# Submerged Aquatic Vegetation (SAV) Reproductive Ecology in the Chesapeake Bay: Evaluating the State-of-the Knowledge and Assessing Future Research Needs

**Report of the STAC Workshop**
**March 6-7, 2007**
**Annapolis, Maryland**

**STAC Publication 07-006**

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The Scientific and Technical Advisory Committee (STAC) provides scientific and technical guidance to the Chesapeake Bay Program on measures to restore and protect the Chesapeake Bay. As an advisory committee, STAC reports periodically to the Implementation Committee and annually to the Executive Council. Since it's creation in December 1984, STAC has worked to enhance scientific communication and outreach throughout the Chesapeake Bay watershed and beyond. STAC provides scientific and technical advice in various ways, including (1) technical reports and papers, (2) discussion groups, (3) assistance in organizing merit reviews of CBP programs and projects, (4) technical conferences and workshops, and (5) service by STAC members on CBP subcommittees and workgroups. In addition, STAC has the mechanisms in place that will allow STAC to hold meetings, workshops, and reviews in rapid response to CBP subcommittee and workgroup requests for scientific and technical input. This will allow STAC to provide the CBP subcommittees and workgroups with information and support needed as specific issues arise while working towards meeting the goals outlined in the Chesapeake 2000 agreement. STAC also acts proactively to bring the most recent scientific information to the Bay Program and its partners. For additional information about STAC, please visit the STAC website at www.chesapeake.org/stac.

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Cover photo of seahorse in an eelgrass (*Zostera marina*) bed provided by Michael Naylor, Maryland Department of Natural Resources.



STAC Administrative Support Provided by:
Chesapeake Research Consortium, Inc.
645 Contees Wharf Road
Edgewater, MD 21037
Telephone: 410-798-1283; 301-261-4500
Fax: 410-798-0816
http://www.chesapeake.org

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**Workshop Steering Committee:**

**Peter Bergstrom (NOAA Chesapeake Bay Office)**
**Lee Karrh (Maryland Department of Natural Resources)**
**Mike Kemp (U. MD Center for Environmental Science, STAC)**
**Evamaria Koch (U. MD Center for Environmental Science)**
**Ken Moore (Virginia Institute of Marine Science)**
**Mike Naylor (Maryland Department of Natural Resources)***
**Becky Thur (Chesapeake Research Consortium)**

**Workshop Chair*



**STAC Publication 07-006**

## TABLE OF CONTENTS

# Executive Summary

## *Overview*

On March 6th and 7th, 2007, members of the Chesapeake Bay Program's Submerged Aquatic Vegetation (SAV) Workgroup, and invited guests, held a workshop designed to characterize our understanding of the modes of reproduction and expansion for Chesapeake Bay SAV. This workshop was intended to help develop an understanding of how the Bay's diverse SAV communities have changed in distribution over time, how they might be expected to reestablish and expand following disturbance, and how resilient each species can be.

Day one of the workshop focused on the reproductive ecology of the high salinity species, *Zostera marina* (eelgrass), in the both the Chesapeake Bay and Long Island Sound. Presentations were given by Chris Pickerell and Bob Orth. Follow-up group discussion touched on topics such as eelgrass community dynamics, seed dispersal, and reproductive strategies. In day two, presentations and discussion transitioned to the reproductive strategies and output of oligohaline and freshwater species, as well as the general seed ecology of aquatic plants. Presentations were provided by Dwilete McFarland, Steve Ailstock, and John Madsen.

Through these presentations and discussions, the group identified gaps in knowledge relative to the reproductive ecology of SAV, and developed research questions specific to filling those gaps. Specific topics included characterizing modes of reproduction for key SAV species, identifying barriers to the germination of seeds and seedling establishment, creating a propagule budget, and understanding mechanisms of SAV expansion. The group also created a summary table for 15 Chesapeake Bay SAV species and 25 separate parameters as a way to organize and synthesize the current state of knowledge. Workshop follow-up measures were detailed, including intentions to re-visit and update the landmark 1978 Stevenson and Confer[1] publication.

## *Workshop Objectives*

1. Review the current state of knowledge of the reproductive ecology of SAV species in the Chesapeake Bay.
2. Identify gaps in knowledge and specific research questions related to these issues.
3. Develop priority research recommendations.
4. Define specific actions to be undertaken by the Chesapeake Bay Program Living Resources Subcommittee's SAV Workgroup.
5. For each SAV species in the Bay, [coontail (*Ceratophyllum demersum*), slender pondweed (*Potamogeton pusillus*), curly pondweed (*Potamogeton crispus*), water stargrass (*Heteranthera dubia*), wild celery (*Vallisneria americana*), southern

[1] Stevenson, JC and Confer, NM. Summary of Available Information on Chesapeake Bay Submerged Vegetation. Fish and Wildlife Service, Office of Biological Sciences FWS/OBS-78/66, August, 1978. 335, 50 fig, 88 tab, 747 ref.

naiad (*Najas guadalupensis*), elodea (*Elodea canadensis*/*Elodea nutalli*), hydrilla (*Hydrilla verticillata*), spiny naiad (*Najas minor*), Eurasian watermilfoil (*Myriophyllum spicatum*), redhead grass (*Potamogeton perfoliatus*), sago pondweed (*Stukenia pectinata*), horned pondweed (*Zannichellia palustris*), widgeon grass (*Ruppia maritima*), and eelgrass (*Zostera marina*)], document important facets of physiology and reproductive ecology in a summary table (see Appendix A).

## *Knowledge Gaps*

1. We need to better understand the factors that affect lateral shoot formation in eelgrass such that we could maximize shoot production in transplants and/or seeding efforts.
2. We need a better understanding of eelgrass flowering ecology, identification of the cues which start seed development, and the extent to which water depth, sediment, and nutrient dynamics affect the height of reproductive shoots.
3. We should think about the relative contribution of clonal versus sexual reproduction roles in bed maintenance and expansion for all SAV species.
4. We should find out how important redox is as a habitat characteristic for selecting potential restoration sites.
5. We should work toward a better understanding of factors that influence seed germination rates and seedling survival, such as environmental stressors.
6. We need to establish a propagule budget for all species, including propagules other than seeds, that accounts for sources and withdrawals.
7. For many species, there is still a need to determine the best time of year for planting seeds for restoration.
8. Through genetics work, we need to achieve a better understanding of population structure, ecotypes, and restoration tracking for all species.

## *Specific Research Questions*

1. What are the sources of variability in seed production, germination, and survival?
2. What is the role of sediment redox in germination and plant recovery?
3. What role does fragmentation play in eelgrass expansion?
4. In eelgrass, what length or amount of rhizome remains viable and contributes to the growth of a new terminal shoot after a clone breaks into multiple combs?
5. Are adult plants more likely than seedlings to survive at borderline conditions?
6. What are the causes of low seed germination rates in the field? What are the most significant barriers to and requirements for seed germination/seedling survival?
7. Are there annual and perennial populations of eelgrass in Chesapeake Bay and if so, how are they different?
8. What are the best methods for determining propagule availability by species?
9. What are the dynamics of SAV reproduction along the outer edges of beds, both nearshore and towards deeper water?
10. Does curly pondweed exhibit two or more growth forms or ecotypes?

## *Recommendations*

The workshop recommendations are divided into two categories below:

### High Priority Research Recommendations:

- More research is needed to quantify the magnitude of the SAV propagule budget in the Chesapeake Bay. This should include seed bank studies, measures of species specific reproductive output and potential, propagule persistence within the sediments, and processes that affect the supply and viability of these propagules.
- Understanding the mechanisms of propagule dispersal and seedling establishment may relieve an important bottleneck to SAV recruitment and restoration. Specific questions of importance include:
    - What are the habitat conditions most conducive or detrimental to propagule establishment?
    - What are the roles of intra- and interspecific competition in bed formation, development and succession, i.e., seedlings versus adults of the same species, multi-species competition, or annuals versus perennials?
    - What role do herbivores play in seed dispersal, fragmentation, and seedling survival?
- The genetics of SAV can be important for understanding population structure, ecotypes, species diversity, positive species identification, and restoration tracking. In general this information is lacking for Chesapeake Bay species and warrants further investigation.

### Recommendations for SAV Workgroup Action:

- Promote within the Chesapeake Bay Program the research agenda and questions identified within these proceedings and support the acquisition of funds through STAC and other sources.
- Revisit the idea of revising the Technical Synthesis document to incorporate habitat requirements for additional parameters, such as sediment redox, water temperature, colored dissolved organic matter (cDOM), and low dissolved oxygen, in addition to interactions between such parameters.
- Coordinate with the CBP Sediment Workgroup to identify nearshore sediment distributions in the Bay conducive to SAV recovery and restoration, towards further refinement of habitat targeting strategies.
- Consider revising the SAV acreage goals for certain tributaries in which the current acreage has already surpassed a very low initial goal.
- Establish better, more consistent SAV monitoring protocols that help to characterize both species distribution and propagule availability.
- Support efforts to coordinate long-term quantitative monitoring of SAV by species in low salinity waters.

# Agenda

**"Submerged Aquatic Vegetation Reproductive Ecology"**
**March 6-7, 2007**
**U.S. Fish and Wildlife Service Chesapeake Bay Field Office**
**Annapolis, Maryland**

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**Objective:**

For each Chesapeake Bay SAV species of interest, [coontail (*Ceratophyllum demersum*), slender pondweed (*Potamogeton pusillus*), curly pondweed (*Potamogeton crispus*), water stargrass (*Heteranthra dubia*), wild celery (*Vallisneria americana*), southern naiad (*Najas guadalupensis*), elodea (*Elodea canadensis*/*Elodea nuttalli*), hydrilla (*Hydrilla verticillata*), spiny naiad (*Najas minor*), Eurasian watermilfoil (*Myriophyllum spicatum*), redhead grass (*Potamogeton perfoliatus*), sago pondweed (*Stuckenia pectinata*), horned pondweed (*Zannichellia palustris*), widgeon grass (*Ruppia maritima*), and eelgrass (*Zostera marina*)], document and synthesize into a proceedings document the following:

1. Mode(s) of reproduction
2. Mechanisms for natural expansion
3. Potential restoration techniques
4. Methods for propagation and establishment
5. Gaps in knowledge related to above issues

**March 6, 2007**

8:30 Check-In & Coffee

8:50 Mike Naylor – Workshop and participant introductions

9:00 Chris Pickerell – Reproductive strategies of eelgrass (*Z. marina*) on Long Island, NY: Implications for restoration

9:45 Bob Orth/Tom Parham/Chris Tanner – Eelgrass in the Chesapeake: Reproductive biology and ecology and implications for restoration

10:30 Group Discussion

12:00 Catered Lunch

1:00 Group Discussion

3:15 Steering Committee meeting/Synthesis of day one; All others adjourn

5:30 Group dinner at Viet-Thai Paradise, 2526 Riva Rd, Annapolis, MD

**March 7, 2007**

8:00 Steve Ailstock – Seed propagation: Methods for *Potamogeton perfoliatus*, *Ruppia maritime*, and *Stuckenia pectinata*

8:45 Dwillette McFarland – Propagule production and success in *Vallisneria americana*: Focus on sexual reproduction

9:30 John Madsen – Life history characteristics of multiple SAV species and the implications for their use in revegetation and natural dispersion

10:15 Group Discussion

12:00 Catered Lunch

1:00 Synthesis Session (information summary, identify desired workshop product(s), and identify next steps)

3:15 Adjourn

# Workshop Report

## *Presentation Summaries – Tuesday, March 6*

### Reproductive strategies of eelgrass (*Z. marina*) on Long Island, NY: Implications for restoration – Chris Pickerell

Following are the results and observations from three different regions of study:

| High Energy (Long Island [LI] Sound & Gardiners Bay) | |
|---|---|
| Habitat Characteristics: | Good water quality; high wave energy[2]; One continuous or two bands of eelgrass typically exist parallel to shore; lots of erosion occurs on the inshore edges of these beds; sediment in beds ranges from fine sand to a hard packed peat/gravel |
| Eelgrass Bed Reproductive Dynamics: | Rhizome expansion is the primary method of natural bed maintenance; seeds are held down and retained in the downstream eddy adjacent to meadows at some sites; seeds within beds are retained in depressions at the base of adult plants and germinate. If the primary shoots are lost, seedlings take their place. |
| Threats to Survival: | Hermit crabs prey opportunistically on seeds/seedlings beyond bed edges creating a “wall” of grass around the perimeter of most meadows, but impacts on eelgrass population unknown; shallow-water areas with no sand accumulation do not recruit seedlings because the sediment is too hard (gravelly peat); waves |
| Restoration Strategies: | Seeding does not work unless to infill existing beds (increase genetic diversity) or as a screening tool to check site suitability for adult transplants; rock planting[3] of adult planting units |
| Results: | Unprotected (outside of an existing canopy) seedlings do not typically survive > 1 year |

| Low Energy / Sheltered Bay (Peconic Bay, East end) | |
|---|---|
| Habitat Characteristics: | Shallow; poor water clarity; high temperatures (from 26-28ºC); low currents; few waves; fine sediments |
| Eelgrass Bed Reproductive Dynamics: | Seeds are a significant factor in recruitment both within and outside of meadows; some sites forced into annual expression; seed dispersal to open areas by drifting reproductive shoots and/or floating seeds |
| Threats to Survival: | High temperatures and low light in late summer; High percent organic matter could also be a factor |
| Restoration Strategies: | Broadcast seeding, seed buoys and transplants |
| Results: | Shallow meadows may be more persistent than adjacent deeper meadows (~ 1 m deeper); high summer mortality; very low success here |

[2] Eelgrass was found at Mulford Point where it was thought that wave energy was too high. Mulford Point has a very strong tidal current (a few knots).
[3] Pickerell et. al. 2007. In Preparation.

| Coastal Lagoon (South Shore Estuary Reserve) | |
|---|---|
| Habitat Characteristics: | Poor water clarity; wind-generated waves; shallow, variable temperatures but high (> 28ºC) in sites distant from inlet; low light (summer); variable sediment |
| Eelgrass Bed Reproductive Dynamics: | Generally better water quality, lower wave energy, and cooler water increases seedling recruitment. plants look much more like Chesapeake Bay plants (short with lots of lateral shoots); high natural recovery rate by rhizome expansion, lateral shoot initiation and seedling recruitment; maximum growth in fall |
| Threats to Survival: | High temperatures; low light in late summer; wave disturbance |
| Restoration Strategies: | Combination of seeding and transplants is likely to do well, with seeding as most efficient method |
| Results: | Plan to use seed buoys and broadcasting and compare results |

*Overall Lessons Learned:*

- The LI eelgrass seed collection window runs from June through August
- Don't count successes until you have plants in September
- Regular and long-term monitoring is critical, including temperature
- There has been a paradigm shift in restoration site-selection from creeks and harbors to bays and LI Sound because of better success. This may be related to wave energy and/or percent organic matter in sediment, temperature, water clarity and water quality
- Opportunistically obtain adult shoots from "blow-outs", i.e., wrack
- Helpful to track plots with little rocks for record keeping (density, shoot #, diver, etc.), and perform intensive test plot monitoring to predict spread
- Seed recruitment in high-energy sites is not good and there is an inability to get long-term survival (> 1yr) of seedlings at any site regardless of methods; cannot restore in sheltered embayments within Peconic Estuary

*Questions & Answers:*

Q1. How do you perform site selection?

A1. We consider fetch data, boating and shellfishing activities, Fred Short's Preliminary Transplant Suitability Index (PTSI) model[4], similar bottom types and exposures, and test plots (1 $m^2$ circular plots of adult plants perpendicular to shore). The PTSI model worked pretty well in the Peconic Estuary, but was skewed by high temperatures so we had to adjust the temperature data by leaving the very warm creeks with eelgrass (Noyack and Bullhead) out of the model.

Q2. How long have your adult transplants lasted? Why has there been more success with that over seeds?

A2. Transplants have lasted 3 years in both the Peconic and Long Island Sound Estuaries, If transplants are lost, losses typically occur during August or September. The high initial biomass of adult plants allows them to survive through the winter and into the next growing season. Seedlings tend to die-off at most sites as temperatures peak in August or September and there isn't enough biomass to hold them over through this stressful period.

[4] Short, F.T. and D.M. Burdick. 2005. Eelgrass Restoration Site Selection Model. CD-ROM and Manual. CICEET, University of New Hampshire, Durham, NH.

Q3. Is wasting disease a constant in all 3 environments?
A3. Wasting disease appears to show up in cooler, deeper, higher salinity sites where there is no epiphytic fouling. But, maybe fouling covers it up at other sites. We are just starting to investigate that now. We do not think it is the cause of die-off.

Q4. Can you talk more about the annual form of eelgrass? What are those habitats like? What is the flowering proportion in these areas?
A4. The western-most Peconic Estuary does not have complete annual expression, but more than in our unprotected bays. Annual beds produce seed and revegetate, so it is not true annual expression. The seedlings die off later so it seemed similar to annual expression because of the scale on which it was occurring. The beds look fine in June but epiphytes increase in July and August. About 10-15% of shoots flower each year.

Q5. Have you looked at dissolved oxygen levels?
A5. No. There is not a lot of water movement at the fine sediment sites so we guess that dissolved oxygen is not high.

Q6. How do seedlings die?
A6. We will visit a site and they are just gone, as if overnight! We expected to count lateral shoots because they looked so good before and so we were not going back every day/week to check on them. They have no rhizome to find so they just disappear.

Q8. How much sediment deposition is occurring at the sheltered site?
A8. Not much at all from May - June. We can still see the outline in the sediment of where we set down the PVC grid from our previous monitoring visit.

Q9. What happens to plants at the sheltered site that do not disappear annually? Do they foul?
A9. There is fine sediment floccing and a little bit of fouling. We may have lost the bed last year but it still seems to be there. It looks bad in July and August with lots of epiphytes and macroalgae in place. The temperature gets up to 28ºC at this site. The bed has existed there at least since 1930. Expansion occurred in ’01-02.

Q10. Are there more fine sediments and organic matter in the deeper part of the sheltered site?
A10. Yes, the sediments are finer and percent organic matter is higher.

## Eelgrass in the Chesapeake: Reproductive biology and ecology and implications for restoration – Bob Orth/Tom Parham/Chris Tanner

*Orth: Overview of Eelgrass Reproduction*[5]

Eelgrass reproduces by both sexual and asexual reproduction. Vegetative growth measures about 25-30 cm/yr. Eelgrass is monoecious like most terrestrial plants, unlike most SAV which are dioecious (e.g. wild celery). In order to guard against self-fertilization, pollen is not released at the same time that the stigma is receptive. Eelgrass uses hydrophilous, or submerged pollination[6].

Seeds measure approximately 3.5 x 1.5 mm and are produced at an average rate of 8,000 seeds per square meter up to millions per acre in the Chesapeake Bay. Overall, there is high variability in seed production in space and time (range of 1 – 6,000/$m^2$, average 500/$m^2$). The average percent viability of any given seed set is unknown. Blue crabs are a significant predator of eelgrass seeds and the high number of hermit crabs in the coastal bays may also be a problem.

Seeds typically experience a dormancy period of 5-10 months before germination around October in Chesapeake Bay; however, this can be shortened to a month or less with vernalization. But how is it induced naturally? In a laboratory setting, seeds germinate as well in water with no dissolved oxygen as they do in normal sediment, which suggests that redox is a major driver of germination[7]. Both salinity and temperature are also significant factors in determining eventual percent germination.

Under certain calm conditions, seed dispersal may occur via an attached air bubble that allows buoyant travel over short distances (tens of meters). Reproductive shoots can travel by floatation over much longer distances (hundreds of meters to tens of kilometers, with 109 km being the maximum documented). Seed establishment is affected by sediment topography and bioturbators. Loose (broadcast) seeds do not tend to move far from where they settle[3a]. And, for in-situ restoration projects, the number of germinating seedlings stays between 1-5 % regardless of seed broadcast density and site.

*Marion: Eelgrass Restoration by Seeds in Chesapeake Bay*

The Virginia Institute of Marine Science (VIMS) rented an eelgrass seed harvester in 2004, and then built a new kind of harvester in 2005. The harvester did not appear to

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[5] For a full reproductive phenology, see Orth, R.J. and K.A. Moore. 1983. Seed germination and seedling growth of *Zostera marina* L. (eelgrass) in the Chesapeake Bay. Aquat. Bot. 15:117-131.
[6] Cox, P.A. 1993. Water-pollinated plants. Scientific American. 269:68-74.
[7] Moore, K.A., R.J. Orth, and J.F. Nowak. 1993. Environmental regulation of seed germination in *Zostera marina* L. (eelgrass) in Chesapeake Bay: Effects of light, oxygen, and sediment burial depth. Aquat. Bot. 45:79-91.
[3a]Orth, R. J., M. L. Luckenbach, and K. A. Moore. 1994. Seed dispersal in a marine macrophyte: Implications for colonization and restoration. Ecology. 75:1927-1939.

have a significant negative impact on donor beds. VIMS harvested a record 7 million seeds per day in 2004 using the mechanical harvester.

VIMS, the Maryland Department of Natural Resources (MD DNR), and St. Mary's College of Maryland (SMCM) conducted multiple experiments to determine optimal eelgrass seed storage techniques. Experiments during the first year specifically addressed different water and air treatments. Seeds were aerated to prevent germination during storage. The use of raw (unfiltered) river water was deemed detrimental due to the build-up of organic matter, thus, a filtered recirculation water system was most successful for keeping seeds clean.

In the second year of experiments, the groups tested salinity and temperature effects. The best survival was obtained using cool, higher salinity water. The salinity effect was greatest in warm water, such that there was very low seed germination at 12 or 20 ppt, even among "good" viable seeds, at the end of the storage period. When water temperatures were kept cool (3-5ºC), salinity did not have a significant negative effect over the 12-30 ppt range. The fall velocity[8] of a seed in water was a good predictor of its quality; the best seeds fell at 5 cm/sec or greater. The VIMS laboratory work was supported by SMCM planting experiments, which had much higher seed survival and germination in protected burlap bags in the field than in the greenhouse. Chris Tanner reported that the highest recruitment of seeds in SMCM experiments was achieved at a medium planting depth of 1cm, or optimal redox.

When VIMS tested recruitment of new shoots from seed using an adapted buoy method (from Pickerell et al. 2006[9]) in 2004, there was only 1% recruitment in the spring. In contrast, there was a much higher germination success from fall broadcasts of stored seed, possibly indicative of some kind of summer stressor limiting seeds recruited in the spring. VIMS believes the issue is timing rather than the buoys themselves because of the similar results obtained from spring and summer broadcasts, while fall resulted in the highest germination rates. VIMS decided not to pursue buoys further and MD DNR got similar results testing seed buoys.

In 2005, VIMS tested a planting sled designed by Michael Traber, Scott Nixon, and Stephen Granger[10] of Rhode Island. The gel matrix pump worked better than broadcasting and hand-injection for percent seedling establishment in the Piankatank River. In the Virginia coastal bays (e.g., Spider Crab Bay) the machine also did well, but overall hand-injected seeds by SCUBA did best. This may be partly due to the high-density clustering of seeds that result with hand-injection. VIMS tested their own

[8] The fall, or terminal velocity, is the particular falling speed for any given object moving through a fluid medium of specified physical properties, at which the drag forces and buoyant forces exerted by the fluid on the object just equal the gravitational force acting on the object. The object falls at a constant speed, unless layers of different physical properties are encountered. Glossary of Meteorology. 2nd Ed. 2000. American Meteorological Society. http://amsglossary.allenpress.com

[9] Pickerell, C. S. Schott, and S. Wyllie-Echeverria. 2006. Buoy-deployed seeding: A new low-cost technique for restoration of submerged aquatic vegetation from seed. SAV Technical Notes Collection (ERDC/TN SAV-06-2), U.S. Army Engineer Research and Development Center, Vicksburg, MS.

[10] Patent No. US 6718890 B1, issued April 13, 2004, for an "Underwater seagrass seeding apparatus."

mechanical seed injector to establish some test plots in fall of 2006. It worked to satisfaction in initial trials, but needs more work to optimize seed planting depth.

*Questions & Answers:*

Q1. Why were there such low numbers resulting from the York River planting and burial trials?
A1. The low germination resulting from all planting methods in the York River at Mumford may have been due to a disturbance issue; there is a different sediment structure in the York. In contrast, the coastal bays experience a high level of bioturbation, but also had highest germination. An abundance of seedlings develop around the worm tubes of the polychaete, *Diopatra cuprea*[11], which probably helps germination.

Q2. Do you pretest seed lots prior to field study?
A2. Yes.

Q3. How do you determine distance of seed dispersal?
A3. By counting seedlings from where they are released to where you see the first seedling. You can most easily do this at unvegetated sites that have been bare for some time.

Q4. Has anyone counted seeds after dispersal?
A4. Only in cores (C. Pickerell). Most of the sites tested have no other nearby seed sources, and they don't move very far.

Q5. Looking at seed banks, what is the variability at the time of germination?
A5. We sample in November to gain an appreciation of the dynamics of seed banks.

Q6. How are seed primordia data obtained?
A6. By using a dissection microscope to look at the spathe. But we don't know what the cue is to start development.

Comment 1: We need an experiment that germinates seeds in the lab and then puts them in the field as a jump start to flowering. Can we compare these with a more southern annual population?

Comment 2: Reproductive shoots last year in Maryland were about half the normal height so the harvest boat couldn't be used by MD DNR for seed collection. VIMS also sees variability across sites in the height of reproductive shoots. Maybe water depth, sediment, and nutrient dynamics are important factors.

[11] Harwell, M.C. and R.J. Orth. 2001. Influence of a tube-dwelling polychaete on the dispersal of fragmented reproductive shoots of eelgrass. Aquat. Bot. 70:1-7.

## General Discussion from Day 1

*Thread 1: Sources of variability in seed production, germination, and survival.*

Why do SAV flower? Nobody knows. Other aquatic plants need to be at the right stage (at change in nutrient status of plant and transition from prostrate to erect growth stage). Can it be a change of stage or $O_2$ tension (as they approach the surface), or is it change in nutrients, such as the internal N and P tissue pool? Higher plants are photoperiodic, i.e., the phytochrome system triggers floral initiation and first differentiation of meristematic tissue after 15-hour days and nutritional maturity. Then it depends on a lot of other things, like temperature and nutrition. The phytochrome system tends to be ignored in aquatics, i.e., light is <u>not</u> a factor in SAV germination. To see primordia in January suggests that *Zostera* is a short-day plant. The flowering of an annual and a perennial are very different.

Seeds can grow under atypical conditions. Chris Pickerell had some eelgrass seeds germinate in a lab holding tank where the water's salinity reached about 40 ppt due to inadvertent evaporation. When fresh water was added back, the water clouded and the shoots died!

In 2006, the reproductive shoot height of eelgrass seemed to be half of what was seen in the previous year. Why? Bob Orth thinks this was due to high temperatures in 2005. Lee Karrh said that the only tall plants that MD DNR found in 2006 were at sites that had not been harvested before, such as in the Big Annemessex. They are not sure why, but speculate that it may not have been related to harvesting history. The best seedling recruitment in 2006 in Virginia appeared to be in areas where all adult plants were lost—the adults may suppress seedling establishment due to competition.

Are there any animals that move eelgrass seeds around by eating them (fish or birds), as they do for lower salinity species? Bob Orth is not sure yet, as the concept has not been tested in Chesapeake Bay. There were not a lot of waterfowl in Virginia when seeds were available in the past, but now there are resident Mute Swans and they should be tested for seed ingestion.

*Thread 2: What role does fragmentation play in eelgrass expansion?*

As a theme, we should think about the relative contribution of clonal versus sexual reproduction roles in bed maintenance and expansion for all SAV species.

Bob Orth stated that lower salinity plants can spread by vegetative fragments, but that this is probably rare for eelgrass. Mike Kemp asked about the possibility of dispersal of whole plant fragments that later sink and root, as happens in low salinity SAV – does eelgrass do this? Probably not because plants are more buoyant in salt water. Bob does not think eelgrass shoots will sink before they wash ashore as wrack or die.

Nancy Rybicki positioned propagule traps in the mesohaline (8 – 15 ppt) at 5 – 7 sites every couple of weeks for two years on the Virginia side of Potomac River, spread out over a wide area. The purpose was to trap plant fragments along the bottom. She was curious if plants from the transition zone were getting down that far. There is probably no chance for eelgrass to be there, as the closest population was Dameron Marsh. Should we propose to do propagule trap work for eelgrass? We could determine that the fragments are buoyant for "x" number of days and then start to sink through mid-water.

Can the fragments dislodged by cownose rays act as dispersants of viable shoots? Probably not. Laura Murray suggested that the simple thing to do is to put fragments in a tank in the lab and see how long it takes them to sink. If they grow after sinking, how long does that take?

Large peaty chunks can dislodge and move in the Long Island high energy sites. We should look after storm events for broken off chunks in the Chesapeake Bay and evaluate that relative to the other dispersal mechanisms. Maybe it is one small class of dispersal events we have overlooked. If the goal is to identify the distribution range, we should first look at dispersal of reproductive shoots from natural systems as the main thrust.

We should consider the nutritional status of a plant at the time the fragmentation occurs or of the fragments themselves. Dwilette McFarland has preliminary evidence that nutritional status has something to do with how fast the fragments sink.

*Thread 3: The role of sediment redox in germination and plant recovery.*

O2 inhibits germination in the sediments and sulfides may also play a role. We should find out how important redox is in terms of evaluating restoration sites. Perhaps the enhanced eelgrass seed germination at low DO that we see in the lab does not happen in the field. Low sediment and/or water column dissolved oxygen (DO) could cause meristematic death (kill rhizomes) at some sites in adult plants. This could be a factor in the loss of seedlings at Chris Pickerell's transient sheltered site. A 3-dimensional plot of temperature, redox, and salinity would be helpful for locating sites with the potential for the best germination or seedling survival. Mike Naylor proposed experiments on the effects of low DO on eelgrass. It would be easier to lower DO in the water by bubbling in nitrogen gas, but how would we test this in sediment? If a terminal meristem dies from low DO, can a new one form? We are not sure.

How much recovery in the Chesapeake Bay after the mass defoliation in 2005 occurred because of variations in whether the meristem survived and was able to regenerate? Work of Ken Moore and others suggests (Greve et al. 2003[12]; Borum et al. 2005[13]) that low DO in sediments can raise sulfide levels with the meristematic tissue of eelgrass and

[12] Greve, T.M., J. Borum, and O. Pedersen. 2003. Meristematic oxygen availability in eelgrass (*Zostera marina*). Limnol. Oceanography. 48:210-216.

[13] Borum, J., O. Pedersen, T.M. Greve, T.A. Frankovich, J.C. Zieman, J.W. Fourqurean, and C.J. Madden. 2005. The potential role of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass, *Thalassia testudinum*. J. Ecol. 93:148-158.

other seagrasses causing plant die-off. Unpublished research by Moore also suggests that as genets of clonal plants consisting of interconnected shoots experience a negative carbon balance they get smaller and smaller as they use up their combined carbon stores. If the thinned clones survive the summer they are usually able to recover and expand again. Seedling genets may not have enough of a combined reserve capacity to withstand extreme summer conditions and therefore may be more susceptible to summertime dieback in the Chesapeake Bay region. The adult plants in test plots may not survive under these same stressful conditions because, as with seedlings, their carbohydrate reserves may be reduced compared to those of shoots in established beds. Preliminary results of research by J. Jarvis suggests that recovery of eelgrass in 2006 in areas of the lower bay completely denuded of vegetation in 2005 was from both seedlings and rhizomes. This implies that some living rhizome meristematic tissue must remain in the sediment and that seeds can play an important role in initial recovery of beds following defoliation. Meristematic tissue is not likely being poisoned.

There are two different issues to investigate: What is happening to newly established plants versus established beds? What are the needs of each type of plant for being able to survive similar stressors? There was agreement with Katie (Preen) Busch's suggestion that conditions may be quite different when we are trying to establish new plants on bare bottom compared to adding plants to existing beds from natural recruitment. Physical characteristics of a bed can give the plants better water quality, such as when there is a vegetation canopy. This may indicate that there is a critical mass required for an initial patch of SAV in marginal areas to begin to modify the environment, i.e., in the upper Chesapeake Bay where water quality and water clarity are poor.

*Thread 4: Rhizome health*

Often only a small section of rhizome appears healthy in eelgrass. How far back from the primary shoot do you see living rhizome on established beds? A lot of it appears dark and unhealthy at end of the summer. Are carbohydrates translocating to shoots? Are carbohydrates moving from older to younger leaves? Do we have ecotypes such that in Long Island translocation happens at 26-28ºC, but in the Chesapeake Bay it occurs closer to 30ºC? Are there enzymatic adaptations? Freshwater plants can grow in water up to 34-35ºC. Chris Pickerell stated that the brown/rust colored rhizomes are still viable, but not the blackened ones. What is the contribution of physiological integration of clones within the first growing season? After a clone breaks into multiple combs, what length or amount of rhizome remains viable and contributes to the growth of a new terminal shoot?

*Thread 5: Are adult plants more likely than seedlings to survive at borderline conditions?*

We can culture plants with extensive rhizome mats, especially with fertilization within 4-5 months. But it is very expensive to keep them alive to two year plants and then transplant them to the field. What effect does flowering have? Does flowering deplete below-ground stores just before a plant enters into the hot summer period?

And if seeds cannot survive in a given location, should we even plant adult plants there, where conditions are not suitable for seedling recruitment and any growth would have to be by bed expansion? Chris Pickerell recommends that we may need to have adult eelgrass plants in high energy sites to shelter any seedlings.

*Thread 6: Causes of low seed germination rates in the field*

Why are seed germination rates so low in the field? What is happening to the seeds? We should consider investigating what the barriers are to seed germination. Monitoring is expensive, but these answers are just as important and need to be funded. Chris Pickerell is working with Dr. Jerry Churchill (Adelphi University, NY) to understand the process of ovule development in eelgrass and answer specific physiological questions about seed production.

Are predators a large factor? How much loss is due to seed predation versus failure to germinate or washout into deep water, etc.? As far as we know, blue crabs do not seek out eelgrass seeds; however, there are so many crabs that they must bump into the plants fairly often, and may eat the seeds opportunistically. Should we hold seeds until very late into the fall or even March before restoration to eliminate the possibility of blue crab predation? Becky Thur (CRC) suggested experiments on seed predation using wire cages to exclude crabs. Occasionally mud crabs are seen on eelgrass flowers. They are active at night on Long Island, as detected by low-light video and could be taking seeds then (Chris Pickerell).

Are high water temperatures a major factor in loss of seed viability? We need to distinguish between the natural seed budget and what effects temperature may be having. Has anyone done a seed budget to see what is in the sediment in early fall compared to what comes up as seedlings? No. The variability on tenths of square meters is phenomenal and so one cannot extrapolate accurately to a larger area. It appears that eelgrass does not have a prominent seed bank. Jessie Jarvis (VIMS) took cores recently and found eelgrass seeds in only 9% of them, of which none were viable. Matthew Harwell has some eelgrass seed core data for the Bay as well[14]. Looking for seeds in cores is very tedious work. Is it possible to get a seed budget for all of the Bay species? Seed bank studies are necessary! Bob Orth suggested that we need a graduate student to document seed banks for various species.

Lee Karrh said Stan Kollar had redhead grass (*Potamogeton perfoliatus*) seeds in sediment in an aquarium that germinated after a long period of time, so we know that redhead grass has a viable seed bank. Dormancy varies by species. Some lack it altogether, and in others it is short. It is a complex topic.

Laura Murray thinks that the spread of redhead grass in the Choptank River is from fragments rather than seeds, however; satellite colonies are only found near adult plants. Nontidal creeks and impoundments containing SAV act as seed banks as well, if the

[14] Harwell, M.C. and R.J. Orth. 2002. Seed bank patterns in Chesapeake Bay eelgrass (*Z. marina* L.): A bay-wide perspective. Estuaries. 25:1196-1204.

seeds are transported either by flow or by waterfowl. Mike Naylor noted that there is an abundance of sago pondweed (*Stukenia pectinata*) in impoundments on the Eastern Shore of Maryland.

*Thread 7: Annual versus perennial populations in eelgrass.*

Should we start considering our eelgrass populations as annual because they rely so much on seed? Eelgrass in North Carolina tends to be annual or perennial. According to Jud Kenworthy, the annual eelgrass there makes all of the reproductive shoots. Will we see these in the Chesapeake Bay as the water gets warmer? The North Carolina annual populations are probably maintained by seeds from perennial beds in deeper water.

*Thread 8: Revisions to the eelgrass row of the SAV summary table (Appendix X)*

Mike Naylor handed out his draft table summarizing what we know by SAV species about its reproduction. We reviewed this for eelgrass and filled in gaps, including adding some new columns and revising others.

Discussion for eelgrass metrics included:

- Salinity: Eelgrass grows in South Bay at up to 33 ppt, not 22 ppt as listed in the Chesapeake Bay SAV Identification Guide[15]
- Growing season column: Should this refer to the optimal, possible, or critical periods of eelgrass growth? Should we include a defoliation period? Should it be in terms of months or temperatures? The critical stress period appears to be roughly July 15-September 15. Optimal growth temperatures for vegetative growth are approximately 15-20ºC.
- Animal Interactions: Brant, and probably mute swans eat them, but seeds cannot pass through waterfowl intact, as far as we know. Research is needed to verify this. Do snails promote SAV survival? According to Chris Pickerell, they appear to in Long Island beds. But in the Chesapeake, it appears that blue crabs eat *Bittium varium* as fast as we add them. The Long Island snails also cannot recruit onto bare bottom between the plants.

[15] Bergstrom et al. 2006. Underwater grasses in Chesapeake Bay & Mid-Atlantic Coastal Waters: Guide to Identifying Submerged Aquatic Vegetation. Maryland Sea Grant. 72 p.

## *Presentation Summaries – Wednesday, March 7*

### Seed propagation: Methods for *Potamogeton perfoliatus*, *Ruppia maritima*, and *Stuckenia pectinata* – Steve Ailstock

Variation in the ambient conditions of the mid-Bay of the Chesapeake results in many unknowns for field work. Beds of SAV disappear and reappear, but they also change in species composition. A restoration seed mix would yield a greater likelihood of success, regardless of seasonal variation. All of the seeds should grow in a good or normal year. A mix can be customized depending on the restoration site, for instance, by adding in eelgrass (*Zostera marina*) for more saline water or wild celery (*Vallisneria americana*) seeds for fresher water. The standard mix includes redhead grass (*Potamogeton perfoliatus*), widgeon grass (*Ruppia maritima*), and sago pondweed (*Stuckenia pectinata*). The restoration cost using this mix is derived from what it would take to sod a one-acre yard ($14.375/acre).

*Reproductive Potential*

Counts of reproductive potential, i.e., the number of plants per unit area and flowering stems per plant, are HIGHLY VARIABLE! These three species are incredibly capable of producing an incredible number of seeds. 2006 was the first year that reliable flowers on *S. pectinata* were obtained in the lab.

**Table 1. Table of reproductive potential for three mesohaline Chesapeake Bay SAV species. Data reproduced from full presentation (see www.chesapeake.org/stac/SAVEcologyWorkshop.html).**

| Reproductive Potential | *Potamogeton perfoliatus* | *Ruppia maritima* | *Stuckenia pectinata* |
|---|---|---|---|
| **Plants per unit area** | Highly variable | Highly variable | Highly variable |
| **Flowering stems per plant** | Highly variable | Highly variable | Highly variable |
| **Inflorescence per flowering stem** | 2.4 | 2.6 | 1.2 |
| **Flowers per inflorescences** | 5-12 (mean of 9) | 2 | 4-12 (mean of 6) |
| **Carpels per flower** | 4 | 4 | 4 |
| **Ovules per carpel** | 1 | 1 | 1 |
| **Seeds per inflorescence** | 20-48 | 8 | 16-48 |
| **Seeds per flowering stem** | 48-115 | 20.8 | 20-59 |

*Seed Harvesting and Storage*

- Collected plant material composts readily. You can kill an entire seed lot in a matter of hours if you are not careful. Wrack needs to be processed and chilled very quickly.

- A week can make a tremendous difference in the developmental biology for flowering. See the full presentation for species-specific floral development data over time.
- There is a narrow seed harvest window, with main collections occurring from late July to early August. Bad weather (i.e., hurricanes) can wipe out an entire year of collection if you wait too long and poor pollination can lead to poor seed set, as seen in 2006. We did our first collection of *S. pectinata* in August 2006 at Kent Island.
- Seed harvesting collects seeds that mature at the same moment in time and that are the biggest seeds, etc. This selectivity can affect genotypes of restored populations.
- We harvest the upper 1/3 of the water column and store as wrack for 6-9 months at 4ºC to retain viability in *P. perfoliatus* and *R. maritima*. Seeds stored in this manner have reduced germination rates (12-25%) as compared to extracted seeds because of seed germination in the cold. There is more loss of germination in *R. maritima*.
- Direct seed deployment after collection has not worked in the past due to hurricane wash-outs at Poplar Island.
- A seed agitator is used to separate seeds from the wrack after storage.

*Seed Testing and Germination*

- A standard seed test consists of five petri plates containing 50 seeds each
- *P. perfoliatus* seeds germinate best after cold storage for 6 – 9 months in 15ppt salinity with aeration, and then submersion in fresh water.
- *R. maritima* stored at 4ºC experienced a lot of germination while in storage. It was a challenge to hold it dormant. The best results were achieved with storage at 21ºC. Most of the remaining seeds germinated after storage within a week.
- The second processing (agitation) of *P. perfoliatus* wrack produces seeds with equally good germination as the first collection. The additional storage time gives more seeds time to mature.
- *S. pectinata* had low germination rates in the first experiments, but a little better for seeds taken from the second processing.
- Germination rates are very consistent and fast in warm temperatures, and are directly related to temperature for *P. perfoliatus* and *R. maritima*. The *S. pectinata* germination rate is a bit longer, but this may have resulted from less than optimal storage conditions.
- So when is the best time to plant seeds? When water is warm, the seeds germinate faster, but there are also more herbivores and murkier water. The opposite is true for cold water planting. It is unclear which scenario results in more restored plants.
- In *P. perfoliatus* and *R. maritima* germination, the shoot comes out first, then the root (opposite of terrestrial seeds). This has implications for transport in these species, since the germinated seeds may move around before settling in one place. It is not clear why the unrooted, germinated seeds do not float to the surface once photosynthesis fills the aerenchyma, as happens in eelgrass. These seeds are

possibly heavier than eelgrass seeds, and/or the fresher water provides less buoyancy.

- In *S. pectinata* germination, the rhizome comes out with the shoot and then the root system comes off of the first node as an adventitious root. Because of this, structural complexity and burial depth would seem especially important for recruitment of this species.
- We are still successfully germinating older seeds (circa 2003) with up to 50% germination rates, so the rates are slowing over time, but seeds are still viable.

The next steps for this research group include identifying barriers to and requirements for seedling establishment, and devising a seed distributions system for planting seeds, such as seed tapes or pellets or some type of injector.

*Discussion of Seed Bank Implications*

Some seeds do not germinate until after a few years have passed, but their sample sizes are small. Court Stevenson looked for seeds in Choptank River sediment cores (4 cores per site) and tested seed viability. He found that most *R. maritima* and horned pondweed (*Zannichellia palustris*) seeds were viable. Steve Ailstock thinks the higher germination rates of these seeds in fresh water may be akin to fire ecology, wherein seeds are adapted to sprout after a big storm with a freshet that lowers salinity and that may have also wiped out the parent plants.

Bob Orth asked, rather than a seed bank acting as a source of new plants, what about seeds from waterfowl, or fragments washing in from upstream populations?

Peter Bergstrom said that there is another possibility for *E. canadensis* (elodea): some areas such as the Magothy River seem to have beds every year, some of which are fairly deep. The beds may expand when salinity drops and then we notice them.

Mike Naylor suggested that instead of picking seeds out of cores, we could try simply spreading out the collected sediment to see what grows.

## Propagule production and success in *Vallisneria americana*: Focus on sexual reproduction – Dwilette McFarland

Dwilette's 2006 technical note publication[16] and this presentation provide a comprehensive update on the information learned about *V. americana* reproductive ecology since the 1988 publication on the same topic by Korschgen and Green[17]. Following are a few notable items taken from the presentation:

- There are both northern (has winter buds) and southern ecotypes of *V. americana*, but we do not know if the two overlap and where or how they may differ in response to environmental factors.
- Cold treatment and scarification are known to effect germination in the northern ecotype of *V. americana*[18].
- Some seeds might germinate in the same year that they are produced.
- Some authors describe *V. americana* as non-dormant.
- Nutrient (nitrogen and phosphorus) additions to seed capsules can result in greater above-ground biomass and seed production. Nitrogen is generally the limiting nutrient.
- For pollination to occur, the female flower needs to reach the water's surface. Also, the water cannot be moving too fast nor can there be too much wind.
- A seed bank study on Lake Onalaska, WI, was conducted to determine if the lake could be naturally repopulated by *V. americana* after a dieback in late 1980's; it was caused by drought-induced low water levels and high water temperatures (over 30°C), which causes *V. americana* to stop making tubers
    - The study was comprised of 74 sites from which 10cm cores of the top 5 cm (~2 L total volume) of sediment were taken.
    - 15 aquatic macrophyte species were recorded, with *V. americana* having the greatest abundance, distrubution (36 of 74 cores), and density.
    - The greatest number and densities of all species were in cores that had come from the center or just south of existing beds; there was a similar pattern for *V. americana* alone, which seemed to follow the pattern of water flow. The seeds collected from these areas also showed the best germination.
    - Dwilette concluded that there is potential for wild celery to expand back to its former extent using seeds. She would like to see more standardized methods for testing seed banks; the seedling emergence method she used seems to give results similar to separating and identifying seeds.

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[16] McFarland, D. 2006. Reproductive ecology of *Vallisneria americana* Michaux. SAV Technical Notes Collection (ERDC/TN SAV-06-04). Vicksburg, MS: U.S. Army Engineer Research and Development Center.

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

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

Research Needs for *V. americana*: One important focus of future research should be identifying the barriers to seedling establishment. We also still need additional information on the effects of sediment density, water movement, and bioturbation on plant distribution and dispersal.

*Questions & Answers:*

Q: How much did scarification help the germination rates, given that we already get about 85% germination in Chesapeake *V. americana* seeds without it?
A: Dwilette did not conduct the study but speculates that scarification may be needed in some populations and not others. The single population studied had only about 40% germination without scarification.

Q: What is the purpose of the gel around wild celery seeds?
A: The gel matrix in *V. americana* seed pods could aide in the clumping of seedlings, but may also help seedlings to burst out of pods. It is something that has not been well characterized. When pods are stored in water, the gel dissolves within about a day.

**Life history characteristics of multiple SAV species and the implications for their use in revegetation and natural dispersion – John Madsen**

- A 1978 paper by van der Valk and Davis[19] describes in great detail the dynamics of marsh vegetation, with particular attention paid to the role of seed banks. The results of their study demonstrate how an "environmental sieve" places restrictions on what can species will germinate and when. This "environmental sieve" can include conditions of varying rainfall patterns, disease, water level changes, animal interactions, temperature variation, seed inputs, etc. Often we do not know what all those conditions are.

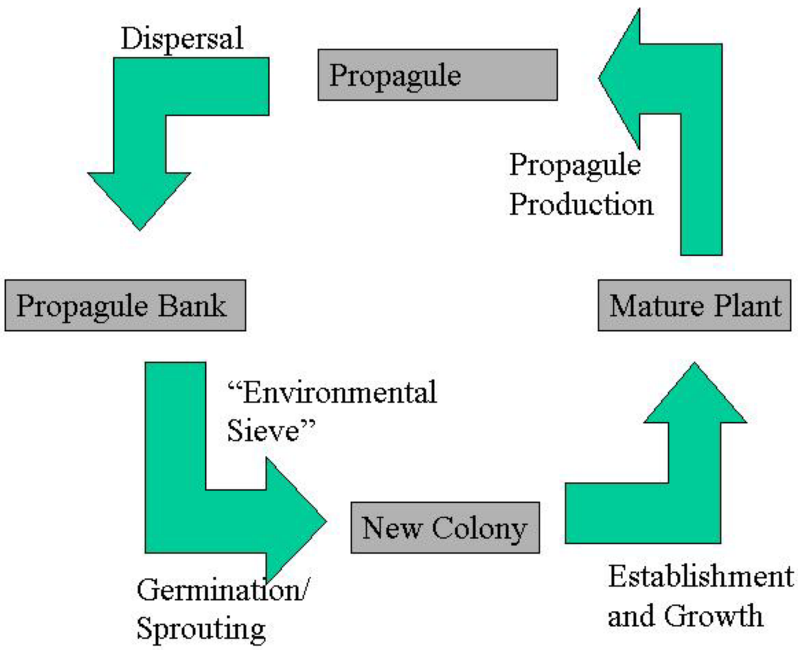


**Figure 1. Depiction of a generic vegetative cycle in which a plant population is self-sustaining through the use of propagules/a propagule bank, and germination of new colonies is under the selective pressure of an "environmental sieve."**

- SAV can reproduce sexually using seeds and/or asexually by clonal growth and/or vegetatively via propagule production. Forms of vegetative propagules can include axillary turions produced at apical meristems located at the top of the plant, and winter buds or tubers produced in association with the rhizomes.
  - Under nitrogen limitation, there is a considerable increase in axillary turion production and corresponding decrease in below ground reproductive structures in monoecious *Hydrilla verticillata* (hydrilla).
- Reproductive structures must be resistant to survive harsh environmental periods or disturbances and dormancy. For instance, *Myriophyllum spicatum* (Eurasian watermilfoil) seeds stored up to 250 days dry can still achieve 65% germination[20].

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[19] A.G. van der Valk and C.B. Davis. 1978. The role of seed banks in the vegetation dynamics of prairie glacial marshes. Ecology. 59:322-335.
[20] Standifer, N.E. and J.D. Madsen. 1997. The effect of drying period on the germination of Eurasian watermilfoil seeds. J. Aquat. Plant Management 35:35-36.

- Monoecious *H. verticillata* employs two different reproductive strategies, as it is able to produce both tubers and turions. While tubers can survive in undisturbed sediments over four years after being produced, turions germinate readily and expire after one year[21].
- Dioecious *H. verticillata* propagates only vegetatively in the U.S., primarily via tuber formation. When propagule emergence times for the two biotypes were compared, the degree-day method for predicting tuber sprouting was found to be very consistent, even between geographically separated California and Texas-based experiments[22].

- Most aquatic plants can regenerate from stem fragments (allofragments) formed by mechanical damage, wind, or waves. Some species may propagate via autofragments, which are formed by an abscission layer on stem and have carbohydrate stores just like propagules. As a result, they may overwinter.
- There are four life history types: annual, herbaceous perennial, evergreen perennial and woody perennial (not found in SAV).
  - Annuals overwinter by seed and only grow for one season, e.g., *Najas* spp. is an early annual (March-June) that germinates in December to February.
  - Herbaceous perennials overwinter as vegetatively-produced propagules, have a vegetative shoot that grows for a single season, and commonly grow clonally, e.g., *Stuckenia pectinata* (horned pondweed) biomass peaks in July, tuber biomass germinates in early spring, new tubers form in July, and then the plant senesces.
  - Evergreen perennials have green shoots which survive all year, often make no vegetative propagules, and spread extensively via clones and stem fragment dispersal, e.g., *M. spicatum* is omnipresent but with phenology that can vary significantly over its range, interannually, and between locations because of lower tolerance to very warm water conditions.
- The above life history types fall into categories of competitors, ruderals, and stress tolerators (Grime CSR Classification[23]):
  - Competitors favor low stress and low disturbance environments (herbaceous perennial rhizome and tuber species, and evergreen perennials);
  - Ruderals respond well to disturbance (annuals, herbaceous perennial tuber and turion species); and
  - Stress tolerators thrive in high stress but low disturbance environments (evergreen perennials).
  - There is no SAV when both stress and disturbance are high.

[21] Van, T.K. and K.K. Steward. 1990. Longevity of monoecious Hydrilla propagules. J. Aquat. Plant Management. 28:74-76.
[22] Spencer, D.F. et al. 2000. Emergence of vegetative propagules of *Potamogeton nodosus*, *Potamogeton pectinatus*, *Vallisneria americana*, and *Hydrilla verticillata* based on accumulated degree-days. Aquat. Bot. 67:237-249.
[23] Grime, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. The American Naturalist. 111:1169-1194.

- There can be phase shifts from turbid to clear water states in lakes.  Such a shift can be initiated by a drawdown, which stimulates growth of both SAV and EAV (emergent aquatic vegetation) from seed banks.
    - This was demonstrated in Lake Onandaga, WI, where nothing else had worked to clear up the highly eutrophic and highly calcified environment.
    - After the drawdown, secchi depth tripled, *M. spicatum* frequency declined from 90% to 11%, native plants frequency increased from 28% to 100% and grew in diversity.
    - A hay bale wavebreak was used to shield nearshore plantings along with exclosures.

*Questions and Answers:*

Q1: Why are exclosures needed in many systems?
A1: Exclosures are needed where a system is very degraded and changed by humans, and where you may need to exclude herbivores such as carp (Mike Smart).

Q2: Court Stevenson asked about the role of mycrorrhizal symbionts.
A2: Unknown.

Q3:  Has there been a switch from more to less invasive forms of exotic plants, as happened with Chesapeake Eurasian watermilfoil?
A3: It is hard to document in fresh water because data sets are not as extensive, but they have seen this shift, possibly mediated by herbivores, changes in water clarity, water level, etc.  Mike Naylor added that SAV in the Chesapeake still has shifts from native to hydrilla, as well as in the other direction. This can be caused by short increases in salinity that knock back hydrilla.  The effect of salinity on wild celery may depend on light levels.

Court Stevenson said that we need more long-term quantitative monitoring of SAV by species in low salinity.  Peter Bergstrom said he is talking to the National Estuarine Research Reserve (NERR) sites in the Chesapeake Bay about enhancing and standardizing their SAV monitoring.  MD DNR is establishing SAV transects in “core beds” in that tend to be present every year.

## General Discussion from Day 2

*Thread 1: Workshop products*

Products envisioned from this workshop include the proceedings document encompassing presentations and notes, and a detailed summary table for reproductive characteristics of all Bay species.

There was also discussion of a follow-up document covering the last ten years of new knowledge in SAV reproductive ecology. This could take the form of a broad revision of the 1978 Stevenson and Confer paper and unpublished updates to that version. Following a defined template for each species, authors would produce an 8-10 page summary of the reproductive biology for their species, in addition to a complete literature references section. Volunteers were solicited for writing summaries for all of the species in the summary table.

*Thread 2: SAV Genetics*

Genetics work has been very lacking from this workshop because it is lacking in general! Nancy Rybicki and colleagues at the USGS have done some preliminary work on protocols for DNA fingerprinting of four freshwater species, *Egeria densa*, *H. verticillata*, *Elodea canadensis*, and *Elodea nutelli*. There is ongoing *V. americana* work at the UMCES Appalachian Lab by Katia Engelhardt in conjunction with Maile Neel at the University of Maryland, College Park. There has also been some interest in the topic within the USDA Agricultural Research Service. This type of work can lead to a much better understanding of population structure, ecotypes, restoration tracking.

*Thread 3: Funding Strategies/Justification*

We should try to solicit attention and/or funding on a species-by-species basis rather than SAV as a whole. The timing of global changes can lend extra importance to the need for this information.

Tom Parham suggested placing any products in the context of the SAV Workgroup's SAV Strategy and the Bay Program's efforts to help remove the Chesapeake Bay from the EPA 303(d) "impaired waters" list.

SAV habitat requirement refinements can influence policy and management for de-listing waters. If a Bay segment meets the SAV acreage goals, it can be de-listed despite marginal water quality. If local governments know that they can de-list waterways based upon SAV acreage increases rather than reductions in nutrients/sediments, then we might be able to garner more immediate attention to the need for additional SAV research and restoration.

Identifying limiting factors that kill SAV, either by ending reproduction, inhibiting seed survival, or killing seedlings, can help impose more stringent water quality attainment standards. We must take into account the interactions between stressor conditions like temperature, low dissolved oxygen, low light, etc. For instance, we can develop maximum water temperatures and exposure thresholds for certain species or determine target dissolved oxygen levels for SAV survival.

*Thread 4: Revision of SAV Habitat Requirements*

The habitat requirements as they are now were set up to maintain then-current SAV acreage to the 1-meter depth contour. Supporting new additional SAV restoration beyond 1 meter may require further restrictions. Should we revise the Technical Synthesis document[24]? Do we also need to revisit the SAV acreage goals? The Upper Patuxent River is at 1000% of its attainment goal only because a small acreage goal was set initially.

*Thread 5: Reproductive Success Index and Propagule Budget*

Steve Ailstock suggested that we create a "reproductive success index" for each SAV species, similar to the striped bass juvenile index that has engaged grass roots support. The index would indicate how well each species was reproducing that year (sexual and asexual). For example, in a given year, what is the reproductive output of *V. americana* on the Susquehanna Flats compared to two years ago? This should include vegetative propagules.

Mike Kemp added the need for periodic assessments of the seed banks for each SAV species. He thinks the Choptank River seed bank was built up enough by 1996 to allow *R. maritima* to respond to light availability; prior to that year, there was no relationship between percent cover and secchi depth. One reason may have been that there was not a sufficient seed bank. Every year that bank gets tapped and in bad years, you dig further into it. Several bad years in a row might eliminate a seed bank. This justifies the need for assessing seed banks and identifying local sources of propagules.

Steve Ailstock stated that we should expand this idea to a propagule budget covering all possible sources of propagules. It is incredibly difficult to know what is in the bank and account for withdrawals. This budget would examine all deposits to the propagule bank, including propagules other than seeds. We can measure propagule formation and seed output with species distribution and biomass data. We could choose some sites as target areas with a lot of transects. MD DNR is already doing that this year with the modified oyster tong method developed by Nancy Rybicki. One big next step would be to identify the means to characterize propagule availability.

*Thread 6: Additional Research Needs*

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[24] Batiuk, R. et al. 2000. Chesapeake Bay Submerged Aquatic Vegetation Water Quality and Habitat Based Requirments and Restoration Targets: A Second Technical Synthesis. Chesapeake Bay Program, Annapolis, MD. (http://www.chesapeakebay.net/pubs/sav/index.html)

Can we learn more about SAV reproduction along the outer edges of beds, both nearshore and towards deeper water? We only know a little bit about this for some species.

We need expanded SAV monitoring by species. No transects are planned for the Choptank River this year, which tends to have highly variable SAV cover over time. Peter Bergstrom calculated the interquartile range of SAV area by segment over time for the SAV trends report, and the lower Choptank River was one of the most variable segments. Mike Naylor took names for a workgroup to devise better SAV monitoring protocols, both for species distribution and propagule availability.

*Thread 7: Curly Pondweed*

The group discussed the growing season for curly pondweed. MD DNR staff will look for a summer dieback in the Chesapeake this year. There may be two ecotypes; it may be invasive in reservoirs and less invasive in tidal water. These two variants might have different growth patterns.

# Workshop Attendees

(* = steering committee)

| | |
|---|---|
| Mike Naylor* | Maryland Dept. of Natural Resources |
| Ken Moore* | Virginia Institute of Marine Science |
| Peter Bergstrom* | NOAA Chesapeake Bay Office |
| Mike Kemp* | UMCES Horn Point Laboratory |
| Becky Thur* | Chesapeake Research Consortium |
| Evamaria Koch* | UMCES Horn Point Laboratory |
| Nancy Rybicki | U.S. Geological Survey |
| Tom Parham | Maryland Dept. of Natural Resources |
| Lee Karrh | Maryland Dept. of Natural Resources |
| Justin Reel | Rummel, Klepper & Kahl, LLP |
| Mark Lewandowski | Maryland Dept. of Natural Resources |
| Bob Murphy | Ecosystem Solutions Inc. |
| Laura Murray | UMCES Horn Point Laboratory |
| Steve Ailstock | Anne Arundel Community College |
| Chris Tanner | St. Mary's College of Maryland |
| Bob Orth | Virginia Institute of Marine Science |
| Scott Marion | Virginia Institute of Marine Science |
| Patrick Kangas | University of Maryland |
| Dale Booth | UMCES Horn Point Laboratory |
| Ben Anderson | DE Dept. of Natural Resources & Environmental Control |
| Dwilette McFarland | U.S. Army Corps of Engineers, ERDC |
| Elizabeth Zinecker | University of Maryland |
| R. Jay Ugiansky | USDA, NRCS, National Plant Materials Center |
| Ed Schenk | U.S. Geological Survey |
| Kathryn (Preen) Busch | Maryland Dept. of Natural Resources |
| Rebecca Golden | Maryland Dept. of Natural Resources |
| Michael Smart | U.S. Army Corps of Engineers, ERDC |
| John Madsen | Mississippi State University |
| Chris Pickerell | Cornell University |
| Court Stevenson | UMCES Horn Point Laboratory |
| Jessie Jarvis | Virginia Institute of Marine Science |
| Erin Shields | Virginia Institute of Marine Science |
| Nina Fisher | Independent Technical Writer |

# Appendix A - SAV Summary Table

| Species | Common Name | Salinity Range | Native | Distribution | Growing Season | Aboveground Biomass |
|---|---|---|---|---|---|---|
| *Ceratophyllum demersum* | coontail | 0 – 8[1] | Y | Increasing | March/June[2] - Oct[3] | Jan – Dec |
| *Potamogeton pusillus* | slender pondweed | 0 – 8 | Y | | April – Oct | April – Oct |
| *Potamogeton crispus* | curly pondweed | 0 – 8 | N | | March – June? | Sept – June? |
| *Heteranthera dubia* | water stargrass | 0 – 8 | Y | Increasing | April – Oct | Year round |
| *Vallisneria americana* | wild celery | 0 – 12 | Y | Increasing | April – Oct | April – Oct |
| *Najas guadalupensis* | southern naiad | 0 – 10 | Y | | April – Oct | April – Oct |
| *Elodea canadensis / Elodea nuttalli* | elodea | 0 – 14? | Y | | April – Oct | Jan – Dec |
| *Hydrilla verticillata* | hydrilla | 0 – 9 | N | Increasing | May – Oct | May – Oct |
| *Najas minor* | spiny naiad | 0 – 10 | N | | April – Oct | April – Oct |
| *Myriophyllum spicatum* | Eurasian watermilfoil | 0 – 14 | N | | April – Oct | Jan – Dec |
| *Potamogeton perfoliatus* | redhead grass | 2 – 19 | Y | | April – Oct | March – Nov |
| *Stuckenia pectinata* | sago pondweed | 0 – 20 | Y | | May – Oct | Jan – Dec |
| *Zannichellia palustris* | horned pondweed | 0 – 22 | Y | | Feb – June/fall? | Feb – June |
| *Ruppia maritima* | widgeon grass | 0 – 22 | Y | Variable | May – Oct | Jan – Dec |
| *Zostera marina* | eelgrass | 10 – 36 | Y | Decreasing | Jan – Dec | Jan – Dec |

[1] Bourn, W.S. 1932. Ecological and physiological studies on certain aquatic angiosperms. Cont. Boyce Thompson Inst. 4:425-496.
[2] Best. E.P.H. and J.T. Meulemans 1979. Photosynthesis in relation to growth and dormancy in *Ceratophyllum demersum*. Aquat. Bot. 6:53-65.
[3] Best, E.P.H. 1979. Growth substances and dormancy in *Ceratophyllum demersum*. Physiologia Plantarum 45(4):399-406.

| Species | Optimal Growth Temp | Dormancy | | Mature Fruit Present | Germination | | Meristem (apical/basal) |
|---|---|---|---|---|---|---|---|
| | | Y/N | Cues | | Time/Temp | Cues | |
| *C. demersum* | 25ºC[4] | Y[5] | Cold | Summer - Fall[6] | | | |
| *P. pusillus* | | Y | Cold | July - Sep | | | Apical |
| *P. crispus* | | Y | Warm | August | | | Apical |
| *H. dubia* | | Y | Cold | July – Oct | 8°C[7] | | Apical |
| *V. americana* | | Y | Cold | Aug – Oct | 15°C? | Cold – warm? | Apical |
| *N. guadalupensis* | | Y | Cold | | | | Apical |
| *E. canadensis / E. nuttalli* | | N | | | | | Basal |
| *H. verticillata* | 30°C | Y | Cold | Rare | 15°C | Cold – warm | Apical |
| *N. minor* | | Y | Cold | | | | Apical |
| *M. spicatum* | 25°C | N | | July – Sept | 15 – 25°C | Cold – warm | Apical |
| *P. perfoliatus* | | Y | Cold | Aug – Sept | 60 – 80°F | Cold – warm + fresh | Apical |
| *S. pectinata* | | | | Aug – Sept | 60 – 80°F | Cold – warm | Apical |
| *Z. palustris* | | Y | Cold | April – July/Sept | | | Apical or both |
| *R. maritima* | | | | July – Aug | 50 – 80°F | Cold – warm | Apical |
| *Z.marina* | 15-20°C | Y | Warm/cold | May – June | Oct – Dec | Redox; temperature | Both |
| | | | | | | | Basal |

[4] Best, E.P.H. 1986. Photosynthetic characteristics of the submerged macrophyte *Ceratophyllum demersum*. Physiologia Plantarum. 68(3): 502-510.
[5] Sculthorpe, C.D. 1967. The biology of aquatic vascular plants. Edward Arnold Ltd., London. 610 pp.; Best 1979.
[6] http://www.plants.usda.gov
[7] For stem germination.

| Species | Expansion Rate (lateral) | Seeds | | | |
|---|---|---|---|---|---|
| | | **Y/N** | **# Per Plant** | **Longevity** | **Optimal Burial Depth** |
| *C. demersum* | 0.025 – 0.041 g ash free dry wt/d[8] | Y | 1 seed/fruit[9], 300 seeds/over 100 kg fresh weight of plant material[10] | Several years[11] | |
| *P. pusillus* | | Y, rare | | | |
| *P. crispus* | | Y | 1,445/m$^2$ | | |
| *H. dubia* | | Y | | | |
| *V. americana* | | Y | 50 – 200/capsule | Transient | < 2 cm |
| *N. guadalupensis* | | Y | 10,696/m$^2$ | | |
| *E. canadensis / E. nuttalli* | | | | | |
| *H. verticillata* | 4 cm/d (40 m/yr) | N | Rare | Rare | |
| *N. minor* | | Y | | | |
| *M. spicatum* | 3.9 cm/d (38.9 m/yr) | Y | 60/flowering stem | Decades | < 2 cm |
| *P. perfoliatus* | | Y | 48 – 115/flowering stem | | < 2 cm |
| *S. pectinata* | | Y, rare | 20 – 59/flowering stem | | < 2 cm |
| *Z. palustris* | | Y | 799,630/m$^2$ | | |
| *R. maritima* | | Y | 21/flowering stem | Decades? | < 2 cm |
| *Z. marina* | 26 cm/yr | Y | 31 – 200/shoot | Transient | 1 cm |

[8] Best 1986.
[9] Stevenson, JC and Confer, NM. Summary of Available Information on Chesapeake Bay Submerged Vegetation. Fish and Wildlife Service, Office of Biological Sciences FWS/OBS-78/66, August, 1978. 335, 50 fig, 88 tab, 747 ref.
[10] Wyman, C. and D.A. Franko. 1986. Germination of *Ceratophyllum demersum* seeds in asceptic liquid culture. Proc. Okla. Acad. Sci. 66: 27-29.
[11] Costa, N.V., D. Martins, and C.C. Martins. 2005. Dormancy break of *Ceratophyllum demersum* seeds. Planta daninha. 23(2):187-191.

| Species | Reproductive Modes | | | | | Can Seeds Act As Waterfowl Vectors? | Sediment Tolerance |
|---|---|---|---|---|---|---|---|
| | **Tubers** | **Turions** | **Winter Buds** | **Auto-fragment** | **Overwinter** | | |
| *C. demersum* | N | N | N | Y | Y | Y[12] | 20 – 32% organic[13] |
| *P. pusillus* | N | Y | N | N | N | | |
| *P. crispus* | N | Y | N | N | N | Y | |
| *H. dubia* | N | N | N | N | Y[14] | | |
| *V. americana* | N | N | Y | N | N | Y | |
| *N. guadalupensis* | N | N | N | N | N | | |
| *E. canadensis / E. nuttalli* | N | N | N | N | Y | | |
| *H. verticillata* | Y | Y | N | N | N | N/A | < 20% organic |
| *N. minor* | N | N | N | N | N | | |
| *M. spicatum* | N | N | N | Y | Y | Unlikely | < 20% organic |
| *P. perfoliatus* | N | N | Y | N | N | Y | |
| *S. pectinata* | Y | N | N | N | N (rarely Y) | Y | |
| *Z. palustris* | N | N | N | N | N | Y | |
| *R. maritima* | N | N | N | N | Y | Y | |
| *Z. marina* | N | N | N | N | Y | N | < 5% organic, 35% silt/clay |

[12] Stollberg, B.P. 1950. Food habits of shoal-water ducks on Horicon March, Wisconsin. 1950. J. Wildlife Manage. 14(2):214-217.; Sculthorpe 1967.
[13] Chambers, P.A. and E.E. Prepas. 1990. Competition and coexistence in submerged aquatic plant communities: the effects of species interactions versus abiotic factors. Freshwater Biol. 23:541-550.
[14] Plants overwinter with stems lying flat on bottom. New growth emerges from nodes in spring.

| Species | Important Animal Interactions | | Habitat Limiting Factors |
|---|---|---|---|
| | **Negative** | **Positive** | |
| *C. demersum* | Eaten by Chinese grass carp (*Ctenopharyngodon idella*) and Brazilian snail (*Marisa cornuarietis*)[15] | Presence of freshwater snail, *Planorborbis planorbis*[16] increases plant length and plant life | Moderate to high N & P concentrations and slow moving water |
| *P. pusillus* | | | |
| *P. crispus* | | | |
| *H. dubia* | | | |
| *V. americana* | | | |
| *N. guadalupensis* | | | |
| *E. canadensis / E. nuttalli* | | | |
| *H. verticillata* | | | Light availability |
| *N. minor* | | | |
| *M. spicatum* | | | Light availability |
| *P. perfoliatus* | | | |
| *S. pectinata* | | | |
| *Z. palustris* | | | |
| *R. maritima* | | | |
| *Z. marina* | Seed predation by hermit and blue crab waterfowl; cownose ray damage | Diapetra tubes[17] and other burrowing organisms facilitate seed entrainment; Epiphytic grazers (e.g., snails) | |

[15] Stevenson and Confer 1978; Chapman, V.J., J.M.A. Brown, C.F. Hill, and J.L. Carr. 1974. Biology of excessive weed growth in the hydro-electric lakes of Waikato River, New Zealand. Hydrobiologia. 44(4):349-363.
[16] Presence of this freshwater snail increased plant length and life of plant both by releasing N and P to the water and by epiphytic grazing. Underwood, G.J.C. 1991. Growth enhancement of the macrophyte *Ceratophyllum demersum* in the presence of the snail *Planorbis planorbis*: the effect of grazing and chemical conditioning. Freshwater Biol. 26:325-334.
[17] Harwell, M.C. and R.J. Orth. 2001. Influence of a tube-dwelling polychaete on the dispersal of fragmented reproductive shoots of eelgrass. Aquat. Bot. 70:1-7.