ERDC/TN SAV-07-3
December 2007

Using Seeds to Propagate and Restore *Vallisneria americana* Michaux (Wild Celery) in the Chesapeake Bay

by Kenneth A. Moore and Jesse C. Jarvis

PROBLEM: Loss of submerged aquatic vegetation (SAV) has been significant in many coastal and estuarine systems such as the Chesapeake Bay where SAV die-offs have been observed in marine, brackish, and freshwater SAV communities (Orth and Moore 1983). Large-scale replanting using whole plants can be cost-prohibitive, and the use of plants taken from wild populations for propagule supply may not be possible or desirable. The use of SAV seeds for restoration has been increasingly demonstrated to be an effective method for replanting large areas of bottom, especially for marine species such as eelgrass, *Zostera marina* (Pickerell et al. 2005; Orth et al. 1994, 2006; Granger et al. 2002). Only more recently have techniques been developed to use seeds in the restoration of some brackish and freshwater SAV species (Ailstock and Shafer 2004, 2006). The commercial production of freshwater SAV for waterfowl habitat has been ongoing for many years in the Midwest and northern prairie regions. However, propagation has for the most part used over-wintering buds or tubers, and costs associated with these methods are high (Korschgen and Green 1988). Although a great deal of research has been conducted on freshwater and brackish SAV, much is still unknown as to the factors affecting the utility of seeds of species such as *Vallisneria americana* Michaux as an option for SAV restoration, especially in tidal, estuarine habitats.

PURPOSE: This technical note reviews the results of studies investigating the use of seeds of *V. americana* for restoration in the Chesapeake Bay, including aspects of the collection, storage, germination, and seedling growth. The goal is to improve restoration success of this species.

BACKGROUND: *Vallisneria americana* (Figure 1)[1] is a clonal, perennial species with ribbon- or tape-like leaves that grow from a basal meristem. The leaves bear a distinct central longitudinal stripe; leaf tips are bluntly rounded, margins entirely too conspicuously toothed (Lowden 1982, Catling et al. 1994). It is a dioecious plant that can reproduce asexually through the spreading of rhizomes and stolons, which produce vertical short shoots consisting of rosettes of leaves (see McFarland (2006) for

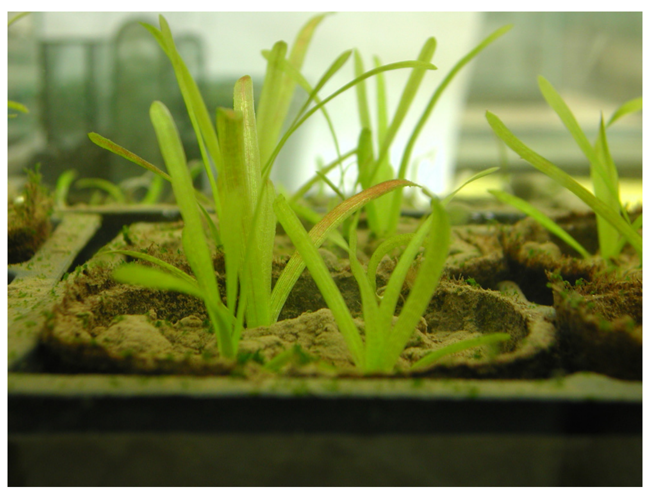


Figure 1. *Vallisneria americana* (Michx.) is a dioecious perennial species of submerged aquatic vegetation in Chesapeake Bay. These specimens are transplants grown from planted seeds.

[1] All photographs by K. A. Moore and J. C. Jarvis.

a complete review). The species is broadly distributed along the Atlantic and Gulf of Mexico and interior areas from Nova Scotia to South Dakota (Lowden 1982; Korschgen and Green 1988; Adair et al. 1994) and can tolerate a wide range of environmental conditions (McFarland 2006). In most areas the plants produce over-wintering buds that allow for repopulation the subsequent spring. In estuarine conditions both salinity and turbidity can reduce plant biomass, growth, and reproduction including winter bud formation (French and Moore 2003).

Figure 2. Pistillate flowers elongate to the water surface while staminate flowers break from the base of the shoot and float to the surface. Pollination (shown here) occurs when the staminate flower, propelled by water currents or wind, dips into the depression created by the pistillate flower and pollen is deposited onto the stigma (Sculthorpe 1967; Kaul 1978).

In the Chesapeake Bay system, *V. americana* was historically dominant (Davis 1985). In freshwater and brackish regions of the Chesapeake Bay, flowering typically occurs from July to August (Figure 2), with fruits maturing by September to October (Figure 3). The fruits consist of seed pods that contain hundreds of seeds in a gelatinous matrix (Figure 4). Campbell (2005) found approximately 168 seeds per pod in Chesapeake Bay beds, with approximately 20 to 40 percent of the shoots producing fruits. Seed production varied with shoot density, the former ranging from <1,000 m^{-2} to 16,000 m^{-2}.

Figure 3. Mature seed pods produced by *V. americana*.

SEED HARVEST: In the Chesapeake Bay, *V. americana* is found in monotypic beds or mixed with other species in tidal freshwater regions in the upper bay and its tributaries, especially the Susquehanna Flats region and the Potomac River system (Moore et al. 2000). Generally seed pods are well formed with seeds beginning in late August and continuing through October. Typically seeds in seed pods are more immature in the late summer (August-September) than early fall (September-October). Immature seeds are typically light in color compared to dark brown mature seeds (Figure 5) and their germination success is reduced (McFarland 2006). Seed development may, however, vary from year to year, even within a single bed. Therefore, source beds should be monitored for seed development and density of reproductive shoots. While early October may be the optimum time for seed development in the Chesapeake Bay, seed pod detachment and loss may be rapid over periods of one week or less due to natural detachment at

Figure 4. Mature *V. americana* seeds in a seed pod. The deteriorated condition of the outer wall of the seed pod occurred after 6 months of storage at 4 °C in non-aerated conditions.

Figure 5. Mature *V. americana* seeds. Seed color ranges from white, to light brown, to dark brown. As seeds mature their color becomes darker, therefore the darkest seeds have the greatest viability.

the end of the seed production cycle or storms. Water fowl may also consume a large proportion of the seed pods in any one area over a few days (Perry and Deller 1996). Ailstock and Shafer (2006) recommend multiple collection sites and repeated seed harvesting over several weeks to preclude the potential for poor seed harvest during any one year for other SAV species. These guidelines are similarly appropriate for *V. americana.*

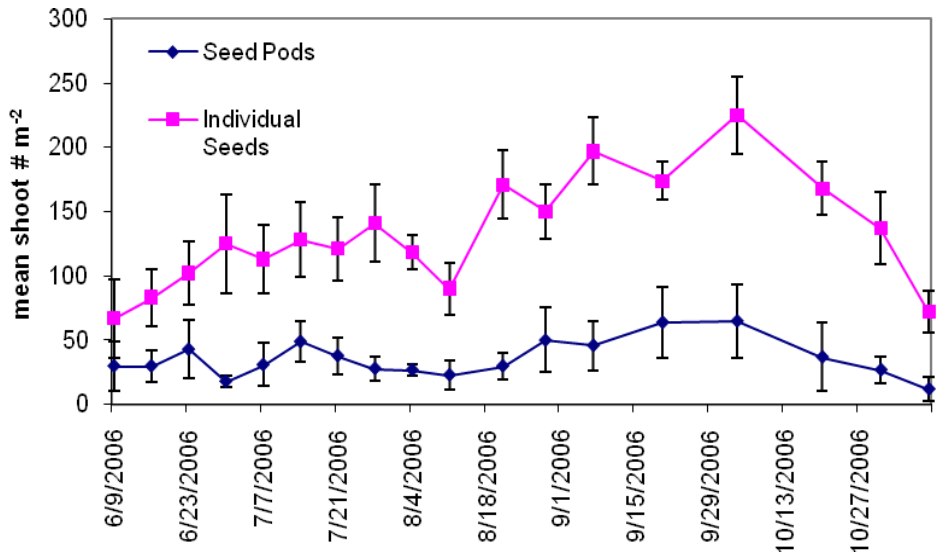
Seed pods can easily be harvested by hand, especially at lower tidal levels. Usually an individual can harvest several hundred pods per hour by simply wading through a bed with an attached floating mesh bag tied to a string or small rope. The seed pods are usually visible near the surface and can be harvested by hand and placed into the floating bag. At the end of the season, the pods may be lower in the canopy and periphyton fouling may mask their appearance and make harvesting difficult. Therefore seed pod harvesting efficiency can vary greatly from day to day depending on water depth and other conditions. Seed pods appear to sink and remain within the beds and little wrack containing pods can be found with other species (Ailstock and Shafer 2006). Once harvested, the seed pods can be placed in insulated coolers filled with water from the site and transported to the storage facility. Seed pods can remain in coolers at ambient temperatures (~25 ºC) for one to two days, especially if the water is aerated to prevent decomposition.

SEED STORAGE: In this project, seed pods were stored in the dark at 4 ºC for 6 months or longer in sealed containers with no aeration (Baskin and Baskin 1998). Unpublished data and other anecdotal evidence suggest that seed viability decreases over time even when stored at 4 ºC or lower. However, a few viable seeds have remained even after storage for 2-3 years in the dark at 3-4 ºC (Campbell 2005). Seed viability can be tested using tetrazolium staining (Lakon 1949, Grabe 1970). Seed embryos are removed from their seed coats by slicing the seed down the middle and removing the embryo from the seed coat with tweezers. Once the seed is removed from the seed coat, it should be soaked in a 1-percent tetrazolium chloride (tetrazolium) solution for 24 hr before examination on a dissecting scope at 10X magnification. Positive tests (live embryos) occur when greater than 50 percent of the embryo is stained red (Association of Official Seed Analysts (AOSA) 1981). Due to some variability in staining of aquatic macrophyte seeds (Madsen and Boylen 1989), at least 50 seeds should be used in viability testing (Baskin and Baskin 1998).

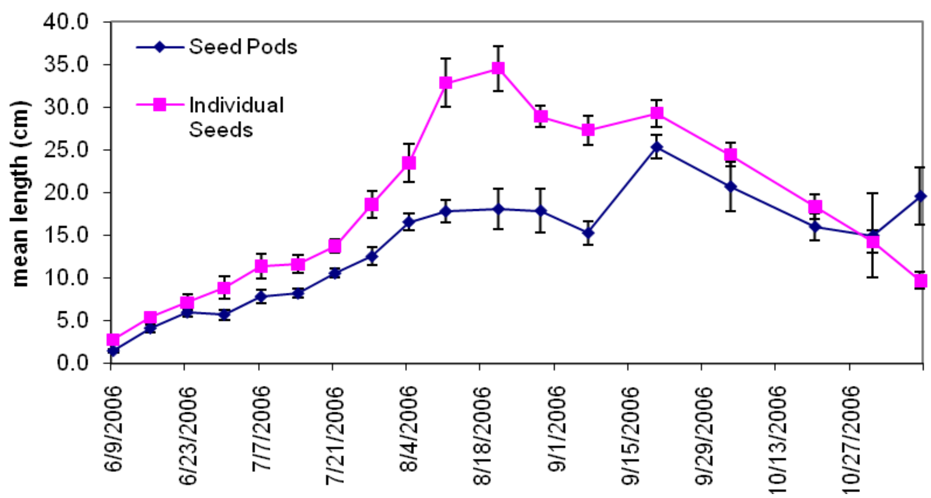
SEED PLANTING: In the field, seeds can be planted by simple dispersal of the intact seed pods or by dispersal of masses of individual seeds that have been removed from the pods. The pods will break down over time in storage and the seed can easily be removed by gently cutting or tearing the pods apart by hand (Figure 4). Seeds or pods can be dispersed into ponds or the field at any time after collection. Although cold storage has not been found necessary for seed germination (McFarland 2006, Ferasol et al. 1995), under ambient field conditions germination will not begin to occur in this region until the following spring when water temperatures exceed 13 ºC (Campbell 2005). Warmer temperatures can significantly reduce the mean time to germinate (MTG). Campbell (2005) observed that the MTG for seeds held in cold storage at 4 ºC decreased from 12 days when placed in water at 22 ºC to 6 days at 29 ºC. The percentage of seeds that germinated also increased from approximately 70 to 90 percent. Therefore, planting seeds during warmer periods will decrease the length of time that seeds are subject to predation or other potential losses.

Campbell (2005) found that germination in *V. americana* seeds increased significantly at salinities less than 5 psu, organic matter content less than 3 percent, burial depths less than 15 mm, and in sandy substrates. Campbell also observed that germination was much higher for seeds in water (70-90 percent) versus seeds planted in sediments (< 20 percent). This is particularly important as many SAV seed germination studies are conducted using water treatments (e.g., Muenscher 1936, Moore et al. 1993) and these germination rates may be useful for comparative purposes only. One negative effect of in-water germination is that although the seeds are negatively buoyant, the seedlings will float. Therefore, some degree of incorporation into sediment will be necessary for either nursery pond or field seed dispersal. Since this sediment incorporation can be accomplished by natural bioturbation processes acting on the seeds prior to germination, it is suggested that seeds be dispersed early enough to allow for these natural processes to occur. Seeds can be dispersed by hand in the fall, or can be dispersed using buoy-deployed seeding techniques (Pickerell et al. 2006), paralleling natural processes. Mechanical seed planters have been used to plant seeds of eelgrass (*Zostera marina*) (Orth et al. 2006), but there have been no published reports of mechanical planting of *V. americana* seeds.

EXPERIMENTS IN PONDS AND RIVER FIELD SITES: Recently, an experiment was conducted in nursery ponds to compare the results of dispersing individual seeds or entire seed pods of *V. americana*. Seed pods were collected, as described previously, from the Potomac River, a tributary to the Chesapeake Bay. Prior to experimentation seed viability was tested using tetrazolium staining. On average, *V. americana* seed pods contain 168 seeds (Campbell 2005). For the individual seed treatments, seeds were counted, with 168 seeds equaling one seed pod. Equal numbers of seeds (2500 m^{-2}) were dispersed as either individual seeds or seed pods directly into two freshwater ponds in the fall immediately after harvesting (Figure 10a). For the seed pod treatment, intact seed pods were distributed randomly throughout the pond area and gently patted into the sediment. For the seed dispersal treatment, the seed pods were first gently broken apart by hand in a small amount of water in a container to remove the seeds, the seed pods were then discarded and the seeds randomly distributed by hand across the pond treatment area and gently patted into the sediment. At approximately weekly to bi-weekly intervals, a 0.25-m^2 quadrat was randomly placed on the bottom and all shoots were counted and shoot lengths measured (centimeters). The dispersed seeds resulted in significantly greater numbers of seedlings (Figure 6a) and these seedlings reached greater maximum size than those produced by the seed pod dispersal treatment during the first growing season (Figure 6b). This was due in part to the clumping effect of the seedlings that germinated from the pod dispersal that may have had a negative effect on seedling establishment.



a. Number of germinated and surviving seedlings for two restoration methods. Treatments consisted of planting either individual seeds or whole seed pods in freshwater nursery ponds. Results are reported as mean (±SE) shoots m^{-2}, n = 3.



b. *V. americana* shoot lengths for two restoration methods. Treatments consisted of planting either individual seeds or whole seed pods in freshwater nursery ponds.

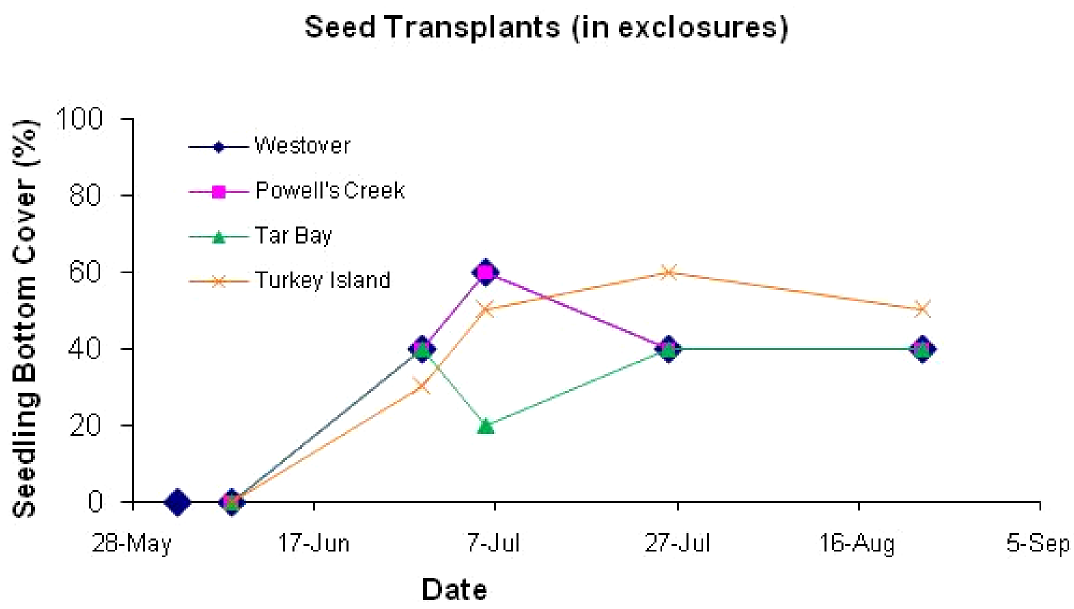
Figure 6. Comparisons of germinated and surviving seedlings (a.) and shoot lengths (b.).

For the study in the James River, *V. americana* seeds were obtained from the Potomac River, MD in October by harvesting seed pods by hand as described above. Seed pods were kept in river water and were refrigerated in the dark at 4 ºC until being transplanted the following April. Seeds were dispersed at four transplant sites in the tidal James River in 4- by 10-m areas both inside and outside of fenced exclosures (Figure 7). Treatments consisted of intact seed pods and an equal number of seeds that had been removed from the pods as described earlier. Seeding rates were approximately 2,500 seeds/m^{-2}. The seed pods were gently broken apart by hand and the seeds removed just prior to planting. Both the intact seed pods and separated seeds were randomly dispersed onto the bottom by divers and lightly patted into the sediments within each treatment area at each study site. Germinated seedlings and whole plants were checked by divers for size and abundance at monthly intervals. Plant basal bottom cover was estimated by divers on a 0- to 100-percent scale within each 4- by 10-m transplant area. Maximum shoot lengths were measured in centimeters.

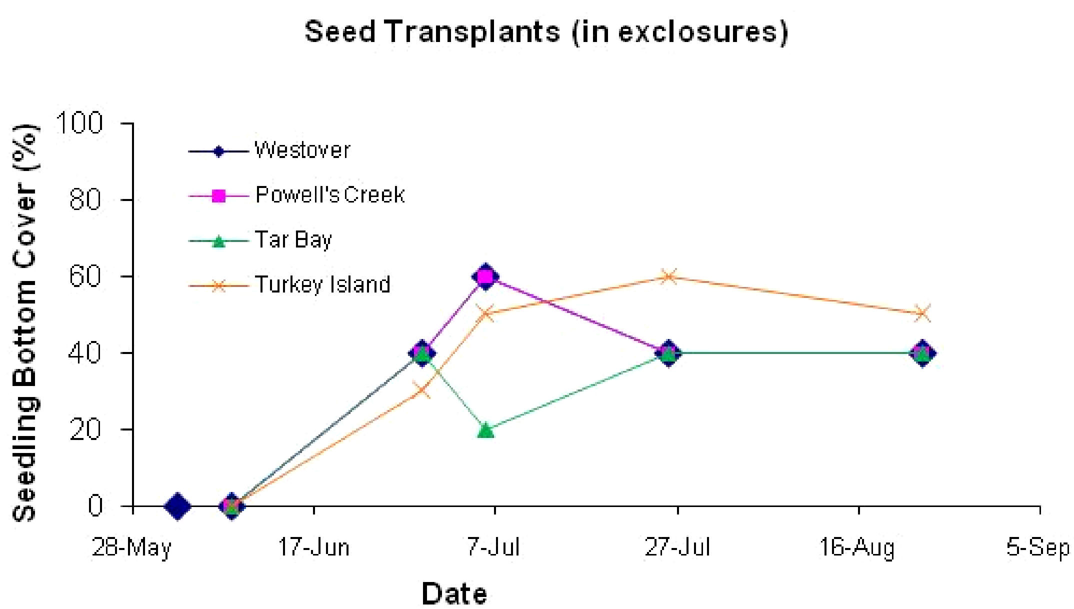
Figure 7. SAV restoration exclosure on the Upper James River. Exclosure fencing consists of 1.5-in. PVC poles and 1-in. mesh polyethylene aquaculture netting.

The results indicated that by the end of the initial growing season within the protective exclosures, the separated seed treatments resulted in 40-60 percent bottom cover by seedlings compared to 20-40 percent for the seed pod dispersal (Figure 8). Seed transplanting outside of the exclosures resulted in an initial germination and growth period, followed by nearly complete loss of seedlings through herbivory (Figure 9). Although initial germination of seeds outside of the exclosures was comparable to those inside the exclosures, once the seedlings reached approximately 10 cm in height, they were grazed to a height of 1-2 cm.[1] One strategy for successful growth and survival of *V. americana* in these high turbidity, but historically vegetated, regions of the James River, is the capacity of the plants to elongate so that the leaves can reach closer to the water surface to gather adequate light for photosynthesis. When subject to continual cropping, the plants may rapidly use up stored reserves and die.

[1] Personal observation, 2006, Kenneth A. Moore, Professor Marine Science, Virginia Institute of Marine Science, Gloucester Point, VA.

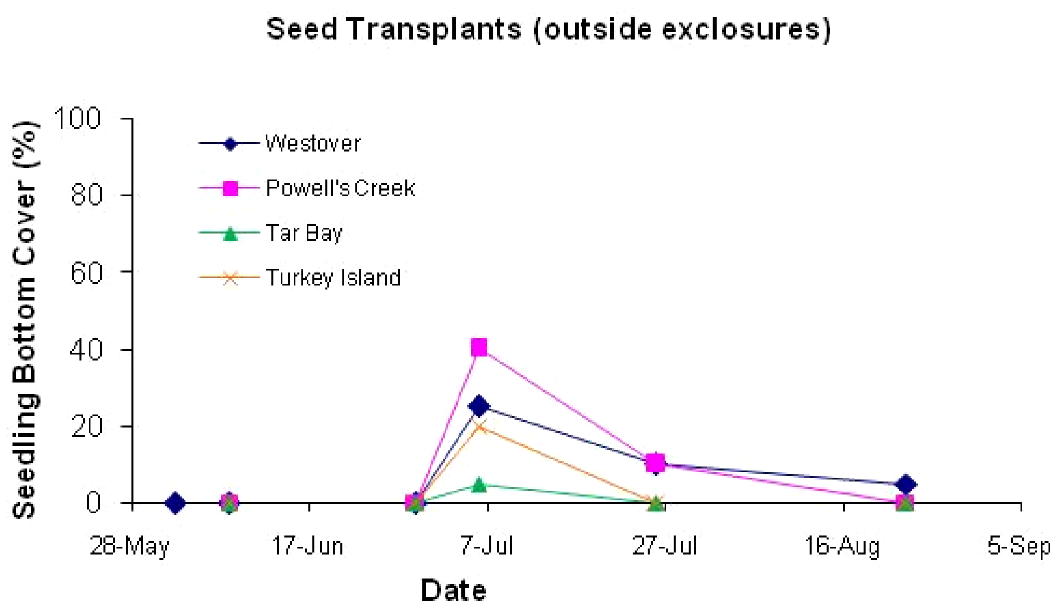


a. Results of the exclosure-protected seed transplant experiment located at four sites in the tidal James River, VA. SAV abundance presented as transplant area percent bottom cover.

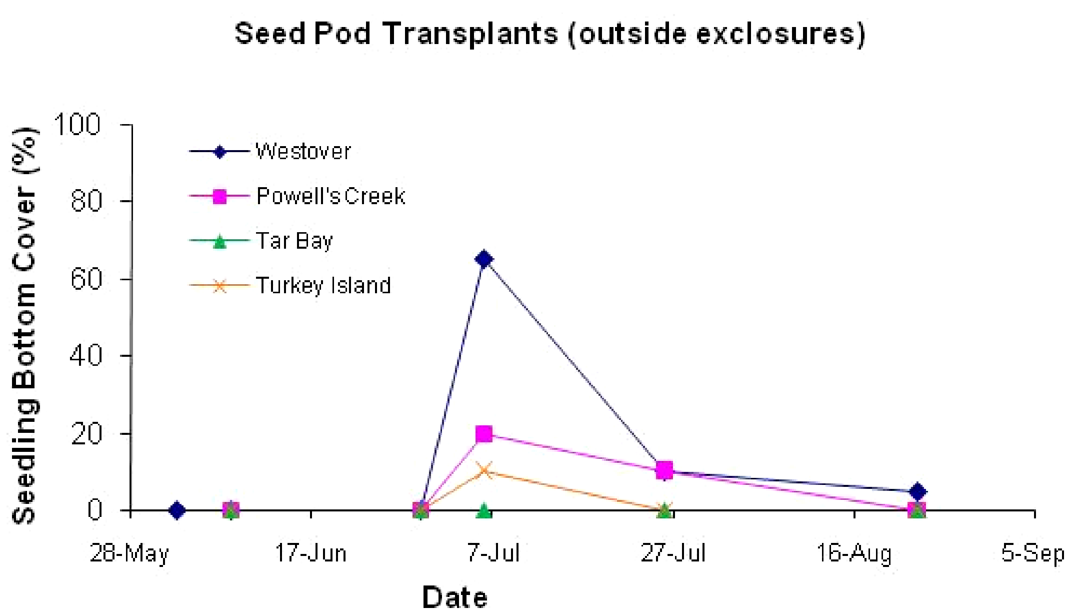


b. Results of the exclosure-protected seed pod transplant experiment located at four sites in the tidal James River, VA. SAV abundance presented as transplant area percent bottom cover.

Figure 8. Experiment results at four James River sites for seed transplants (a.) and seed pod transplants (b.).



a. Resulting transplant area bottom cover of *V. americana* from seeds directly desposited on the sediment in unprotected (no exclosure) areas at four sites in the James River, VA.



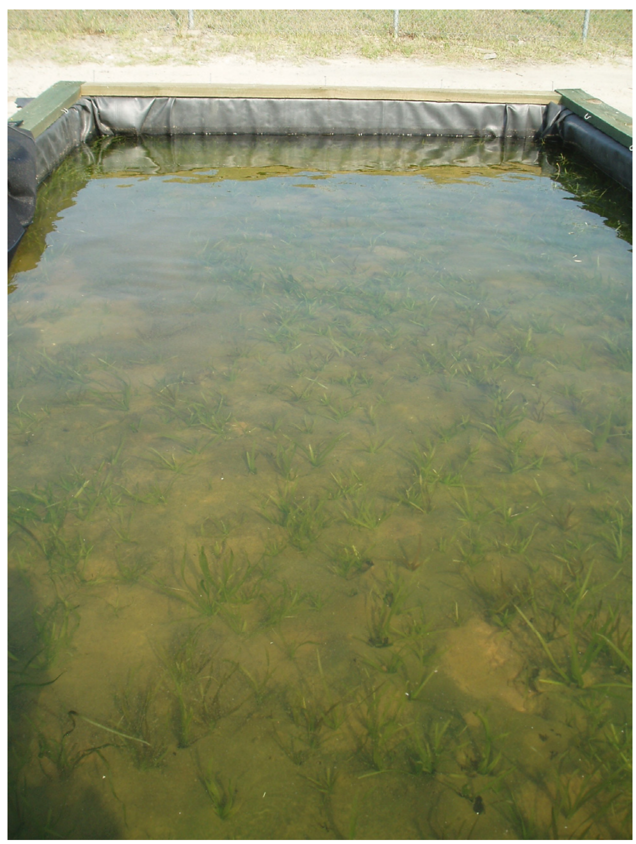
b. Resulting transplant area bottom cover of *V. americana* from seed pods directly desposited on the sediment in unprotected (no exclosure) areas at four sites in the James River, VA.

Figure 9. Transplant area bottom cover from seeds (a.) and seed pods (b.) at four sites in the James River, VA.

SEEDLING ESTABLISHMENT AND USE IN RESTORATION: Restoration of *V. americana* using seeds either individually or dispersed as intact seed pods can be an alternative to the direct planting of shoots. As with all direct seeding approaches used for SAV restoration, there are the benefits of reduced labor efforts and removal of the requirement for in-water restoration by divers or other personnel with water skills (Orth et al. 2006). Similarly, because of the low rates of germination success and the potential for herbivory or other losses of seedlings, the success rate of seedlings produced by transplanted seeds can be very low. For example, Campbell (2005) observed that between 1,000 and 16,000 seeds/m^2 were produced in established *V. americana* beds in the Chesapeake Bay. Presumably these are for natural bed maintenance and provide a seed source for bed recovery from diebacks. Seed dispersal at these rates over large restoration areas would generally not be possible. In field studies in the James River, seeding rates of approximately 2,500 seeds/m^2 were used with success. Typically, restoration to 100-percent bottom cover requires approximately 3 years of growth (Moore et al. 2005). An intermediate step to large-scale restoration includes using seeds to produce smaller, scattered founder colonies that would then re-propagate nearby unvegetated areas naturally. Similar approaches are currently being used for the restoration of *Zostera marina* (eelgrass) beds (Orth et al. 2006).

Direct seeding in the field shows that initial protection from herbivory by exclosure fencing would also be necessary. Another option to direct seeding of wild grown seeds would be to develop freshwater nursery ponds for the future harvesting of seeds, whole shoots, and overwintering buds. Costs can be reduced by developing simple, shallow ponds using wooden timbers and commercial pond liner (Figure 10). SAV can be grown from seeds and whole shoots using small static ponds (3 ft by 8 ft by 16 ft) filled with dechlorinated water and sand substrate. Water is periodically added to replace that

a. SAV growout ponds at Gloucester Point, VA. Ponds are constructed from 6- by 6-in. pressure-treated timbers and lined with commercial pond liner. Shade cloths reducing ambient light by 50 percent are used to reduce algal growth.



b. SAV growout pond containing *V. americana*.

Figure 10. SAV growout ponds.

lost by evaporation and to flush the ponds if they become cloudy from algal growth. Thirty to fifty percent neutral density shade cloths (Figure 10a) assist in keeping algal growth low and keeping summertime water temperatures at 30 ºC or less.

POINTS OF CONTACT: For more information, contact Dr. Kenneth A. Moore, (moore@vims.edu), The Virginia Institute of Marine Science, Gloucester Point, VA 23062, or Dr. Deborah Shafer (Deborah.J.Shafer@usace.army.mil), U.S. Army Engineer Research and Development Center, 3909 Halls Ferry Road, Vicksburg, MS 39180. This technical note can be cited as follows:

> Moore, K. A., and J. C. Jarvis. 2007. *Using seeds to propagate and restore* Vallisneria americana *Michaux (wild celery) in the Chesapeake Bay*. SAV Technical Notes Collection (ERDC/TN SAV-07-3). Vicksburg, MS: U.S. Army Engineer Research and Development Center.

REFERENCES

Adair, S. E., J. L. Moore and, C. P. Onuf. 1994. Distribution and status of submerged vegetation in estuaries of the upper Texas coast. *Wetlands* 14:110-121.

Ailstock, M. S., and D. Shafer. 2004. *Restoration potential of Ruppia maritima and Potamogeton perfoliatus by seed in the mid-Chesapeake Bay*. ERDC/TN EL-04-02. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

__________. 2006. *Protocol for large-scale collection, processing, and storage of seeds of two mesohaline submerged aquatic plant species*. ERDC/TN EL-06-03. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Association of Official Seed Analysts (AOSA). 1981. Rules for testing seeds. *Journal of Seed Technology* 6:1-126.

Baskin, C.B., and J. Baskin. 1998. Ecologically meaningful germination studies. In *Seeds: Ecology, biogeography, and evolution of dormancy and germination*, 5-26. London: Academic Press.

Campbell, J. J. 2005. Influence of environmental factors on the seed ecology of *Vallisneria americana*. MS Thesis, The College of William and Mary, School of Marine Science, Gloucester Point, VA.

Catling, P. M., K. W. Spicer, M. Biernacki, and J. Lovett-Doust. 1994. The biology of Canadian weeds. 103. *Vallisneria americana* Michx. *Canadian Journal of Plant Science* 74:883-897.

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

Ferasol, J., L. Lovett-Doust, J. Lovett-Doust, and M. Biernacki. 1995. Seed germination in *Vallisneria americana*: Effects of cold stratification, scarification, seed coat morphology and PCB concentration. *Ecoscience* 2:368-376.

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

Grabe, D. F. 1970. Tetrazolium testing handbook for agricultural seeds. Association of Official Seed Analysts.

Granger, S., M. Traber, S. W. Nixon, and R. Keyes. 2002. *A practical guide for the use of seeds in eelgrass (Zostera marina) restoration. Part 1. Collection, processing, and storage*, ed. M. Schwartz. Narragansett, Rhode Island: Rhode Island Sea Grant.

Kaul, R. B. 1978. Morphology of germination and establishment of aquatic seedlings in Alismataceae and Hydrocharitaceae. *Aquat. Bot*. 5:139-147.

Korschgen, C. E., and W. L. Green. 1988. *American wildcelery (Vallisneria americana): Ecological considerations for restoration*. Report 19. Washington, DC: U.S. Department of the Interior, Fish and Wildlife Service.

Lakon, G. 1949. The topographical tetrazolium method for determining the germinating capacity of seeds. *Plant Physiology* 24:389-394.

Lowden, R. M. 1982. An approach to the taxonomy of *Vallisneria* L. (Hydrocharitaceae). *Aquatic Botany* 13:269-298.

Madsen, J. D., and C. W. Boylen. 1989. Eurasian watermilfoil seed ecology from an oligotrophic and eutrophic lake. *J. Aquat. Plant Manage.* 27:119-121.

McFarland, D.G. 2006. *Reproductive ecology of Vallisneria americana Michaux*. ERDC/TN EL-06-04. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Moore, K. A., R. J. Orth, and J. F. Nowak. 1993. Environmental regulation of seed germination in *Zostera marina* L. (eelgrass) in Chesapeake Bay: Effects of light, oxygen, and sediment burial. *Aquatic Botany* 45(1):79-91.

Moore, K. A., D. L. Wilcox, and R. J. Orth. 2000. Analysis of abundance of submersed aquatic vegetation communities in the Chesapeake Bay. *Estuaries* 23:115-127.

Moore, K. A. B. B. Neikirk, B. A. Anderson, and J. Campbell. 2005. *Water quality conditions and restoration of submerged aquatic vegetation (SAV) in the tidal freshwater James River 2003-2004*. Special Report No. 389 in Applied Marine Science and Ocean Engineering. Gloucester Point, VA: Virginia Institute of Marine Science.

Muenscher, W. C. 1936. *Storage and germination of seeds of aquatic plants*. Bulletin No. 652. Cornell University Agricultural Science Experiment Station.

Orth, R. J., and K. A. Moore. 1983. An unprecedented decline in submerged aquatic vegetation (Chesapeake Bay). *Science* 22:51-53.

Orth, R. J., M. Luckenbach, and K. A. Moore. 1994. Seed dispersal in a marine macrophyte: Implications for colonization and restoration. *Ecology* 75:1927-1939.

Orth, R. J., J. Bieri, J. R. Fishman, M. C. Harwell, S. R. Marion, K. A. Moore, J. F. Nowak, and J. van Montfrans. 2006. A review of techniques using adult plants and seeds to transplant eelgrass (*Zostera marina* L.) in Chesapeake Bay and the Virginia Coastal Bays, In *Proc. Conf. Seagrass Restoration: Success, Failure, and the Costs of Both. March 11, 2003. Sarasota, Florida*, ed. S. F. Treat and R. R. Lewis, 1-17.

Perry, M. C., and A. S. Deller. 1996. Review of factors affecting the distribution and abundance of waterfowl in shallow-water habitats of Chesapeake Bay. *Estuaries* 19:272-278.

Pickerell, C. H., S. Schott, and S. Wyllie-Echeverria. 2005. Buoy-deployed seeding: Demonstration of a new eelgrass (*Zostera marina* L.) planting method. *Ecological Engineering* 25:127-136.

Pickerell, C. H., S. Schott, and S. Wyllie-Echeverria. 2006. *Bouy-deployed seeding: A new low-cost technique for restoration of submerged aquatic vegetation*. ERDC/TN EL-06-02. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Sculthorpe, C. D. 1967. *The biology of aquatic vascular plants*. New York: St. Martin's Press.