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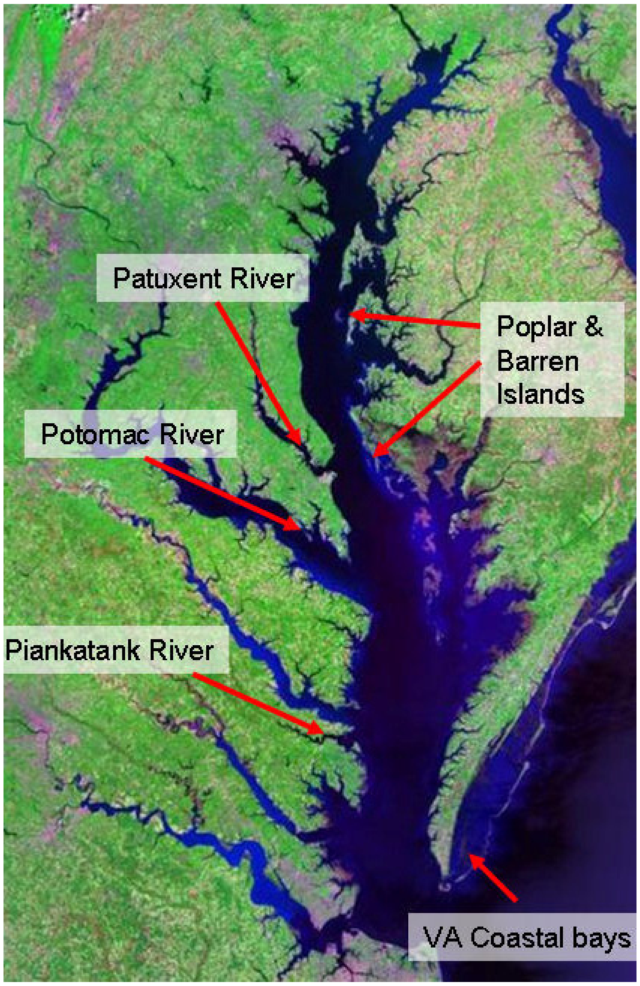
Submerged Aquatic Vegetation Restoration Research Program

Large-Scale Submerged Aquatic Vegetation Restoration in Chesapeake Bay

Status Report, 2003-2006

Deborah J. Shafer and Peter Bergstrom

June 2008



Locations of large-scale SAV seed planting projects in the Chesapeake Bay, 2003-2006.

Environmental Laboratory

Submerged Aquatic Vegetation Restoration
Research Program

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Deborah J. Shafer

Environmental Laboratory
U.S. Army Engineer Research and Development Center
3909 Halls Ferry Road
Vicksburg, MS 39180-6199

Peter Bergstrom

National Oceanic and Atmospheric Administration
Chesapeake Bay Office
410 Severn Avenue
Annapolis, MD 21403- 2524

Final report

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Abstract: In 2003, the U.S. Army Engineer Research and Development Center (ERDC) and the National Oceanic and Atmospheric Administration Chesapeake Bay Office began a comprehensive research effort to restore submerged aquatic vegetation (SAV) in the Chesapeake Bay region. The effort employed an agricultural approach to restore under-water grasses by using seeds to produce new plants and mechanical equipment to plant seeds and harvest. Since this research initiative began, an average of 33 acres/yr of SAV has been planted in the Chesapeake Bay, compared to an average rate of 9 acres/yr during the previous 21 years (1983–2003). New techniques and equipment developed as part of this research have introduced the capability to collect and disperse millions of eelgrass seeds. These results demonstrate these programs' success in developing tools and techniques necessary to plant SAV at scales unattainable with technologies existing only a few years ago.

Contents

Figures and Tables

Figures

Tables

Preface

The work reported herein was performed by the U.S. Army Engineer Research and Development Center (ERDC) and the National Oceanic and Atmospheric Administration (NOAA) Chesapeake Bay Office. This document was prepared by Dr. Deborah J. Shafer, Environmental Laboratory (EL), ERDC, and Dr. Peter Bergstrom, NOAA.

The NOAA Chesapeake Bay Office works to help protect and restore the Chesapeake Bay through its programs in fisheries management, habitat restoration, coastal observations, and education, and represents NOAA in the Chesapeake Bay Program.

The work by Dr. Shafer was performed under the general supervision of Dr. Morris Mauney, Jr., Chief, Wetlands and Coastal Ecology Branch; Dr. David J. Tazik, Chief, Ecosystem Evaluation and Engineering Division; and Dr. Elizabeth C. Fleming, Director, EL.

COL Richard B. Jenkins is Commander and Executive Director of ERDC. Dr. James R. Houston is Director.

Unit Conversion Factors

Multiply	By	To Obtain
acres	4,046.873	square meters
cubic yards	0.7645549	cubic meters
degrees (angle)	0.01745329	radians
degrees Fahrenheit	(F-32)/1.8	degrees Celsius

Executive Summary

In 2003, the U.S. Army Corps of Engineers (USACE) Engineer Research and Development Center (ERDC) and the National Oceanic and Atmospheric Administration (NOAA) Chesapeake Bay Office (NCBO) began to lead a comprehensive submerged aquatic vegetation (SAV) restoration research effort in the Chesapeake Bay region involving numerous Federal, state, local, and private partners and stakeholders. These two Federally funded research programs represent the largest single coordinated research effort to date to develop, evaluate, and refine protocols suitable for large-scale SAV restoration.

An agricultural approach to the restoration of underwater grasses has been employed through the use of seeds to produce new plants and mechanical equipment to harvest and plant seeds. Seeds are typically the most cost effective method for the production of all major domesticated crop plants. Similarly, seeding has the potential to offer the most cost effective approach for restoring large, genetically diverse, self-maintaining populations of underwater grasses. Since this research initiative began, a total of 133 acres of SAV has been planted in the Chesapeake Bay, an average of 33 acres/year. By comparison, during the previous 21 years (1983–2003), approximately 189 acres of SAV were planted, an average rate of 9 acres/year. New techniques and equipment developed as part of this research have introduced the capability to collect and disperse millions of eelgrass seeds (e.g., 10 million in 2004).

These results demonstrate these programs’ success in developing tools and techniques necessary to plant SAV at scales that would have been unattainable with technologies existing only a few years ago. Furthermore, the costs to conduct these plantings are falling as the understanding of the limiting factors is increased and as technology development advances. Although seedling establishments rates were lower than expected, due to the large numbers of seeds distributed, even low rates of initial seedling establishment can result in large numbers of seedlings per acre. Problems seem to lie more with site selection than in planting techniques. Ongoing and future research funded in part through these programs will improve

existing site selection models and contribute to increased success of SAV planting efforts.

Despite the considerable progress that has been made, it is clear that the Chesapeake Bay Program's goal of planting 1,000 acres of SAV by 2008 will not be achieved. Given the current technology, it seems that the targeted SAV restoration acreages established by the Chesapeake Bay Program are unrealistic, and may need to be re-evaluated. Nevertheless, establishing this goal has had a strong positive impact on SAV restoration in the Chesapeake Bay by stimulating the development of innovative new techniques and technologies to advance the capabilities of SAV restoration to heretofore unprecedented levels.

1 Introduction

Background

Submerged aquatic vegetation (SAV) performs many important ecosystem functions, including wave attenuation and sediment stabilization, water quality improvement, primary production, food web support for secondary consumers, and provision of critical nursery and refuge habitat for fisheries species, as well as for the attachment of epiphytic organisms (Fonseca et al. 1998; Orth et al. 2006a). Over the last few decades, there have been global declines in SAV abundance that could have widespread deleterious effects on coastal and estuarine ecosystems (Green and Short 2003; Orth et al. 2006a). Anecdotal information suggests that historically, extensive SAV beds covered the coastal bays and many areas of the lagoons and estuaries within the Chesapeake Bay. In the 1930s, the combined effects of eelgrass wasting disease and a strong hurricane caused unprecedented declines in SAV abundance and distribution throughout the bay, from which some areas have not recovered (Koch and Orth 2003).

SAV is widely recognized as an aquatic habitat vital to the health of the Chesapeake Bay, and its restoration has long been an important goal of the Chesapeake Bay Program and its partners. Recent improvements in water quality offer the potential for restoration of areas that once supported extensive SAV beds; however, natural recolonization in some areas has been limited, either by a lack of propagules, or other environmental factors affecting SAV colonization. In its document, the *Strategy To Accelerate the Protection and Restoration of Submerged Aquatic Vegetation in the Chesapeake Bay* (2003), the Chesapeake Bay Program established a bay-wide goal of 185,000 acres of SAV by 2010 (Figure 1), and identified a variety of actions to increase SAV populations in the Bay. These actions included improving water quality, promoting recolonization, and planting 1000 acres of SAV by December 2008.

Most early SAV restoration efforts used whole plants collected from the wild, rather than seeds or commercially propagated plants. In the Chesapeake Bay, SAV planting efforts began in 1984 with planting whole eelgrass (*Zostera marina*) plants, using sods, cores or bareroot plants (Orth et al. 2006b). Major limitations of using whole plants include availability of suitable donor sites, time and labor needed for harvest and transport, and impacts and recovery rates of donor sites following plant harvest.

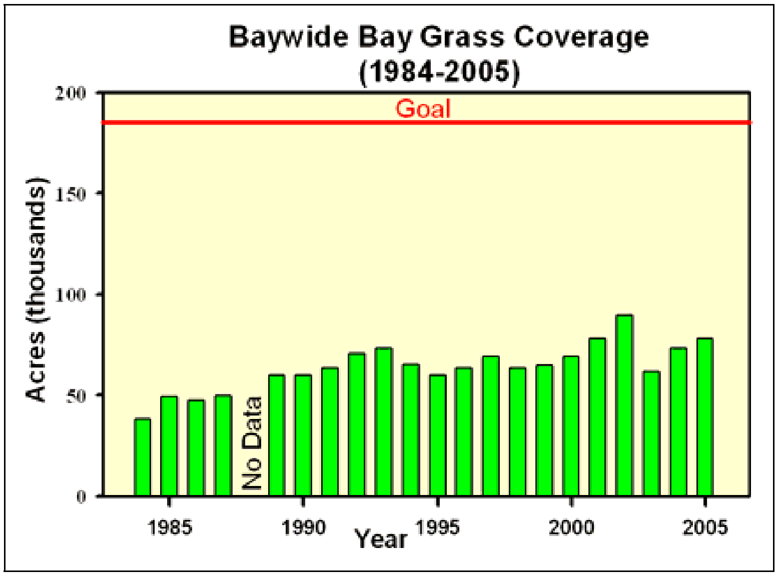

Figure 1. Annual acreage estimates of SAV coverage within the Chesapeake Bay from 1984 to 2005, in comparison to the baywide goal of 185,000 acres established by the Chesapeake Bay Program. (Source: http://www.dnr.state.md.us/bay/sav/coverage.asp).

Seeds offer an efficient and cost effective method for providing new plants for large-scale plantings and hence are used for the production of all major domesticated crop plants. Similarly, seed propagation offers the most cost effective approach for restoring large, genetically diverse, self-maintaining populations of underwater grasses. Seeds are thought to be particularly important in the establishment of new SAV patches far from the parent source, and in recolonization of disturbed areas (Orth et al. 2006d). In the Chesapeake Bay, small patches of eelgrass (*Z. marina*) have been observed at distances of up to 100 km from existing source beds (Harwell and Orth 2002). However, their use in SAV restoration had been limited. Small scale experiments with eelgrass seeds were initiated in the Chesapeake Bay in 1987 (Orth et al. 2006b). The results of early eelgrass seed planting experiments in the Lynnhaven, York, James, Rappahannock, and Piankatank Rivers from 1987–2003 were promising enough to indicate that seeds should be the focus of most large-scale efforts, at least for eelgrass. Of the 70 individual sites planted with eelgrass seeds in those four rivers, 80 percent of the sites had plants survive for 1 year or more; at three sites (4 percent) plants survived for 5 years or more (Orth et al. 2006b).

Although planting methods have improved, SAV restoration remains an extremely labor-intensive and costly endeavor, with a variable track record of success. These concerns, along with high costs and logistical constraints, had limited SAV restoration to small projects, typically on a scale of tens or hundreds of square meters, before 2003 (Fishman et al. 2004; Orth et al. 2006b). Approximately 100,000 additional acres of SAV are needed to reach the ambitious SAV restoration goals established by the Chesapeake Bay Program (Figure 1). While other actions identified by the Chesapeake Bay Program include improvements in water quality intended to promote natural SAV recolonization, the *Strategy To Accelerate the Protection and Restoration of Submerged Aquatic Vegetation in the Chesapeake Bay* (Chesapeake Bay Program 2003) also calls for planting 1,000 acres of SAV by December 2008. It was clear that the attainment of such an ambitious planting goal would require developing new SAV planting tools and techniques to conduct SAV restoration at much larger scales than had been previously attempted. Large SAV beds are also thought to be more stable and resilient to stress than small beds (Wilcox et al. 2000), so large restoration plots may be more successful than smaller ones.

Benefits

The major benefits of large-scale SAV restoration include overall improvements in ecosystem health, higher levels of ecosystem functions, and increased habitat availability for critical fisheries resources. State-of-the-art technical standards and guidance for planning, implementation, and monitoring of SAV restoration projects will provide resource managers with the necessary tools to help meet targeted SAV restoration goals. The research and technology demonstrations accomplished under this program will contribute to improved success rates and predictability for SAV restoration projects, not only in the Chesapeake Bay region, but in other areas that have experienced loss of SAV habitat.

Objective and Scope

The objective of this work is to summarize the status of the comprehensive, multi-level SAV research and restoration effort that began in 2003, led by the U.S. Army Corps of Engineers (USACE) Engineer Research and Development Center (ERDC) and the NOAA Chesapeake Bay Office (NCBO), involving numerous Federal, state, local, and private partners and stakeholders within the Chesapeake Bay region. This was one of the first coordinated efforts to develop, evaluate, and refine protocols suitable for large-scale SAV restoration.

Due to the large numbers of groups involved, this work reports only on the Federal expenditures on this effort by USACE and NOAA. No details are provided on an effort done from 2002–2005 to plant about 20 acres of SAV in the Potomac River as mitigation for SAV beds destroyed by the Woodrow Wilson Bridge replacement, since that effort was not part of this program.

2 Research Program Authorization, Planning, and Implementation

In 2003, the U.S. Army Corps of Engineers (USACE) Engineer Research and Development Center (ERDC) and the NOAA Chesapeake Bay Office (NCBO) began to plan and implement their respective research programs to promote the development of innovative new tools and techniques for the large-scale restoration of SAV throughout the Chesapeake Bay region. The authorization for the Corps of Engineers research initiative was included in fiscal year (FY) 2003 Omnibus Appropriations Bill language for the GI Research and Development funding line item. The language of the bill specifically directed that the USACE was "to conduct investigations, assessment, and demonstrations on large-scale submerged aquatic vegetation restoration techniques and technologies … within the Chesapeake Bay, MD," with $500,000 provided (Senate Report 107–220, p. 24). The authorization for the NCBO research initiative was also in FY03 (Senate Report 107–218): "The Committee recommendation includes $3,500,000 for Chesapeake Bay Studies [at the NOAA Chesapeake Bay Office], of which $500,000 is for sea grass restoration." Table 1 lists annual funding levels for each agencies respective research program during the period from 2003–2006. The USACE SAV Restoration Research Program was led by Dr. Deborah Shafer, Research Marine Biologist, and the NCBO SAV Restoration Research Program was led by Dr. Peter Bergstrom, Fisheries Biologist.

Table 1. Federal funding levels for the USACE and NCBO SAV Restoration Research Programs from 2003-2006.

Fiscal Year	USACE		NCBO	
	Funding Level	No. Projects Funded	Funding Level	No. Projects Funded
FY03	$340,000	3	$550,000	4
FY04	$160,000	3	$810,000	5
FY05	$730,000	7	$678,000	3
FY06	$500,000	6	$380,000	3

USACE SAV Restoration Research Program

An initial organization meeting was held at the Baltimore District Corps of Engineers (COE) offices on 16 April 2003; representatives of Engineering Research and Development Center, Baltimore District COE, NOAA Chesapeake Bay Office, and the Maryland Department of Natural Resources (MDDNR) were present. The purpose of this initial meeting was to provide a forum for open discussion and exchange of ideas on the types of demonstration projects that could be accomplished under the program. Specific objectives of this meeting were to establish and prioritize a list of research needs relating to SAV restoration in the Chesapeake Bay, and suggest several possible locations for demonstration projects to be conducted in the fall of 2003. The group met again in May and June 2003 to refine the coordination of funding from different sources.

Through out this program, insights were solicited from both internal and external stakeholders, including other Federal and state agencies, academia, and non-government organizations that have an interest in Chesapeake Bay habitat restoration. A Cooperative Research Agreement was developed between the U.S. Army Corps of Engineers and Maryland Department of Natural Resources to assist in the execution of demonstration projects and to ensure a source of seed material for future planting efforts.

At the initial organization meeting, six major research topics were identified (in order of priority):

1. Issues related to plant supply and propagation (action 3.2 in CB SAV strategy)
2. Evaluating new equipment and techniques for SAV planting
3. Use of seeds and seedlings as planting material
4. Improving site selection criteria, i.e., habitat requirements other than light (e.g., sediments, wave energy)
5. Seedbank and propagule dynamics
6. Role of interspecific competition in restoration success.

Based on these identified research needs, the Corps of Engineers Submerged Aquatic Vegetation Research Program was divided into two major research focus areas. The first, Innovative Technologies for SAV Production and Planting, involves applied research to support the development of techniques for large-scale production of plants and propagules, including seeds. Research conducted under this focus area addresses topics 1, 2, and 3 above. Due to limited funding, initial efforts were focused on a single SAV species, eelgrass (*Z. marina*). However, with increased funding levels, it became possible to expand the list of species considered to include wild celery (*Vallisneria americana*), redhead grass (*Potamogeton perfoliatus*), sago pondweed (*Stuckenia pectinata*), and widgeongrass (*Ruppia maritima*).

The second major focus area of the research program, Engineered SAV Habitats, involves research to determine wave energy tolerances for mature and developing SAV beds of various species, as well as the design of structures that may facilitate the establishment and restoration of SAV beds. This focus area was developed to address research needs identified in topic number 4 above. External proposals were accepted through the existing Broad Agency Announcement (BAA).

The BAA is a means of soliciting proposals for basic and applied research and may be used by agencies to fulfill their requirements for scientific study and experimentation directed toward advancing the state-of-the-art or increasing knowledge. All proposals received were reviewed and ranked by a panel of internal and external reviewers on the following factors:

1. Relevance to identified research needs
2. Experimental design
3. The offeror's capabilities, related experience, facilities, techniques, or unique combinations of these
4. The qualifications, capabilities, and experience of the proposed principal investigator, team leader, and other key personnel who are critical to the achievement of the proposal objectives
5. Reasonableness of costs
6. Offeror's previous performance history.

Internal proposals were also solicited within the ERDC; internal proposals were also evaluated and ranked based on the criteria described above.

NOAA Chesapeake Bay Office SAV Restoration Program

Dr. Peter Bergstrom, as the NOAA Project Officer, worked closely with the SAV Funding Coordination group described above, other NCBO staff, and the Chesapeake Bay Program's (CBP) SAV Workgroup in the spring of 2003 to develop and revise program priorities, also based on priorities in the CBP's SAV Strategy. Four priority areas, identified based on the CBP SAV Strategy for the 2003 competition, were revised slightly in subsequent years. Not all of these priorities had projects submitted or funded. A new priority, integrated restoration involving SAV near oysters, was added in 2004.

The program priorities published in 2003–2006 were as follows:

1. Large-scale SAV planting
2. Site assessments for future SAV planting
3. Applied research to enhance success of planting SAV from seed
4. SAV propagation
5. Integrated restoration (e.g., restoring SAV with oysters) (added in 2004).

In each year of the competition, the priorities and application instructions were published in the Federal Register, and the applications were each reviewed by three qualified reviewers outside of NCBO with no conflicts of interest. Proposals were reviewed and scored on the strength of the written proposals alone. Scoring was conducted on a scale of 1 - 100 (100 being the best possible score) based on five published criteria:

1. Relevance and applicability of proposal to program goals (30 points)
2. Technical merit (30 points)
3. Overall qualifications of applicants (10 points)
4. Project costs (20 points)
5. Outreach and education (10 points).

These technical evaluations were then reviewed by NCBO staff, and recommendations for funding were made by the Director of NCBO. The projects were funded as Cooperative Agreements that allowed for productive cooperation between the Project Officer and the recipients. The total amount of funding and the number of projects funded varied among years (Table 1).

3 Large-Scale Restoration Planting Projects

Background

Due to the labor-intensive and costly nature of SAV planting projects involving manual transplanting of SAV plants, large-scale SAV restoration required the development of new, more efficient and cost-effective tools and techniques. The large-scale SAV planting projects accomplished by these research programs employed an agricultural approach grasses through the use of seeds to produce new plants and mechanical equipment for seed harvest and planting. Seeds are typically the most cost effective method for providing new plants for large-scale plantings and hence are used for the production of all major domesticated crop plants. Similarly, seeding has the potential to offer the most cost effective approach for restoring large, genetically diverse, self-maintaining populations of underwater grasses. Although the use of seeds in submerged aquatic vegetation restoration has been limited to date, interest is increasing. The harvest and sowing of eelgrass seeds (*Z. marina*) has recently emerged as a viable way to plant and restore large acreages (Granger et al. 2002; Orth et al. 2003; Pickerell et al. 2005), although the use of seeds for eelgrass restoration may not be suitable for all sites.

Although mechanized equipment for farming terrestrial crops has been available for more than a century, the development of mechanized equipment for the harvest and planting of underwater plants remains in its infancy. Nevertheless, employing mechanized equipment for the planting and restoration of SAV beds holds the potential for rapidly and cost-effectively planting larger acreages of SAV than would ever be possible through manual means. Therefore, a major focus of the both research programs has been to develop and test mechanical tools[1] for the planting

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[1] Tests of one type of mechanized planting equipment, the JEB paddlewheel planting boat (Seagrass Recovery, Inc., Ruskin, FL) which plants individual whole shoots, are not reported here because these tests were not funded by either of these research programs. Results of the 2001 trials of this boat in Virginia were reported in Fishman et al. (2004), while results of 2003 tests in Maryland were reported in Bergstrom et al. (2004). Both studies, funded by the NOAA Restoration Center through the Chesapeake Bay Foundation (CBF), concluded that the boat did not offer compelling advantages compared to hand planting, either because many shoots came out of the sediment immediately after planting (Fishman et al. 2004) or the boat was unable to plant shallow enough for the plants to survive in murky water (Bergstrom et al. 2004). Mechanical planting of seagrass sods, with roots, rhizomes and associated sediments have proven to be more successful (Paling et al 2001a, 2001b, Uhrin et al. 2008), however, no tests of this type of equipment were funded under either of these research programs.

of SAV plants and seeds, and to harvest seed-bearing shoots for use in seed-based restoration efforts.

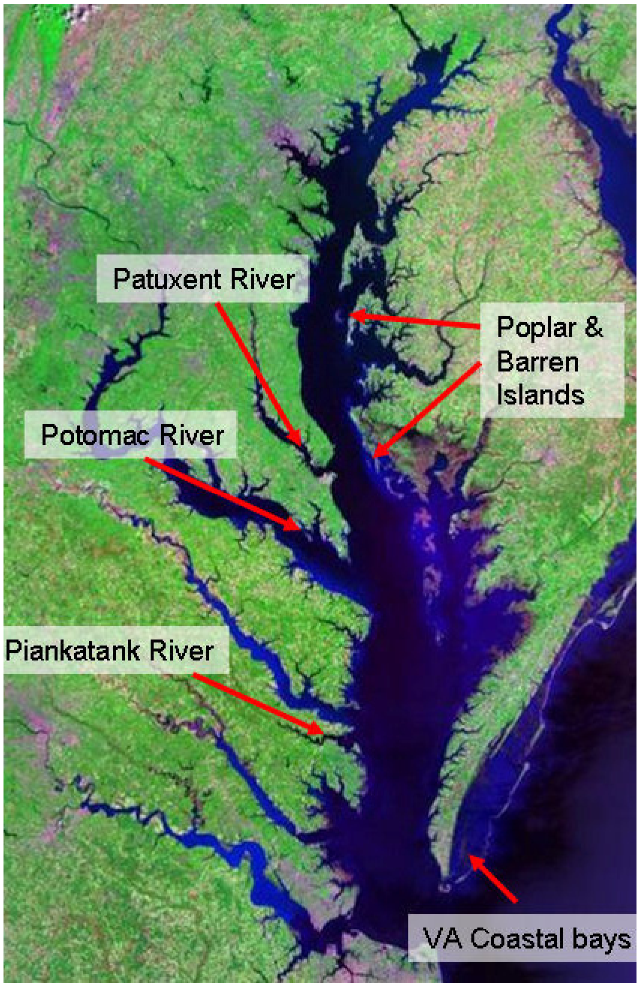
Funded Projects

A number of large-scale SAV restoration projects using seeds were funded beginning in 2003 (Table 2). Most of the emphasis was placed on the polyhaline SAV species *Z. marina*. A total of 101 acres on the Potomac, Patuxent, and Piankatank Rivers (Figure 2) were planted with this species during the period from 2003 to 2006 (Table 2). This report includes the results of these three projects in detail. Considerably less effort was given to other SAV species. In 2004, 12 acres were planted at Poplar Island, MD (Figure 1) with seeds of two mesohaline SAV species, *Potomogeton perfoliatus* and *R. maritima* (Table 2). In 2005, 3 acres were planted at Barren Island (Figure 2) with *R. maritima* seeds (Table 2). One additional project, funded in 2006 (but not planted until 2007), involved seeding two mesohaline SAV species on the Choptank River, MD. The last row in Table 2 lists some projects funded by USACE, NCBO, and other sources; some of these were done opportunistically when extra seeds became available. Thus, those results are not reported here.

Table 2. Large-scale SAV planting projects conducted in the Chesapeake Bay from 2003 to 2006.

Location	Species	Funding Recipient	Funding Duration	No. Acres Planted	Funding Source(s)
Piankatank River, VA	*Z. marina*	VIMS	2003-2005	40	NCBO, KCF
Potomac River, MD	*Z. marina*	MD DNR	2003-2006	37.25	USACE, KCF
Patuxent River, MD	*Z. marina*	MD DNR	2003-2005	23.75	NCBO, KCF
Poplar Island, MD	*P. perfoliatus* *R. maritima*	AACC	2004	12	USACE, MPA
Barren Island, MD	*R. maritima*	AACC	2005	3	USACE
Various	Various	Various	2004-2005	16.5	USACE, NCBO, KCF, NAIB, ACB

Abbreviations: VIMS (Virginia Institute of Marine Science); MD DNR (Maryland Dept. of Natural Resources); UMCES (University of Maryland Center for Environmental Science; KCF (Keith Campbell Foundation); MPA (Maryland Port Authority); NAIB (National Aquarium in Baltimore); ACB (Alliance for the Chesapeake Bay).



SAV zonation within the Chesapeake Bay is typically described as occurring in three zones: the lower bay between the mouth of the bay and Smith Island; the middle bay between Smith Island and Kent Island, and the upper bay between Kent Island and the Susquehanna Flats. These zones roughly correspond with the polyhaline (18–25 psu), mesohaline (5–18 psu), and oligohaline (0.5–5.0 psu) salinity zones, respectively (Orth and Moore 1984). Polyhaline SAV species include eelgrass (*Zostera marina*) and widgeongrass (*Ruppia maritima*), which are the dominant SAV species in the lower bay (Orth and Moore 1984). Mesohaline SAV species include widgeongrass, sago pondweed (*Stukenia pectinata),* redhead grass *(Potamogeton perfoliatus),* and horned pondweed (*Zanichellia palustris).* Oligohaline SAV species include wild *celery (Vallisneria americana),* and the non-native Eurasian watermilfoil *(Myriophyllum spicatum)* and hydrilla (*Hydrilla verticillata*) (Bergstrom et al. 2006).

Figure 2. Locations of large-scale SAV seed planting projects in the Chesapeake Bay, 2003-2006.

Methods

Eelgrass planting projects

Planting locations in Virginia were chosen based on past eelgrass distribution and water depth (Orth et al. 2007d). The sites used in the Piankatank River (Burton Point, Moore's Creek, Healy Creek, Osprey [south of Iron Point, Rte. 3 bridge], Gwynn's Island, and Milford Haven), were all locations of historical eelgrass beds. Water depths at all sites were 1 m or less at Mean Low Water (MLW). In Maryland, planting locations were determined using a Geographic Information System (GIS) based SAV restoration site selection model developed by the MDDNR (Parham and Karrh 1998). Data layers included in the site selection model were shoreline, water quality, bathymetry, historic SAV coverage, and prohibited clam dredging areas (Parham and Karrh 1998).

Two methods of seed collection tested in all three eelgrass seed planting projects were: (1) hand collection by divers, and (2) use of a mechanical harvester. In 2003, divers collected eelgrass reproductive shoots by hand over a 3-week period in April and May. This method proved to be very expensive in terms of work-hours required, so mechanical harvesters were used in subsequent years. In Maryland, a commercial aquatic plant harvester (Figure 3) (M. J. McCook & Associates, La Plata MD) was used in 2004 and 2005. In Virginia, a custom made harvester designed by VIMS (Figure 4), was used beginning in 2005 (Orth and Marion 2007). The design and construction of the VIMS harvester was funded as part of one of the applied research projects described in Chapter 4. Most of the eelgrass seeds were collected in Tangier Sound and nearby rivers in Maryland, and in Mobjack Bay in Virginia.

Two methods tested for dispersing eelgrass seeds in all three projects were: (1) immediate deployment of the reproductive shoots in floating mesh bags (seed buoys) (Figure 5) in areas suitable for planting (Pickerell et al. 2005, 2006), and (2) separation and storage after harvest and broadcast in the fall (Orth and Marion 2007). The utility of using seed buoys containing freshly collected eelgrass reproductive shoots for eelgrass seedling establishment and restoration was first demonstrated in Long Island, NY by Pickerell et al. (2005). The seeds are gradually released in a manner that mimics natural dispersal patterns (Pickerell et al. 2005, 2006).

Figure 3. Commercial aquatic vegetation harvester used for the collection of eelgrass reproductive shoots (photo by VIMS).

Figure 4. Portable boat-mounted aquatic reproductive shoot harvester designed by VIMS scientists (photo by VIMS).

Figure 5. Floating seed buoys filled with eelgrass reproductive shoots (photo by MDDNR).

See Pickerell et al. (2006) for details on the construction and deployment of floating seed buoys. Seed buoys were tested in the Piankatank in 2004 only (Orth et al. 2007d), and were not tested in 2005 because previous results at that location (Table 3) were not promising. The seed buoys were tested in the Patuxent and Potomac Rivers in both 2004 and 2005, but were only used in the Potomac in 2006 (Lewandowski et al. 2006, 2007; Preen et al. 2006).

Eelgrass seed broadcasting has been shown to be effective because seeds are rapidly incorporated into the sediments and generally do not move far from where they settle (Orth et al. 1994). In the fall of 2003 in Maryland (Patuxent and Potomac River sites), and in all 3 years of the Piankatank River planting in Virginia, seeds were broadcast by hand in a series of 25 m radius (0.485 acre) plots (Lewandowski et al. 2006; Preen et al. 2006; Orth et al. 2007c). Within each plot, seeds were distributed in a series of concentric rings in an effort to achieve an even seed distribution.

Table 3. Initial seedling establishment rates for selected SAV plantings in the Chesapeake Bay.

FY	Location/Sources*	Method	No. Sites	No. Acres	No. Seeds/ Acre	Initial Seedling Establishment Mean % (Max %)
Planting Projects Funded by ERDC and NCBO						
2003	Piankatank River, VA[1]	Broadcast	1	4.5	375,000	0 (0)
	Patuxent River, MD[3]	Broadcast	1	3	300,000	0 (0)
	Potomac River, MD[2]	Broadcast	1	3	300,000	0 (0)
2004	Piankatank River, VA[1]	Broadcast	4	5	750,000	0.57 (1.38)
		Seed Buoys	4	20	3,200,000	0.03 (0.06)
	Patuxent River, MD[3]	Broadcast	3	0.75	112,500	0 (0)
		Seed Buoys	4	14.5	1,905,000	0.05 (0.10)
	Potomac River, MD[2]	Broadcast	3	1	300,000	0.06 (0.16)
		Seed Buoys	3	20	2,400,000	0.20 (0.47)
2005	Piankatank River, VA[1]	Broadcast	6	10	500,000	0.75 (3.79)
	Patuxent River, MD[3]	Broadcast	3	5.5	368,500	8.4 (13.2)
	Potomac River, MD[2]	Broadcast	3	2	400,000	0.38 (1.14)
		Seed Buoys	3	10	4,510,000	1.8 (6.22)

* Sources: [1] – Orth et al. 2007c, [2]- Lewandowski et al. 2006, 2007, [3]- Preen et al. 2006 (results through 2005 only).

The hand-broadcasting method proved to be slow, and an even seed distribution was difficult to achieve (Lewandowski et al. 2006; Preen et al. 2006). Beginning in 2004, a specially designed seed sprayer broadcast apparatus developed by MDDNR staff and C&K Lord, Inc. (Figure 6) was used at Patuxent and Potomac sites (Lewandowski et al. 2006; Preen et al. 2006). This mechanical seed sprayer, mounted to a boat, is capable of evenly dispersing seeds at suitable densities (100,000 to 300,000 seeds/ acre) at the rate of 10 min/acre. In the Patuxent, it was used to broadcast seeds in the Fall of 2004 at the Hungerford Creek, Parrans Hollow, and Solomons Island locations and in 2005 at Jefferson Patterson Park, Hungerford Creek, and Myrtle Point locations (Preen et al. 2006). In the Potomac, the seed sprayer was used at Piney Point and St. George Island (Lewandowski et al. 2006).

To use the seed sprayer, the area of bottom to be planted was multiplied by the desired planting density to determine the total number of seeds necessary. The volume of seeds needed to achieve the desired seeding density was determined based on the percent of viable seeds of the total volume.

Figure 6. Mechanical seed sprayer used for eelgrass seed dispersal (photo by MDDNR).

The flow of the seed sprayer mechanism was then calibrated and adjusted to distribute seeds uniformly at the desired density. Seeds were loaded into the seed broadcast machine and expelled into the water column (Lewandowski et al. 2006; Preen et al. 2006; Orth et al. 2007c).

An independently funded mitigation project involving the planting of approximately 22 acres of adult SAV plants (eelgrass, redhead, widgeongrass, and sago pondweed) at two sites on the lower Potomac was done as part of a mitigation package for the impacts of the new Woodrow Wilson Bridge. This project used adult plants and would have presented an opportunity to directly compare the effectiveness of two different approaches to large-scale restoration, planting adult plants vs. seeds. Due to a nearly complete loss of the eelgrass plantings conducted as part of the mitigation project, this objective was not realized. Because the mitigation planting was not funded by or under the control of either ERDC or NCBO, the results are not reported in detail here; some of these details can be found in Lewandowski et al. (2006). A study of the eelgrass transplants on the Virginia side of the river by USGS suggested that low salinity during the high flow years immediately after planting, 2003–2004, was one of the causes of failure of the eelgrass transplants (Schenk and Rybicki 2006).

Mesohaline planting projects

Seeds of two mesohaline SAV species, redhead grass (*P. perfoliatus*) and widgeongrass (*R. maritima*), were planted at Poplar Island in 2004; *R. maritima* seeds were planted at Barren Island, MD in 2005 (S. Ailstock, personal communication). Seed-bearing wrack (floating plant material containing detached stems with seeds) of both species was collected in Marshy Creek near Kent Narrows at the northern end of Eastern Bay in the summer, held in cold storage at 4 °C, then put in floats with wire mesh bottoms at the planting sites in the fall of the same year. Wrack was placed in the baskets where currents would move the seeds to new areas, and extracted seeds and unconfined wrack in the dead end arms that would not be good places for seed dispersal by currents. At Poplar Island, floats, wrack, and seeds were distributed on 23 October 2004, in a total of 12 acres (half in the Notch and half in wetland cell 4DX). At Barren Island, floats containing wrack and seeds were deployed on 21 September 2005 in a 3-acre area in a shallow cove on the west side of the island protected by a new breakwater. The floats with wire mesh bottoms allowed the seeds to settle out as they ripened, similar to the mesh bag technique used for eelgrass (e.g., Pickerell et al. 2005, 2006).

Results and Discussion

Total acres planted

A total of 133 acres of SAV have been planted since the initiation of this research initiative (Table 4), an average of 33 acres/year. By comparison, during the previous 21 years (1983–2003), approximately 189 acres of SAV was planted, for an average annual rate of 9 acres/year (Orth et al. 2006b). These results demonstrate the success in developing tools and techniques necessary to plant SAV at scales that would have been unattainable with technologies existing only a few years ago. Despite the considerable progress made in improving the technology and ability to plant at larger scales, it seems unlikely that the goal of planting 1,000 acres of SAV by 2008 established by the Chesapeake Bay Program will be achieved. However, the establishment of this goal has had a significant impact on SAV restoration in the Chesapeake Bay by stimulating the development of innovative new techniques and technologies to advance the capabilities of SAV restoration to heretofore unprecedented levels.

Table 4. Acres of SAV planted by year, state and funding source in the Chesapeake Bay, 2003-2006.

	State		Funding Source			
FY	MD	VA	NCBO	USACE	Other*	Total
2003	1.0	4.5	5.5	0	0	5.5
2004	37.0	25.7	42.0	20.0	0.7	62.7
2005	41.5	5.0	13.3	27.3	6.0	46.6
2006	6.8	11.5	10.6	7.7	0	18.3
Totals	86.3	46.7	71.3	55.0	6.7	133.0

* "Other" includes Keith Campbell Foundation, National Aquarium in Baltimore, and Alliance for the Chesapeake Bay.

Eelgrass seed collection results

Mechanical harvesters proved to be faster and more cost-effective than hand-collecting eelgrass reproductive shoots, and resulted in minimal damage to the source beds (Orth and Marion 2007). In the spring of 2003, approximately 2.3 million eelgrass seeds were collected by hand by MDDNR staff and volunteers (Lewandowski et al. 2006).

VIMS also collected about 2.5 million seeds by hand with divers in the spring of 2003 (Orth and Marion 2007). Using a commercial mechanical harvester in 2004 approximately doubled the number of seeds collected in both states to 10 million (Orth and Marion 2007). Numbers of seeds harvested in 2005 in either state, when VIMS started using a mechanical harvester of its own design in Virginia waters, were not available (Lewandowski et al. 2006; Preen et al. 2006; Orth and Marion 2007). The work-hours expended each year on seed collection were also not available. There is also the potential for qualitative differences in the seed material collected by hand versus machines, however no tests were conducted to examine this possibility. Because the machine is non-selective, it is likely that a larger proportion of the seeds collected are immature, and incapable of germination, whereas divers may be able to visually select more mature reproductive shoots for harvest.

Although effective, water depth proved to be a limiting factor for the commercial vegetation harvester, which was designed to harvest nuisance plants and could not always operate at high tide if the eelgrass reproductive shoots were short. The commercial harvester was also expensive and cumbersome to transport from one location to the next. This led to the development of a more portable, adjustable system with improved operational characteristics better suited to the harvest of eelgrass reproductive shoots. This system (Figure 3) has been used in Virginia by VIMS since 2005, but has not yet been tested for use in the collection of seed-bearing plant material of other submerged aquatic grass species.

Eelgrass seed processing and storage

The ability to mechanically harvest huge volumes of plant material poses new logistical problems. One of the biggest challenges to seed planting success involves the ability to process, and store large volumes of seeds under conditions that maintain seed viability and prevent premature germination. In some cases, unknown factors have resulted in large seed losses (Orth and Marion 2007). This proved to be particularly problematic with the *Z. marina* seeds collected and stored at the Piney Point facility and subsequently planted at the Potomac and Patuxent River sites. In 2003, 11 percent of the seeds collected were viable at planting, while in 2004, only about 7 percent of those seeds remained viable at planting (93 percent mortality) (Lewandowski et al. 2006). Changes in storage methods were made at Piney Point to more closely resemble the methods used in Virginia; this increased the survival of seeds at Piney Point to 20 percent in 2005 (Preen et al. 2006; Table 5). In 2006, the survival of stored seeds in Maryland increased to 87 percent (Lewandowski et al. 2007). This emphasizes the need for a greater understanding of the conditions under which seeds may be safely collected and stored, a topic that was addressed by a number of the applied research projects described in Chapter 4.

Seed dispersal

From 2003 to 2006, approximately sixteen million eelgrass (*Z. marina*) seeds were dispersed at three sites on the Piankatank, Potomac, and Patuxent Rivers (Table 5). A total of 101 acres was planted at densities ranging from 40,000 to 1,050,000 seeds/acre (Table 5). More than 130,000 widgeongrass (*R. maritima*) seeds were dispersed across an area approximately 3 acres in size at Barren Island, MD (Table 5). More than one million seeds of widgeongrass (*R. maritima*) and redhead grass (*P. perfoliatus*) (522,720 seeds of each species) were dispersed across an area 12 acres in size at Poplar Island, MD (Table 5).

Table 5. Total numbers of SAV seeds planted at each of the large-scale planting projects during the period from 2003 to 2006.

Site/Sources*	Method	No. Seeds	No. Acres	Density (seeds/acre)
Polyhaline SAV Species (*Z. marina*)				
Piankatank River, VA[1]	Spring Seed Bags	3,200,000	20	150,000
	Fall Seed Broadcasting	1,625,000	19.5	40,000-100,000
Potomac River, MD[2]	Spring Seed Bags	7,035,500	31.45	110,000-800,000
	Fall Seed Broadcasting	1,224,000	6.25	100,000- 1,050,000
Patuxent River, MD[3]	Spring Seed Bags	1,905,000	14.5	121,000-245,000
	Fall Seed Broadcasting	781,000	9.25	67,000-150,000
Mesohaline SAV Species (*P. perfoliatus* and *R. maritima*)				
Poplar Island, MD[4]	Mesh Floats	1,045,440 (522,720 of each species)	12	43,560 for each species
Barren Island, MD[4]	Mesh Floats	130,680	3	43,560
* Sources: [1] – Orth et al. 2007c, [2]- Lewandowski et al. 2006, 2007, [3]- Preen et al. 2006 (results through 2005 only), [4] - S. Ailstock, personal communication.				

A total of 36 acres were seeded using the broadcast method during the period from 2003 to 2006 (Table 5). Seed buoys were used to seed 66 acres with eelgrass seeds in the Chesapeake Bay during the period from 2004 to 2006 (Table 5). A similar method was used to seed approximately 15 acres with *R. maritima* and *P. perfoliatus* seeds. Immediate deployment of the seed buoys eliminates the need to store seeds during the summer, which reduces the number of seeds lost, and decreases the labor and expense involved in seed transport, processing, and storage (Lewandowski et al. 2006). However, experience with large-scale deployments (>1000 units) revealed a number of logistical constraints (Lewandowski et al. 2006; Orth et al. 2007b). These included: (1) obtaining permits to deploy floating bags in navigable waters, (2) the handling of significant volumes of plant material, numbers of individual units, and the weight of the holding blocks, and (3) the physical removal of the units, made more difficult by accumulation of macroalgae on the bags.

Plant establishment and survival

Eelgrass seedling establishment rates were very low at all sites. Averaged by river, the rates were less than 1 percent for all rivers and years, although some individual sites in the Piankatank had survival rates up to 3.8 percent from fall broadcast seeding (Table 3). However, due to the large numbers of seeds distributed, even low rates of initial seedling establishment can result in large numbers of seedlings. For example, in the Piankatank River, seeds were broadcast at an average density of 750,000 seeds/acre (Table 3). An average seedling establishment rate of 0.57 percent would yield more than 4,200 seedlings/acre. Earlier fall broadcast tests in the Chesapeake Bay, and tests of seed buoys in other estuaries, had still higher establishment rates (Table 3). Estimates of eelgrass seedling establishment rates under ambient conditions in the field are rare, but limited data (Harrison 1993) suggest that they are also low (approximately 10 percent). Therefore, the rates of initial seedling establishment observed for artificially distributed seeds in the Chesapeake Bay may be within the range of natural seedling establishment rates.

For the three projects described here, most of the eelgrass seedlings that resulted from either planting method did not survive the following year (Lewandowski et al. 2006, 2007; Preen et al. 2006). The combination of high water temperatures (>30 °C), high light attenuation by epiphytes, and low ambient light levels, in the summers of 2004 and 2005 were probably responsible for the loss of most eelgrass seedlings and adult plants. However, at some sites, notably the Cherryfield Point and St. George Island sites on the Potomac River in Maryland, eelgrass seedlings recruited from 2004 and 2005 plantings have persisted for multiple years, albeit at very low densities, and were still present in October of 2007 (L. Karrh, personal communication).

Seedling establishment rates of mesohaline SAV species at Poplar and Barren islands could not be determined, but were probably also very low (S. Ailstock, personal communication). No seedlings were found on a 2005 return visit to the 12 acres that were planted with seeds at Poplar Island in October 2004. There was also little suitable shallow water habitat in the area planted, especially in Cell 4DX. Seeds from the same batch germinated in the lab. At Barren Island, where 3 acres were planted on 21 September 2005, no plants could be found on a return visit about 10 months after planting, on 17 July 2006. During that time, the bottom sediments changed from firm to very soft, so those new sediments may have buried any plants that grew from seeds there.

Although the survival of these large-scale *Z. marina* plantings from seeds in the Chesapeake Bay was low, the same methods have been employed with greater success in Virginia's southern coastal bays, mainly in South

Bay near Wreck Island (Orth et al. 2006c). This project was mainly funded through the Virginia Coastal Zone Program's Seaside Heritage Program. The maximum eelgrass seed germination rate in South Bay was 5-10 percent versus <1 percent in the Chesapeake Bay (for more information, see URL: http://www.deq.virginia.gov/coastal/vshp/homepage.html).

An area in South Bay approximately 21 ha in size that was planted with eelgrass seeds from 2001–2004 had developed 38 percent eelgrass cover visible in aerial photographs taken in 2004, not counting new eelgrass plants growing outside the original plots that were planted (Orth et al. 2006b). By contrast, none of the recent Chesapeake eelgrass plantings have produced beds dense enough to be seen in aerial photographs. The higher success rates in Virginia's coastal bays show that these large-scale *Z. marina* seed harvest and distribution methods can work in some locations. Ongoing research is trying to determine why there was better success in the coastal bays.

The better survival rates of eelgrass planted from seed in the Virginia coastal bays illustrates the general pattern, that SAV planting success rates are usually site-specific, for reasons not entirely understood. This pattern is shown clearly in a summary of survival rates from small scale eelgrass planting done by VIMS at 230 sites over 1983–2003 (see Table 1 in Orth et al. 2006b). While about half of the small scale planting sites had survival less than 1 year, about 16 percent had survival for 5 years or more, including some surviving over 10 years. Several of the small and large-scale eelgrass planting sites still had plants present in 2007: 3 sites on the James River, East River (Mobjack Bay), Little Creek (Lynnhaven River), and all the Virginia coastal Bay sites (R. Orth, personal communication).

Small-scale planting projects involving mesohaline SAV in the Chesapeake Bay have also had mixed and site-specific success, with many sites surviving less than 1 year, some lasting for 1–3 years, and a few sites lasting longer (P. Bergstrom, unpublished data). The longest known success of a mesohaline planting project is up to 8 years, from a planting of wild celery and redhead grass in Shallow Creek at the mouth of the Patapsco River done from 1999–2003. The project and its estimated costs were described in Bergstrom (2006), and the beds that resulted have been mapped in the VIMS aerial survey since 2004 as they expanded from 0.01 ha at planting to cover 0.85 ha in 2006 (Orth et al. 2007c) and probably a larger area in 2007, based on ground surveys (P. Bergstrom, unpublished data).

The higher germination and survival rates of eelgrass planted from seed in the Virginia coastal bays, using very similar planting methods and extensive site selection, suggests that the site selection criteria used in the selection of planting sites in the Chesapeake Bay are either too lax (suggesting that SAV was planted where it could not survive) or incomplete. For example, wave exposure and sediment quality are not currently included in site selection process. It is important to note that the current SAV habitat requirements developed in 1992 and modified in 2000 (Batiuk et al. 1992; Dennison et al. 1993; Kemp et al. 2004) were developed primarily for established populations of plants and highlighted five key water quality factors: light, turbidity, chlorophyll a, nitrogen, and phosphorus. Established plants have developed into beds with altered sediment characteristics (e.g., higher percentages of silt and clay, and organic content) and biogeochemistry (e.g., nutrient recycling), and they can increase water clarity in a relatively short time due to the baffling effects of the leaves (Moore 2004). Established plants might therefore be expected to have requirements for persistence that would be less stringent than those required for establishment, especially in areas that have been un-vegetated for decades and where sediment properties may be very different. Establishing new beds either from seeds or transplants is likely to require water quality conditions that exceed those of existing established beds (Fonseca et al. 1998). Recent data suggest that eelgrass seedlings may also have a lower lethal temperature tolerance than older, established plants (Orth et al. 2007a; R. Orth, unpublished data).

In addition, other habitat characteristics can influence plant establishment that had not been considered earlier, e.g., wave exposure, sediment mobility, temperature, and salinity (Koch 2001). To become more accurate predictors of planting success, SAV habitat requirements may need to be refined to account for short-term, episodic events. They may need to include more factors such as wave exposure, sediment quality, and temperature, and they may need to be made more stringent (requiring greater water clarity to consider an area suitable for SAV planting). Interannual variability in climate and water quality conditions also plays a critical role in initial establishment and survival of planted SAV; thus, planting efforts may need to be repeated over multiple years to achieve success.

Cost estimates

Cost estimates for the various methods and locations are shown in Table 6. These costs include normal, recurring costs associated with seed collection, processing, storage, and dispersal, such as staff labor, utilities, boat support, and expendable supplies. These costs do not include the additional costs required for one-time capital purchases of major equipment, such as tanks, pumps, chillers, etc. Seed germination rates and subsequent survival are also not considered. Note that the costs for all methods and locations declined from 2004 to 2005. Since these costs are expressed as costs per viable seed, this decrease is due largely to improved storage and processing techniques that resulted in increased seed viability in 2005.

Table 6. Cost estimates* for various methods of SAV planting in the Chesapeake Bay, 2003-2005.

Site/Sources**	Method	Cost per Seed or Adult Plant	Cost per Acre Planted	Density (units/acre)
Piankatank River, VA[1]	Spring Seed Bags	\$0.0034	\$680	200,000
	Fall Seed Broadcasting	\$0.0025	\$500	200,000
Potomac River, MD[2]	Spring Seed Bags	2004-\$0.022 2005-\$0.007	2004-\$4,473 2005-\$1,468	200,000
	Fall Seed Broadcasting	2004-\$0.335 2005-\$0.027	2004-\$67,085 2005-\$ 5,489	200,000
Patuxent River, MD[3]	Spring Seed Bags	2004-\$0.022 (none 2005)	2004-\$4,473 (none 2005)	200,000
	Fall Seed Broadcasting	2004-\$0.335 2005-\$0.028	2004-\$67,085 2005-\$ 5,533	200,000
Potomac River, MD[2]	Hand planting adult plants in pairs	\$2.35	\$25,592	10,890
Shallow Creek, Patapsco River, MD[4]	Hand planting adult plants singly	\$4.50-6.50	\$33,750 - \$99,000	7,500-15,000

* Includes normal, recurring costs associated with seed collection, processing, storage, and dispersal, such as staff labor, utilities (MD only), boat support, and expendable supplies. These costs do not include additional costs required for one-time capital purchases of major equipment, such as tanks, pumps, chillers, etc. Both states used actual labor costs, which varied by state.

**Sources: [1] – Orth et al. 2007c, [2] – Lewandowski et al. 2007, [3] – Preen et al. 2006, [4] – Bergstrom 2006

The cost effectiveness of using seeds for SAV restoration, especially the spring seed bags, compared to fall seed broadcasting and transplanting adult plants, is readily apparent (Table 6). Deployment of seed bags containing freshly collected reproductive shoots in spring appears to be the least expensive option for seeding since this avoids the need for expensive processing and storage, and the potential for significant seed mortality in storage. This approach lends itself well to situations where volunteers can be used to perform many of these tasks (Pickerell et al. 2005, 2006).

Fall seed broadcasting was 17 times more expensive than seed bag deployment (Table 6). Additional limitations of fall seed broadcasting include the necessity for highly specialized infrastructure to process and store seed material at the appropriate temperature and salinity, and the associated costs of maintaining these facilities (Lewandowski et al. 2007). Although the costs per seed for fall seed broadcasting were higher in some cases, preliminary results suggest that seedling establishment rates may be higher than those of the seed bag method. In 2004, seeds broadcast by hand in the Piankatank had a much higher spring 2005 establishment rate (0.57 percent) than those seeds released from the buoy deployed bags (0.03 percent, Orth et al. 2007c). This may be due to the fact that seeds dispersed from seed bags deployed in the spring are exposed to field based mortality factors for 5 months longer than seeds broadcast in the fall (Orth et al. 2007b).

Compared to the seed methods, hand planting of adult plants had much higher costs per unit (since the units are much larger), but costs per acre that are comparable to the costs of fall seed broadcast. This assumes a fairly low planting density for the whole plants; 7500/acre is roughly one shoot every 2 feet, and survival might be higher with denser planting. Costs for planting whole plants would be much higher if the number of shoots per acre were increased in an effort to increase survival.

4 Applied Research To Increase the Success of Large-Scale Planting

Background

The development and testing of many of the innovative new techniques and tools used in the large-scale planting projects described in Chapter 3 was done as part of the applied research funded by both the USACE and NCBO. The applied research covered a range of topics, all related to improving the success and cost-effectiveness of SAV restoration in the Chesapeake Bay. Many involved finding the best methods for harvest, processing, storage, and planting of seeds and other propagules (Table 7). Due to the limited use of seeds and vegetative propagules for SAV restoration before this research program began, no suitable protocols for the collection, processing, storage, and subsequent distribution of large numbers of SAV seeds and vegetative propagules existed.

Table 7. Applied SAV research projects involving seeds and other propagules in the Chesapeake Bay, 2003-2006.

Title	Species	Funding Recipient	Funding Duration	Funding Source(s)
Restoration of Two Mesohaline Submerged Aquatic Plant Species By Seed In the Mid-Chesapeake Bay	*P. perfoliatus* *R. maritima*	AACC*	2003-2006	USACE
Reproductive Success of Seed Yields from Natural and Cultured SAV Populations	*P. perfoliatus* *R. maritima* *S. pectinata*	CBEC & AACC	2003-2004	NCBO
Production and Planting of Vegetative Propagules for Restoration of Redhead Grass and Sago Pondweed	*P. perfoliatus* *S. pectinata*	UMCES	2005-2006	USACE
Use of Seeds in the Large-Scale Propagation and Restoration of *Vallisneria aAmericana*	*V. americana*	VIMS	2005-2006	USACE
Emerging Issues in the Restoration of Eelgrass with Seeds in the Chesapeake Bay	*Z. marina*	VIMS	2003-2006	NCBO
Achieving Critical Thresholds for Large-Scale SAV Restoration	*Z. marina*	VIMS	2005-2006	USACE
Comparative Test of Mechanized and Manual Planting of Eelgrass Seeds	*Z. marina*	VIMS	2005	NCBO
Restoring Eelgrass from Seed: A Comparison of Planting Methods	*Z. marina*	VIMS	2005-2006	USACE

* AACC (Anne Arundel Community College); CBEC (Chesapeake Bay Environmental Center); UMCES (University of Maryland Center for Environmental Science); VIMS (Virginia Institute of Marine Science).

Use of Seeds and Other Propagules in SAV Restoration

This section summarizes the currently available information on collection, processing, storage, and planting of SAV seeds and other propagules for use in restoration projects.

Propagule collection

In most cases, seeds must be collected from wild plants, and suitable seed collection sites need to be identified well in advance. Once plants have begun to flower, weekly assessments of multiple sites may be required to evaluate flower development and determine the optimum time for seed collection to ensure the highest numbers of mature seeds per unit effort (Ailstock and Shafer 2004, 2006; Orth et al. 2007b). In the Chesapeake Bay, eelgrass (*Z. marina*) begins to flower during late winter and early spring; mature seeds are released from mid-May to early June (Silberhorn et al. 1983). In freshwater and brackish regions of the Chesapeake Bay, *V. americana* typically flowers from July to August; seed pods mature by September to October (Moore and Jarvis 2007). Some SAV species, such as *P. perfoliatus*, may exhibit two distinct flowering cycles, one early in the summer and the other toward the middle to end of the seasonal growth cycle (Titus and Hoover 1991; Spencer and Ksander 1996; Ailstock and Shafer 2004).

Seed production can be extremely variable, both temporally and spatially (Silberhorn et al. 1983; Orth and Moore 1986). Typically, the optimum collection window may last only 1–2 weeks at any individual location. Within a region, the stage of reproductive development may vary up to several weeks among different sites. Cooler than normal water temperatures during the spring may delay the development of mature seeds for up to several weeks. Conversely, above normal water temperatures during the spring may accelerate seed development and release (Orth et al. 2007b). Collecting throughout the reproductive cycle optimizes diversity and ensures that phenotypes that mature both early and late are captured in the collection process. While this will reduce seed harvest efficiency, the presence of a diversity of phenotypes may be important for improving plant establishment under variable environmental conditions (Ailstock and Shafer 2006).

Depending on the quantity of seeds needed and the morphology of the reproductive shoots, seed-bearing shoots may either be harvested by hand, or mechanically. Harvesting seed-bearing shoots by hand is labor intensive and limited by weather and water quality conditions. In some situations, hand harvesting may require the use of self-contained underwater breathing apparatus (SCUBA). However, if storage space is limited, hand harvesting may be the best option for producing the largest number of seeds (Orth et al. 2007b).

There are two options for hand collection of seeds. The first uses seeds retained in detached stems that accumulate as wrack along shorelines following anthesis (Figure 7) (Ailstock and Shafer 2004, 2006). While this method offers the advantage of a seed source that would otherwise be unavailable for natural reproduction, there are also disadvantages. Seed yields from wrack are lower than those of material harvested during active growth since many seeds from the wrack have already been dispersed. A second disadvantage is that the availability of wrack is heavily influenced by weather. Sites along the windward facing shorelines of protected coves can yield abundant wrack in some years; the same location may yield little if any wrack when storms disperse the broken stems to open water environments (Ailstock and Shafer 2006).

Figure 7. Hand-collecting SAV seeds from wrack (photo by S. Ailstock).

Figure 8. Seed-bearing shoots harvested directly from plants and placed in mesh baskets (photo by S. Ailstock).

A more reliable method for hand-harvesting seeds is to collect reproductive shoots directly from actively growing beds before stem detachment (Figure 8). For *R. maritima* and *P. perfoliatus*, this technique leaves the majority of the photosynthetic stems and many reproductive stems intact, thereby minimizing potential adverse effects on the population (Ailstock and Shafer 2006). Up to several hundred *V. americana* seed pods per hour can be harvested by simply wading through a bed at lower tidal levels with an attached floating mesh bag tied to a string or small rope (Moore and Jarvis 2007).

A variety of mechanical cutter/seed harvesters designs (described in Chapter 3) have been successfully used to collect seed-bearing shoots of *Z. marina* with minimal damage to the source beds (Orth and Marion 2007). In 2004, a commercially available vegetation cutter designed for nuisance aquatic vegetation was tested, but it was found to be too cumbersome and inflexible for ideal use in *Zostera* beds, especially in deeper beds when water levels are high. A series of new designs developed and tested at VIMS have improved the operational characteristics and capacity of the equipment (Orth and Marion 2007). Using this equipment, it is now possible to collect millions of eelgrass seeds (e.g., 10 million in 2004).

The collection and distribution of over-wintering propagules (e.g., buds and tubers) can provide an alternative to seeds for restoration of some mesohaline SAV species, such as *P. perfoliatus* and *Stuckenia pectinata*. For *P. perfoliatus*, winter bud formation occurs in the fall and early winter months, with peak numbers produced during the period from October through December (Murray et al. 2007). In contrast to *P. perfoliatus* buds, *S. pectinata* tubers are produced throughout the year under natural conditions, with peak numbers in June and October.

Once collected, seed-bearing plant material must be transported to the processing and storage facility. Small volumes of plant material can be transported using portable coolers filled with ambient seawater (Moore and Jarvis 2007) or large plastic baskets (Ailstock and Shafer 2006). Transporting large quantities of heavy, wet plant material poses a number of logistical challenges that must be overcome, including preventing seed desiccation, and preventing the composting that could generate heat levels sufficient to cause seed embryo mortality during transport.

Seed processing

Reproductive structures in the collected plant material occur in various stages of development ranging from immature flowers to seed stalks from which mature seeds have already detached. Seed processing (Figure 9) is a method of isolating the mature seeds from the stems and other less developed reproductive structures (Benech-Arnold and Sanchez 2004; Raghavan 2000).

Figure 9. Seed processing equipment; large-scale outdoor seed processing tanks for *Z. marina* (left, photo by VIMS); wringer washing machine provides agitation to facilitate seed separation (right, photo by S. Ailstock).

Immediately following collection, seed-bearing plant material is typically placed in large aquaculture tanks, plastic swimming pools, or similar containers (Figure 9, left) and held for some period of time to allow the seeds to be released (Ailstock and Shafer 2006; Orth and Marion 2007). Conditions in the holding tanks have to be monitored for appropriate salinity, water turnover, and temperature (Orth and Marion 2007). The duration of seed release is a function of the stage of development at which seeds were harvested, water temperature, and the amount of material in the storage tanks (Orth and Marion 2007). Agitation may be used to accelerate the seed release process (Figure 8, right) (Ailstock and Shafer 2006; Orth and Marion 2007). For some species, such as *R. maritima* and *P. perfoliatus*, seed release may be essentially complete within 4 to 5 days (Ailstock and Shafer 2006). For *Z. marina*, this process can take from 4 to 6 weeks following collection to complete (Orth and Marion 2007). After all seeds have been released, seeds will need to be separated from the decaying non-reproductive plant material. Traditional labor-intensive techniques involve the manual removal of decomposing plant matter from the tanks and sieving to separate seeds from other plant material (Ailstock and Shafer 2006; Orth and Marion 2007). For larger volumes of material, separation has been accomplished using a system that combines a diaphragm pump capable of moving slurries of solids with passive water flow (Orth and Marion 2007).

Propagule storage

If seeds are not to be dispersed immediately (e.g., Pickerell et al. 2005, 2006), they must be stored under conditions that maintain viability. Before the start of these research programs, little information existed in the literature on the specific conditions for SAV seed storage. For *Z. marina*, large variations in the survival rates of stored seeds have been noted. In some cases, high rates of seed mortality have occurred during storage, but factors responsible for seed death were not well understood (Orth and Marion 2007). Consequently, determining the optimum conditions required to maintain seed viability in storage has been a critical component of many of the applied research projects funded under these programs.

Figure 10. Eelgrass seed storage facilities at VIMS (photo by VIMS).

Two methods are currently used to store seeds for restoration projects involving all SAV species. The first involves short-term storage and incubation of the seeds in flow-through aquaculture tanks to facilitate ripening (as described above) and subsequent distribution at times known to be conducive to seed germination and seedling establishment (Figure 10) (Orth and Marion 2007). Long-term storage would provide the opportunity to have seeds available whenever they are needed. Factors regulating seed viability in storage include temperature, dissolved oxygen and salinity. The length of time that seeds may be safely stored without significant reductions in viability varies, depending on storage conditions and SAV species (Table 8).

Table 8. Storage conditions for propagules of selected Chesapeake Bay aquatic macrophytes.

Species	Propagule	Storage Conditions	Sources
V. americana	Seeds	71% of seeds stored at ambient temperatures germinated within 2 months in a greenhouse at temperatures of 18-21 °C during the day and 13-16 °C at night. Pre-chilling enhances germination, but is not an absolute requirement. Percent germination was 76%, 87%, and 82% for seeds cold stored at 1-3 °C in the dark for 2, 5, and 7 months, respectively. A few viable seeds have remained even after storage for 2-3 years in the dark at 3-4 ºC.	Muenscher 1936, Baskin and Baskin 1998, Campbell 2005, McFarland 2006, Moore and Jarvis 2007
Z. marina	Seeds	To maximize survival of seeds during storage, a) remove as much organic material as possible; b) store seeds in re-circulating water tanks at a salinity of 20-30 PSU; c) maintain temperatures below 24° C; d) avoid a thick layer of seeds (> 3-5 cm) that might promote very low oxygen levels near the bottom; e) aerate the tanks without disturbing the seed layer; f) use shade-cloth over the tanks to reduce algal growth; and g) use a UV sterilizer to prevent growth of microorganisms.	Orth and Marion 2007
R. maritima	Seeds	Cold storage improved germination at all salinities tested, but also induced premature germination during storage	Ailstock and Shafer 2006
P. perfoliatus	Seeds	Seeds must be stored under cold conditions to remain viable in storage. Longer cold storage improved germination without affecting viability. Aeration during storage was also important for retaining viability.	Ailstock and Shafer 2006
P. perfoliatus	Buds	*P. perfoliatus* over-wintering buds require an extended cold (4° C) period in order for effective germination and subsequent growth to occur. Buds can be stored in the cold and dark for up to 8 wks with little or no decrease in the rate of germination or of subsequent plant growth.	Murray et al. (in prep.)
S. pectinata	Tubers	Stored in the cold (4° C) and dark for up to 8 wks with little or no decrease in the rate of germination or of subsequent plant growth. When stored for 12 wks, *S. pectinata* germination rate decreased by 50%; longer storage resulted in little or no germination.	Murray et al. (in prep.)

Propagule germination

Figure 11. Germinating redhead grass (*P. perfoliatus*) seeds (photo by S. Ailstock).

Factors affecting SAV propagule germination (Figure 11) include temperature, salinity, light, burial depth, and redox status (Table 8) (Orth and Moore 1983; Moore et al. 1993; Campbell 2005; Ailstock and Shafer 2006; McFarland 2006; Moore and Jarvis 2007).

In the Chesapeake Bay, eelgrass (*Z. marina*) seeds begin to germinate in the fall, and germination continues through spring (Orth and Moore 1983). During the summer months, seed dormancy is thought to be induced by high water temperatures (Orth and Moore 1983; Moore et al. 1993). Shallow burial of seeds into the sediments (up to 25mm) appears to be essential for germination (Moore et al. 1993). Although the results of some studies indicate that low salinities may stimulate *Z. marina* seed germination (Churchhill 1983; Hootsmans et al. 1987), in the Chesapeake Bay, salinity is thought to play a minor role in eelgrass germination in the field (Orth and Moore 1983; Moore et al. 1993). The longevity of seeds in the natural seedbank appears to be relatively short (1 year) (Moore et al. 1993).

In contrast to *Z. marina*, which requires cool water temperatures for seed germination, the highest rates of germination of seeds of the freshwater to brackish SAV species, *V. americana* were observed at temperatures of at 22–29 °C (Table 7) (Campbell 2005; Moore and Jarvis 2007). Germination of *V. americana* is also significantly increased in sandy substrates, at low salinities (<5 psu), low organic matter content (<3 percent), and shallow burial depths (<15 mm) (Campbell 2005; Moore and Jarvis 2007).

R. maritima is capable of germinating under a wide range of salinities (Ailstock and Shafer 2006), as might be expected for such a euryhaline species (Kantrud 1991). However, the highest germination rate was achieved when seeds stored at 21 °C at 15 ppt were placed in fresh water. Cold storage improved germination at all salinities tested, but also induced premature germination during storage (Ailstock and Shafer 2006). This premature germination reduces effective seed yield for restoration since these seeds are lost for plantings. Future efforts are focusing on identification of a more precise set of storage conditions that will minimize premature germination without significant loss in germination potential.

For some mesohaline SAV species, such as *R. maritima* and *P. perfoliatus*, preliminary data suggests that salinity may play an important role in regulating the time of seed germination to coincide with low salinities (Table 9). Rapid reductions in salinity generally occur following severe storms with heavy precipitation, conditions that may cause stress of established plants (Kantrud 1991). Thus, some mesohaline SAV species may use salinity cues to promote seed germination at times when mature plants are stressed, a mechanism that minimizes competition between generations of plants by providing an abundance of replacement plants at times when suitable habitats are most abundant. Thus, under normal salinity conditions, a small proportion of the seeds may exhibit slow, continuous germination, while most will remain dormant until an event that lowers salinity and favors seedling germination (Ailstock and Shafer 2006).

Table 9. Germination conditions for propagules of selected Chesapeake Bay aquatic macrophytes.

Species	Propagule	Germination Conditions	Sources
V. americana	Seeds	Cold storage is not necessary for seed germination. Warmer temperatures can significantly reduce germination time. Germination time for seeds held in cold storage at 4 °C decreased from 12 days at 22 °C to 6 days at 29 °C, along with a corresponding increase in % germination from ~ 70% to 90%. Germination is also significantly increased in sandy substrates at salinities <5 psu, organic matter content < 3%, and burial depths <15 mm. Under field conditions germination will not begin in this region until spring when water temperatures exceed 13 °C.	Muenscher 1936, Ferasol et al. 1995, Campbell 2005, McFarland 2006, Moore and Jarvis 2007
Z. marina	Seeds	In the field, germination occurs during the cooler months (fall through spring). The highest percentage of seeds germinated at water temperatures ranging from 0-10 °C. High water temperatures (> 20 °C) induce seed dormancy.	Orth and Moore 1983, Moore et al. 1993
R. maritima	Seeds	R. maritima is capable of germinating under a wide range of salinities; highest germination rate was achieved when seeds stored at 21°C at 15 ppt were placed in fresh water. Cold storage improves germination at all salinities tested, but also induced premature germination during storage.	Ailstock and Shafer 2006
P. perfoliatus	Seeds	Germination of seeds stored at 4°C with aeration is rapid with most seeds germinating within 7 days of inductive treatments. Salinity is an important factor regulating germination. Only 7.5% of seeds stored at 15 psu germinated within 3 wks if transferred to the same salinity at 21°C. In contrast, those same seeds show 85.5 % germination when transferred to 0 psu water at 21°C.	Ailstock and Shafer 2006
P. perfoliatus	Buds	For buds collected in Dec., a 6-wk cold treatment yielded higher germination rates, but did not affect the germination rate for buds collected in Feb.	Murray et al. (in prep.)
S. pectinata	Tubers	S. pectinata tubers produced in warm months did not germinate unless they were cold-treated, whereas additional cold-treatment was unnecessary for germination of propagules collected in Dec and Feb).Germination rates were 50-100% in both direct planted and cold-treated propagules.	Murray et al. (in prep.)

Planting techniques

Seed broadcasting has been shown to be effective for eelgrass because seeds are rapidly incorporated into the sediments and generally do not move far from where they settle (Orth et al. 1994). Hand-broadcasting has been widely used in a number of the large-scale eelgrass planting projects described previously. However, for very large sites, the hand-broadcasting method is time-consuming, and an even seed distribution is difficult to achieve (Lewandowski et al. 2006; Preen et al. 2006). To increase efficiency and reduce the amount of time required for planting, a mechanical seed sprayer was developed that is capable of evenly dispersing seeds at suitable densities (100,000 to 300,000 seeds/acre) at the rate of 10 min/acre (Lewandowski et al. 2006; Preen et al. 2006). The mechanical seed sprayer represents a significant improvement over hand-broadcasting for large-scale seeding projects. Both methods are described in detail in Chapter 3. However, in an attempt to increase seedling establishment rates, a number of alternative techniques were investigated on a smaller scale as part of the applied research funded under these programs.

Typically, only about 10–15 percent of broadcast seeds survive to the seedling stage (Harwell and Orth 1999). Seed predation by decapod crustaceans and other animals can be significant (up to 65 percent of seeds consumed), and has been identified as one of the causes of low eelgrass seedling establishment (Fishman and Orth 1996). Shallow burial of the seeds below the sediment surface has the potential to increase the success of seed planting efforts by reducing their availability to predators.

A mechanical planter capable of injecting seeds 1–2 cm below the sediment surface was designed and constructed by scientists at the University of Rhode Island (URI) (Traber et al. 2003) (Figure 12). This machine uses a gel matrix to deliver the seeds. An alternative design that does not require a gel matrix for seed delivery was also designed and tested by VIMS (R. Orth, personal communication).

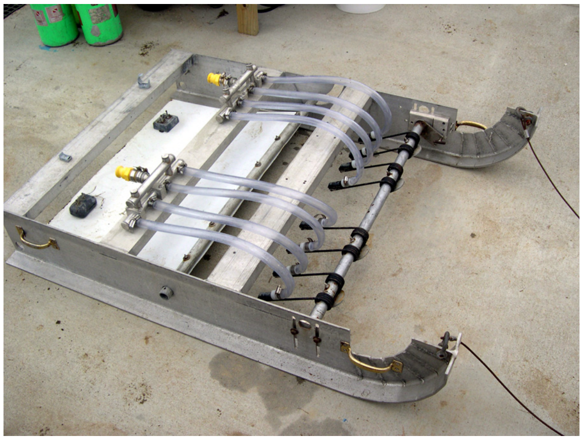


Figure 12. Mechanical seed injector planter designed by scientists at the University of Rhode Island to inject eelgrass seeds below the sediment surface (photo by VIMS).

In a comparison of seedling establishment rates using the URI seed injection sled with traditional hand broadcasting, seedling establishment rates for machine planted seeds were up to four times greater than hand-broadcast seeds at the Piankatank site (Orth et al. 2007a). However, this effect was not consistent among sites, possibly due to differences in sediment characteristics and energetic regimes (Orth et al. 2007a). This suggests that the potential for effective use of the machine planter should be evaluated on a case-by-case basis.

Other considerations include differences in the amount of time, labor and equipment required for each method. An anchored second boat with an electric pull system is necessary to pull the planting machine back to the anchored boat as the seeds are being injected into the bottom, thus ensuring a constant delivery and more even distribution of seeds. Submerged objects such as rocks, tree stumps, or old pilings, as well as high wind conditions, can interfere with the efficient operation of the planter. The broadcast method requires only one individual to disperse the seeds, and can be conducted under more compromising weather conditions. With hand broadcast there is little control over where seeds eventually settle, which will depend on sediment surface features (Orth et al. 1994), while seeds placed into the sediment with the seed injection sled can be more evenly distributed and are less affected by sediment features.

The URI seed injection sled requires a pre-made gel matrix for seed delivery, which must be kept cool during the entire process. The seed injector designed by VIMS, which does not require a gel matrix, has been tested in Spider Crab Bay in Virginia's Coastal Bays (Figures 13 and 14).

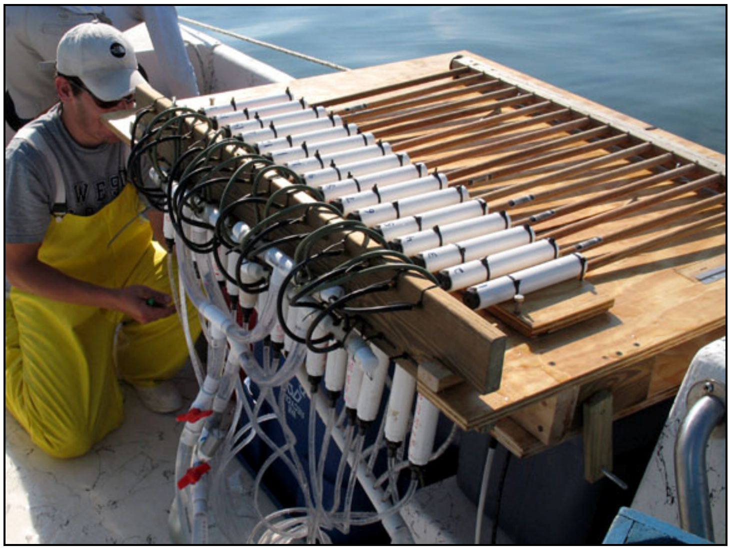


Figure 13. Seed delivery apparatus that controls the rate of seed introduction into individual tubes feeding the seed injector (photo by VIMS).

Figure 14. Seed injector suspended behind the boat prior to deployment, which holds spring-loaded planting tines that inject seeds below the sediment surface (photo by VIMS).

Preliminary results (October 2007) show that numerous seedlings germinated from seeds were planted by this injector, but data analysis to compare its germination rates to those resulting from seeds that were broadcast nearby is incomplete (R. Orth, personal communication).

Other planting techniques, such as covering eelgrass seeds with burlap bags or clay balls (encapsulation) in both small and large-scale restoration projects have had varying degrees of success. In some studies, the highest seedling establishment occurred where seeds were protected in burlap bags (Harwell and Orth 1999). In the most recent tests of the effects of eelgrass seed encapsulation at four sites in the York River, none of the encapsulated seeds germinated, while all of the plots with un-encapsulated seeds had some germination (R. Orth, personal communication). However, a recent study from Korea indicates that germination of *Z. marina* is enhanced by encapsulation with loess (Park and Lee 2007). This substance is believed to reduce bacterial and fungal infections that may lead to early seedling mortality, and also to provide physical protection from seed predators (Park and Lee 2007). Therefore, this technique may warrant further investigation in the Chesapeake Bay.

SAV Habitat Requirements and Site Selection Criteria

A few projects examined other aspects of restoration, including site selection. Table 10 lists the titles of the funded research projects, SAV species included, and the years funded. Their general results are summarized below, and more detailed results of each project are in Appendixes A and B to this report.

Table 10. Applied SAV research projects involving site selection in the Chesapeake Bay, 2003-2006.

Title	Species	Funding Recipient	Funding Duration	Funding Source(s)
Site Assessments for Future, Large-Scale Submerged Aquatic Vegetation Restoration in the Chesapeake Bay	Mainly *Z. marina*	MD DNR & VIMS	2003-2006	NCBO
Wave exposure as an additional parameter for identification of suitable SAV restoration sites	Variety	UMCES	2004-2005	NCBO
Seagrass habitat engineering: the role of wave attenuation structures in SAV bed survival and growth	*Z. marina*	UMCES	2005-2006	USACE

Site assessments for future SAV planting

One of the key parameters used in SAV planting site selection is interpolated water quality. In the Chesapeake Bay, the fixed station water quality monitoring network that was established in 1985 is now supplemented with spatially and temporally intensive monitoring of selected parameters in selected tributaries (for details, see URLs: http://www.chesapeakebay.net/wquality.htm; http://mddnr.chesapeakebay.net/sim/index.cfm). Each tributary gets this enhanced monitoring for 3 years, and the results are being used in 303(d) assessment of impaired water bodies, in addition to being used for site selection for SAV planting. This enhanced monitoring is very expensive; NCBO funding allowed more segments to be assessed sooner than would have been possible with existing funding.

Wave exposure as a site selection criteria

In high wave energy environments, seagrasses are commonly found only in sheltered areas, such as those behind coral reefs or sand bars (Fonseca and Bell 1998; Koch 2001). Seagrass density appears to be a direct function of wave exposure (Fonseca et al. 1983), and it seems likely that an increase in wave attenuation would result in more dense seagrass populations. The main goal of the wave exposure research funded by NCBO (Table 9) is to develop a wave exposure model that can be added to the existing factors used to assess the suitability of future large-scale SAV restoration sites (Koch 2007). Its specific objectives are:

1. To understand the processes that limit the survival of SAV seeds and plants in wave exposed areas
2. To characterize the wave climate in SAV habitats associated with different wave exposure regimes in the Chesapeake Bay
3. To add wave exposure as a criteria for the assessment of site suitability for SAV restoration in the Chesapeake Bay
4. To test the validity of the wave-exposure model when selecting sites for SAV restoration in the Chesapeake Bay.

Progress to date has focused on improving an existing model of wave exposure developed in North Carolina (Koch 2007). This wave model was upgraded to generate wave parameters such as significant wave height instead of a relative wave exposure index. This represents a major improvement of the model, as it can now be directly related to wave data collected with wave gauges. Critical wave heights defined in exceedance plots for the upper and lower Bay in the wave model will be used to geographically define SAV habitats suitable for restoration in the Chesapeake Bay based on wave exposure (in addition to light availability and water quality) and to identify the wave exposure exclusion zones, where wave action should be too high to allow SAV growth except in protected habitats. Experiments in flumes under different simulated wave conditions are also being done to test the model results (Koch 2007).

Preliminary results indicate that wave energy tolerances of seagrasses and other SAV may be species-specific. In the Chesapeake Bay, SAV in the upper Bay were found to have a lower wave exposure tolerance (significant wave height (Hs) < 0.15 m) than species in the lower Bay (Hs < 0.30 m) (Koch 2007). This difference may be a result of plant morphology, sediment characteristics, or an interaction between these features. The upper Bay is dominated by canopy-forming species that occupy the entire water column throughout most of the growing season (e.g., *Myriophyllum spicatum*, *P. perfoliatus, Hydrilla verticillata*), while the lower Bay is dominated by meadow-forming species that only occupy the entire water column when reproductive (e.g., *R. maritima*, *Z. marina*). As the drag exerted on the plants is a function of the area exposed to waves and currents, the plants in the upper Bay are more likely to be dislodged or damaged than SAV species in the lower Bay. Additionally, sediments in the upper Bay tend to be finer than those in the lower Bay, providing less anchoring capacity for SAV. It has been hypothesized that the combination of plant morphology that creates higher drag and sediment characteristics that provide less anchoring potential lead to lower wave energy tolerances in the upper Bay. An ongoing project, funded by NCBO in 2007, is investigating the interaction of sediment type and wave energy in SAV establishment and survival.

The effects of wave attenuating structures on SAV bed survival and growth

A related project, funded by the USACE (Table 10), focused on a comparison of the wave attenuation effects of breakwaters and sandbars and their role in SAV establishment and survival. Breakwaters are typically built along shorelines to attenuate wave energy and minimize shoreline erosion, especially in areas with high relative sea level rise such as the Chesapeake Bay. Therefore, breakwaters have the potential to improve seagrass habitat quality by: (1) reducing wave energy and thereby also improving water quality via reduced sediment resuspension, and (2) reducing sediment movement, allowing seagrasses to become dense and well-established. Indeed, in a highly wave-exposed area (Hs = 1 m) in Japan, *Z. marina* colonized an area following the construction of a breakwater (Dan et al. 1998).

In contrast, the results of a study of two breakwater systems in a much lower wave energy environment in the Chesapeake Bay (Hs < 0.4 m) indicate coastal structures such as breakwaters have the potential to be detrimental to seagrasses over time (Figure 15). While breakwaters produce the reduced wave energy environment that can be beneficial for seagrasses/SAV, they also tend to trap fine and organic particles. As a result, over time, sediments in the protected area become finer and more organic than sediments outside of the breakwater-protected area (Martin et al. 2005). Initially, the deposition of fine and organic sediments in the protected area may enhance seagrass growth, especially if the sediment was relatively sandy and nutrient poor before breakwater construction. However, the continued deposition of fine and organic particles eventually leads to detrimental conditions. As sediments shoreward of the breakwater become finer as a result of wave damping (Martin et al. 2005), they become resuspended at lower wave energy than the coarser sediments offshore of the structure (Figure 15).

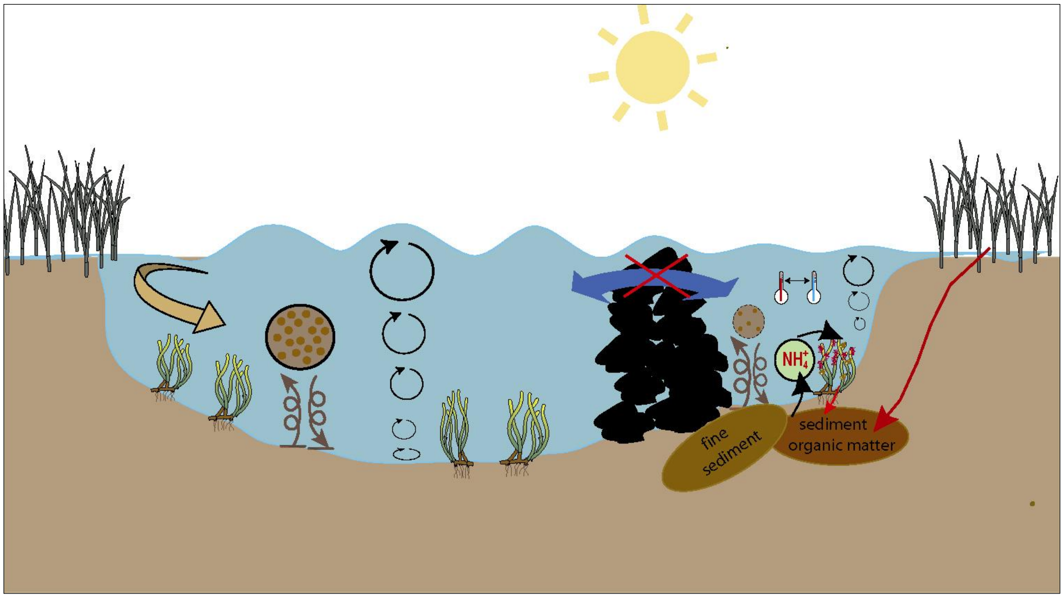


Figure 15. Breakwaters not only attenuate waves but also accumulate fine and organic particles (brown ovals) landward of the structure. These sediments are readily resuspended by small waves leading to increased turbidity. Fine and organic sediments also release more nutrients (NH_4 circle) than offshore sediments, fueling the growth of epiphytes (red cover) on seagrasses, contributing to their loss. Additionally, waters landward of restrictive breakwaters tend to be warmer (blue and red thermometers) than those offshore, leading to elimination of certain species such as *Z. marina* (drawing by E. Koch).

As a result, turbidity in the area protected by the breakwater is increased. Additionally, the fine particles that tend to deposit shoreward of the breakwater usually have a high organic content. Sediments with high organic content (>5 percent) are detrimental to some SAV species (Barko and Smart 1986; Koch 2001). Highly organic sediments also tend to have a higher nutrient content, which may stimulate the growth of epiphytes on SAV leaves in the breakwater-protected area (Figure 15, Koch et al. in preparation). The thickened epiphytic layer contributes to light attenuation and SAV decline. Despite these findings that suggest a potential long-term negative effect of breakwaters on seagrasses, some of the healthiest seagrass beds in the Chesapeake Bay are found shoreward of sand bars, a natural wave-reducing structure (Figure 16a).

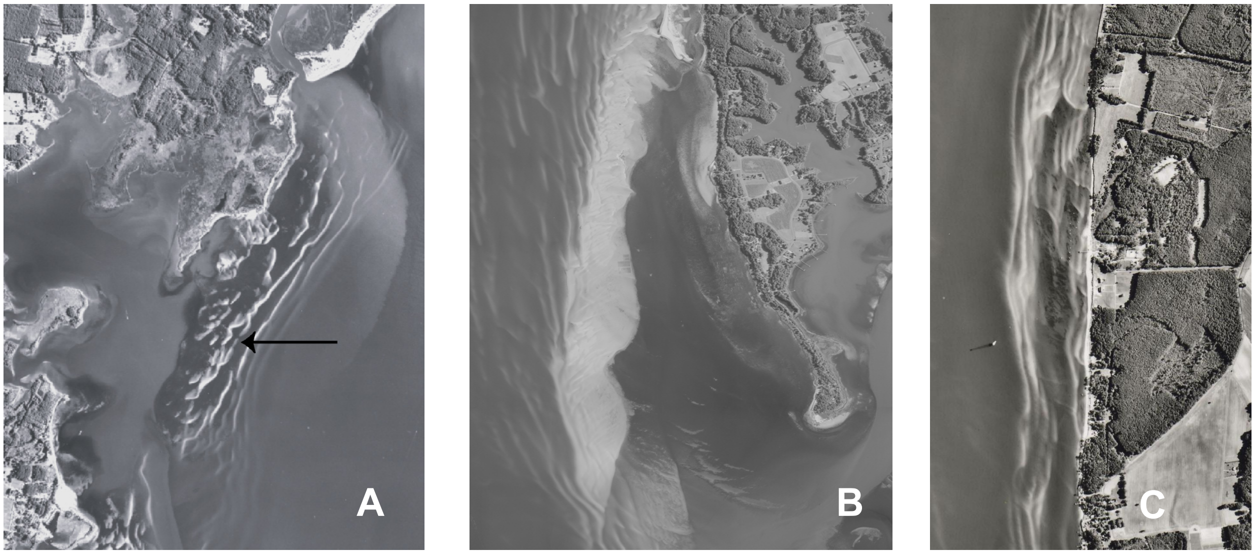


Figure 16. Geomorphological features in wave-exposed areas that provide sheltered habitats where seagrasses (darker areas) can thrive: (A) ridge (arrow), (B) stable sand bar, or where seagrasses may become smothered by moving sediment, (C) unstable sandbar (Fig. by E. Koch; photographic images from VIMS SAV Monitoring Program).

When these geomorphological features are relatively stable over time (slow migration rate), seagrass beds can flourish (e.g., the sand bar off Hungars Creek, VA, Figure 16b), but when they tend to be unstable and migrate at a fast rate, seagrasses in the sheltered area can be buried and die (e.g., the sand bar south of the mouth of Nassawadox Creek, VA, Figure 16c). Why do seagrasses flourish in sand bar protected areas, but not in breakwater protected areas? Ongoing research is testing the hypothesis that, while seagrasses need a stable substrate, i.e., relatively wave-protected areas, some wave exposure is necessary to keep fine and organic sediments at a low level that allows seagrass growth. In sand bars, this limited wave exposure can occur at high tides when waves propagate over the natural wave attenuating structure.

The results of this research will be used to determine the degree of wave attenuation favorable for seagrass establishment and sustainability. Preliminary data suggest that the potential negative effects of breakwaters on SAV can be corrected by: (1) allowing for sufficient flushing and resuspension to allow fine and organic particles to be removed from the site on a regular basis, perhaps by storm events and/or, (2) by allowing coarser particles to be deposited on a regular basis in the protected area (Koch, in preparation). Therefore, less restrictive (less high and/or wider openings) breakwaters are recommended to minimize the deposition of fine and organic particles in the wave-protected area. Another alternative would be to “dilute” the deposition of fine particles with the deposition of coarser particles (sand) that naturally occurs adjacent to sand bars (Koch, in preparation). This could be accomplished by building double-walled submersed breakwaters and filling them with dredged sand. During high tides the sand will then be eroded from the breakwater and deposited in the protected area maintaining a constant “fertilized” state of the sediment that benefits seagrasses without allowing sediments to become excessively fine and organic, thereby limiting seagrass growth.

This information could then be used in the engineering of seagrass habitats for large-scale restoration projects. For example, the U.S. Army Corps of Engineers (Norfolk District) will be dredging a new Federal channel in an existing natural channel (Nassawadox Creek, VA). This process is to be repeated every 4–6 years over a period of 50 years. The preferred plan for disposal of beach-quality material is overboard disposal. If the dredged material (5,000 cu yds) could be positioned just off Silver Beach in the form of a sand bar (similar to that at a creek just south of there), this could reduce wave energy to the point that the existing seagrass bed could expand in density and size; an indirect form of restoring seagrasses. Creating such longshore bars with dredge material to promote SAV growth is being tried in Tampa Bay (Lewis 2002; Cross 2007). Another example is the plan to construct a breakwater at the northern end of Tangier Island to stop shoreline erosion (a proposed Norfolk District Corps of Engineers project). Based on this research, the structure should both attenuate waves to reduce shoreline erosion, and allow sufficient tidal exchange to minimize the accumulation of fine organic sediments that are detrimental to seagrasses. This goal could be achieved by a breakwater attached at one end and extending at a 30 to 45 degree angle from the shoreline. These examples illustrate the emergence of a new field: engineering seagrass habitats for large-scale restoration.

Other Applied Research Projects

Two other projects funded by NCBO (Table 11) focused on less traditional ways to enhance SAV planting success. The first one investigated the feasibility of using oyster restoration to improve water clarity enough to promote survival of SAV planted nearby. The second looked for candidates for probiotics, bacteria that might enhance SAV growth.

Table 11. Other applied SAV research projects in the Chesapeake Bay, 2003-2006.

Title	Species	Funding Recipient	Funding Duration	Funding source(s)
Coupling Oyster and SAV Restoration in South River, Maryland	*P. perfoliatus* *S. pectinata*	MD DNR	2005-2006	NCBO
Breaking barriers in SAV restoration: using plant associated bacteria to enhance restoration success	*P. perfoliatus* *S. pectinata* *Z. marina* *V. americana*	UMCES	2004	NCBO

Using oysters to enhance SAV planting success

A recent modeling study concluded that a tenfold increase in oyster biomass would likely result in improved water quality and substantially increased SAV biomass in the Chesapeake Bay (Cerco and Noel 2007). The goal of this project was to add enough oysters to a small cove on Harness Creek to clear the water sufficiently to permit SAV growth. Past efforts to plant SAV in that cove, starting in 1998, had failed, suggesting that water quality needed to improve before SAV could be established.

Results to date have not achieved the objectives. MDDNR was unable to acquire enough oysters to provide the filtration needed, and many of those planted could not be found on subsequent monitoring visits. Water clarity conditions in the cove did not improve sufficiently to support SAV growth. A monitoring visit on 28 June 2007 found less than 5 percent of the original density of oyster spat planted in September 2006. The year 2006 was also a poor year for SAV in the South River in general. The South River had no SAV mapped anywhere in the river in 2006 (Virginia

Institute of Marine Science [VIMS] aerial surveys, segment SOUMH) and only a few small beds were found during ground truthing in 2007. The project is continuing with other sources of funding. As more oysters are added, the effects of their filtration may become more pronounced. For more details see the Appendix B (p 78).

Can probiotic bacteria enhance SAV growth?

This study characterized the diverse communities of bacteria attached to the leaves and roots of four important Chesapeake Bay SAV species collected from different regions of the Chesapeake Bay (Crump and Koch 2007): (1) eelgrass (*Z. marina*), (2) wild celery (*V. americana*), (3) sago pondweed (*S. pectinata*), and (4) redhead grass (*P. perfoliatus*). Molecular and cultivation techniques were used to discover micro-organisms common to submersed aquatic vegetation (SAV), characterize potential symbionts, and cultivate candidates to use for probiotic technology development.

Attempts to improve the growth of bacteria-free (axenic) SAV in tissue culture by introducing naturally occurring bacteria provided statistically significant results for two of the three mesohaline species tested, but it remains unclear whether these relationships are positive or negative for the plant. A positive relationship suggests that bacteria could serve as probiotics for improving restoration success. For more details, see the Appendix B (p 76), or Crump and Koch (2007).

5 Emerging Issues and Future Directions

Seed Coatings

A recent study from Korea indicates that germination in *Z. marina* is enhanced by encapsulation with loess (Park and Lee 2007). This substance is believed to reduce bacterial and fungal infections that may lead to early seedling mortality, and also to provide physical protection from seed predators (Park and Lee 2007). In the Chesapeake Bay, techniques for covering eelgrass seeds with clay balls or burlap bags in both small and large-scale restoration projects have had varying degrees of success. In some studies, the highest seedling establishment occurred where seeds were protected in burlap bags (Harwell and Orth 1999; Orth et al. 2006b). In a recent test of encapsulated eelgrass seeds at four sites in the York River, none of the encapsulated seeds germinated, while all of the plots with un-encapsulated seeds had some germination (R. Orth, personal communication). The factors responsible for the varying results in Korea and the Chesapeake Bay are unknown. This technique may warrant further investigation in the Chesapeake Bay.

Improving Existing Site Selection Criteria for SAV Restoration

The low rates of seedling establishment rates at the large-scale planting sites indicate that current site selection models that use a minimum light requirement of 22 percent of surface light at 1 m for high salinity zones within the Chesapeake Bay (Batiuk et al. 1992; Dennison et al. 1993; Kemp et al. 2004) may need to be revised upward. As Fonseca et al. (1998) point out, the minimum light requirements of transplanted seagrasses exceed those of established meadows. In mature, established SAV beds, physiological support for stressed shoots may be provided by translocation of stored metabolites from adjacent healthy plants through the network of interconnected underground rhizomes. Individual transplant units typically consist of only one or two SAV plants with attached roots and rhizomes, which are much more vulnerable to short-term fluctuations in environmental conditions because their connections to other plants have been severed. Similarly, the vulnerability of newly established seedlings to stressful environmental conditions is likely to be much greater than that of established plants, due to the lack of substantial stored belowground reserves and the ability to translocate nutrients between adjacent plants.

More recent analyses suggest that Chesapeake SAV in higher salinity regions (mesohaline and polyhaline) may need 33 percent or more of surface light at 1 m rather than 22 percent or more (Bergstrom et al. 2007).

In an effort to further refine the existing site selection criteria, ongoing research is being conducted to support the addition of other site selection parameters such as wave energy thresholds and sediment type. In fact, it seems likely that these two factors may exert an interactive effect on SAV growth and survival due to the effect of sediment type on plant architecture and rooting capacity. Seeds that germinate in natural depressions in compacted peat sediments develop into plants with large above-ground biomass and with small roots due to the organic nature of the peat (Wicks 2005). These plants have high drag and low anchoring capacity and are eventually dislodged (E. Koch, personal observation). However, if compacted peat areas are covered by 2 or more cm of sand, the area is once again suitable for SAV colonization (Wicks 2005). Recent data also suggest that seagrasses in the Maryland Coastal Bays (*Z. marina* and *R. maritima*) may require sediments with more than 65 percent sand content to survive in areas where light is not limiting (Koch et al., in preparation). An influx of sand may also help ameliorate the negative effects on SAV due to the accumulation of fine and organic particles landward of restrictive breakwaters. These results emphasize the potential importance of sand in SAV habitats, especially those in high salinity areas.

Analysis of Sites with Persistent Survival

In-depth monitoring and analysis of the SAV planting sites with the most persistent beds is encouraged, including those in the Virginia coastal bays, as well as sites with natural recolonization, to try to determine any common factors contributing to their success. In particular, this examination should look for possible factors that are not captured in current site selection models. For example, do these sites tend to be closer to the mouths of the creeks or rivers in which they are located than other sites, which might confer some water quality benefits? Do they tend to have lower summer water temperatures, or less exposure to winter scouring, or less disturbance by cownose rays or other species? How do they compare in terms of water depth, fetch distance (maximum distance over which winds can travel to reach the site), and/or sediment characteristics? There are enough of these sites now so that it may be possible to find some common attributes of the most successful sites that may be useful additions to the site selection tools described here.

Chesapeake Bay SAV Restoration Goals

These two Federally funded research programs represent the largest single coordinated research effort to date to improve the status of the science and capabilities for large-scale SAV restoration. Since the beginning of this research initiative, a total of 133 acres of SAV has been planted in the Chesapeake Bay, an average of 33 acres/year. By comparison, during the previous 21 years (1983–2003), approximately 189 acres of SAV were planted, at an average rate of 9 acres/year (Orth et al. 2006b). These results demonstrate the success of this work in developing tools and techniques necessary to plant SAV at scales that would have been unattainable with existing technologies only a few short years ago. Furthermore, the costs of conducting these plantings are on a downward trend as the understanding of the limiting factors increases and as new advances are made in technology development. There have also been substantial breakthroughs in the applied research to support large-scale planting efforts. Although seedling establishment rates were less than expected based on previous work, the higher rates at some sites in some years show that the problems may lie in site selection rather than in planting techniques. Researchers hope to improve site selection models in the near future, using research funded in part through these programs.

Despite the considerable progress made in technology improvement and in the ability to plant at larger scales, it is clear that the goal of planting 1,000 acres of SAV by 2008 established by the Chesapeake Bay Program will not be achieved. In the short-term at least, it seems that the targeted SAV restoration acreages established by the Chesapeake Bay Program are unrealistic, given the current technology, and may need to be re-evaluated. However, it should be recognized that the establishment of this goal has had a strong positive impact on SAV restoration in the Chesapeake Bay by stimulating the development of innovative new techniques and technologies to advance the capabilities of SAV restoration to heretofore unprecedented levels.

Climate Change and the Future of SAV Restoration in the Chesapeake Bay

Climate change is likely to cause shifts in the distribution of seagrasses and changes in community composition due to increased thermal stress, eutrophication, shoreline erosion, and increased frequency and intensity of storms (Short and Neckles 1999). Species near the limits of their ranges of distribution are likely to be particularly vulnerable to thermal stress (Short and Neckles 1999). For example, the temperate seagrass *Z. marina* approaches the southernmost limit of its distribution along the Atlantic coast of North America in the Chesapeake Bay (Koch and Orth 2003). Prolonged water temperatures above 30 °C can cause rapid declines, senescence, and mortality of *Z. marina* (Orth and Moore 1986); high water temperatures were implicated as a major cause of the recent massive eelgrass die-off that occurred in the Chesapeake Bay and Chincoteague Bay in 2005 (Marion and Orth 2007). Eelgrass seedlings may have even lower lethal temperature tolerances (e.g., in late summer of 2005, seedlings at major restoration sites in the coastal bays all died while older plants survived (R. Orth, unpublished data).

Climate modeling results predict that heat waves in Europe and North America will become more frequent, more intense, and will increase in duration by the second half of this century (Meehl and Tebaldi 2004). Although the mechanisms behind plant responses to climate change are complex, it may be reasonable to assume that increases in the frequency, intensity, and duration of extreme summer heat events may cause shifts in the abundance and distribution of *Z. marina* near the southern limits of its range on the Atlantic coast. To date, the largest portion of the funding and effort in these research programs has been expended in the restoration of this species. Of the total of 133 acres of SAV planted in the Chesapeake Bay during the period from 2003 to 2006, more than 100 acres were planted with *Z. marina*. Future restoration efforts may consider placing more emphasis on the restoration of other SAV species that have broader thermal tolerances, and may be better able to tolerate the projected conditions associated with climate change. Alternative approaches could involve the identification, selection, and production of more heat tolerant eelgrass strains, similar to the process currently used in the agriculture industry to develop improved plant cultivars with more desirable characteristics, or the introduction of other more heat-tolerant SAV species.

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Acronyms and Abbreviations

Term	Spellout
BAA	Broad Agency Announcement
CBP	Chesapeake Bay Program
COE	Corps of Engineers
DC	District of Columbia
DNA	deoxyribonucleic acid
EDS	extended deployment system (EDS)
EL	Environmental Laboratory
EPA	Environmental Protection Agency
ERDC	Engineer Research and Development Center
FY	fiscal year
GI	government issue
GIS	geographic information system
MDDNR	Maryland Department of Natural Resources
MLW	Mean Low Water
NCBO	NOAA Chesapeake Bay Office
NOAA	National Oceanic and Atmospheric Administration
PCB	polychlorinated biphenyl
SAV	submerged aquatic vegetation
SCUBA	self-contained underwater breathing apparatus
SF	standard form
TN	Technical Note
TR	Technical Report
TSS	Total Suspended Solids
UMCES	University of Maryland Center for Environmental Science
URI	University of Rhode Island
URL	Universal Resource Locator
U.S.	United States
USA	United States of America
USACE	U.S. Army Corps of Engineers
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
VIMS	Virginia Institute of Marine Science
WWW	World Wide Web
YSI	Yellow Springs Instruments Company (now YSI, Inc.)

Appendix A: Large-Scale SAV Planting Project Summaries

Project Title: "Restoration of Submerged Aquatic Vegetation in the Piankatank River, Chesapeake Bay"

Funding recipient. Bob Orth, VIMS

Total Federal funding. $257,410 (NCBO)

Background and research objectives. The Piankatank River and nearby Milford Haven have been used for a variety of transplant projects by VIMS over the past 2 decades, primarily with eelgrass (*Z. marina)* (Orth et al. 2006). Since some of these projects had some success, at least for a few years, and site selection models showed some sites suitable for SAV planting, VIMS staff chose this site to test new methods for planting eelgrass from seed.

The objectives of this project were to: (1) investigate the applicability of large-scale restoration of sites deemed suitable based on historical distribution and adequate water quality using eelgrass seeds, (2) establish test plots of eelgrass at additional locations to assess site suitability for future large-scale restoration, and (3) document spatial and temporal patterns in water quality that may regulate SAV distribution around the study sites and compare to eelgrass restoration success.

Results

Objective 1. Test the applicability of large-scale eelgrass restoration for increasing acreage of SAV.

VIMS staff collected and successfully spread 4.8 million seeds over 3 years into 39.5 acres at sites that were deemed suitable based on both historical coverage and water quality that apparently met the SAV habitat requirements. Seedlings were readily apparent in most plots in the spring following their distribution, but initial establishment rates were very low (1 percent or less) and plants did not survive through the summer.

Objective 2. Assess the utility of test plots for identifying future sites for large-scale restoration.

Test plots using both adult plants and seeds were successfully established in each of 3 years at several sites that were currently unvegetated, but historically supported eelgrass except for one upriver site. Plants persisted at all sites through the early summer although loss rates varied between sites, plant type, and year, but by the following fall, 1 year after establishment, all plants had died.

Objective 3. Document spatial and temporal patterns in water quality that may regulate SAV distribution around the study sites and compare to eelgrass restoration success.

Water quality measurements from the dataflow cruises showed a relatively low range of values among the test sites, and generally similar values for test plot sites and the natural beds at Gwynn's Island and Healy Creek. Coupled to data from the continuous monitoring of water quality parameters at Burton Point, turbidity and chlorophyll values were within the range of values for the polyhaline SAV habitat requirements for TSS and chlorophyll, and had suitable ranges for salinity and dissolved oxygen. However, continuous temperature data at several of the transplant sites in 2006 revealed temperature variability that could impair eelgrass survival when coupled with turbidity spikes. The natural beds at Healy Creek and Gwynn's Island persisted during this time period, suggesting that transplants may be more sensitive to the cumulative stress of temperature and other factors than are existing eelgrass beds.

Implications for restoration. SAV habitat requirements developed in the early 1990s and modified in 2000 (Batiuk et al. 1992; Dennison et al. 1993; Kemp et al. 2004) were mainly developed for established populations of plants and highlighted five key water quality factors: light, turbidity, chlorophyll a, nitrogen and phosphorus. Established plants have developed into beds with altered sediment characteristics (e.g., higher percentages of silt and clay, and organic content) and biogeochemistry (e.g., nutrient recycling), and they can increase water clarity over relatively short temporal periods due to the baffling effects of the leaves (Moore 2004). Established plants might be expected to have requirements for persistence that would be less stringent than those required for establishment, especially in areas that have been un-vegetated for decades where sediment properties are very different. In addition, other habitat characteristics can influence plant establishment that had not been considered earlier, e.g., wave exposure, sediment mobility, temperature and salinity (Koch 2001).

Data on transplant growth, seedling loss, water quality, spatial and temporal variations in natural bed growth, and distribution and abundance in the lower Piankatank from 2004 through 2006 show that the SAV habitat requirement thresholds for established beds did not indicate suitability for survival of transplanted adult plants or seedlings. The implication is that re-introducing eelgrass will likely require conditions better than those required for simply maintaining existing beds (Fonseca et al. 1998).

Further information. This project is complete and the final report was accepted in August 2007 (Orth et al. 2007e).

Project Titles: "Large-Scale Restoration of Eelgrass (*Z. marina*) on the Patuxent River (NCBO), and Potomac River, MD (ERDC)"

Funding recipient. Mike Naylor, Lee Karrh, and Tom Parham, MDDNR

Total Federal funding. $472,859 (NCBO), $432,240 (ERDC)

Background and research objectives. The Patuxent and Potomac Rivers are near the northern limit of potential eelgrass distribution in the Chesapeake Bay, limited by its salinity tolerances (15–30 psu, Bergstrom et al. 2006). Both rivers supported eelgrass in the past, although none was present at the time of planting. Since site selection models indicated some sites suitable for SAV planting, MDDNR staff selected both rivers to test new methods for planting eelgrass from seed.

In general, the Patuxent planting was funded by NCBO and the Potomac planting by USACE, but in 2006 some of the Potomac work was funded by NCBO. The main objective of both studies was to compare two methods for the dispersal of eelgrass seeds: via seed bags that dispersed seeds naturally in the spring (e.g., Pickerell et al. 2006), and via broadcasting of previously stored seeds in the fall. The effect of seeding density was also tested. In the Potomac, an additional objective was to compare the planting success with eelgrass to that from the eelgrass planting included in the Wilson Bridge mitigation project.

Results and implications for restoration. This project planted a total of 37.25 acres with eelgrass (*Z. marina*) seeds in the lower Potomac River in Maryland, and 25.92 acres of eelgrass in the lower Patuxent River in Maryland.

Five sites in the Potomac and five sites in the Patuxent were initially selected for planting based on a GIS-based SAV restoration site targeting system developed by MDDNR (Parham and Karrh 1998). Site conditions were monitored to understand both the short term variation in water quality and the site-specific differences in water clarity throughout the planting areas. Spatially intensive habitat assessments (DATAFLOW) were conducted twice per month throughout the growing season. DATAFLOW is a shipboard system of geospatial equipment and water quality probes that measures water quality parameters (temperature, salinity, dissolved oxygen, turbidity, and fluorescence) from a flow-through stream of water collected near the water's surface. In addition, continuously recording monitors (YSI 6600 extended deployment system [EDS]) were deployed at each restoration site. Seeds were distributed only once within each plot at each of the five locations (i.e., there was no repeated seeding of the same plots over multiple years). After 2 years of site monitoring and evaluation of test plantings, only three of the five Patuxent sites and four of the five Potomac sites proved to be suitable.

Due to a nearly complete loss of the eelgrass plantings conducted as part of the mitigation project in the Potomac, one of the objectives of the Potomac project (comparison of vegetative planting vs. seeding) could not be achieved. However, both projects improved existing protocols for large-scale collection, processing, and planting of eelgrass seeds, and compared two different methods for distributing eelgrass seeds (seed bags and broadcast).

Seed bags were the most cost effective restoration technique in terms of the cost per seedling produced. Table 3 in the main report (p 13) and the accompanying text give details.

Although early seedling establishment was good at three of the Potomac sites, by 2006, surviving plants were observed at only 1 of the 5 sites (St. George Island). Results in the Patuxent were less encouraging, with no plants surviving as long as 1 year after planting at any site. The combination of high light attenuation by epiphytes, low ambient light levels, and high water temperatures (> 30 °C) in the summer of 2004 and 2005 were probably responsible for the loss of most seedlings and adult plants.

As in a similar eelgrass planting done in the Piankatank in Virginia (see above), the results of these two projects also suggest that the current SAV habitat requirements, based on annual median conditions, do not accurately predict where eelgrass will grow when planted as adult plants or seeds. To become more accurate predictors of planting success, SAV habitat requirements may need to be refined to account for short-term, episodic events, they may need to include more factors such as temperature, and they may need to be made more stringent (requiring more water clarity to consider an area suitable for SAV planting).

Further information. Reports on both projects are available on the MDDNR web site (click "Project Reports") through URLs:
Potomac, Lewandowski et al. 2006:
http://www.dnr.state.md.us/bay/sav/restoration/pot_gen_info.asp
Patuxent, Preen et al. 2006:
http://www.dnr.state.md.us/bay/sav/restoration/pax_gen_info.asp

The NOAA-funded part of the project is not yet complete so no final report is yet available.

Project Title: "Large-Scale Planting of Mesohaline SAV Species at Poplar and Barren Islands, MD"

Funding recipient. S. Ailstock, Anne Arundel Community College

Total Federal funding. ERDC, plus additional funding from the Maryland Port Administration

Background and research objectives. Seeds of two mesohaline SAV species, redhead grass (*P. perfoliatus*) and widgeongrass (*R. maritima*), were planted at Poplar Island in 2004; *R. maritima* seeds were planted at Barren Island, MD in 2005 (S. Ailstock, personal communication). Seed-bearing wrack (floating plant material containing detached stems with seeds) of both species was collected in Marshy Creek near Kent Narrows at the northern end of Eastern Bay. The wrack used at Poplar Island was collected in July 2004; wrack used at Barren Island was collected in July 2005. For both sites, wrack was held in cold storage at 4 °C for the remainder of the summer, then put in floats with wire mesh bottoms at the planting sites in the fall of the same year. Cold storage has been shown to improve seed germination without affecting viability (Ailstock and Shafer 2006). Unconfined wrack, wrack in floats, and extracted seeds were placed near each other in the areas planted (S. Ailstock personal communication). The floats used had wire mesh bottoms, which allowed the seeds to settle out as they ripened, similar to the mesh bag technique used for eelgrass (e.g., Pickerell et al. 2005; 2006). Wrack was placed in the baskets where currents would move the seeds to new areas; extracted seeds and unconfined wrack were placed in the dead end arms, which would not be good places for seed dispersal by currents.

At Poplar Island, floats, loose wrack, and seeds were distributed on 23 October 2004, in a total of 12 acres (half in the Notch and half in wetland cell 4DX). At Barren Island, floats, loose wrack, and seeds were deployed on 21 September 2005 in a 3-acre area in a shallow cove on the east side of the island protected by a new breakwater (38.3343 N, 76.2603 W).

Results and implications for restoration. Seedling establishment rates of mesohaline SAV species at Poplar and Barren islands could not be determined, but were probably very low (S. Ailstock personal communication). No seedlings were found on a 2005 return visit to the 12 acres that were planted with seeds at Poplar Island in October 2004. There was little suitable shallow water habitat in the area planted, especially in Cell 4DX. Seeds from the same batch germinated in the lab. At Barren Island, no plants could be found on a return visit about 10 months after planting, on 7/17/06. During that time, the bottom sediments changed from firm to very soft, so those new sediments may have buried any plants that grew from seeds there.

Appendix B: Applied Research Project Summaries

Project Title: "Restoration Potential of Two Mesohaline Submerged Aquatic Plant Species by Seed in the Mid-Chesapeake Bay"

Funding recipient. S. Ailstock, Anne Arundel Community College

Total Federal funding. $ 54,022 (ERDC)

Background and research objectives. Seeds offer an efficient and cost effective method for providing new plants for large-scale SAV restoration plantings and hence are used for the production of all major domesticated crop plants. The same properties that have made seeds the mainstay of agricultural productivity are also applicable for large-scale wetland restorations. Seeds are particularly well suited for creating underwater grass communities where the constraints of working underwater sharply limit the efficiency of vegetative means of plant establishment. This project involved the development of seed-based propagation protocols for two important mesohaline species of underwater grasses, *Potamogeton perfoliatus* (redhead grass) and *R. maritima* (widgeongrass). These protocols include methods for seed collection, isolation, storage, germination and seedling establishment. The techniques can be adjusted to meet the requirements for different types of restoration and could also be applied to a number of other underwater grass species.

Results and implications for restoration. Seed isolation efficiency for both species ranges between 50 and 75 percent, depending on the time of collection and maturation of the flowers. The conditions regulating seed viability in storage include temperature, dissolved oxygen and salinity. Germination rate and percent germination exhibit normal distribution over a temperature range of 10–27 °C (50–80 °F). Optimal salinity for seed germination varies between species. *R. maritima* seeds germinate over a broad salinity range as might be expected of a species so widely distributed, while the seeds of *P. perfoliatus* germinated best at low salinities capable of stressing parent populations. This adaptation may be important for reducing competition between generations of this perennial plant.

Further information

Ailstock, 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. http://el.erdc.usace.army.mil/elpubs/pdf/eltn04-02.pdf

Ailstock, S. and D. Shafer. 2006. *Protocol for large-scale collection, processing, and storage of seeds of two mesohaline submerged aquatic plant species*. ERDC/TN SAV-06-3. Vicksburg, MS: U.S. Army Engineer Research and Development Center. http://el.erdc.usace.army.mil/elpubs/pdf/sav06-3.pdf

Project Title: Production and Field Planting of Vegetative Propagules for Restoration of Redhead Grass and Sago Pondweed

Funding recipient. L. Murray and M. Kemp, University of Maryland, Horn Point Laboratory

Total Federal funding. $ 176,100 (ERDC)

Background and research objectives. Many submerged aquatic plant (SAV) species produce abundant seeds, but seed viability and germination success are relatively low under most estuarine conditions. Although some SAV species may also reproduce by fragmentation, in many areas there are few local source populations to generate plant fragment propagules. The production and distribution of over-wintering propagules can provide an alternative method for restoration of some mesohaline species. This study investigated techniques for restoring *P. perfoliatus* and *Stuckenia pectinata* in the mesohaline region of the Chesapeake Bay through the use of over-wintering subterranean propagules (buds and tubers). Four separate experiments were conducted, including: (1) natural propagule production and viability, (2) artificially induced propagule production, (3) the effects of salinity and cold storage on propagule viability, and (4) propagule planting methods.

Results and implications for restoration. The results of this study indicate that, under *in situ* (estuarine ponds) conditions, *P. perfoliatus* over-wintering buds require an extended cold period for effective germination and subsequent growth to occur. For this species, natural bud production, germination, and plant growth were highest from buds harvested during cold months (Oct-Feb), when plants were dormant. While the number of propagules produced was highest in October, plant growth from these propagules was greater following either an artificially induced cold storage treatment (4 °C refrigeration) or an extended natural cold treatment (February). Experiments conducted to artificially induce *P. perfoliatus* production of viable buds in summer and early fall support this conclusion, where buds forced with exposure to winter light and temperature had low germination rates without additional cold treatment.

P. perfoliatus buds can be stored in the cold (4 °C) and dark for up to 8 wks with no decrease in the rate of germination or of subsequent plant growth. Under field conditions, these buds have the potential to survive as well, if not better, than mature plants planted with intact roots and rhizomes. *P. perfoliatus* buds may be planted using methods that are less labor intensive than hand planting mature plants with intact root balls and do not require SCUBA or snorkeling. Germination and subsequent growth was strongly affected by salinity, with significantly higher values at low salinities.

In contrast to *P. perfoliatus* buds, *S. pectinata* tubers are produced throughout the year under natural conditions, with peak numbers in June and October. While tuber germination is especially low during warm months, they can be "induced" to germinate with a 6-wk period of cold storage. Mature *S. pectinata* plants can be forced (by lowering temperatures and light) to produce propagules during the "off season" (warm months).

S. pectinata tubers can be stored for at least 8 wks in cold/dark conditions with little decrease in the germination rate. When stored for 12 wks, this germination rate decreased by 50 percent, and longer storage results in little or no germination. Salinity had little effect on propagule germination, but plant growth increased in lower salinities. Healthy plant growth was observed in the various methods of deployment investigated. Therefore, *S. pectinata* propagules can provide a less labor-intensive mechanism for restoring this SAV species.

Further information

Murray, L., W. M. Kemp, and D. Hinkle. (in prep.). *Production and field planting of vegetative propagules for restoration of redhead grass and sago pondweed in Chesapeake Bay*. ERDC Technical Note. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Project Title: "Development of Techniques for the Use of Seeds in the Large-Scale Propagation and Restoration of *Vallisneria americana*"

Funding recipient. K. A. Moore, Virginia Institute of Marine Science

Total Federal funding. $ 110,900 (ERDC)

Background and research objectives. The use of wild harvest seeds for the restoration of *V. americana* was investigated at a range of scales ranging from greenhouse tank experiments, to freshwater pond plantings and finally *in situ* estuarine field trials. In the greenhouse seed germination transplanting success was investigated using a (3x2) factorial design consisting of seeds transplanted into 15 L plastic tubs consisting of either of three sediment types (sand, mud, mixed) and two seed burial depths (2 cm, 10 cm) under ambient light and temperature conditions. Best conditions for germination of individual seeds were found to be at shallow depths and in a sandy substrate. Germination rates ranged from approximately 1—16 percent. A comparison of the germination and growth of *V. americana* seedlings from the dispersal of individual seeds removed from seed pods and seeds that remained within intact seed pods was next undertaken in a freshwater pond experiment. Seeds were dispersed here two ways, either as seeds that had been removed from their pods, or as pods dispersed directly into the ponds. The use of dispersed individual seeds resulted in significantly greater shoot abundances than the direct dispersal of seed pods (225 vs. 65 shoots m^{-2} by September of the first growing season). Shoot growth from seed pod seedlings also lagged behind those of the individual seed treatment and maximum shoot size was generally lower.

Field trials using both direct seed pod and individual seed dispersal techniques were also undertaken in the tidal freshwater James River near Hopewell, Virginia. Both seeds and seed pods were dispersed into three replicate areas that were either protected from herbivory by 1 in. mesh, plastic fencing exclosures or were left unprotected. Seedling success was again greatest in treatments with individual seeds removed from the pods compared to direct dispersal of the pods (40–60 percent versus 20–40 percent basal coverage by the end of growing season). Initial seedling production rates were similar in the unprotected areas to the herbivory-protected areas; however, once the seedlings reached 5–10 cm in length they were cropped and cut off at heights of 1–2 cm. Within a week, the cropped shoots were gone. Seedling coverage at the end of the growing season for the unprotected seedlings was 0–5 percent.

Results and implications for restoration. Direct seeding techniques can be a successful and effective approach to restoration of *V. americana* into unvegetated areas. Although separation of individual seeds from seed pods can improve the seedling success and growth rates, direct dispersal of the entire seed pods can also be successful. Germination percentages increased significantly when oxygen was present, temperatures were >19 °C, salinities were <5psu, sediment organic content was <3 percent, and seed burial depths were <15mm. The presence/absence of light had no significant effect on germination. Sandy substrates provide a potentially better habitat for seed germination success; however muddy substrates are also acceptable. Seed burial may not be necessary for successful seedling production, and excessive sedimentation (burial to depths of 10 cm) resulted in reduced germination rates. Seedling herbivory seems to be a factor limiting initial restoration success. However, plastic mesh exclosure fencing can provide an effective barrier to herbivores in field environments.

Further information

McFarland, D. G. 2006. *Reproductive ecology of* Vallisneria americana *Michaux*. ERDC/TN SAV-06-4. Vicksburg, MS: U.S. Army Engineer Research and Development Center. http://el.erdc.usace.army.mil/elpubs/pdf/sav06-4.pdf

Moore, K. A., and J. Jarvis. 2007. *Use of seeds in the propagation and restoration of* Vallisneria americana *Michaux (wild celery) in the Chesapeake Bay*. ERDC/TN SAV-07-3. Vicksburg, MS: U.S. Army Engineer Research and Development Center. http://el.erdc.usace.army.mil/elpubs/pdf/sav07-3.pdf

Project Title: Technology Development for Achieving Critical Thresholds in Large-scale SAV Restoration

Funding recipient. R. J. Orth, Virginia Institute of Marine Science

Total Federal funding. $ 199,000 (ERDC)

Background and research objectives. Recent studies have shown the potential for restoration of eelgrass (*Z. marina*) from seed (Granger et al. 2002; Traber et al. 2003; Orth et al. 2006). However, restoration of large acreages will require the development of new techniques and equipment for collection and handling large volumes of plant material. The effects of varying planting density and plot size will also need to be evaluated. Therefore, a primary goal of this project was to develop methodologies and infrastructure to fully exploit potential mechanized seed harvesting capabilities. Using these newly developed techniques, a second major goal of this project was to test the effects of seeding density, mixed demography and repeated seeding on bed persistence and expansion.

Efforts to develop new methodologies and equipment included: (1) developing a small, portable grass cutting device based on a larger, commercially available cutting apparatus originally intended for use in the removal of nuisance aquatic vegetation, (2) expansion of storage capacity to facilitate the processing of material from the large-scale collections, (3) enhancing seed separation and processing efficiency, and (4) determining optimal conditions for seed storage.

Previous eelgrass restoration attempts using seeds have been small in size (< 10 m^2 plots) and at extremely low seed densities (approximately 25–50 seeds m^{-2}). In this project, the goal was to substantially increase seeding density at multiple sites, over a period of multiple years, to identify potential critical thresholds in bed establishment and expansion.

Results and implications for restoration.

Mechanical Seed Harvest Equipment. A small, portable grass cutting device consisting of a pair of horizontal, toothed cutting bars driven in opposition by an electric motor was designed and constructed. The mechanism was scaled for use on a small boat that allowed easy deployment and relocation, and the height of the cutting bar was adjustable to target taller reproductive shoots while minimizing removal of vegetative leaves. The cutting device is mounted on the bow of the boast and pushed through the bed, cutting off the top-most reproductive shoots, which are then collected in a net attached directly in back of the cutting device. The bag is easily retrieved and material placed in collecting bags.

Large-Scale Storage Facilities. A plastic swimming pool can be used as a rapid, cost-effective, large-capacity solution for large volumes of plant material. To prevent decomposition of material during the holding phase, all holding tanks should be supplied with running seawater and air lines along the bottom to actively aerate tank contents.

Enhancing Seed Separation and Processing Efficiency. The traditional seed separation process is a labor-intensive process, requiring significant work-hours to remove large volumes of decomposing plant matter from the bottom of tanks and sieving to separate seeds. To handle larger volumes of material, new seed separation techniques were developed. A diaphragm pump capable of moving slurries of solids was used to streamline the material separation process. In addition, separation of seeds from vegetative matter was facilitated using passive water flow through a series of elutriation troughs to obtain a purer seed product that will help reduce risk of mortality-inducing hypoxic-anoxic events.

Determine Optimal Conditions for Seed Storage. Recent efforts have shown that survival rates of seeds vary widely, and are thought to be the result of differences in handling and storage conditions between the time of collection and eventual distribution. A series of experiments were conducted to determine the appropriate environmental conditions for seed storage, including salinity, temperature, and various levels of air flow. The results show that *Z. marina* seeds held under moderate to high salinities, with no or low air flow, and at temperatures of 15–20 °C have the highest survival rates during storage of 3–5 months.

Effects of Seedling Density and Repeated Seeding on Bed Survival. The unexpected dieback of eelgrass in 2005 in the Chesapeake Bay resulted in the loss of many high seed bearing areas, resulting in a much smaller number of seeds than anticipated. As a result, efforts were concentrated on one site that has yielded successful plantings in past years, albeit at lower densities. Seeds were broadcast at seed densities of 200,000 seeds/acre into replicated plots, two of which would receive repeated seedings in subsequent years to assess how important seed input was to bed expansion and not just simply vegetative growth. Initial assessments to date suggest that seed densities chosen in this experiment failed to make significant impact on bed development and that a second year of seeding in two plots has not altered the trajectory of the plot. This suggests that initial seed densities may have been too low to initiate rapid bed expansion.

Further information

Orth, R. J., and S. R. Marion. 2007. *Innovative techniques for large-scale collection, processing, and storage of eelgrass* (Zostera marina) *seeds*. ERDC/TN SAV-07-2. Vicksburg, MS: U.S. Army Engineer Research and Development Center. http://el.erdc.usace.army.mil/elpubs/pdf/sav07-2.pdf

Project Title: “Restoring eelgrass (*Z. marina*) from Seed: A Comparison of Planting Methods”

Funding recipient. R. J. Orth, Virginia Institute of Marine Science

Total Federal funding. $ 90,200 (ERDC)

Background and research objectives. In an attempt to minimize the labor-intensive nature of manual planting methods, mechanical seagrass planters have been developed in both the United States and Australia. These systems have mostly focused on adult plants, and have had varying degrees of success (Paling et al. 2001a, 2001b; Fishman et al. 2004). Each has distinct limitations in their operating procedures (e.g., depth limitations, donor bed locations or requiring plants grown from expensive nursery operations, need for SCUBA divers, weather limitations). Although seagrass seeds have had limited use in seagrass restoration programs, and seedling establishment rates are generally low (Orth et al. 2003), there is increasing interest in developing tools to facilitate SAV planting from seed, because seeds offer the potential for the cost-effective restoration of large, genetically diverse SAV populations. This study describes a new gel-matrix mechanical planter (seed injector) designed for planting eelgrass seeds, and compares the efficiency of this method with hand-broadcasting techniques.

Results and implications for restoration. Each method examined has a specific set of requirements that need to be considered in the restoration process. All methods require an efficient method of storing seeds from the collection period until dispersal. The gel-matrix mechanical seed planter requires an anchored second boat with an electric pulley system that draws the planting machine back to the anchored boat as the seeds are being injected into the bottom. This ensures a constant delivery of seeds yielding the more even distribution of seeds. The machine planter requires a pre-mixed gel matrix for seed, which must be kept cool during the entire process. In this project, 5 gal of gel were required for two 10m lines. This method is probably not appropriate for areas with submerged objects such as rocks, tree stumps, or old pilings, which can interfere with the efficient operation of the planter. High wind conditions and chop can also reduce the efficiency of this approach.

The broadcast method is less equipment and labor-intensive, and requires only one individual to disperse the seeds, either underwater or broadcasting on the surface of the water from a boat. The broadcasting method also has the advantage that it can be conducted under more compromising wind conditions. In this situation, there is little control over where seeds eventually settle, which will depend on sediment surface features (Orth et al. 1994), while seeds placed into the sediment with the mechanical planter can be more evenly spread and are not constrained by sediment features.

Further information

Orth, R. J., S. R. Marion, S. Granger, and M. Traber. 2007a. *Restoring eelgrass* (Zostera marina) *from seed: A comparison of planting methods for large-scale projects*. ERDC/TN SAV-08-1. Vicksburg, MS: U.S. Army Engineer Research and Development Center. http://el.erdc.usace.army.mil/elpubs/pdf/sav08-1.pdf

Project Title: "A Comparative Test of Mechanized and Manual Planting of Eelgrass Seeds"

Funding recipient. Bob Orth, VIMS

Total Federal funding. $24,718 (NCBO)

Background and research objectives. The first task was to design, construct, and test an underwater planter that would inject seeds into the sediment similar to the seed planter machine developed at URI, but not use the gel that seeds are embedded in with the URI machine. The second task involved a comparison of seedling success with eelgrass seeds that had been encapsulated in a clay matrix patented by Wayfarers Inc. and Bob Murphy.

Results and implications for restoration. Under the first task, the new planting injector (not using a gel matrix) was built and tested in fall 2007, and the number of seedlings that result will be counted in April 2008. Preliminary results from the second task show that few encapsulated seeds germinated while most non-encapsulated seeds did germinate and sprout.

Further information. The final report is expected in 2008.

Project Title: Restoration of Eelgrass Communities in the Chesapeake Bay with Seeds: The Emerging Issues

Funding recipient. Bob Orth, VIMS

Total Federal funding. $226,644 (NCBO)

Background and research objectives. The goal of this project was to understand basic ecological principles governing successful establishment of eelgrass seedlings and apply this knowledge to large-scale eelgrass restoration programs. Objectives included: (1) determine the relative importance of seed burial for summer and fall distribution of eelgrass seeds; (2) identify the mechanisms enhancing eelgrass seed germination in protected containers (burlap pouches); and (3) determine whether high initial eelgrass shoot densities limit plant survival.

Results and implications for restoration. Preliminary results from Objective 2 suggest that any benefits from protecting seeds depend of the water depth at the planting site as well as the redox potential. Higher sediment oxygen concentrations appear to inhibit germination, although they can also be too low. The timing of planting within the year is also important; as found in other studies, fall (October) appears to be one of the best times to plant eelgrass seeds. Preliminary results from Objective 3 suggest that initial seedling density affects shoot production, with better production from less dense seeding, most likely because of competition for space and nutrients in the denser planting treatments.

Further information. The final report is expected by April 2008.

Project Title: A Comparison of Reproductive Success of Seed Yields from Natural and Cultured SAV Populations

Funding recipient. Steve Ailstock, AACC

Total Federal funding. $136,582 (NCBO)

Background and research objectives. Despite the rapid advancement of techniques to use seeds for large-scale restoration, the availability of viable seeds remains a required prerequisite. Reliance on seeds from natural populations is problematic since populations have been known to experience substantial yearly differences in reproductive success. It is also not uncommon to experience significant Bay-wide population declines. Agricultural systems minimize these unacceptable fluctuations in seed availability by devoting environmentally controlled acreages exclusively to seed production for major crop plants. This project was designed to determine whether a similar approach for seed production that relied on ambient waters known to support long-term growth of diverse mesohaline species of SAV could produce consistently reliable quantities of seed.

The flow-through tanks for growing SAV were built at Chesapeake Bay Environmental Center (CBEC) in Grasonville, MD, and planted with seeds of sago pondweed, widgeongrass, and redhead grass collected from floating wrack in nearby Marshy Creek, which was also the source of the water pumped through the tanks. The tanks were originally to be built much closer to the water, which would have required the water to be pumped a much shorter distance, but the flooding during Hurricane Isabel in September 2003 dictated that they be relocated to higher ground.

Results and implications for restoration. The system tested in this project for mesohaline SAV seed production that relied on ambient waters known to support long-term growth of diverse mesohaline species of SAV could not produce consistent reliable quantities of seed. Unlike terrestrial systems where the environment can be easily manipulated by the use of soil amendments, fertilizers, irrigation systems and pesticides, aquatic systems are much more difficult to manipulate and the growth environment is ultimately determined by weather and ambient water quality. Part of the problem was that the new location for the tanks proved to be too far from the water source (over 1500 feet) for the pumps that were used, and the pumps kept failing. In addition, the SAV did not grow well in the tanks. Thus, this system as implemented at this site did not appear to be a useful aid to mesohaline SAV restoration.

Further information

Ailstock, M. S., and J. Wink. 2006. *A comparison of reproductive success in seed yields from natural and cultured populations of* P. perfoliatus *and* R. maritima *for applications in large-scale restoration in Eastern Bay*. Final Report to National Oceanic and Atmospheric Administration, Grant NA03NMF4570472. http://noaa.chesapeakebay.net/

Applied SAV Research Projects: Site Selection Criteria

Project Title: "Site Assessments for Future Large-Scale Submerged Aquatic Vegetation Restoration in the Chesapeake Bay"

Funding recipient. D. Goshorn, MDDNR, and R. J. Orth, Virginia Institute of Marine Science

Total Federal funding. $911,652 (NCBO)

Background and research objectives. This project funded part of the water quality monitoring programs that were crucial to selecting sites for SAV planting in both Maryland and Virginia. These programs were mainly funded by other sources, especially the state implementation grants from the EPA CBP, but NCBO funds allowed the two states to do more monitoring to target areas for SAV planting.

Results and implications for restoration. The water quality monitoring data were spatially interpolated and used in a GIS-based restoration site selection tools used in both states to choose SAV planting locations.

Further information. The project is almost complete, with a final report expected by April 2008. The spatially and temporally intensive water quality data that were partially funded by this project, accessible through URL: http://mddnr.chesapeakebay.net/eyesonthebay/index.cfm

Project Title: "Wave Exposure: An Additional Parameter for Identification of Suitable SAV Restoration Sites"

Funding recipient. Evamaria Koch, University of Maryland Center for Environmental Science

Total Federal funding. $133,129 (NCBO)

Background and research objectives. This project examined SAV habitat requirements for wave exposure in all Chesapeake Bay habitats, and resulted in a revised wave exposure model (developed by Mark Fonseca at the NOAA Beaufort Lab, who was an unfunded partner on the project) that can be used in restoration site selection.

Results and implications for restoration.

To date, the principal finding is that different SAV species in the upper and lower Chesapeake Bay have differing wave energy thresholds. The maximum wave height that SAV could tolerate in the upper Bay was $\leq$ 0.15 m, while in the mid and lower Bay this value was 0.30 m.

Further information. The final report is expected by January 2008. See also:

Chen, S.-N., L. P. Sanford, E. W. Koch, F. Shi, E. W. North. 2007. A nearshore model to investigate the effects of seagrass bed geometry on wave attenuation and suspended sediment transport. *Estuaries* 30(2):296–310 (a related project, not funded by this award).

Project Title: "Seagrass Habitat Engineering: Defining the Needed Balance in Wave Attenuation."

Funding recipient. E. Koch, University of Maryland Center for Environmental Science Horn Point Lab

Total Federal funding. $ 172,400 (ERDC)

Background and research objectives. Seagrasses are commonly found in wave-protected areas. In higher wave energy environments, seagrasses can be found in areas sheltered by coral reefs or sand bars. Seagrass density appears to be inversely related to wave exposure (Fonseca et al. 1983). Therefore, increased wave attenuation is likely to result in more dense seagrass populations. Both sandbars and breakwater structures are quite effective in attenuating waves. Breakwaters were initially thought to improve seagrass habitat quality by: (1) reducing wave energy and thereby also improving water quality via reduced sediment re-suspension, and (2) reducing sediment movement, allowing seagrasses to become well-established and dense.

However, preliminary data indicate that restricted water flow and reduced wave energy have the potential to be detrimental to (shoreward) seagrasses in the long run. Fine sediments deposited shoreward of the breakwater become re-suspended at lower wave energy than the coarser sediments offshore of the structure. As a result, turbidity in the area protected by the breakwater is not improved. Additionally, the fine particles that tend to deposit shoreward of the breakwater usually have a high organic content. Sediments with high organic content are believed to be detrimental to seagrasses (Koch 2001), therefore, coastal structures such as breakwaters have the potential to be detrimental to seagrasses unless flushing and re-suspension is sufficient to allow fine and organic particles to be removed from the site on a regular basis, perhaps by storm events. This project proposes to determine the appropriate degree of wave attenuation favorable for seagrass establishment and sustainability.

Results and implications for restoration. The results of this study could be used in the engineering of seagrass habitats for large-scale restoration projects. For example, the U.S. Army Corps of Engineers Norfolk District proposed to dredge a new Federal channel in an existing natural channel in Nassawadox Creek, VA. If sandy dredged material were deposited in the form of a longshore sand bar, this may reduce wave energy sufficiently for existing seagrasses to expand in density and size; an indirect form of seagrass restoration. There are numerous examples of other such opportunities. Appropriately designed breakwater structures can protect the shoreline from further erosion while also creating suitable seagrass habitat. These examples illustrate the emergence of a new field: engineering seagrass habitats for large-scale restoration.

Further information. A final report is expected in 2008. See also:

Koch, E. W., L. P. Sanford, S. Chen, D. J. Shafer, and J. M. Smith. 2006. *Waves in seagrass systems: Review and technical recommendations*. ERDC TR-06-15. Vicksburg, MS: U.S. Army Engineer Research and Development Center. http://el.erdc.usace.army.mil/elpubs/pdf/tr06-15.pdf

Other Applied Research Projects

Project Title: "Breaking Barriers in SAV Restoration: Using Plant Associated Bacteria To Enhance Restoration Success"

Funding recipient. Byron Crump, University of Maryland Center for Environmental Science (UMCES)

Total Federal funding. $66,955 (NCBO)

Background and research objectives. The goal in this project was to characterize the bacteria attached to the leaves and roots of field collected and laboratory-reared eelgrass (*Z. marina*), wild celery (*V. americana*), sago pondweed (*S. pectinata)*, and redhead grass (*P. perfoliatus*) collected from different regions of the Chesapeake Bay. Molecular and cultivation techniques were used to discover micro-organisms common to submersed aquatic vegetation (SAV), characterize potential symbionts, and cultivate candidates to use for probiotic technology development.

Project objectives

- Develop probiotic treatments that use naturally-occurring, plant-growth-promoting micro-organisms to enhance the success of SAV restoration.
- Identify and cultivate candidate plant-growth-promoting micro-organisms associated with leaf and root surfaces of four Chesapeake Bay SAV species.
- Evaluate positive effects of these naturally-occurring micro-organisms by re-introducing them before planting or propagation of SAV.

Results and implications for restoration. This study characterized the diverse communities of bacteria attached to the leaves and roots of four important Chesapeake Bay SAV species. These communities were consistent across plants of the same species within beds suggesting that plants host specialized bacterial communities. Also, these communities were vastly different among plant species, including plants in the same bed. While this lends strength to the idea that plants host specialized and potentially symbiotic bacterial communities, it complicates the search for bacteria that help plants grow because it implies that different plants require different bacteria. However, DNA sequencing efforts identified a few classes of bacteria that were found on more than one plant species, suggesting that within these complex bacterial communities a handful of bacteria may be common across plant species. It is possible that this small fraction of the bacterial communities functions similarly on all plant species.

Attempts to improve the growth of SAV in tissue culture by introducing naturally occurring bacteria provided statistically significant results for two of the three mesohaline species tested (sago pondweed and wild celery) i.e., leaf and rhizome mass was elevated relative to root mass when bacteria were present. Effects of bacteria on eelgrass could not be evaluated because tissue culturing of eelgrass was unsuccessful. This may be a problem with the composition of the media or, alternatively, these plants may be unable to grow in the absence of one or more micro-organisms.

This represents the first piece of evidence that bacteria form mutualistic relationships with SAV. It remains unclear whether these relationships are positive or negative, but if they are positive it suggests that bacteria could serve as probiotics for improving restoration success. Future research in this area will focus on isolating bacteria related to those found on multiple SAV species, and examining the impact of pure-cultured organisms on the growth of SAV.

Further information

Crump, B., and E. Koch. 2007. *Breaking barriers in SAV restoration: Using plant-associated bacteria to enhance restoration success*. Final Report on NOAA Chesapeake Bay Office Grant No. NA04NMF4570415. http://noaa.chesapeakebay.net/

Project Title: "Coupling Oyster and SAV Restoration in South River, MD"

Funding recipient. Lee Karrh, MDDNR

Total Federal funding. $108,195 (NCBO; NA05NMF4571249)

Background and research objectives. The ability of bivalves such as oysters to filter water and, in the process, improve water clarity and possibly enhance SAV growth, is well documented (Leffler 2001; Newell and Koch 2004). Zebra mussel invasions in the United States usually improved water clarity and SAV populations, for example in the Hudson River (Strayer et al. 1999). In Chesapeake waters, some have argued that the explosion of the exotic SAV Hydrilla (*Hydrilla verticillata*) in the Potomac River in the early 1980s was facilitated in part by an explosion of the exotic Asiatic clam, *Corbicula fluminea*, in the same region of the Potomac at about the same time (Phelps 1994), although water quality monitoring data were sparse during the explosions of both species. In the Chesapeake Bay, localized explosions of dark false mussels (*Mytilopsis leucophaeata*) in some mesohaline tributaries of the Chesapeake Bay in 2004 led to localized improvements in water clarity, and SAV increases in some of the areas with improved water clarity (Goldman 2007; Bergstrom, unpublished data).

This research project used a small tidal cove on Harness Creek in the South River, MD, as its study site. A small cove was chosen because the oysters' filtration would have a larger impact on a smaller volume of water. The cove has a fairly narrow entrance so most of the water entering the cove passes over the oyster bar. Two continuous water quality monitoring probes were installed, one on either side of the restored oyster bar, and SAV planting was attempted inside the cove.

Results and implications for restoration. Results to date showed that, while the oysters did appear to improve water quality inside the cove, at least at certain tidal stages, that improvement was insufficient to allow planted SAV to survive in the cove.

Due to limited availability, oysters were placed at lower than expected densities, and thus lower than expected filtration rates. Subsequent monitoring efforts found many fewer oysters than expected based on the initial planting density and usual survival rates. Since few dead oysters (boxes) were found, this suggested that either the monitoring was missing some of the oysters (perhaps because they had shifted), or that some had been removed by poachers.

These results are also complicated by the fact that natural SAV beds have done poorly in the South River in the last few years; no SAV beds were mapped there in 2006, and only one bed was mapped in the river in 2007 (Orth et al. 2007d; unpublished data). The main SAV species being planted in the cove, redhead grass, has not been found recently anywhere in the South River, even though salinity conditions are favorable for it, despite several other attempts to plant it in the South River. The one healthy bed in the river in 2007 was made up of three other SAV species (horned pondweed, widgeongrass, and sago pondweed (Bergstrom, unpublished data).

The project is continuing with other sources of funding. As more oysters are added, the effects of their filtration may become more pronounced.

Further information. The final report is expected by April 2008. Preliminary results are available online, including a final report from an earlier phase of the project that was funded by NOAA's Community Restoration Program, accessible through URL: http://www.dnr.state.md.us/bay/sav/restoration/hc_gen_info.asp

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14. ABSTRACT

In 2003, the U.S. Army Engineer Research and Development Center (ERDC) and the National Oceanic and Atmospheric Administration Chesapeake Bay Office began a comprehensive research effort to restore submerged aquatic vegetation (SAV) in the Chesapeake Bay region. The effort employed an agricultural approach to restore under-water grasses by using seeds to produce new plants and mechanical equipment to plant seeds and harvest. Since this research initiative began, an average of 33 acres/yr of SAV has been planted in the Chesapeake Bay, compared to an average rate of 9 acres/yr during the previous 21 years (1983–2003). New techniques and equipment developed as part of this research have introduced the capability to collect and disperse millions of eelgrass seeds. These results demonstrate these programs' success in developing tools and techniques necessary to plant SAV at scales unattainable with technologies existing only a few years ago.

15. SUBJECT TERMS

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