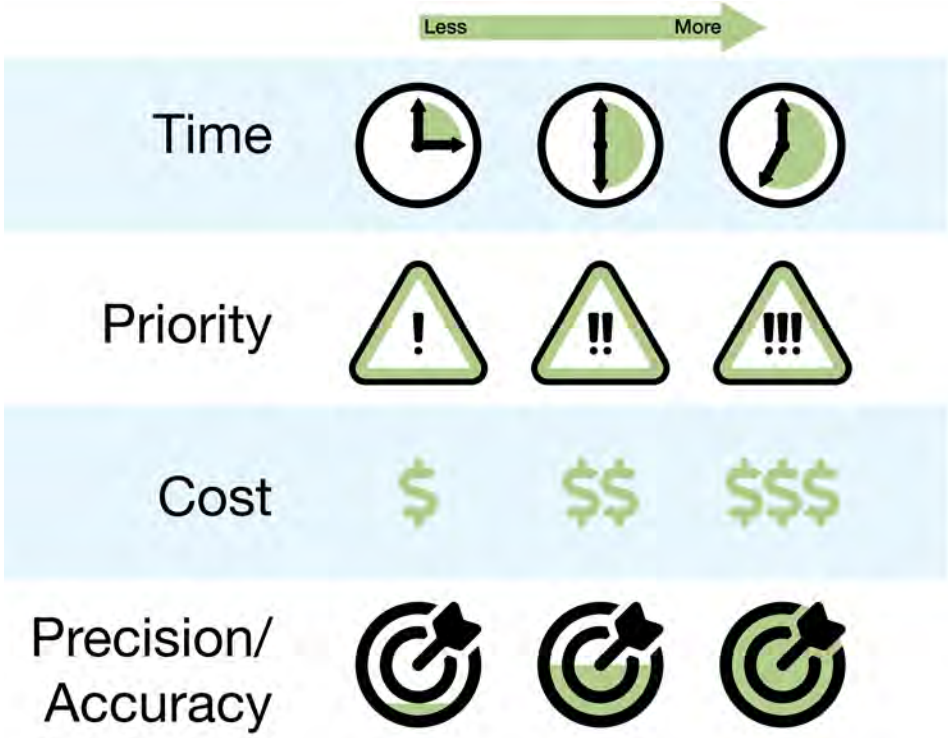


Figure 10 - Icons used throughout this document to indicate for each parameter and method described, the time it takes to measure it, how important it is to measure it, the cost to measure it, and precision and accuracy of the method described.

Once you have measured your high priority parameters, the next step is to compare them to the habitat thresholds for your site's salinity zone to determine if your site is suitable for SAV growth. These quantitative and qualitative habitat requirements are detailed in the next chapter.

Keep in mind that a scientifically rigorous site assessment based on the most accurate and precise methods and many habitat criteria will likely increase your chances of success. But no approach or site is ever "perfect," so try not to let perfect be the enemy of good.

High-Priority Site-Selection Measurements

1. *Previous or nearby SAV presence*

You can determine whether SAV was previously present at a site by using the interactive mapping tool[8] developed by the Virginia Institute of Marine Science (VIMS). The tool can display maps that depict where SAV has been found throughout Chesapeake Bay in every year since 1984. There is also a map layer option that merges all of these occurrences into a single map called an SAV Composite layer. Figure 11 shows the mapping tool

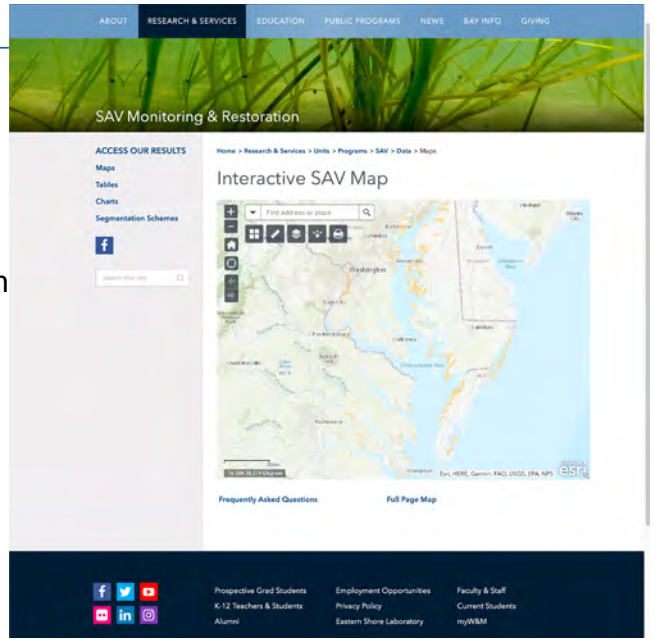


Figure 11 - Mapping tool developed by VIMS that can be used to determine if SAV has ever been present at or near your restoration site.

and the correct layer selection. You can also check for nearby SAV beds (within about 500 m) using the tool. Be aware that as SAV recovers, it is now appearing in some locations not included in the composite layer. So, a visit to the site in person, ideally in the summer when SAV is most abundant, is advised.

2. Water depth

Water depth is a fundamental variable that you must consider when determining where to plant. Compared to most other aquatic plants, such as macroalgae or phytoplankton, most SAV species need a lot of light. Although water quality and clarity are improving in the Chesapeake Bay (thanks to the TMDL and implementation of best management practices, or BMPs), having sufficient light at the bottom where SAV grows can still be a limiting factor for recovery. Most SAV in the Bay grows in depths of 2 meters or less, depending on the clarity in that region. Therefore, a general rule of thumb is to plant in water that is less than 1 m deep at mean low water (MLW) but not so shallow that the bottom will be exposed during the lowest tides. If you are able to also collect water clarity data, you can more precisely estimate the SAV depth limit at your site (see Water clarity section below).

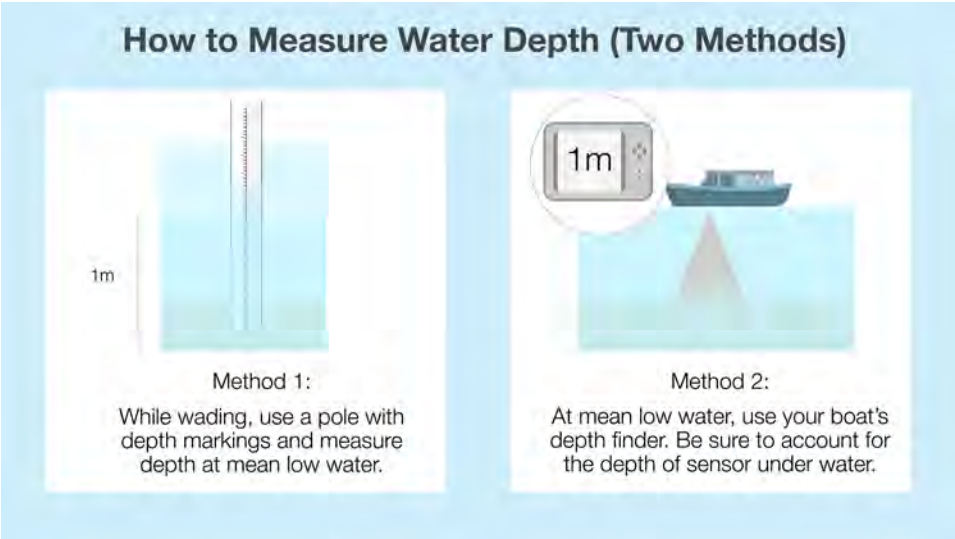


Figure 12 – Two methods for measuring water depth at your potential restoration site.

You can measure water depth using a number of approaches. The most straightforward is to visit your site and measure the depth using a pole with depth markings or your boat's depthfinder (Figure 12). If you use your boat's depthfinder, remember to check how far below the water surface your instrument is attached to the boat and add that length to your depth measurements. Be sure to take the tidal stage and your area's tidal range into consideration when determining your site's MLW depth. If the depth is 1 m at high tide and your tidal range is just under 1 m, the water will only be a few centimeters deep at low tide and, therefore, much too shallow to support SAV. However, if the depth is 1 m at low tide, the water depth at your site should be fine for SAV growth.

You can get an estimate of tidal range at your location using the National Oceanic and Atmospheric Administration (NOAA) PORTS tool[9]. Depending where your site is located, you can choose Chesapeake North or Chesapeake South and then select the tide and current monitoring location on the map that is near your site. The tidal range can be determined from the water level chart. If a water level station is located nearby (within a kilometer or so), you can estimate the long-term average low-water depth at your site with a bit more precision. When you measure the depth at your site using the pole or depthfinder method, note the time. Then, download the nearby station data. You can access these data through the NOAA National Data Buoy Center[10] and MDDNR Eyes on the Bay[11] websites. Calculate the difference between your site's depth and the water level station depth at the time of your measurement and apply that difference to the station data to estimate depth at your site (e.g., if the station depth was 1.5 m and your site was 1 m, subtract 0.5 m from the station depth measurements to estimate depth at your site based on the station data). Then, using the corrected station data, take the average of all of the low tide water level measurements to calculate MLW.

3. Water clarity

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Water clarity goes hand-in-hand with depth as a key parameter constraining SAV growth. The simplest, least expensive measure of water clarity is Secchi depth, which is measured using a weighted black and white plate, known as a Secchi disk, attached to a line marked with depth increments (Figure 14). In the turbid Chesapeake Bay, we typically mark the line in 10-cm increments. You should lower the disk into the water until you can no longer see it, and then raise it up until you can see it once again. Note the depth marking on the rope - this is the Secchi depth. **A general rule of thumb is that SAV grows best when water depth is less than or equal to Secchi depth** - in other words, when you can see the Secchi disk on the bottom. It is important to understand that water clarity at any location in Chesapeake Bay is highly variable. It changes on time scales of hours to days due to storms that wash sediment into the water or stir up the bottom from wind driven waves. Water clarity will also change seasonally as conditions in the Bay become either more or less hospitable to the algae that cloud it. Therefore, the best way to characterize water clarity at your site is to determine the median water clarity over the SAV growing season. This can be done by taking multiple measurements during the growing season, entering them into Excel, and using the median function. Why median instead of mean? Storm events have the potential to result in extremely low Secchi measurements that can skew a mean and make growing season water clarity look worse than it really is. A median is not influenced by extreme values that only occur occasionally.

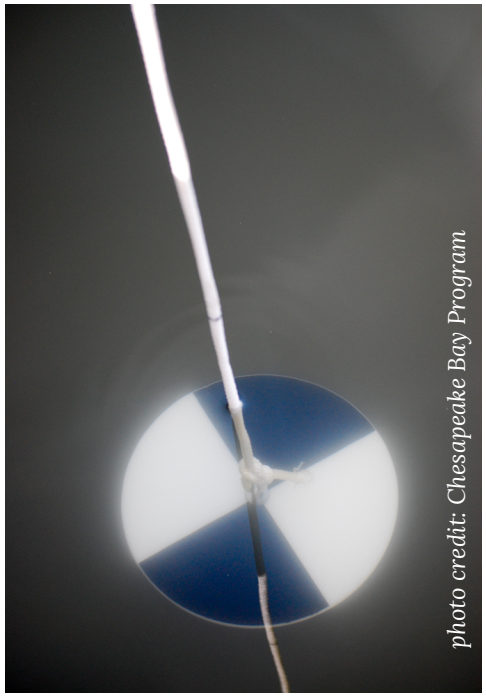

Figure 13 - Secchi disc being lowered into the water to determine water clarity.

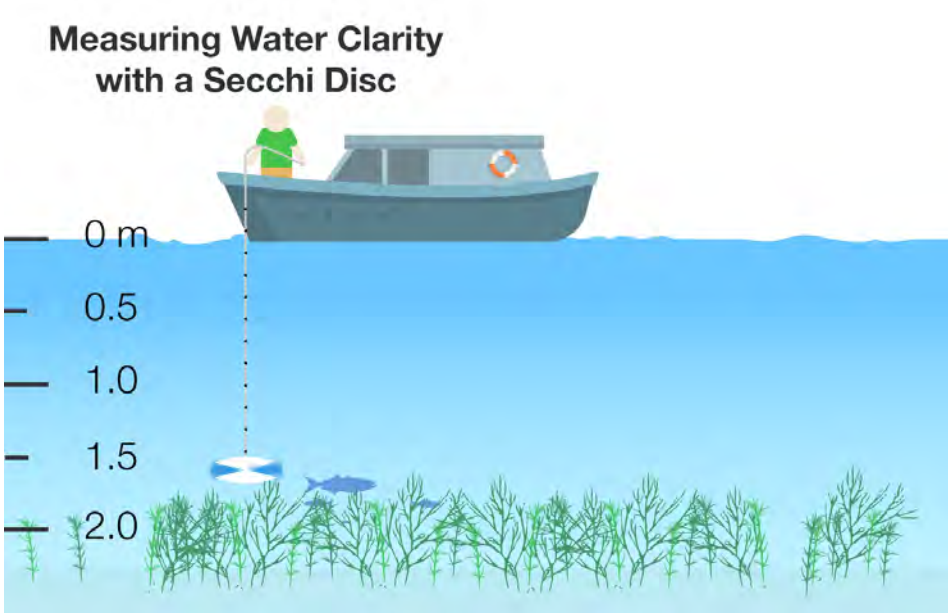

Figure 14 - How to use a Secchi disc to measure water clarity.

A more rigorous estimate of water clarity can be made by calculating the SAV habitat parameter Percent Light through the Water, or PLW. PLW is the percent of surface light which penetrates through the water to reach the bottom. It is calculated using your site depth at MLW and growing season median Secchi depth. PLW is calculated using the following equation:

$$PLW = e^{(-(1.45 \times MLW)/(Secchi\ depth))} x\ 100$$

where e is Euler's number. So for example, if MLW is 1 m and Secchi depth is 0.7 m, PLW = e^(-(1.45 × 1)/0.7)x 100 = 12.6. If you do the calculation using your calculator, you would use the ex function. If you use Excel, you would use the following formula: "=EXP(-(1.45 * 1) / 0.7) * 100."

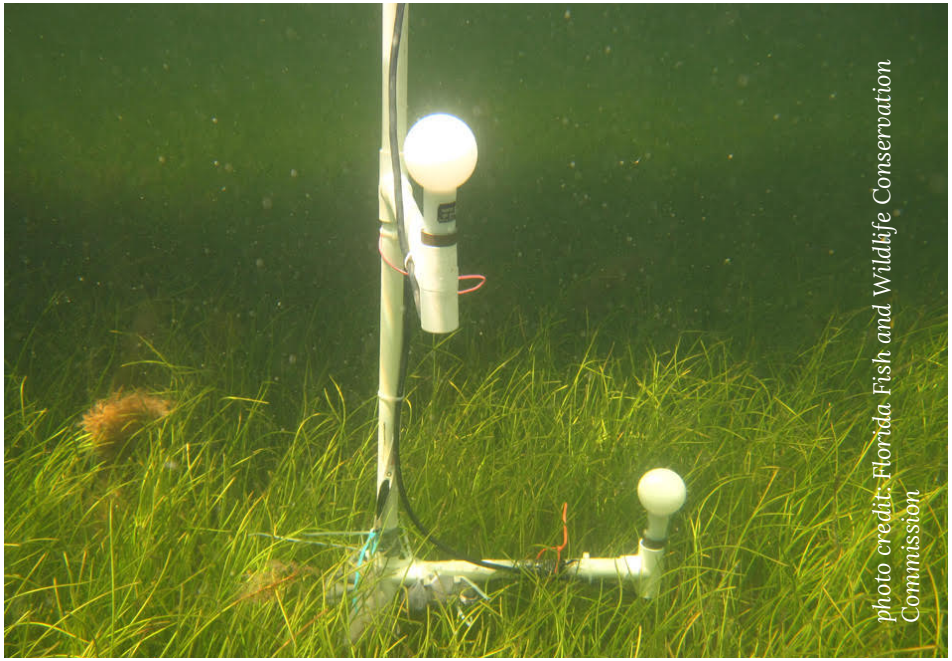

Figure 15 - A light profiler deployed in an SAV bed.

You can also use a quantum light sensor to measure photosynthetically active radiation (PAR). PAR is a direct measure of light within the range of wavelengths that plants use for photosynthesis. We typically use a quantum light sensor to collect a vertical light profile, or a series of light measurements taken at increments throughout the water column. The light profile measurements are then used to calculate the light attenuation coefficient (known as K_d). The equation above traditionally uses K_d to calculate PLW, but scientists have empirically derived a formula (1.45/Secchi depth) to approximate K_d based on Secchi depth since the latter is cheaper and easier to measure. However, if you have access to a quantum light sensor, you should use it to collect a vertical light profile and directly calculate K_d. This way, your PLW estimate will be more accurate and biologically relevant.

To collect a vertical light profile, record the downwelling irradiance just below the surface and at 10 to 20 cm intervals through the water column (or greater intervals in deeper, clear water) until you hit the bottom. To calculate K_d, first calculate the negative natural log (ln) of your light measurement at each depth (I_z) divided by light just below the surface (I_0) using the equation $-\ln(I_z/I_0)$. K_d is the regression coefficient (or slope of the line) for $-\ln(I_z/I_0)$ vs. depth. This sounds complicated but is actually fairly easy to do in a spreadsheet. Figure 16 shows an example of how you would set this up in Microsoft Excel using example data from a shallow site on the Eastern Shore of MD. A quick glance at the data indicate that the water isn't very clear because the light measurements rapidly decrease with depth. You can use the formula "=LINEST(-ln(I_z/I_0) cell range, depth cell range)" to calculate the regression coefficient and, therefore, K_d.

A

	A	B	C	D
1	Sample Depth (m)	Light (umol s-1 m-2)	-ln(Iz/i0) (m)	Regression coefficient (Kd, m-1)
2	0.01	580	=-LN(B2/$B$2)	=LINEST(C2:C9,A2:A9)
3	0.1	488	=-LN(B3/$B$2)	
4	0.2	392	=-LN(B4/$B$2)	
5	0.3	313	=-LN(B5/$B$2)	
6	0.5	199	=-LN(B6/$B$2)	
7	0.75	114	=-LN(B7/$B$2)	
8	1	75	=-LN(B8/$B$2)	
9	1.25	39	=-LN(B9/$B$2)	

B

	A	B	C	D
1	Sample Depth (m)	Light (umol s-1 m-2)	-ln(Iz/i0) (m)	Regression coefficient (Kd, m-1)
2	0.01	580	0.00	2.16
3	0.1	488	0.17	
4	0.2	392	0.39	
5	0.3	313	0.62	
6	0.5	199	1.07	
7	0.75	114	1.63	
8	1	75	2.05	
9	1.25	39	2.70	

Figure 16 - Two versions of the same spreadsheet. A shows the formulas needed to calculate Kd from a vertical light profile and B shows the results.

4. Salinity

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Salinity is one of the most important parameters that determines which SAV species can grow at your site. Salinity can be measured either with a salinity refractometer or a

conductivity/salinity probe. You can measure salinity at the site or collect samples for later analysis. Samples should be collected in a pre-labeled bottle and placed in a cooler with ice until analysis. The maximum holding time when testing for salinity is 28 days.

Figure 17 - Using a dropper to place a water sample on a refractometer to measure salinity.

If using a refractometer to measure salinity, collect a water sample in a pre-labeled bottle from approximately 0.3 m below the surface. To determine salinity, rinse your dropper with sample water three times and then use the dropper to rinse the refractometer with the water sample. Then apply drops from the water sample onto the refractometer (Figure 17) and hold it up to light to read and record salinity as parts per thousand (o/oo) using the scale located on the right-hand side of the view scope. If you are using a probe to measure surface samples on site, place the probe at least 0.5 m beneath the surface of the water, wait for the probe to stabilize, and then record the reading.

5. *Water velocity and waves*

Hydrodynamic forces, such as waves and currents, can play an important role in restoration success, particularly as fragile seedlings become established. Most Chesapeake Bay SAV species do well in protected embayments but can also thrive in moderately exposed environments. However, a site that is regularly exposed to large waves or strong currents is usually unsuitable for SAV growth because seedlings and shoots are scoured or uprooted. The challenge for SAV site assessment is that waves and currents are difficult to measure without expensive equipment or advanced technical knowledge. We recommend that you, instead, conduct a semi-quantitative hydrodynamics assessment.

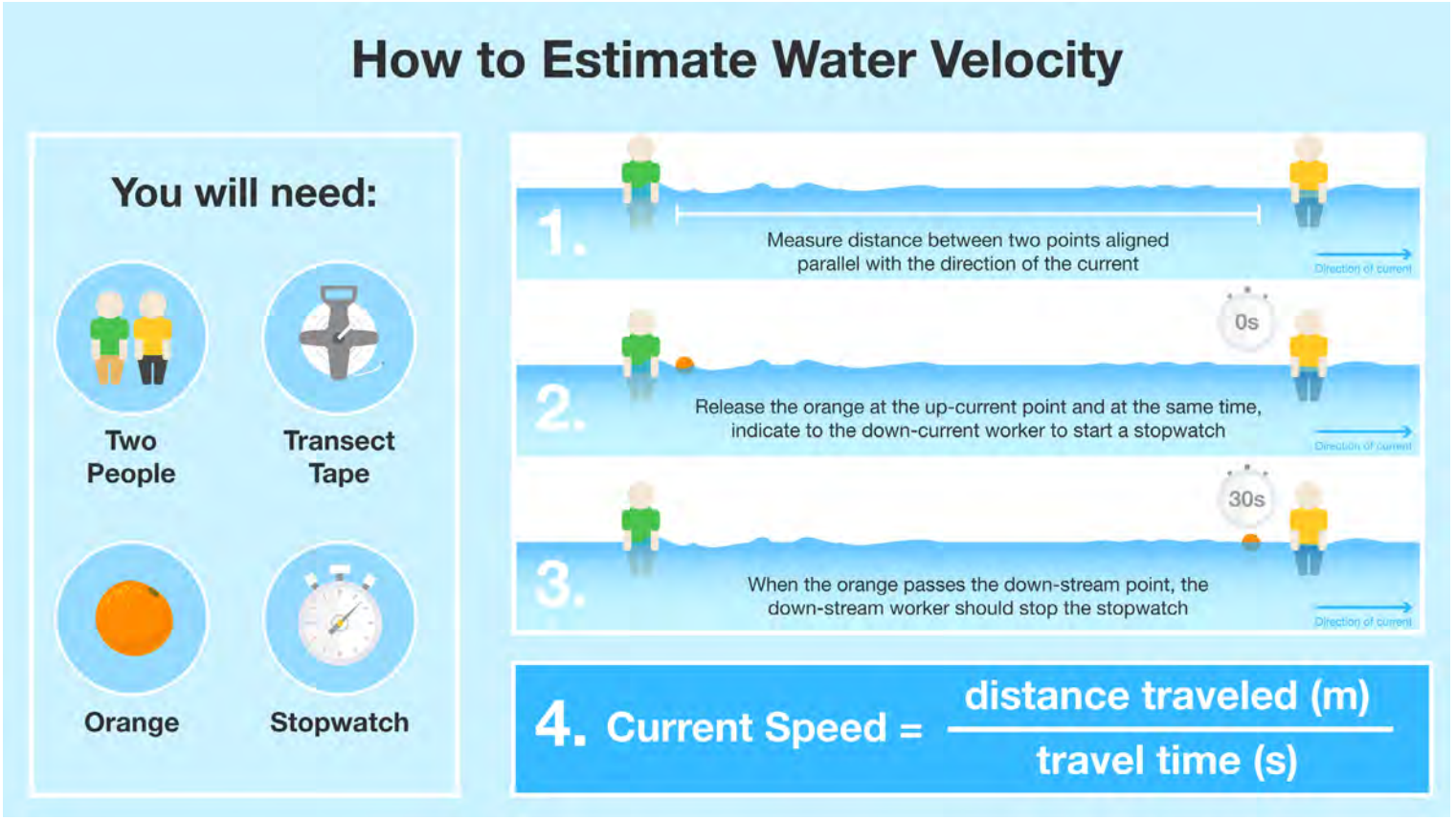

Figure 18 - A simple method to estimate water velocity.

A simple approach to roughly estimate current speed is to measure the time it takes an object that floats just below the water surface to travel a fixed distance (Figure 18). An orange works well for this purpose. You need at least two people for this task. First, measure the distance between two fixed points oriented parallel with the dominant current direction using a transect tape. One person should release the orange at the up-current point and at the same time, indicate to the down-current worker to start a stopwatch. When the orange passes the down-stream point, the down-stream worker should stop the stopwatch. Current speed is equal to the distance traveled (meters) divided by the travel time (seconds). You should repeat this several times across several tidal cycles and at different tide stages, if possible, because currents are primarily the product of tidal motion, which is quite variable across daily and monthly lunar cycles. Restoration practitioners advise that current speeds should not exceed 30 cm/s.

Risk of excessive wave exposure should be assessed qualitatively by noting the wave environment at your site under a range of wind conditions (e.g., calm, light breeze, gale), as well as by noting the presence of eroded shorelines nearby. If you frequently observe white caps and waves breaking on the shoreline, or the shoreline is heavily eroded in the vicinity of your site, then your site is probably too exposed to support SAV. However, you should also check whether SAV is growing at similarly exposed sites in your region. If SAV is present at similar environments in your area, it may indicate that hydrodynamic conditions at your site will be hospitable for SAV.

6. *Sediment organic content and grain size*

The amount of organic matter in bottom sediments can also play an important role in SAV growth and survival. Generally, greater sediment organic content is associated with higher concentrations of sulfide, which is toxic to SAV. Sulfide is especially prevalent in brackish and high-salinity water. Typically, meso- and polyhaline SAV species are unable to survive when sediment organic content exceeds 5%. This is

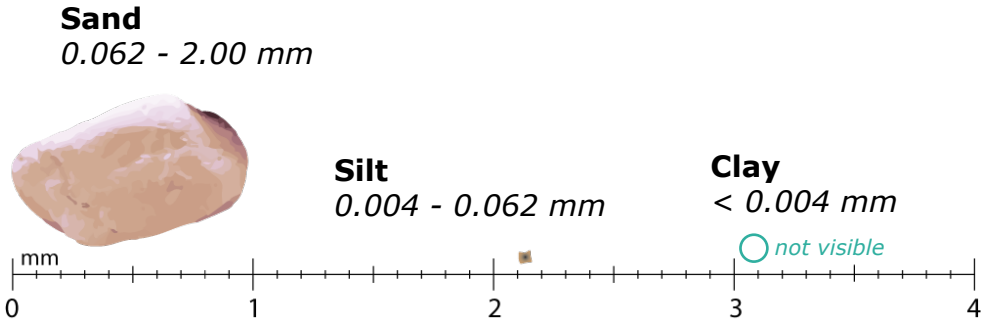

Figure 19 - A 35x magnification of sand, silt and clay particles. Even at this magnification, clay particles are not visible.

difficult to assess without expensive analytical equipment, but you can easily collect a sediment sample and send it to an analytical laboratory for organic content analysis for less than $10-$20 per sample (additional information about labs can be found in Appendix B). You should ask the laboratory how much sediment is needed for the analysis and what type of container you should use. You can also qualitatively assess whether excessive sulfide is an issue by examining the sediment texture, color, and smell. Fine-grained, black sediment that smells like rotten eggs indicates high sulfide concentrations and should, therefore, be ruled out as an SAV restoration site.

Sediment grain size also affects sulfide accumulation and, therefore, SAV survival. Grain size can be divided into three categories based on sediment particle diameter: sand, silt, and clay (Figure 19). The latter two are collectively called "mud" and include all particles <63 µm. Grain size does not appear to constrain SAV growth in freshwater, however, meso- and polyhaline species tend to thrive when the mud content is <20%.

You can qualitatively assess sediment grain size for restoration site suitability as the easiest, but not as precise, method. To do this, note the texture of the sediment and how compact it feels underfoot. If it feels very gritty and compact, the sediment is likely sandy. If it feels smooth and loose or "mucky," the sediment probably consists of mostly mud and is therefore unsuitable for SAV restoration in meso- and polyhaline regions.

You can determine sand and mud content semi-quantitatively if you have a few pieces of basic equipment and a land-based space in which you can process the sample. First use a spoon, scooper, or a 50 ml syringe with the end cut off to collect a small (about 10 cm^3 is sufficient) sediment sample and bring it back to your lab space. Use a 63 µm sieve to separate the sand and mud by wet-sieving the sample. To wet-sieve the sample, place the sieve over a pre-weighed glass beaker labeled "mud" and use a squirt bottle filled with fresh water (distilled or deionized if possible) to rinse the fine particles through the sieve into the beaker. You can use a scoopula or spoon to manually agitate the sediment to facilitate this process. It can also be helpful to place the sieve in a funnel and use a ring stand to hold the funnel over the beaker. The sieving is complete when the water draining from the sieve is clear. At this point, scrape and rinse the sediment remaining in the sieve into another pre-weighed beaker labeled "sand." Then let the samples evaporate in a fume hood or cabinet. When the samples are completely dry, weigh the samples and subtract the original beaker weight to obtain the sand and mud weights. Calculate % mud by dividing the mud weight by combined mud and sand weight and multiply this number by 100 (% mud = sand/(sand+mud)*100). The accuracy of your results will not be research-grade (more rigorous analysis requires a laboratory drying oven) but your data will be good enough to adequately assess site suitability.

7. *Temperature (eelgrass)*

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Water temperature is a key site selection variable for eelgrass, which cannot tolerate extended hot periods (Figure 20). Current data suggests that other Bay species are not as vulnerable to increasing water temperatures, but areas that appear hot and stagnant may not be suitable for restoration of other species as well. Eelgrass becomes stressed when temperatures reach 25° C (77° F) and dieback occurs when they exceed 30° C (86° F). More specifically, eelgrass survival is greatest when the water temperature is less than 30° C (86° F) 93% of the time.

You can check continuously measured temperatures at a nearby water quality or weather monitoring station, but water temperature can be spatially variable so the monitoring station temperature may be different from temperatures at your site.

Water temperature can be measured discretely using an armored glass thermometer probe, or continuously using an inexpensive sensor. When measuring discrete water temperature, place the probe or thermometer at least 0.5 m beneath the surface of the water, wait for the probe to stabilize, and record your temperature reading and the depth at which it was measured. Temperature sensors, such as the HOBO Pendant, can be purchased for less than $50. They collect measurements at user-programmed fixed intervals for up to several months or longer. Follow the sensor instructions to program and prepare your instrument for deployment. The sensor can be deployed by strapping it to a piling below the low-water line or to a concrete block deployed on the Bay bottom (be sure to deploy it where it won't interfere with navigation and attach a line/small buoy to the block so you can find it later). Zip ties usually work well for attaching these small, lightweight sensors to a fixed object. If you plan to conduct eelgrass restoration, we highly recommend deploying a

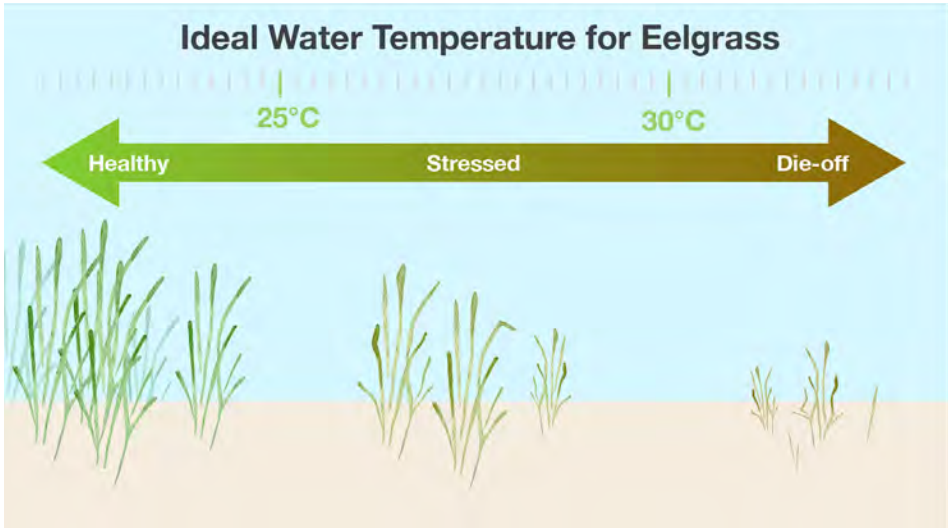


Figure 20 - Eelgrass is sensitive to warm temperatures

continuous sensor, if feasible for your organization, and pairing temperature measurements with water clarity measurements. Data suggests that eelgrass can survive elevated temperatures if water clarity is very high.

Low-priority site-selection measurements

1. Nutrient concentration

Nutrient concentration is indirectly linked to SAV growth because it often controls phytoplankton and macroalgae abundance and, therefore, the amount of light reaching SAV leaves. Although measuring nutrient concentration is less important than directly assessing water clarity, nutrient data can sometimes provide information that water clarity measurements cannot. For example, a site may have good water clarity but nonetheless be unsuitable for SAV growth because high nutrient concentrations can cause epiphytic algae to grow on the surface of SAV leaves. Epiphyte growth is difficult to quantify, so water column nutrient concentration may be your best option for determining whether excessive epiphytic algae will be an issue at your restoration site. If your site has generally good water clarity, we recommend measuring nutrient concentration if feasible to ensure that epiphytic algae are unlikely to be a problem. However, if your site has poor water clarity, nutrient measurements are not necessary because light is already insufficient to support SAV growth.

We typically analyze water samples for the nutrients nitrate+nitrite (NO_3^- + NO_2^-), ammonium (NH_4^+), and phosphate (PO_4^{3-}). You can determine nutrient concentrations by sending filtered water samples to an analytical laboratory or analyzing the samples yourself with a test kit. Nutrient concentrations determined via analysis at a professional laboratory will be the most accurate (additional information about labs can be found in Appendix B). As long as you follow proper sample collection, filtering, and storage procedures, your data will be research-quality and you could even work with the scientific and management community to contribute to regional environmental monitoring databases. Laboratory nutrient analysis costs range from about $8-$12 per sample, depending on the lab and analyte. If samples are being sent to an outside lab, please follow the sample collection instructions issued by the lab. Typically, you will need to filter the water samples to remove particulate matter so that only dissolved nutrients are measured. The easiest way to filter water is using a syringe to push the water through a filter and into a vial. You should ask the analytical laboratory what type of vial, sample size, and filter (material, diameter, and pore size) they prefer. Researchers often use plastic or glass "scintillation" vials to store filtered samples, which can be organized with a vial rack.

If you would like to analyze the samples yourself, there are a few different types of non-research grade nutrient test kits that can nonetheless give you a good sense of nutrient concentrations at your site. Most test kits come with reagent packets that change the color of the water. The amount of nutrient dissolved in the water affects the intensity of the color change. You then either use a portable instrument to analyze the water color to determine nutrient concentration automatically, or visually compare the water to a color swatch or color wheel to estimate nutrient concentration. We do not recommend aquarium nutrient test strips because they are designed to only coarsely measure a much wider range of concentrations than typically seen in the tidal waters of Chesapeake Bay.

Test kits that rely on visual color assessment are less expensive but also less precise. They range from about $50-$200, and you need to purchase a different test kit for each nutrient you plan to analyze. Examples include the Hach NI-14, Lamotte 3354, Hanna HI 713, and Hach PO-19, but you can find several other options through a quick internet search.

The instrument approach is more expensive but also more accurate than the visual test kit. If within the scope of your budget, we recommend a multi-parameter colorimeter, such as the Hach DR900. The nice thing about these is that you can test for many different nutrients and other analytes using just

one instrument as long as you have the correct reagent. A full list of solutes and reagents that are compatible with the Hach DR900 is available here[12]. A multi-parameter colorimeter can be purchased for about $1,500-$1,600 and reagents cost a few cents to a few dollars per sample, depending on the analyte and whether you purchase in bulk. You can also purchase colorimeters that only test for one analyte for about $400-$600 each. However, if you plan to test for more than one analyte, the multi-parameter instrument is more cost-effective.

When purchasing reagents, it is important to look for a product designed to measure a nutrient concentration range that you would expect to see at your site. "Low-range" and sometimes "mid-range" reagents are usually most appropriate for tidal Chesapeake Bay waters, whereas "high-range" reagents are better for non-tidal streams, where nutrient concentrations are often higher. You can determine typical nutrient concentrations in your area using the Chesapeake Bay Program water quality database[13].

When collecting water samples for laboratory or test kit analysis, try to avoid churning up the bottom sediment with your boat or feet. Rinse the sample bottle and cap with sample water three times, being careful not to touch the inside of the bottle or cap. Fill the bottle by lowering it into the water with the cap on and then uncapping it below the surface to fill it. Then remove the sample bottle from the water and cap it. Analyze the sample immediately or place the bottle in a cooler with ice. Samples must remain cool (≤ 6° C / 42.8° F) until they are analyzed within a few hours. Colorimeters and visual test kits are usually very user friendly and include detailed instructions. Make sure to carefully follow the instructions that come with your respective kit. You should also be cognizant of safety protocols, as the reagents often contain hazardous chemicals. If you find that your nutrient concentrations are consistently equal to the minimum or maximum concentration of the range listed on your reagent packet, the nutrient concentrations at your site are probably outside of the reagent range. In this case, you should purchase reagent with a higher or lower range, as appropriate.

2. Chlorophyll a concentration

Chlorophyll a (chl a) is a pigment that plant cells use for photosynthesis. Its concentration in the water serves as a proxy for phytoplankton concentration. Because phytoplankton concentration affects water clarity, which, in turn, affects SAV growth, chl a is another indirect indicator of SAV habitat suitability. Since you should already be collecting water clarity data, chl a concentration is a secondary, low-priority site assessment variable. However, chl a is commonly measured in water quality monitoring programs. You might consider collecting chl a samples if your organization also plans to contribute to regional environmental monitoring efforts, or if you want to generate a more complete picture of the water quality dynamics at your site.

Water for chl a samples can be collected following the procedures described above for nutrients. All samples should be stored in a cooler and kept out of the sunlight for the duration of field sampling. To facilitate this, opaque bottles can be used or samples can be wrapped in aluminum foil to keep out the light. Samples will also have to be filtered using a slightly different protocol and analyzed by an analytical laboratory (additional information about labs can be found in Appendix B). We typically either use a vacuum pump or a syringe to filter chl a samples. The simplest approach is to use a 50 ml syringe, filter holder, and filter. First rinse the syringe three times with sample water. Use forceps or tweezers to place the filter onto the filter holder, then attach the filter holder to the end of the syringe. Next, use the syringe to push water through the filter. Remove the filter holder from the syringe before withdrawing the plunger to add additional sample water. Color on the filter generally indicates a sufficient sample for analysis. Record the volume of water pushed through the filter on your data collection sheet.

Sometimes it is difficult to push sufficient water through the filter using a syringe, in which case a hand vacuum pump and filtering rig is a better option. If you prefer this approach, rinse

the filter holder and filtering rig three times with sample water. Use the hand vacuum pump to push water through the filter and record the volume of water passed through the filter on your data collection sheet.

You should ask the laboratory what type of filter (material, diameter, and pore size) they prefer and how they want you to package the filter. Typically, we fold the filter in half with the filtrate facing inwards, and wrap it with a small square of aluminum foil to keep light out. If properly packaged and frozen (-20°C), chla samples can be stored for up to three and a half weeks. Samples should be mailed overnight on ice or delivered in-person so that they arrive at the analytical laboratory as soon as possible.

3. Total suspended solid concentration or turbidity

Turbidity is a measure of light scatter due to particulates in the water, and total suspended solids (TSS) is a direct measure of particulate concentration. Because turbidity and TSS are linked to water clarity, which, in turn, affects SAV growth, they are another indirect indicator of SAV habitat suitability, similar to nutrients and chl a.

Turbidity can be analyzed using a turbidity kit, like the LaMotte 7519, or a multiparameter colorimeter like the Hach DR900, whereas TSS typically needs to be analyzed by a professional laboratory (additional information about labs can be found in Appendix B). Regardless of which variable you plan to measure, the first step is to collect sample water. As with nutrient and chl a samples, avoid churning up the bottom before collecting a sample. Rinse the sampling bottle and cap with sample water three times, being careful not to touch the inside of the bottle or cap. Fill the bottle by lowering it into the water with the cap on and removing the cap below the surface to fill it. Then remove the bottle from the water and cap it. Place the sample bottle in a cooler with ice until analysis. The maximum sample holding time for turbidity is 24 hours.

If measuring turbidity with a test kit, please follow the instructions that come with the respective kit. If you plan to have your samples analyzed for TSS, you should filter the samples following the procedure described for chl a, filtering until you see a significant color change on the filter. We recommend using the hand vacuum pump approach for TSS because pushing sufficient water through a syringe can be difficult. You need to use pre-weighed filters because TSS is determined based on the change in weight before and after filtering. Ask the laboratory if they can provide pre-weighed filters, or, alternatively, what type of filters they recommend.

Using water quality monitoring data to assess site suitability

Although directly measuring environmental conditions at your particular site is preferable, you can also use data from a nearby water quality monitoring station to more generally assess regional restoration suitability. The Maryland Department of Natural resources maintains a database for its shallow-water continuous monitoring program in Maryland waters, which you can access through the Eyes on the Bay[11] website. The U.S. Environmental Protection Agency Chesapeake Bay Program monitors water quality bimonthly at fixed stations throughout the bay. These data are available through the online DataHub[14]. Water level, temperature, and data for other physical variables can be accessed through the National Data Buoy Center[15] web portal. You can also find water quality data from the Chesapeake Monitoring Cooperative through the Chesapeake Data Explorer[16].

Measurement frequency and duration

The SAV habitat criteria for water clarity, nutrients, chl a, and TSS are based on growing season (March-November for eelgrass and April-October for all other species) median values. Therefore, you should measure these variables multiple times across this period and use the median of those values to compare your data to the habitat thresholds for your salinity zone. One measurement per month will give you a rough idea

of how water quality varies throughout the growing season at your site, but at least two measurements per month is better. A greater number of measurements increases the likelihood that you will capture short-term events that significantly affect water clarity, such as algal blooms, storms, and floods. Because depth, salinity, and hydrodynamic conditions can vary quite a bit across multiple time scales, we recommend also measuring these variables at least one or two times per month throughout the SAV growing season. Sediment organic content is not likely to change substantially over time, so one or two observations made during the summer, when sulfide concentrations are usually highest, is sufficient. As previously stated, continuous temperature measurements are ideal for prospective eelgrass restoration sites so that you can capture the frequency and duration of stressful temperatures, which are just as important, if not more so, as average temperature conditions in predicting the probability of eelgrass survival.

Other considerations

Other general considerations are 1) access for volunteers or whoever is involved in the restoration effort, 2) proximity to other restored or natural SAV communities, and 3) proximity to an armored shoreline.

If volunteers are being used as part of your planting and/or seed collection team, it is important to keep site accessibility in mind. Sites that can't be easily reached by a short walk should be ruled out to reduce the potential for injuries and liabilities.

Proximity to existing SAV communities is not only an indicator that your location is hospitable to SAV, it also increases the amount of pollination sources available to your SAV bed. This can increase your bed's genetic diversity, making it more resilient to both short and long-term disruptions in the local environment. The prospects for long-term survival are therefore increased.

While we do not suggest that you automatically rule out a potential restoration site near an armored shoreline, studies have shown that they can have a negative impact on SAV growth, particularly bulkheads. If all other parameters look good at a potential site near an armored shoreline, it may still be a suitable site, but if other site options exist that don't have armored shorelines nearby, they may be a better bet for long-term project success.

Parameter	Method	Equipment/Facilities	Priority	Cost	Time	Accuracy & Precision
Salinity	Collect discrete measurements	Refractometer		$		
Salinity	Collect discrete measurements	Handheld YSI		$$$		
Salinity	Check publicly available monitoring data	Computer or device with internet access		$		Depends on distance to station
Depth	Soundings	Boat with depth finder or pole with length markings		$		
Depth	Check publicly available gauge data	Computer or device with internet access		$		Depends on distance to station
Previous SAV presence	Check composite SAV layer on VIMS interactive map	Computer or device with internet access		$		
Nearby SAV presence	Check most recent SAV layer on VIMS interactive map	Computer or device with internet access		$		
Nearby SAV presence	Visually inspect field site	None		$		
Sediment grain size	Qualitatively estimate sand/ mud content	None		$		
Sediment grain size	Seive and dry sediment samples	64 um sieve, beakers, scale, lab space		$$		
Temperature	Collect discrete measurements (digital)	Probe or sonde	*	$$		
Temperature	Collect discrete measurements (analog)	Thermometer	*	$		
Temperature	Check publicly available continuous monitoring data	Computer or device with internet access	*	$		
Temperature	Deploy temperature logger	Temperature logger	*	$$		
Waves	Visually estimate site exposure	None		$		

Parameter	Method	Equipment/Facilities	Priority	Cost	Time	Accuracy & Precision
Current velocity	Measure transport time of floating object	Transect tape, floating object, stopwatch		$		
Water clarity	Collect discrete Secchi disc measurements	Secchi disc		$		
Water clarity	Collect discrete vertical PAR profiles	PAR sensor and handheld meter		$$$		
Water clarity	Check publicly available monitoring data	Computer or device with internet access		$		Depends on distance to station
Nutrients	Analyze discrete samples using test kit	Nitrate, ammonium, and phosphate test kits or colorimeter and reagents		$-$$		-
Nutrients	Collect, filter, and send discrete samples to professional laboratory for analysis	0.45 um filter and syringe		$$		
Chl-a	Collect, filter, and send discrete samples to professional laboratory for analysis	0.7 um 47 mm glass fiber filter, hand vacuum pump, and filtration unit		$$		
TSS	Collect, filter, and send discrete samples to professional laboratory for analysis	Pre-weighed glass fiber filter, hand vacuum pump, and filtration unit		$$		
Nutrients, chl-a, TSS	Check publicly available monitoring data	Computer or device with internet access		$		Depends on distance to station
Sediment organic content	Visually estimate organic content	None		$		
Sediment organic content	Send to professional laboratory for analysis	Sample container or plastic bag		$$		

* Temperature is a high-priority variable for eelgrass only

6

General and Specific SAV Habitat Criteria for Restoration Species

Water depth: A general rule of thumb is to plant in 1 m of water (mean low water) or less. SAV can grow in deeper water if the water is clear, but for restoration purposes, it is advised to stay shallow enough to ensure adequate light penetration for seeds to grow and thrive.

Sediment velocity and waves: In general, plantings should occur in calm water with relatively low water velocity and wave exposure to prevent scouring or dislodging of transplants or seedlings. Restoration experts suggest only planting where water velocities do not exceed 30 cm/s.

Sediment grain size and organic content: SAV growth and survival declines when sediment organic content >5%. Freshwater SAV do not appear to be constrained by sediment grain size. Meso- and polyhaline SAV growth and survival is highest when mud content (particles < 63 µm) is <20%.

photo credit: Chesapeake Bay Program

Tidal Fresh and Oligohaline Restoration Sites

Salinity: 0-5 ppt.

Suitable species to use for SAV restoration:

Wild celery
(*Vallisneria americana*)

Site Selection Methods and Thresholds

Water Clarity - PLW: **>13%**

Total Suspended Solids: **<15 mg/l**

Chlorophyl-a: **<15 µg/l**

Dissolved Inorganic Nitrogen **- NA**

Dissolved Inorganic Phosphorus **- <0.02 mg/l**

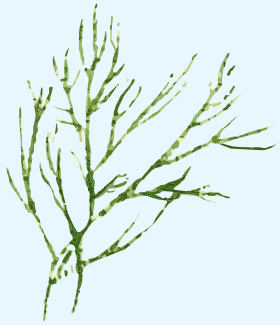
Silt and Clay **- <20%**

Organic Matter **- <5%**

Mesohaline Restoration Sites

Salinity: 5-18 ppt.

Suitable Species to use for SAV restoration:



Sago pondweed
(*Stuckenia pectinata*)

Redhead grass
(*Potamogeton perfoliatus*)

Widgeon grass
(*Ruppia maritima*)

Site Selection Methods and Thresholds

Water Clarity - PLW: **>22%**

Total Suspended Solids: **<15 mg/l**

Chlorophyl-a: **<15 µg/l**

Dissolved Inorganic Nitrogen **- 0.15 mg/l**

Dissolved Inorganic Phosphorus **- <0.01 mg/l**

Silt and Clay **- <20%**

Organic Matter **- <5%**

Polyhaline Restoration Sites

Salinity: greater than 18 ppt.

Suitable species to use for restoration in the Polyhaline

Widgeon grass
(*Ruppia maritima*)

Eelgrass
(*Zostera marina*)

Site Selection Methods and Thresholds

Water Clarity - PLW: **>22%**

Total Suspended Solids: **<15 mg/l**

Chlorophyl-a: **<15 µg/l**

Dissolved Inorganic Nitrogen - **0.15 mg/l**

Dissolved Inorganic Phosphorus - **<0.02 mg/l**

Silt and Clay - **<20%**

Organic Matter - **<5%**

Current Velocity - **>10 and <100 cm/s**

photo credit: Jonathan Lefcheck

7

Collecting Seeds or Plants

While planting can be done using seeds or whole plants, restoration via seeding is recommended in this guidance document because whole plant restoration is expensive and labor-intensive. Additionally, seeds can be easily harvested by hand and restoration via seeds rather than adult plants may result in more genetically diverse and, therefore, more resilient restored SAV beds (Reynolds et al. 2012a and 2012b). However, we provide some basic guidance for whole-plant restoration as whole-plant restoration may be preferred in some situations.

Seed collection

When deciding where to collect seeds, it is important to aim for high genetic diversity so that your restored SAV bed is robust and resilient. You can increase the chances of creating a diverse SAV bed by collecting from diverse donor beds and gathering seeds from a few different locations (spaced about 20 m apart if the bed is large enough) within each donor bed. Spreading out seed collection in such a manner also helps ensure that excessive harvest stress is not placed on a single

photo credit: Michael Norman

Seeds and Seed Pods

Wild celery
(Vallisneria americana)

Widgeon grass
(Ruppia maritima)

Redhead grass
(Potamogeton perfoliatus)

Sago pondweed
(Stuckenia pectinata)

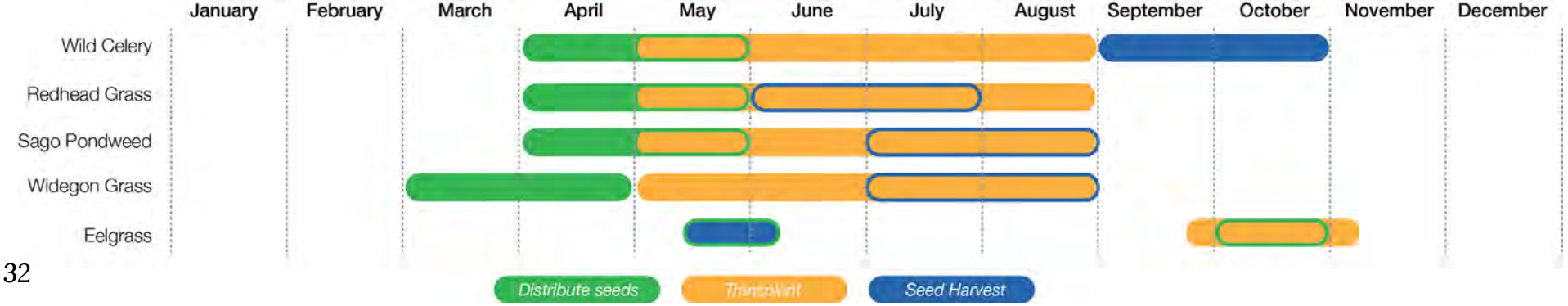
Eelgrass
(Zostera marina)

donor bed or donor area. Collecting plants that have adapted to conditions that are similar to those at the restoration site will also increase the likelihood of success. As an example, individuals which have developed genetic adaptations to withstand high salinity at a salty donor site would be well-suited for a higher salinity restoration site. To increase the likelihood of transplanting into similar conditions, you should avoid moving propagules too far beyond the original donor bed (i.e., stay within the same region of the bay and within the same tributary if feasible) and **you should not use or buy propagules sourced from outside the Chesapeake Bay and its tributaries**.

It is also important to consider the age and stability of the potential donor bed. You should not collect seeds or plants from recently recovered sites as those beds may not yet be resilient enough to survive excessive seed removal. Instead, use the VIMS interactive SAV map to identify SAV beds that have been present and densely populated (70%-100% density) for at least 5 years. Please refer to the section in this manual on regulatory requirements prior to collection.

The first step in the process is to closely monitor potential donor beds for flowering and subsequent seed development. With the exception of eelgrass, the SAV species included in this manual produce seeds on reproductive shoots that extend almost or fully to the water surface. Eelgrass seeds develop in spathes, or modified leaves that cover the reproductive structure, on reproductive shoots at various heights throughout the water column. The donor site should be visited several

Figure 21 – Transplant, seed harvest, and seed distribution schedule for SAV restoration species. Eelgrass seeds can be distributed immediately or held until fall.



times during the typical fruiting period (see species-specific dates below) to ensure that seeds are collected when mature but before they detach and disperse, or before waterfowl descend upon them (you've got to get them before the migrating ducks do!). To determine if SAV seeds are ready to

Figure 22 - Harvesting redhead grass seed material by hand. Harvested material is placed in plastic bushel baskets for transport

harvest, collect about twenty seed pods, clusters, or spathes from various locations in the donor bed. When the majority contain mature seeds (described on the following pages), it's time to harvest.

Although large-scale SAV restoration projects may require a mechanical harvester to collect enough seeds, hand harvesting seeds, seed pods, and/or other reproductive material is adequate for the smaller restoration projects discussed here. With the exception of eelgrass, seed-bearing structures develop at the top of long, thin peduncles, or stems, near the water surface. They are easily visible and can be collected by detaching the top third or less of the stem (Ailstock and Shafer 2006). One must look a little lower in the water column for eelgrass seeds. You can collect seeds from canoes or kayaks, while wading, or hanging over the side of a small boat (Figure 22). The seed-bearing structures can be placed into buckets, mesh bags, or plastic bushel baskets during collection and transported to the processing or storage facility like that (Figure 23). If possible, seed collection should occur within multiple donor beds across several site visits to increase seed diversity and to ensure that too much harvest stress isn't placed on one donor bed.

Figure 23 - Harvested redhead grass seed material ready to be taken to a facility for processing

Wild celery (Vallisneria americana)

Figure 24 - (A) a wild celery flower and developing seed pod, (B) mature seed pods at the water's surface, and (C) mature seeds.

Wild celery seed pods originate below the flower once fertilized and resemble a green bean or vanilla bean (Figure 24 A). The pods are found at the end of a cylindrical peduncle at or near the water surface and elongate and thicken as they mature (Figure 24 B). Seed pod development should be closely monitored in September to early October. To determine if the multitude of seeds within the pod are mature, break the pod open lengthwise (Figure 24 C). Bright green seeds are not yet mature; seeds that are beginning to or have turned brown and are slightly gelatinous looking are ready for harvest. Not all seed pods mature at exactly the same time, so harvest once the majority of your test pods are mature.

Sago pondweed (Stuckenia pectinata)

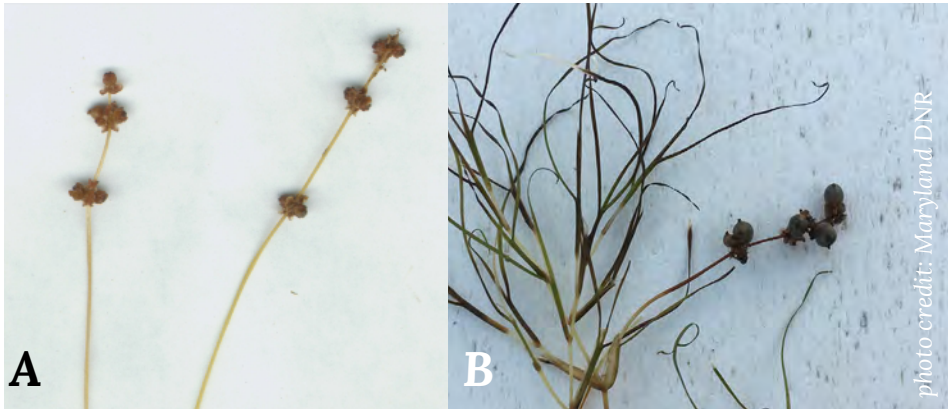


Figure 25 - (A) immature sago seeds and (B) mature seed pods.

Sago pondweed seed clusters loosely resemble an elongated bunch of grapes and can usually be seen at or above the water surface, especially if you harvest at low tide. Immature sago pondweed seed clusters appear as bright green clusters where individual seeds are not yet fully separated (Figure 25 A). They should be harvested in July/August, once the seeds are distinguishable and appear robust and brown (Figure 25 B).

Redhead grass (Potamogeton perfoliatus)

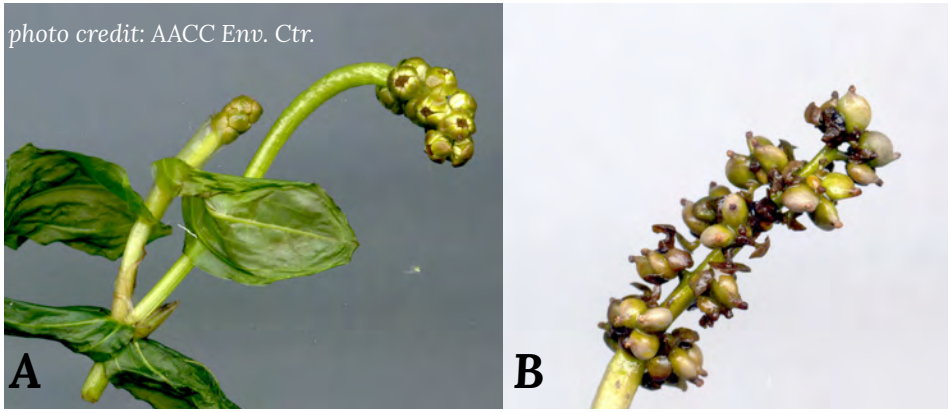


Figure 26 - (A) a flowering redhead grass stalk and a smaller stalk with immature seeds that are not ready for harvest and (B) mature fruit.

Redhead grass seeds appear similar to sago pondweed seeds – much like an elongated bunch of grapes at or near the water's surface; fortunately it is very easy to tell the difference between the two species based on their leaf shape. Redhead grass seeds start out in tight, green clusters where individual seeds are not fully distinguishable (Figure 26 A). They should be harvested in June/July once the seeds are differentiated and appear swollen and brown (Figure 26 B).

Widgeon grass (Ruppia maritima)

Figure 27 - (A) a widgeon grass stalk and a smaller stalk with immature, green fruit and mature, darker fruit and (B) a closeup of mature fruit.

Widgeon grass seeds should be harvested in July/August. Like the others, seeds are easily observed near the surface and are very distinct once mature. Seeds occur in groups of four or five and resemble a stick-figure hand with bulbous ends. Once the bulbous ends are fat and brown, seeds are mature (Figure 27 B).

Eelgrass (Zostera marina)

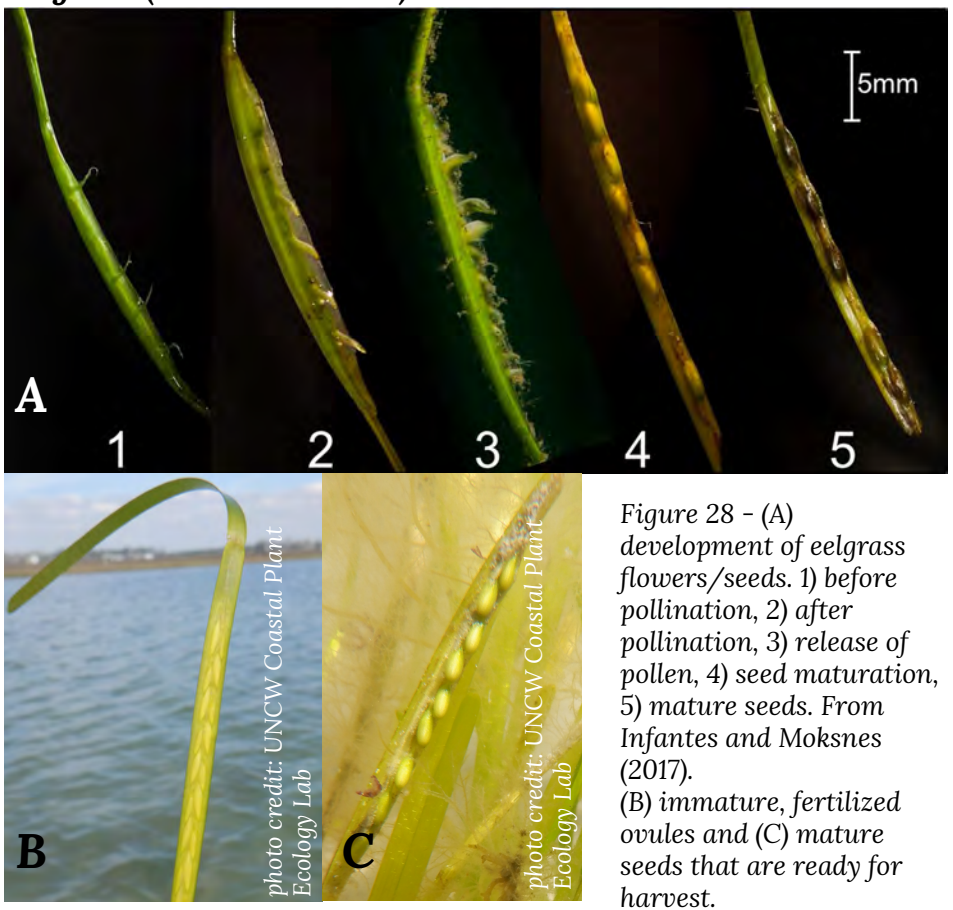


Figure 28 - (A) development of eelgrass flowers/seeds. 1) before pollination, 2) after pollination, 3) release of pollen, 4) seed maturation, 5) mature seeds. From Infantes and Moksnes (2017).
(B) immature, fertilized ovules and (C) mature seeds that are ready for harvest.

Eelgrass seeds are the earliest to mature in Chesapeake Bay and harvesting should take place around the third week of May, depending on donor bed assessments. Eelgrass seeds develop in spathes on reproductive shoots at various heights throughout the water column and loosely resemble oval-shaped peas in a pod when mature. To gauge seed maturity, assess the size, color, and space between the seeds within the spathe. Newly fertilized ovules will appear as alternate, thin, light green structures in the spathe (Figure 28 B). As the fertilized ovules mature, they will grow larger, get darker, and the space between each seed will expand so that they are no longer positioned in an alternate fashion. Once there is space between

each seed, they are ready to harvest and the darker the seed, the closer they are to fully mature (Figure 28 C). Maturation of the seed will continue in storage as long as the spathe remains attached to the shoot to continue receiving nutrients. When the majority of seeds checked are darker and swollen, the seeds are ready for harvest, especially if the spathe appears to be missing some. Seeds mature quickly, so it is important to check the donor bed frequently, particularly after a series of hot days. See Figure 28 A for reproductive development of eelgrass.

Plant collection and transplanting

Whole-plant restoration may still be a feasible option for small restoration projects, as whole plants are hardier than seedlings. There are several methods for collecting whole plants. The simplest approach is to carefully pull plants from the sediment, making sure that the stem, rhizome, and roots stay intact. Once the plant is removed, shake the plant underwater to remove sediment clinging to the roots. Transport the plants to the restoration site in a bucket of water or wrapped in wet newspaper, then transplant the bare-root plant to the restoration site by digging a small hole in the sediment, placing the roots and rhizome in the hole, and tamping it down to stabilize. This approach works particularly well for wild celery.

Similarly, the "plug method" involves collecting plant material in cores or plugs together with surrounding sediments using a 10-15 cm diameter PVC tube (Fonseca et al. 1998). The tube is inserted into the sediment and capped underwater to create a vacuum so that the plant, roots, rhizomes, and sediment are removed in one intact unit. Another cap is placed on the bottom for transport to the restoration site. Plants and sediment can also be placed into peat pots before transport or collected using a sod cutter and stabilized with mesh fabric to decrease the cost of PVC cores.

A third approach is to dig whole plants with a shovel, use a sieve to remove the surrounding sediment, and attach plants or bundles of plant fragments to the sediment surface at the restoration site with biodegradable bamboo staples. The fragment approach is particularly effective for sago pondweed and redhead grass.

SAV restoration that transplants whole plants from a donor bed to a restoration site does not involve processing or storage. Rather, the plants are collected and then immediately transplanted to the restoration site.

8

Processing and Storing Seeds

Seed processing and storage protocols are specific to each SAV species, as detailed below.

Wild celery *(Vallisneria americana)*

Seed pods should be manually separated from the peduncle by hand either in the field during collection or upon returning to the storage facility. Whole pods can then be stored over the winter at 4º C (39.2º F) submerged in ambient water in sealed bags or containers (Moore and Jarvis 2007). Entire seed pods can then be dispersed the following spring, or seeds can be manually removed prior to dispersal by cutting or tearing the pod open. Alternatively, you can maintain whole pods in an aerated tank at ambient temperature for 6-8 weeks to allow the pods to begin to decompose. Then, separate the seeds from the pod and store the pure seed at 4°C (39.2º F) following the same method described above. Field trials suggest that dispersing seeds may produce more seedlings than whole pods.

photo credit: Michael Norman, Anne Arundel Community College

The Chesapeake Bay Foundation's Grasses for the Masses program provides step-by-step grow-out methods for wild celery (*Vallisneria americana*) in a simple homemade grow-out system for 10-12 weeks. Appendix C summarizes these methods. They could potentially be scaled up for a larger scale grow out or possibly applied to other tidal fresh species.

Redgead grass (*Potamogeton perfoliatus*), Sago pondweed (*Stuckenia pectinata*), and Widgeon grass (*Ruppia maritima*)
The three species used for mesohaline restoration projects can all be processed using the same method. A custom built aeration system, named "the turbulator" by its developers, can be used to separate mature seeds from harvested redhead grass, sago pondweed, and widgeon grass plant material. The system consists of a large aquaculture tank outfitted with a 2-inch bottom drain and the components necessary for constant, vigorous aeration. The design of the system consists of foam swim noodles, a wire and mesh framework that fits tightly into a round tank, and a series of eight adjustable PVC air-ports powered by three 5-horsepower wet/dry shop vacuums. The shop vacs are necessary to create enough airflow to vigorously agitate the water and detach seeds from the harvested material. Each air port is equipped with a separate shutoff valve so that the operator can turn air on and off as needed to maximize turbulence in the tank. The bottom of the tank is lined with wire and plastic mesh to separate the seeds from the plant material. Foam swim noodles are used to create a gasket between the mesh and the tank. The entire PVC-mesh aeration/turbulation apparatus can be removed for cleaning and inspecting the tank. See Figure 29 for turbulator design details and Figure 30 for an example budget breakdown.

Prior to turbulation, however, the plant material should be stored in shallow bins (Figure 31) for 5 -7 days at 20-23°C (68-73.4º F); this is a necessary step and part of the after-ripening process. During this time, the plant material should be turned and moistened periodically to prevent the build-up of temperatures and to aid the after-ripening process of the seed. Next, place 2-3 plastic bushel baskets of plant material into the turbulator for aeration and seed separation. Once the plant material is distributed evenly around the tank, turn the air on for 15-20 minutes. It is helpful to have multiple people present to turn the shop vacs on simultaneously and to open or close the shut-off valves when necessary to maximize turbulation.

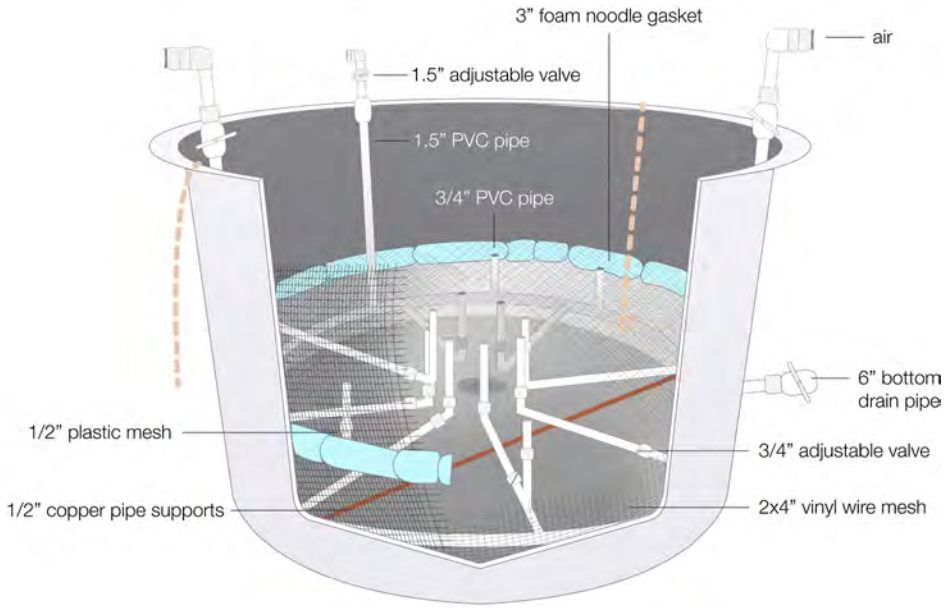


Figure 29 - Schematic of the Turbulator.

Budget Item	Quantity	Cost/Item	Cost
Seed collection baskets	20	$30	$600
Aquaculture tanks	8	$300	$2400
6' round turbulator tank	1	$1400	$1400
Sieves	8	$20	$160
Turbulator supplies	1	$400	$400
Storage supplies	1	$500	$500
Piping for plumbing tank	1	$20	$20
Nozzled hose	1	$75	$75
Planting supplies	1	$100	$100
Shop Vacs with extra tubing	3	$100	$300
Spat bags	8	$8	$64
Structural platform for turbulator	1	$500	$500

TOTAL: $6519

Figure 30 - Example budget for building a turbulator and processing seed. Information provided by Elle Bassett, Miles-Wye Riverkeeper, in 2021.

Figure 31 - Harvested plant material is stored in plastic bins for 5 to 7 days at 20-23°C (68-73.4° F). Redhea grass material pictured here.

Figure 32 - Harvested material being processed in the Turbulator. Redhead grass shown here.

During the turbulation process, dislodged seeds will fall out of the wrack, pass through the mesh, and settle on the bottom of the tank while the wrack itself is trapped above the mesh. Figure 32 shows an active turbulator. After 15-20 minutes of turbulation, turn off the shop vacs and remove the processed wrack from the turbulator. The processed wrack should be placed back into an empty storage bin to continue the after-ripening process. Once 4 - 6 batches of wrack have been processed, remove the aeration components of the turbulator, drain the tank, and collect the seed-rich material. To collect the seed-rich material, attach a 3 mm mesh bag placed inside a 1.5 mm mesh bag to the tank's 2-inch bottom drain and open it up. Seeds and fine plant material will be trapped in the mesh bags as the water passes through. Then to completely separate the fine plant debris from the seeds, sieve the material through a series of five wire and plastic screens ranging in size from 1.6-14 mm mesh. The screens can be attached to wooden frames to construct the sieves.

After the first process of the wrack material is complete, return the material to the bins for further after-ripening. The second process is typically done 7 - 10 days later, or whenever the remaining seeds show signs they are ready for removal. Second process yields often compare to or exceed the first process. After the second process, look closely at the material to determine the amount of remaining seeds and whether an additional process is required.

Once seeds are refined and ready for storage over winter, perform seed counts to determine the number of seeds per gram. With that information, divide the seeds and store them as lots at some known quantity (i.e., containers 1-5 each contain 100,000 seeds; containers 6-10 each contain 200,000 seeds). Storage containers with pre-determined amounts of

seeds simplify preparation the following spring when gathering equipment and supplies to conduct your restoration project.

Storage for all seeds should not exceed 30-40% of the volume of the container and the container is then filled to about 1-inch from the top with the proper salinity water (see below).

Redhead grass seed storage: Isolated seeds can be stored submerged in aerated containers at 10 ppt salinity (using Instant Ocean or Hawaii Salts) water at 4º C (39.2º F). Storage should be in food-grade polypropylene containers with lids, either ½ or 1-gallon in size. Drill a 1-inch hole in the lid to accommodate a sponge stopper and aeration tubing for a 1-inch airstone. The airstone and tubing should be attached to a standard aquarium airpump.

Sago pondweed seed storage: Isolated seeds can be stored submerged in aerated containers at 10 ppt salinity (using Instant Ocean or Hawaii Salts) water at 4º C (39.2º F). Storage should be in food-grade polypropylene containers with lids, either ½ or 1-gallon in size. Drill a 1-inch hole in the lid to accommodate a sponge stopper and aeration tubing for a 1-inch airstone. The airstone and tubing should be attached to a standard aquarium airpump.

Widgeon grass seed storage: Isolated seeds should be stored submerged at 4º C (39.2º F) at a salinity of 30 ppt to prevent premature germination, but note that germination rates after high-salinity storage are low if planting also occurs in high-salinity water. Widgeongrass seeds do not require aeration, but storage should be in food-grade polypropylene containers with lids, either ½ or 1-gallon in size. Drill a 1-inch hole in the lid to accommodate a sponge stopper for venting and gas exchange.

Eelgrass (*Zostera marina*)

Eelgrass reproductive material is first stored in an aerated tank with a drain in the bottom center and with flow-through seawater ideally kept below 25º C (77º F). The tank may be equipped with a shade cloth if located outdoors to reduce temperatures (Granger 2002; Orth et al. 2005; Marion and Orth 2010). Tanks should be stirred daily to flush anoxic water, which can develop as fine organic material accumulates on the bottom and decomposes. 1-inch nylon-coated wire mesh can be placed atop the floating wrack to force submergence and prevent desiccation. In three to six weeks, after reproductive shoots have begun to release seeds, the seeds are then separated from the wrack.

To separate seeds from the wrack, first remove large vegetative fragments using 1-cm mesh screens passed through the water column just above the bottom. Following this, one approach is to vigorously stir the tanks, allowing the dense seeds to accumulate in the middle on the bottom around the center drain. Seeds are collected by draining the tank onto a 1-mm mesh screen, stopping just after all seeds have drained to leave the lighter organic detritus in the tank. Alternative approaches could be tailored to a particular tank set up. The resulting seed product, which still typically contains fine plant material, sand, and shell fragments, should be further separated by resuspending the plant material in a flume and siphoning the seeds from the bottom (Marion and Orth 2008) or by hand-stirring in a 5-gallon bucket and decanting the plant material and overlying water (Granger et al. 2002). Additional separation using a sieve stack can further separate larger shell fragments and smaller sand grains from the seeds.

Widgeon grass can also be processed in this manner and this alternative method may be preferred if building a turbulator is not in your restoration project budget.

Seeds are then stored in aerated tanks or tubs with recirculating, cool (<20º C/68º F) seawater until they are dispersed in the fall of the same year (Granger et al. 2002; Marion and Orth 2010). Seed layer thickness should be less than 3-4 cm to prevent anoxic water accumulation. Eelgrass seed dispersal should take place in October. Over-summering the seeds in cool, aerated tanks and then dispersing them in the fall decreases the likelihood of predation prior to germination. Eelgrass seeds are the only seeds discussed in this manual that aren't overwintered prior to dispersal.

9

Testing Seed Viability and Germination Rates

If possible, seed viability should be assessed prior to dispersal. You don't want to spend your time and resources to disperse seed that will not grow. If you skip this step, you will not be able to determine whether a restoration failed due to poor seeds or poor environmental conditions. Having seed viability numbers will also give you an idea of the density of seeds you need to disperse to get the desired density of plants in your SAV bed. Viable seeds are "living" seeds that have the potential to germinate. There are several tests for seed viability including the tetrazolium assay, the crush test, and the fall test (Sawma and Mohler 2002; Marion and Orth 2010). These are described in further detail on the following pages.

photo credit: AACC Environmental Center

Tetrazolium Assay
The tetrazolium assay consists of soaking a representative subsample of seeds in 1% tetrazolium chloride for 24 hours and then counting the number of viable seeds (seed embryos must be removed from seed coats with a scalpel prior to soaking), which will be stained red or sorrel, under a dissecting scope. To be considered viable, at least 50% of the seed must be stained red. Non-viable seeds will not stain.

Crush Test
The crush test consists of gently squeezing a representative sub-sample of seeds with forceps and recording how many are firm (viable) and how many deform and break under pressure (non-viable).

Fall Test
Finally the fall test consists of dropping seeds in a graduated cylinder filled with seawater and measuring fall velocity (cm/s). Viable eelgrass seeds have been found to fall at >/= 5 cm/s in a salinity of 20 (Marion and Orth 2010). Velocity varies with water density (salinity) and among species so specific rates may need to be empirically determined for your system. However, the difference in fall rate between viable and bad seeds is typically fairly obvious – viable seeds fall rapidly, non-viable seeds do not.

Direct Testing
Germination rates can be directly tested by placing 30-50 viable seeds submerged in water with temperature and salinity comparable to anticipated planting conditions in 3-5 replicate petri dishes (Ailstock et al. 2008a). Germination is indicated by seed coat rupture and growth of the cotyledon, or embryonic leaf. For species with a natural seedbank, seeds that fail to germinate immediately may require additional germination triggers such as changes in temperature or salinity, reflecting natural variation in germination triggers in the population.

To determine how many seeds you have, you can calculate the number of seeds per unit weight or volume of "pure" seed and seeds combined with other material (Granger et al. 2002). This information can then be combined with estimates of seed viability and germination rates to aliquot appropriate quantities (**at least ~100,000-200,000 viable germinating seeds per acre**) of seeds for broadcasting.

10

Dispersing Seed

After seeds are collected, processed, and checked for viability and germination rates, they can be dispersed by hand either from a boat or by walking in shallow water (Orth et al. 2005). One-hundred to two-hundred thousand viable, germinating seeds should be distributed per acre, so if viability and germination rates are each 50%, at a minimum you should distribute at least 400,000 actual seeds per acre. Mixing seeds with sand is recommended as it facilitates more even spreading and the weight of the sand stuck to the seed encourages the seed to sink in place.

Seeds can also be broadcast by a custom-built mechanical seed sprayer mounted to a boat to evenly distribute seeds. You will need to work with a fabricator to design a sprayer that will fit your boat, but essentially it functions to automatically suspend the seeds in water and distribute seed-water mixture in streams from the rear of the boat.

photo credit: Maryland Department of Natural Resources

An alternative processing and dispersal approach is the buoy method, which was developed using eelgrass and has only been tested using that species. With this method, illustrated in Figure 33, reproductive material is placed into mesh bags directly after harvesting and attached to a floating buoy anchored to the bottom with a concrete block. The reproductive material is left in the mesh bags to release seeds over the next 4-5 weeks. An advantage of this approach is that land-based seed processing and storage facilities are not required because the seeds do not have to be separated and stored over winter. It also more closely mirrors the natural timing of seed dispersal, which may allow more time for seed distribution and root establishment in the sediment. However, the buoy-deployed seeding approach also becomes more difficult to carry out as the scale of the project increases because transporting and deploying the concrete anchors can be time and resource-intensive.

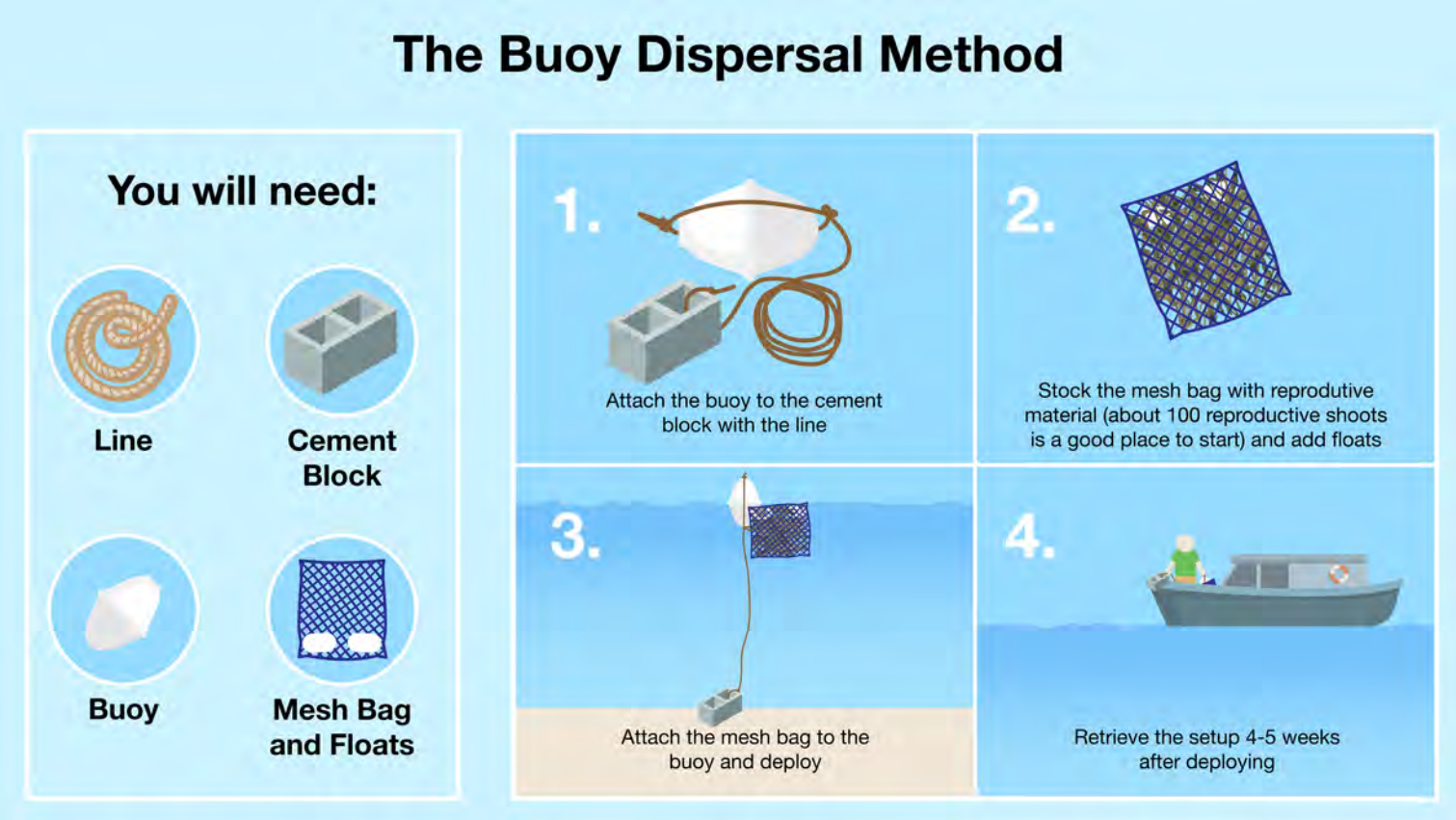


Figure 33 - An alternative processing and dispersal approach called the buoy method.

Optimizing success

Spreading risk: Regardless of the species or restoration method, planting projects are inherently risky due to uncertainty associated with storms, droughts, scour events, salinity fluctuations and other environmental disturbances. However, restoration practitioners can plan to spread the risk of plant loss over space and time to increase the probability of success. One approach is to plant at multiple sites to increase the chance that at least a subset of the sites will support SAV growth (van Katwijk et al. 2009). Similarly, several within-site plots can be planted at a distance of 10's -100's meters and multiple tidal depths. To the extent possible, planting should also occur over multiple years and on different dates within the same year so that, again, at least a subset of the plantings will occur when conditions favor SAV germination and growth. Since the environment is inherently dynamic, optimal planting depths or locations may also vary over time and space. Just like genetic diversity increases population resilience, these

risk-spreading approaches help to increase the resilience of a restoration program.

Restoration practitioners can also harness the ecosystem engineering properties of SAV beds to increase the chances of restoration success (Figure 34). The extent to which SAV modifies the water flow characteristics of a site is a function of the number plants and the size of the bed, where larger beds with more plants tend to create calmer conditions (Luhar et al. 2008). Evidence suggests (van Katwijk et al. 2015) that survival of resorted beds is directly related to the initial number of shoots or seeds planted. Planting a minimum of 1,000-10,000 shoots or seeds increases the chances that restored SAV beds can reach the critical mass required for ecosystem engineering properties to take effect.

Similarly, restoration projects can harness the "nursery bed effect," where a less abundant species is co-planted with a more abundant species to provide shelter from wave and current exposure. This method would be used when the

Figure 34 - Ecosystem engineering properties of SAV beds.

objective is to increase species diversity within an SAV bed, rather than SAV acreage.

Exclosures: If grazing or disturbance by waterfowl, fish, turtles, or rays is expected (and it usually is!), the construction of

Figure 35 - A very robust DC Department of Energy and Environment exclosure set up.

exclosures around your beds should be considered. In the Chesapeake Bay region, these are typically constructed using stakes (often PVC) and heavy snow or construction fencing (see Figure 35 for an example). If waterfowl are suspected grazers, caging to cover the top of the exclosure can also be installed. It is important to verify with your regional US Coast Guard office if permits are required prior to installing exclosures.

11

Monitoring and Success Criteria

Monitoring and success criteria of SAV restoration sites largely depend on restoration site planting goals.

If you dispersed seeds, you should aim to quantify plant growth and survival. Collect data on-site by surveying at least two transects per restoration area. Surveys will be most successfully conducted by snorkeling. Along each transect, lay a PVC quadrat (0.5 x 0.5 m is a good size) at fixed intervals and visually estimate the percent cover of restored SAV within the quadrat and the number of shoots per quadrat. See Figure 36 for an example of a quadrat. It helps to drill holes in the PVC quadrat so that it sinks, and attach a small buoy to a quadrat corner with about 2-2.5 m of line so that you can easily find and retrieve the quadrat after placing it on the bottom. If the transect approach is too labor-intensive for your group, you can also map the perimeter of the restored SAV bed with a handheld GPS unit or smartphone. Once you map the perimeter, conduct random point checks within the perimeter using a PVC quadrat and collect the same data as you would along a transect.

photo credit: Maryland Department of Natural Resources

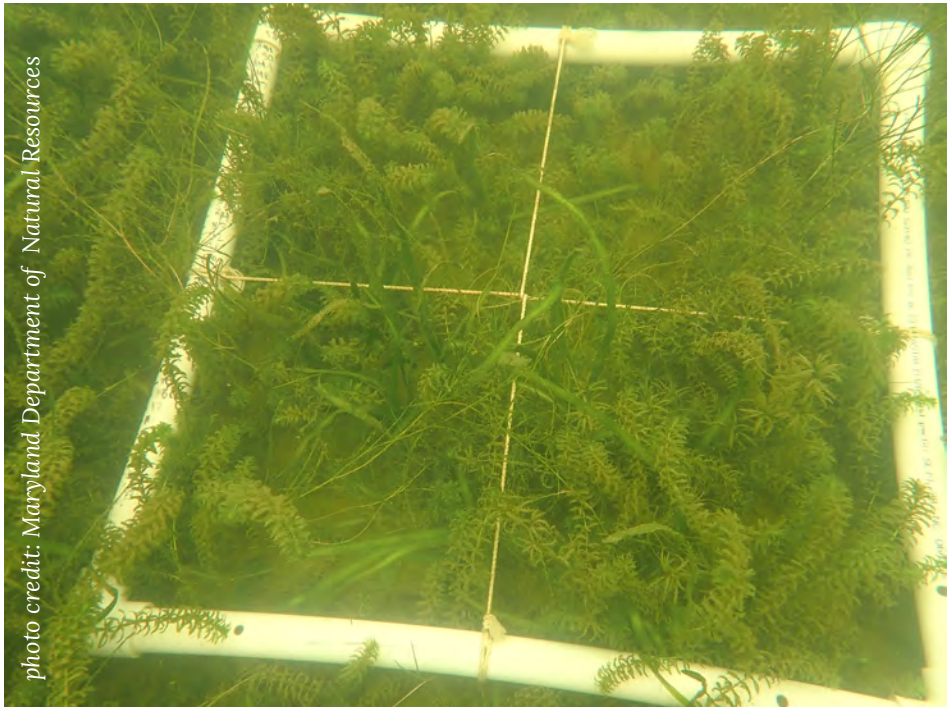


Figure 36 - A PVC quadrat deployed in the field.

If you transplant whole plants and record the coordinates of each planting unit, you can simply return to each unit to record survival metrics, at least within the first few months of planting. However, as the plants spread over time, the transect or perimeter mapping approach will more effectively capture bed expansion. Publicly available spatial data from annual aerial monitoring can also be used to assess coverage, but be aware that SAV at the site needs to be dense enough to be visible from a plane. Also be aware that these data are typically not released until the year after aerial photos are taken. Other metrics should be determined based on the restoration goals (see examples in Figure 37).

Restoration sites should be monitored at least once during the first year after planting but ideally several times during the SAV growing season (March-November for eelgrass; April-October for all other species) to track plant growth and survival. We recommend two monitoring trips during the SAV growing season in subsequent years. Although post-restoration monitoring frequency and duration is typically limited by funding availability, monitoring should be continued for as long as feasible. If you are interested in the long-term establishment of the bed, monitoring for 3-5 years is recommended to capture initial exponential growth, natural mortality, and eventual stabilization. Even longer monitoring is recommended if you want to assess whether ecological functioning is restored, as many of the relevant biogeochemical processes develop slowly over time. For example, you may need to collect data for a decade or longer if your goal is to capture changes in carbon and nutrient cycling (McGlathery et al. 2012; Bell et al. 2014).

Planting Goals	Example Metrics
Reproduction	Reproductive shoot density and seed production rates
Restore ecosystem functions	Nutrient transformations
Restore habitat	Fish/invertebrate abundance and diversity
Understand restoration success	Variables representing the SAV habitat requirements
Reduce erosion	Sediment deposition
Increase species diversity	Number of species present at restoration site
Environmental outreach	Number of people engaged

Figure 37 - Examples of metrics that could be used to monitor an SAV restoration project with different planting goals.

12

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Appendix A - Web Links

1. Chesapeake Bay SAV Restoration Methods: Literature review: *https://greenfinstudio.com/wp-content/uploads/2021/11/SAV-Lit-Syn_Final.pdf*

2. SAV Identification Key: *https://dnr.maryland.gov/waters/bay/Pages/sav/key.aspx*

3. Existing Chesapeake Bay Watershed Statutes and Regulations Affecting Submerged Aquatic Vegetation: *https://www.chesapeakelegal.org/guides-resources/report-existing-chesapeake-bay-watershed-statutes-and-regulations-affecting-submerged-aquatic-vegetation/*

4. Maryland Department of Natural Resources Natural Resources Article 4-213: *http://mgaleg.maryland.gov/2020RS/Statute_Web/gnr/4-213.pdf*

5. Title 4 Virginia Administrative Code 20-337-30: *https://law.lis.virginia.gov/admincode/title4/agency20/chapter337/section30/*

6. D.C. Department of Energy & Environment: *https://doee.dc.gov/service/wetland-and-stream-permits-water-quality-certifications*

7. Baltimore District Website: *https://www.nab.usace.army.mil/Missions/RegulatoryA/Permit-Types-Process/Nationwide-Permits/*

8. Virginia Institute of Marine Science mapping tool: *https://www.vims.edu/research/units/programs/sav/access/maps/index.php*

9. National Oceanic and Atmospheric Administration (NOAA) PORTS tool: *https://tidesandcurrents.noaa.gov/ports.html*

10. NOAA National Data Buoy Center: *https://www.ndbc.noaa.gov/*

11. MDDNR Eyes on the Bay: *http://eyesonthebay.dnr.maryland.gov/*

12. Hach DR900 compatible solutes and reagents: *https://www.hach.com/dr900-multiparameter-portable-colorimeter/product-parameter-reagent?id=15684103251*

13. Chesapeake Bay Program water quality database: *https://www.chesapeakebay.net/what/downloads/cbp_water_quality_database_1984_present*

14. Chesapeake Bay Program DataHub: *https://datahub.chesapeakebay.net/Home*

15. NOAA National Data Buoy Center: *https://www.ndbc.noaa.gov*

16. Chesapeake Monitoring Cooperative Chesapeake Data Explorer: *https://cmc.vims.edu/#/home*

Appendix B - Laboratory Services

There are several analytical laboratories in the Chesapeake region associated with colleges and universities. You can bring water and sediment samples to these labs for analysis. There are fees associated with these services and you should contact you lab of choice prior to bringing samples to ensure that you are following the correct sampling and storage protocols.

photo credit: Chesapeake Bay Program

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UMCES Horn Point

The Horn Point Analytical Services Laboratory provides a wide range of water quality analyses for investigators from the University System of Maryland and around the world. All analyses follow strict QA/QC procedures. Details on methodology and equipment are available upon request. The Analytical Services personnel can also assist in providing supplies and equipment for field sampling and collection

Services:

Nutrient Analyses
Dissolved (fresh, brackish, or seawater samples)

- Nitrate and nitrite
- Nitrite
- Ammonium
- Phosphate
- Silicate
- Total nitrogen (filtered or unfiltered)
- Total phosphorus (filtered or unfiltered)

Particulate

- Nitrogen, carbon and phosphorus
- Total Suspended Solids

Please contact Erica Kiss at 410-221-8317 or ekiss@umces.edu

Pigment Analyses
Pigment analyses by HPLC

- Chlorophylls a,b,c
- Full suite of carotenoids, xanthophylls, and chlorophylls
- HPLC Methodology upon request.

Pigment Analyses by fluorometry

- Chlorophyll a
- Phaeophytin

Please contact Meg Maddox at 410-221-8375 or mmaddox@umces.edu

Pricing:

Please contact Meg Maddox at 410-221-8375 or mmaddox@umces.edu

Process for accepting samples:

- Requisition form for use with sample analyses found here: https://www.umces.edu/sites/default/files/SampleReq-Form_0.pdf

Contact:

Meg Maddox at 410-221-8375 or mmaddox@umces.edu

UMCES CBL

Nutrient Analytical Services Laboratory is a full-service laboratory that is part of the University of Maryland Center for Environmental Science Chesapeake Biological Laboratory and provides analytical support to researchers of the University of Maryland, state and federal agencies, and private sector in water column chemistry, particulate and sediments, and other chemistries.

Services:

Water Column Chemistry
- Orthophosphate
- Ammonium
- Nitrite
- Nitrite and Nitrate
- Silicate
- Total Dissolved Nitrogen and Phosphorus
- Total and Dissolved Organic and Inorganic Carbon

Particulates & Sediments
- Carbon and Nitrogen
- Phosphorus
- Biogenic Silica
- Total Suspended Solids and Total Volatile Solids
- Chlorophyll a and Phaeopigments
- Sediment Size Analysis

Other Chemistries
- Hardness – EDTA Titrimetric Method
- Carbonate Alkalinity
- Biochemical Oxygen Demand (BOD)
- Dissolved metals
- Sulfate, Chloride, and Bromide

Methods for the services: https://www.umces.edu/nasl/methods

Pricing:

Most prices are based on a pre-determined hourly charge or a negotiated contract. Factors that affect prices include type of analyses requested and if pre-analysis sample processing is requested. For specific price information, contact:

Jerry Frank
146 Williams St.
Solomons, MD 20688
410-326-7252
frank@umces.edu

Process for accepting samples:

Please contact NASL staff before sampling so that we can ensure that you have appropriate equipment and sample containers, and that you are familiar with the proper sampling methodology.

Contact:

Jerry Frank
Manager of Analytical Services
Advanced Senior Faculty Research Assistant
410-326-7252
frank@umces.edu

Address:
Chesapeake Biological Laboratory
Nutrient Analytical Services
146 Williams Street
Solomons, MD 20688
Attn: Jerry Frank

Staff: https://www.umces.edu/nasl/contact-us

Virginia Institute of Marine Science

Services:

Dissolved Nutrients
- Ammonium
- Nitrate/Nitrite
- Nitrite
- O-Phosphate
- Silica
- TDN/TDP
- TDS

Particulates
- Chlorophyll/Pheophytin
- Biogenic Silica
- Particulate Phosphorus
- Particulate Inorganic Phosphorus
- PC/PN
- POC/PN
- TSS/TFS

Solids
- Total Solids (Percent Moisture)
- TOC/TN
- TOC/TN (pre-dried)
- Grain Size
- Grain Size/RSA
- Organic Matter
- Filtering
- Enterococcus (MPN)

Pricing:

Dissolved Nutrients
- Ammonium $8.80
- Nitrate/Nitrite $8.80
- Nitrite $8.80
- O-Phosphate $8.80
- Silica $8.80
- TDN/TDP $21.85
- TDS $7.05

Particulates
- Chlorophyll/Pheophytin $21.35
- Biogenic Silica $22.20
- Particulate Phosphorus $15.50
- Particulate Inorganic Phosphorus $14.85
- PC/PN $23.10
- POC/PN $24.10
- TSS/TFS $12.80

Solids
- Total Solids (Percent Moisture) $9.70
- TOC/TN $25.75
- TOC/TN (pre-dried) $24.10
- Grain Size $23.40
- Grain Size/RSA $33.50
- Organic Matter $12.60
- Filtering $6.20
- Enterococcus (MPN) $31.25

Lab maintenance fee of $1.40 per sample will be charged.

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Process for accepting samples:
Contact Carol Pollard, pollard@vims.edu

Contact:
Carol Pollard
pollard@vims.edu
(804) 684-7213
Laboratory Manager, Analytical Services Center

Appendix C - Grasses for the Masses Growth Chamber Set-up Summary

Grasses for the Masses Materials Checklist

Total List for 1 Growth Chamber

- 1 black plastic tub (approx. 3x2 ft)
- 1 powerhead pump
- 1 submersible heater
- 2 swing arm desk lamps
- 1 thermometer
- 1 powerstrip with surge protector
- 3 small plastic growth trays (kitty pans)
- 1 bag of wild celery seed pods
- All-purpose sand (not play sand- it is too fine in texture; NOTE: buy the smallest bag of all-purpose sand that is available at your local garden store. We suggest 25 lbs because it is the smallest bag we could find – you will have leftovers.)
- Top soil or garden soil, NOT potting soil. Preferably soil from your yard.
- 1 - 75 watt light bulb (incandescent or compact fluorescent)
- Bucket or similar container (5-gallon, 2.5-gallon, etc). This is to air out tap water for 24 hours before introduction into the system.

NOTE: It is extremely important to plant your seeds right away! Before planting, they should be stored in a refrigerator.

<u>Growth chamber set-up:</u>

1. Place tub in a location with a stable temperature, near an electrical outlet or window. Please do not move your growth unit once it is set up and keep in mind that you will need to leave the system (lamp, filter, heater, etc.) on constantly for the duration of seed germination.
2. Fill large black tub halfway with tap water and set up the pump & filter. Place the powerhead pump into the tub of water (needs to be underwater before you turn it on) and plug pump into the power strip. Please ensure that a drip loop is in place to prevent water from running into the socket/strip.
3. Allow the filter pump to bubble/run for 24 hours before planting grass seeds to remove chlorine from the tap water.
4. Set the heater to 78º F by turning the knob at the top, place horizontally in the water and then plug it into the power strip. Place the heater in the path of the water pump, so the pump will push water over the heater and help circulate the heat. Let the heater run for several hours before adding the seed trays, to allow water to reach optimal temperature of 78 degrees.
5. Set up thermometer on the side of the tub.
6. Fill a bucket or large pitcher with water and allow to sit for 24 hours. (This will once again allow any chlorine present in tap water to evaporate. You will use this during the next step)

Planting the seeds:

Mixing soil and sand

1. Fill each small tray about halfway with a 25:75 ratio of top soil and all-purpose sand mixture and firmly pack mixture down. Do not use fine playground sand or potting soil.
2. Keep about six handfuls of the mixture aside for use on the top of the trays.

Prepare your seeds

1. Take seeds out of the refrigerator and transfer the seeds to a small container of the remaining dampened sand/soil mixture using a spoon to scoop the seeds.
2. Spread sand/soil and grass seeds mixture across the top of each tray.
3. To help keep the seeds in place, sprinkle a handful or two of extra sand over top of the seeds in each tray, then or spray or mist the top of the sand in the trays.

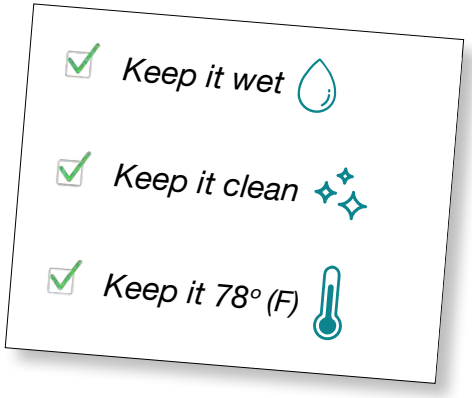
Immerse your seeds - You want to disturb the seeds as little as possible. It is very important you take your time submerging the trays into the water.

1. After the seeds are in the trays, fill each tray with aired water (from the bucket/pitcher you let sit out 24 hours or the tub you let bubble for a day) to let the sand/ soil mixture saturate. You can also cover each tray with paper towels, plastic bags, or plastic wrap to minimize disturbance.
2. Turn off your powerhead pump before you add the seed trays to the large tub to reduce disturbance.
3. Slowly lower each tray into the larger tub of water, allowing the water to slowly sink in and saturate the trays (try not to disturb the top layer and seeds).
4. Once the trays are submerged, pour or ladle your bucket of water into the tub slowly (it helps to push the trays over to one side and pour in water from the other side).
5. If the bucket of water does not fill the tub all the way, fill bucket again and let water sit for a day and then add it to tub. Repeat until the water is above the powerhead pump and above the kitty pan edges
6. Once the water is above the powerhead you can turn the pump back on. (you will want to keep the water above this level, keep the water bucket by the tub and add as needed).

Set-up and attach lamp

1. Place lamp clamps on the side corners of the black tubs or on the table the kit is resting on.
2. Adjust the lamp to hover about 6 to 10 inches from the water to shed light evenly over the seeds.
3. Use a regular 75 Watt bulb or a 75 Watt Compact Fluorescent bulbs for the lamp (you do not a need flood or a heat lamp bulb)
4. Leave the lamp on day and night for optimal growth during the germination period. Once your grasses sprout you may turn the lamp off at night. (You can see sprouts as early as one week after planting, but as late as 21 days.)

Daily maintenance:



Check water level

- It is important to keep the water level a couple inches above the outflow of the filter pump and kitty pan edges to allow for circulation and oxygenation of the seeds.

- If you leave your grasses unattended for a few days, make sure there is enough water to stay above the trays and filter for the duration of your absence.
- We suggest letting water air (sit out in a bucket/pitcher) for a day before adding it to the tub, again this will help reduce chlorine in your grass systems.
- Please note: If the water level drops below the filter pump intake or the heater, they will break and an electrical shock could result.

Clean filter and algae

- As algal growth builds, you may see a green or oily sheen on the plants, sand, water surface, and/or tub. Stir the surface of the water with a spoon or fork—this will promote oxygen to rid algae. Also check filter to make sure it is not clogged; it may need a rinse in the sink.
- Use any tool you see fit (turkey baster, hands, toothbrush, plastic fork) to gently wipe algae off of the plant stems. A fine mesh net works well for skimming the water surface.
- The tub can be wiped with a cloth or sponge. DO NOT USE BLEACH or other cleaning agents.
- The filter pump will need to be rinsed in water as it accumulates build-up. Check it periodically but remember that it houses beneficial bacteria, so don’t use any cleaners or rinse it too often. Simply unplug and remove the filter attachment and rinse in the sink.
- Please DO NOT add any living creatures such as snails, fish, etc. to your grasses system. We do not want to risk adding any exotic species to the rivers when we plant.

Monitor temperature

- The water temperature should be at a constant 78 degrees F. Adjust the heater accordingly to maintain optimal temperature.

Preparing for planting:

- Disassemble the light, the filter pump, and the heater and clean with a dilute vinegar solution and scrub brush.
- Drain water from the tub but keep a small amount of water in the trays to keep the plants wet. Cover your grasses (in the small trays) with wet strips of newspaper to retain the moisture and minimizes splashing.