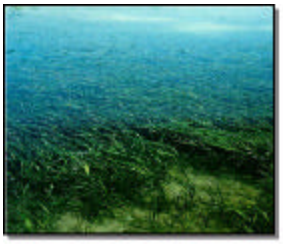

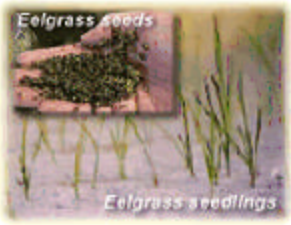

Welcome to the

Submerged Aquatic Vegetation Propagation Workshop

3-4 September 2003
Baltimore, MD

US Army Corps of Engineers

Sponsored by: US Army Corps of Engineers Research and Development Center, Environmental Laboratory

SUBMERGED AQUATIC VEGETATION PROPAGATION WORKSHOP
3-4 September, 2003
The Maritime Institute Conference Center, Baltimore, MD

Sponsored by:

US Army Corps of Engineers, Engineer Research and Development Center, Environmental Laboratory

Schedule of Events
for
WEDNESDAY, September 3, 2003

HOURS FROM	HOURS TO	TOPIC	SPEAKER
9:00	**10:00**	**REGISTRATION**	
10:00	**10:20**	**Welcome and Opening Remarks**	D. Shafer
10:20	**10:40**	**USACE Role in SAV Restoration in Chesapeake Bay**	M. Mendelsohn
10:40	**10:50**	**BREAK**	
10:50	**13:50**	**Session 1: Use of Seeds in SAV Restoration Planting**	B. Abadie
10:50	11:10	Culture of Eelgrass (*Zostera marina*) for Restoration Projects	C. Tanner
11:10	11:30	Eelgrass Restoration in Chesapeake Bay: Are Seeds the Way to Go?	R. Orth
11:30	**12:30**	**LUNCH**	
		Session 1: Use of Seeds in SAV Restoration Planting (continues)	
12:30	12:50	Habitat Restoration and Planting Strategies Using Eelgrass Seeds	S. Granger
12:50	13:10	Buoy-Deployed Seeding: A New Approach to Restoring Seagrass Using Seeds	C. Pickerell
13:10	13:30	Reproductive Potential of Natural Populations of *Ruppia maritima* and *Potamogeton perfoliatus* by Seed in the Mid-Chesapeake Bay	S. Ailstock
13:30	13:50	Question & Answer Session 1	B. Abadie
13:50	**14:05**	**BREAK**	
14:05	**15:25**	**Session 2: Techniques for SAV Plant Propagation**	M. Fritz
14:05	14:25	Propagation and Reproduction of SAV Transplant Stock for Ecosystem Restoration	M. Smart
14:25	14:45	Applications and Limitations of Micropropagation for the Production of Underwater Grasses	S. Ailstock
14:45	15:05	Bay Grasses in Classes	M. Lewandowski
15:05	15:25	Question & Answer Session 2	M. Fritz
15:25	**16:40**	**Plenary Discussion: Survey Questions #1 - #2**	D. Goshorn
16:40	**17:00**	**ANNOUNCEMENTS/ADJOURN**	D. Shafer

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Schedule of Events
for
THURSDAY, September 4, 2003

HOURS FROM	HOURS TO	TOPIC	SPEAKER
8:30	**9:00**	**REGISTRATION**	
9:00	**9:30**	**Wednesday Recap and Thursday Overview**	D. Shafer
9:30	**10:30**	**Session 3: Feedback Loops in SAV Restoration: Does Existing SAV Enhance Future Planting Success?**	R. Orth
9:30	9:50	Use of Colonizing Species of Submersed Aquatic Vegetation as Nurse Crops in Restoration Projects	L. Murray
9:50	10:10	Founder Colonies for Restoration of Aquatic Plant Communities in Unvegetated Freshwater Ecosystems	M. Smart
10:10	10:30	Question & Answer Session 3	R. Orth
10:30	**10:45**	**BREAK**	
10:45	**11:30**	**Plenary Discussion: Survey Question #3**	D. Goshorn
11:30	**12:30**	**LUNCH**	
12:30	**14:25**	**Session 4: Future Directions in Large-Scale SAV Production**	D. Shafer
12:30	12:50	Eelgrass Restoration in Chesapeake Bay: The Emerging Issues with Large-Scale Restoration Using Seeds	R. Orth
12:50	13:10	~~Processes for Developing Large-Scale Commercial Production of Submerged Aquatic Vegetation (Fresh and Brackish)~~	~~W. Skaradek~~
13:10	13:30	The Adaptation and Application of Modern Agricultural Production Practices to SAV Restoration	T. Mazzaccaro
13:30	**13:45**	**BREAK**	
		Session 4: Future Directions in Large-Scale SAV Production (continues)	
13:45	14:05	Chesapeake Bay Foundation Presentation	B. Street
14:05	14:25	Question & Answer Session 4	D. Shafer
14:25	**16:30**	**Group Discussions**	
16:30	**17:00**	**Closing Remarks/ADJOURNMENT**	D. Shafer

Submerged Aquatic Vegetation Propagation Workshop
Presenter Bios

Deborah Shafer

Ms. Shafer is a Research Marine Biologist with the U.S. Army Corps of Engineers, Engineer Research and Development Center, Environmental Laboratory in Vicksburg, MS. She is currently the SAV Research Program Manager and the Lead for this SAV Propagation Workshop.

Mark Mendelsohn

Mark Mendelsohn has been a Biologist for the Army Corps of Engineers Baltimore District for 11 years. He has done work with oysters and Poplar Island for the past 10 years. Previously, he was an engineer at Westinghouse.

Chris Tanner

Dr. Christopher Tanner is a professor of biology at St. Mary's College of Maryland, located on the shore of the St. Mary's River estuary. Originally from the West Coast, Dr. Tanner received his BA in biology at Occidental College in Los Angeles and his Ph.D. in Marine Botany from the University of British Columbia where he worked on the ecology and systematics of green macroalgae. Dr. Tanner is currently the co-director of the St. Mary's River Project, a federally funded project supporting long term monitoring of water quality in the St. Mary's River estuary and watershed and research on SAV, oysters and other estuarine species. Dr. Tanner has been working with students on eelgrass restoration in the St. Mary's and lower Potomac Rivers for the last 7 years. He has also been working on research funded by the Wilson Bridge Mitigation Program and the Chesapeake Bay Trust to develop methods for the propagation of eelgrass in culture. This year, he is collaborating with the Maryland Department of Natural Resources to develop culture facilities at the Piney Point Aquaculture Facility and grow eelgrass for the Wilson Bridge SAV mitigation work in the lower Potomac.

Bob Orth

Bob Orth is a professor of Marine Science at the Virginia Institute of Marine Science. He received a PhD from the University of Maryland, a MS from the University of Virginia, and a BA from Rutgers University. Dr. Orth's research focuses on the biology and ecology of seagrasses, principally in the Chesapeake Bay. His current emphasis is on habitat restoration and conservation and understanding the principles and processes governing the persistence, alterations, and dynamics of these plant communities.

Stephen Granger

Steve Granger is a research scientist at the University of Rhode Island's Graduate School of Oceanography. He received a Bachelor's Degree in Zoology from UVM in 1976 and a Master's Degree in Oceanography at URI in 1990. He has spent 22 years working with Scott Nixon on various projects concerning nitrogen enrichment of coastal waters and the ecological impact on near shore habitats such as seagrass.

Submerged Aquatic Vegetation Propagation Workshop
Presenter Bios

Chris Pickerell

He has a BS in Biotechnology from RIT and a MS in plant and soil science from Cornell University. Chris has worked for Cornell Cooperative Extension of Suffolk County's, Marine Program for the last 11 years. His work involves managing all of CCE's salt marsh and SAV restoration and monitoring programs. Current work focuses on adapting existing techniques and developing new techniques for restoring eelgrass to the waters around Long Island.

Steven Ailstock

Steve Ailstock is the Chair of the Biology Department and Director of the Environmental Center at Anne Arundel Community College. His research interests are submerged aquatic plants, wetlands creation, and Phragmites.

Mike Smart

Mike Smart is an Aquatic Plant Ecologist for the Army Corps of Engineers Research and Development Center, stationed in Lewisville, Texas. He is the Ecological Technology Area Leader for the Aquatic Plant Control Research Program and Director of the Lewisville Aquatic Ecosystem Research Facility. He conducts research on aquatic plant ecology and ecosystem restoration.

Mark Lewandowski

Mark Lewandowski is a Natural Resources Biologist for MD DNR – Tidewater Assessment. He is the coordinator of the Bay Grasses in Classes Program.

Laura Murray

Laura Murray received a BS in Marine Science and a MS in Science Education from the University of West Florida and a Ph.D. in Wetlands Ecology from the College of William and Mary. She served on the Biology faculty at Salisbury University for 12 years. Since 1993, she has been a research associate professor at the University of Maryland, Center for Environmental Science, Horn Point Laboratory. Her research interests have included the impacts of nutrients on submersed aquatic vegetation growth and survival. Recently, her research efforts have included restoration ecology of SAV.

Bill Street

Bill Street is on staff at the Chesapeake Bay Foundation.

Tony Mazzaccaro

Tony Mazzaccaro is a professor at the University of Maryland Eastern Shore in Princess Anne, MD.

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Funding Authorization

FY 2003 Omnibus Appropriations Bill

(GI Research and Development)

$500 K was provided *"to conduct investigations, assessment, and demonstrations on large-scale submerged aquatic vegetation restoration techniques and technologies. Appropriate demonstration activities should be considered within the Chesapeake Bay, MD."*

(from p. 24, Senate Report 107-220)

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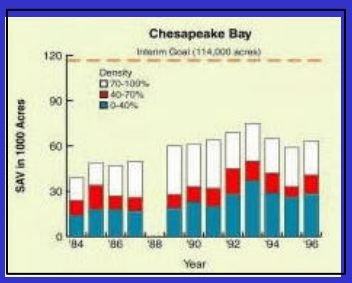
Engineer Research and Development Center

Why is SAV Restoration Important?



Source: Chesapeake Bay Program
www.epa.gov/maia/html/es-habitat.html

- *Of the more than 600,000 acres of SAV historically present in Chesapeake Bay, less than a tenth remains*
- *More than 50% lost since the 1960's*
- *More than 20 SAV species have declined*
- *Although some increases in recent years, still far below targeted goals*

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Why is SAV Restoration Important?

SAV Performs many ecosystem functions:

- **wave attenuation**
- **sediment stabilization**
- **water quality improvement**
- **primary production**
- **provide critical habitat structure**

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Problems

- Traditional approaches to SAV planting are extremely labor-intensive and costly, with a variable track record of success
- Significant investments in research and demonstrations must be made to improve our understanding of SAV restoration techniques
- Managers and stakeholders need guidance on selection of most appropriate methods for large-scale SAV restoration

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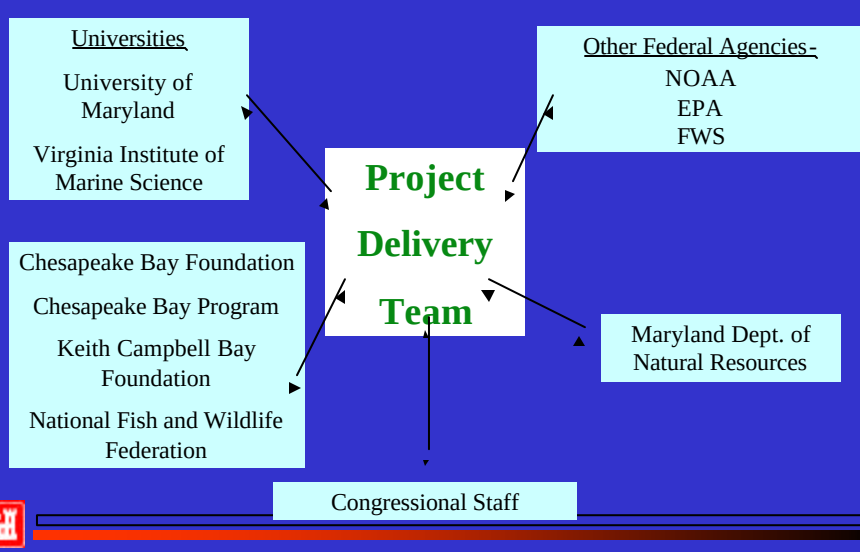
Program Coordination



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Program Focus Areas

SAV Production and Planting *(FY 03)*

Potential New Focus Areas

- Engineered SAV Habitats
- SAV Assessment Methods

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Initial Program Focus Area: SAV Production and Planting

Source: University of Rhode Island

Many SAV restoration projects rely on whole plants collected in the wild

Source: University of Rhode Island

Availability of donor sites?

Donor site impacts?

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FY03 Activities

1. Expand Eelgrass Seed Collection and Storage Capability
   Piney Point Aquaculture Facility (MDDNR)
2. Multi-species Pilot Scale Test Planting
   Poplar Island (Anne Arundel CC)
3. Demonstration Planting: Potomac River (MDDNR)
   Comparison of eelgrass plants vs. seeds
4. Regional Workshop (Sept. 3-4, Baltimore, MD)

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Information Transfer

- Goal: Each planting project/work unit documented in the peer-reviewed literature
- Workshop Proceedings
- Results will be incorporated into **guidance document** on selection of appropriate methods for SAV restoration
- **Web links** and information on ongoing projects

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Benefits

- Contribute to the status of the science of SAV restoration
- Provide practical guidance on selection of appropriate methods for SAV restoration
- Improved coordination between Corps and other stakeholders involved in SAV restoration
- Results directly applicable to regions outside Chesapeake Bay

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Links to Other Corps Programs

- Section 206 Aquatic Ecosystem Restoration
- Section 204 Beneficial Uses of Dredged Materials
- Section 227 Shoreline Erosion Control
- Ecosystem Management and Restoration Research Program (EMRRP)
- Aquatic Plant Control Program
- Regional Sediment Management Program (RSM)
- Dredging Operations and Environmental Research Program (DOER)

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Future Directions

- **Demonstration projects need a minimum of 2 years monitoring in order to evaluate success**
- **Additional funding would enable us to expand the scope and direction of the program to include a wider variety of plant species, planting techniques, and locations throughout the Bay**
- **National Workshop planned for FY 04**
- Dependent on availability of future funding …

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SAV Propagation Workshop

September 3-4, 2003

- **Availability of planting stock is often a critical bottleneck in SAV restoration projects**
- **If we are to meet targeted restoration goals, we must find an economical way to produce and plant large numbers of plant propagules!**
- **The large-scale production of plant propagules must be matched to the needs of those involved in the planting**

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Workshop Objectives

- ***Exchange information on the status of the science with respect to SAV propagation and planting***
- ***Develop species-specific management recommendations on selection of appropriate methods for SAV planting and propagation***

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Workshop Format

- **Series of technical presentations organized into 4 sessions**
- **2 sessions per day**
- **Lunch (provided in Main Bldg)**
- **Afternoon discussion sessions**
- **Workshop Questionaire**

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Acknowledgements

Thanks to the members of the Workshop Steering Committee—

Mike Fritz, Dave Goshorn, Bob Orth, Madeline Broadstone, Bill Dennison

Special Thanks to Antisa Webb

US Army Corps of Engineers

Engineer Research and Development Center

SAV Propagation Conference

U.S. Army Corps of Engineers
Baltimore District

USACE Role in SAV Restoration in the Chesapeake Bay

September 3, 2003

Mark Mendelsohn

SHALLOW CREEK

- Mitigation for small navigation dredging project in Baltimore. (Near Beth Steel)
- Species mitigated for was Eurasian millfoil
- USFWS did the work. USACE paid
- Area characterized by Secchi of .5 meter
- Salinity ranges of 2.5 to 15 ppt
- Mute Swans

Shallow Creek (cont.)

- 3 species planted: redhead, wild celery, and sago pondweed-Grown at AACC and USDA
- June 1999, 2,000 shoots of each species. Unfenced. Cost was $12,7000
- June 2000, 4,000 shoots of 3 species, Fenced. Cost was $18,100
- September 2001, 600 shoots of redhead, Fenced, Cost was $3,950
- Cost did not include monitoring

Shallow Creek Results

- It depends on WQ, salinity and swans.
- Redhead did best
- Wild Celery did OK
- Sago pondweed didn't do well
- For details call Peter Bergstrom now at NOAA

Poplar Island

- 1140 acre man-made island using clean dredged material in mid-bay.
- First planting in Spring 2003 outside of constructed site.
- Fall Planting in 2003 inside the site and outside planned and funded by WES.

Poplar Island

- Steve Ailstock AACC is lead
- Redhead planted will be 1,044 lbs of seed
- Rupia will be 1,956 lbs of seed
- Cost is $41,000
- Channel area in site is around 4 acres.

Isle of Wight

- Project constructed for purpose of replacing saltmarsh and as a site for beneficial use of dredged material. Near Ocean City.
- SAV developed in site after EIS was completed
- SAV transplanted out of the site using new “sod” technology

Isle of Wight (cont.)

- 4 by 5 foot areas 10 inches deeps were moved
- 4/10ths of an acre transplanted to approximately 1 mile
- Eel grass and Widgeon grass
- Seems to be working

Saxis 206 Island Aquatic Ecosystem Restoration

- Proposed by Norfolk District - Near MD and VA border
- Not approved yet by CENAD
- Ready to go into plans and specs
- Purpose of project is restoring beach , dunes, riparians and intertidal wetlands

Saxis 206 Island Aquatic Ecosystem Restoration

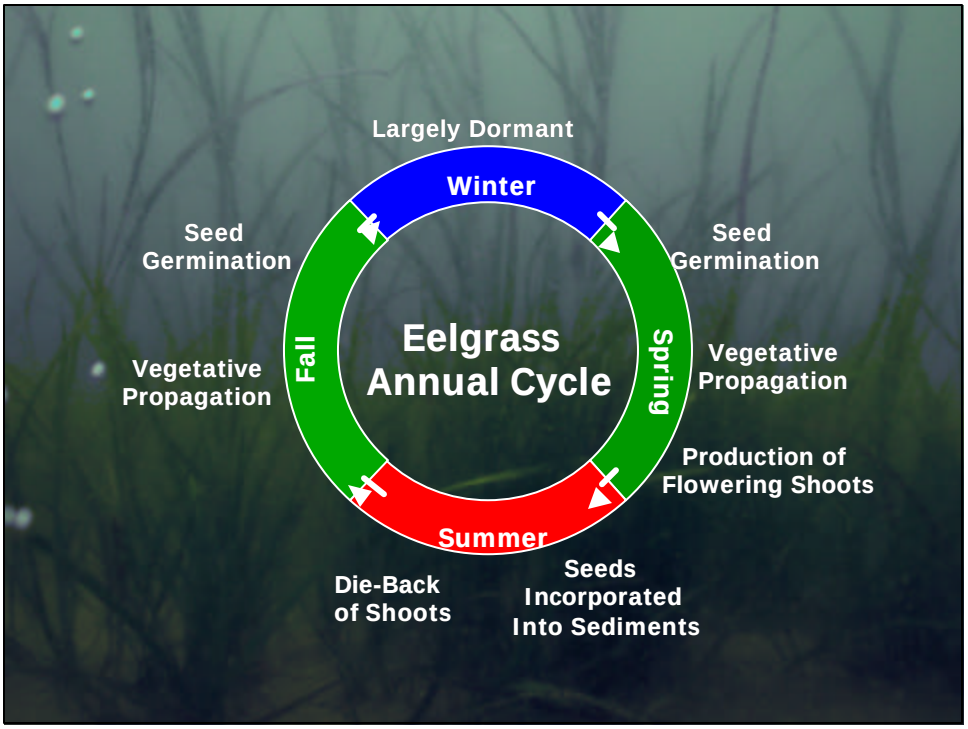
- Breakwaters will be constructed suitable for 1.3 -8 acres of SAV colonization
- District may seed - but lots of propagules floating around in area

Woodrow Wilson Bridge Mitigation

- Location is Potomac River on DC beltway
- Mitigation required by Corps permit
- Some transplants came from Maryland Coastal Bays

CULTURE OF EELGRASS (*ZOSTERA MARINA*) FOR RESTORATION PROJECTS

Christopher Tanner
St. Mary's College of Maryland

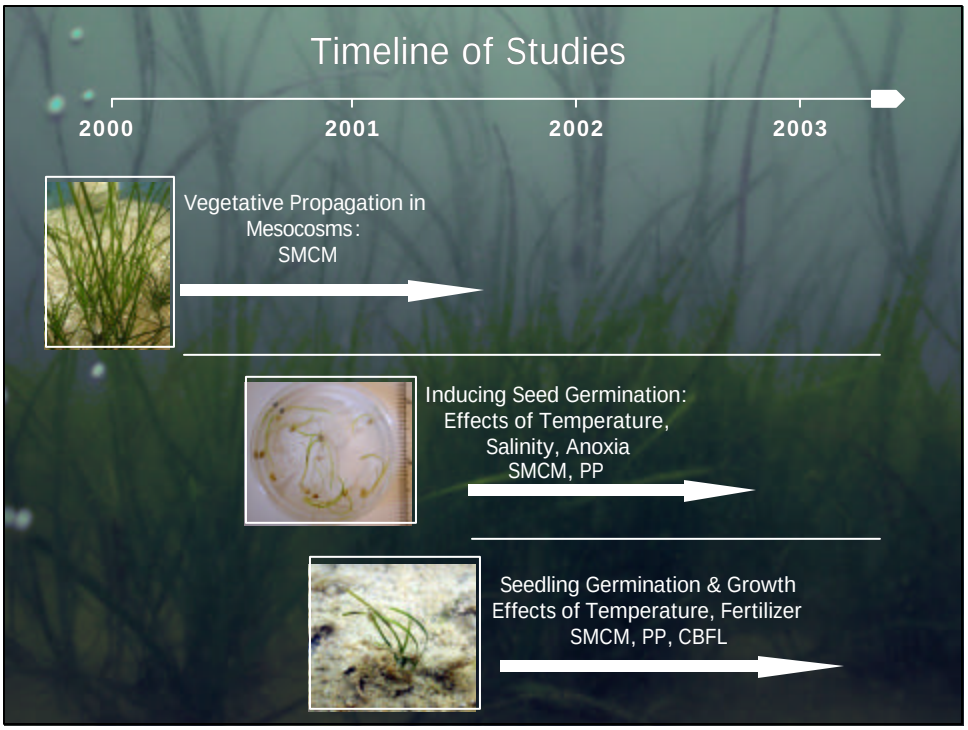


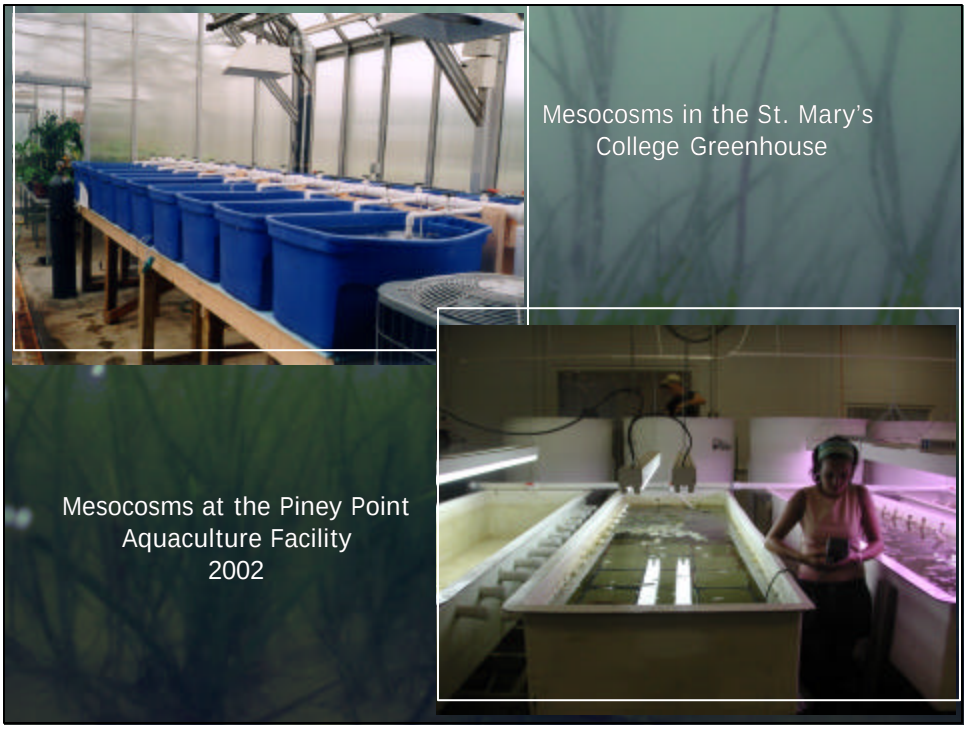
Research Goals

To develop methods for land-based propagation of eelgrass

- Investigate eelgrass vegetative propagation under culture conditions
- Determine whether eelgrass seeds can be induced to germinate early and seedlings grown to size for outplanting

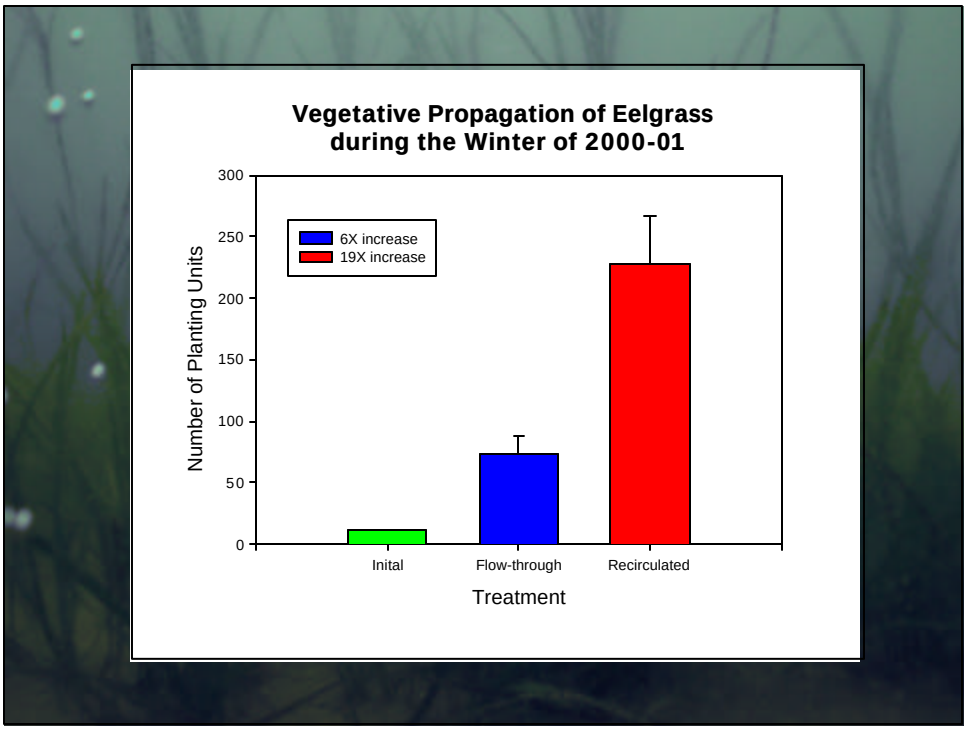
Eelgrass Planting Unit


Timeline of Studies
2000
2001
2002
2003
Vegetative Propagation in Mesocosms: SMCM
Inducing Seed Germination: Effects of Temperature, Salinity, Anoxia SMCM, PP
Seedling Germination & Growth Effects of Temperature, Fertilizer SMCM, PP, CBFL

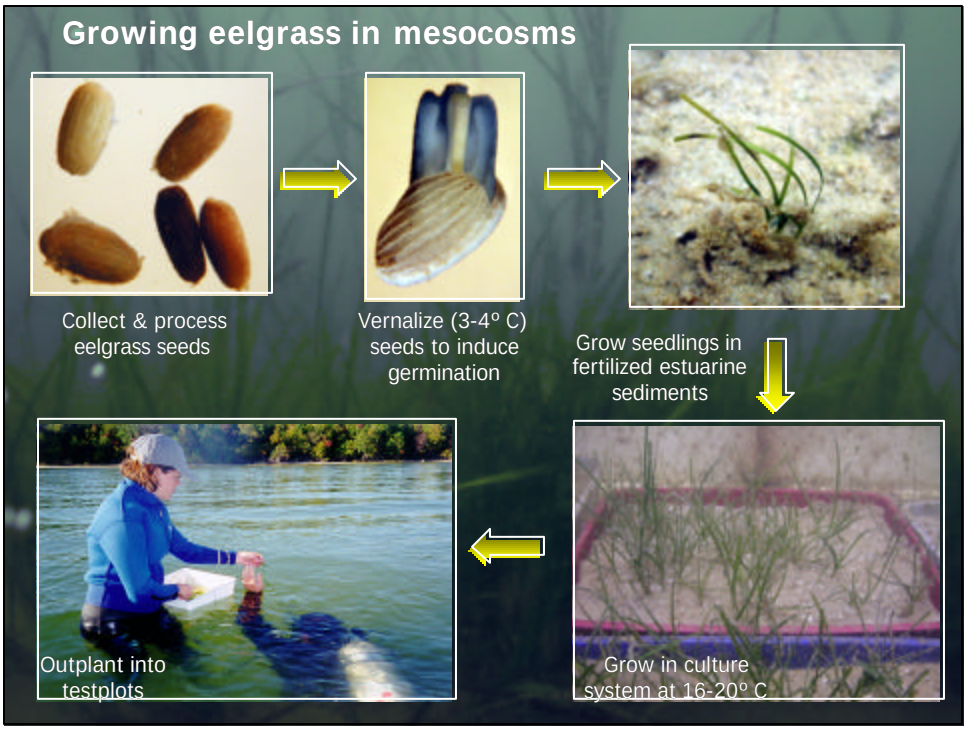

Mesocosms in the St. Mary's College Greenhouse
Mesocosms at the Piney Point Aquaculture Facility 2002

Mesocosms at the Chesapeake Bay Field Laboratory 2002
Mesocosms at the Piney Point Aquaculture Facility 2003

Vegetative production of new shoots


Vegetative Propagation of Eelgrass
during the Winter of 2000-01
Number of Planting Units
300
250
200
150
100
50
0
6X increase
19X increase
Inital
Flow-through
Recirculated
Treatment

Seedling grown under ambient conditions
Seedling grown under controlled conditions



Seed Germination Experiment

Soil-free culture held at 14° C (SMCM; July-September, 2001)

Treatment	% Germination	% Seedling Survival	Days to Germination
14-16 ppt	43	20	7-13
Sterilized, 14-16 ppt	27	20	13-27
Scarified, 14-16 ppt	60	27	10-27
Hypoxic, 14-16 ppt	100	100	?-29*
0 ppt	93	50	3-27
5 ppt	87	47	3-27
10 ppt	33	20	10-27
15 ppt	40	13	10-28
20 ppt	7	0	28-29
25 ppt	7	0	27

*Initial germination of scarified seeds was not observed

Seed Germination and Seedling Growth Experiment

Planted in peat pots with natural sediments and placed in mesocosms with flow-through ambient estuarine water (SMCM, Fall 2001). Results 127 days after planting.

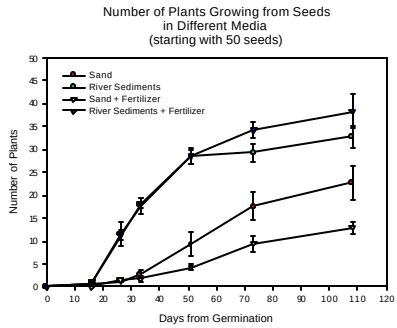
Storage Conditions	% Pots with Seedlings*	% Survival**	Maximum Shoot Height (cm)
4º C	90.3 ± 5.4	61.1 ± 6.6	22.6 ± 1.6
14º C	59.7 ± 4.0	25.0 ± 2.6	22.9 ± 1.1
Ambient Temp	33.5 ± 5.7	10.9 ± 2.1	16.9 ± 1.6
Seedlings	47.2 ± 11.5	59.7 ± 18.7	20.9 ± 3.5

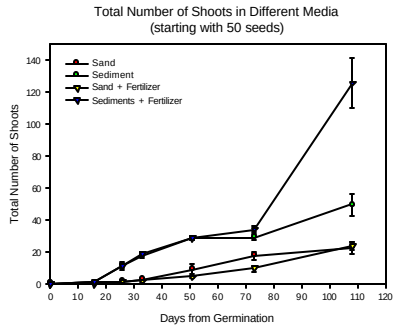
Values respresent means ± the standard error
*Five seeds were planted per peat pot
**Percent survival based on the number of shoots per pot, which could include new turions

Effects of sediments and fertilizer on seedling growth

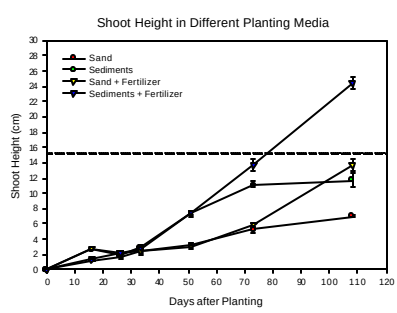
(SMCM, Fall 2001)

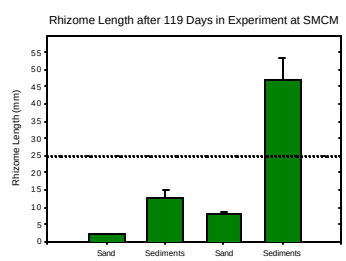
Fall 2001 Seedling Growth Experiment SMCM



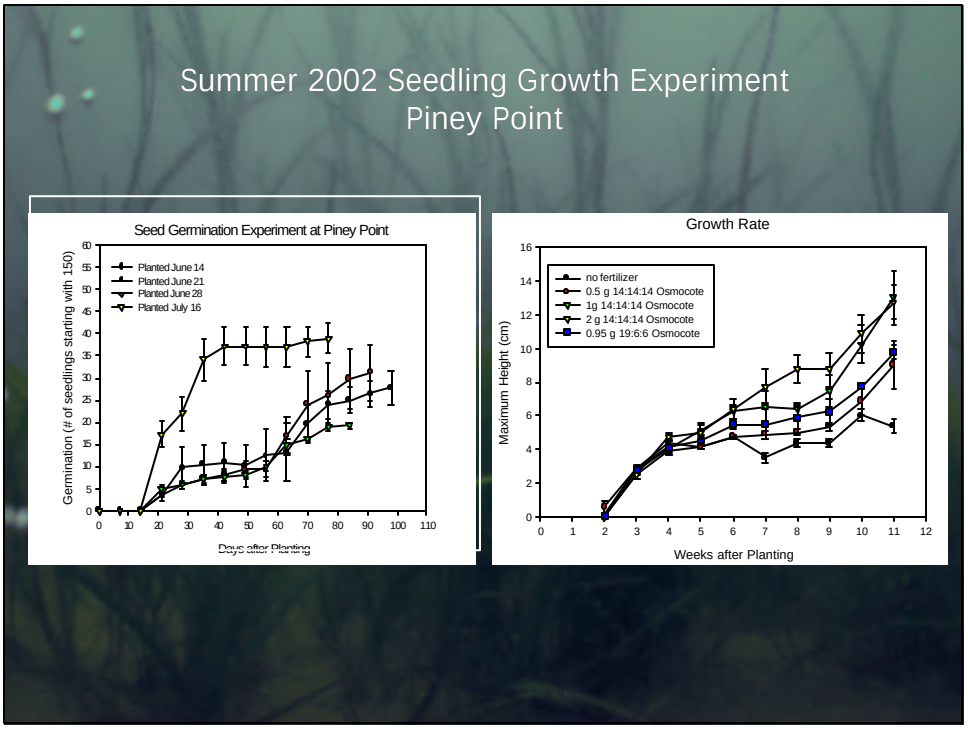


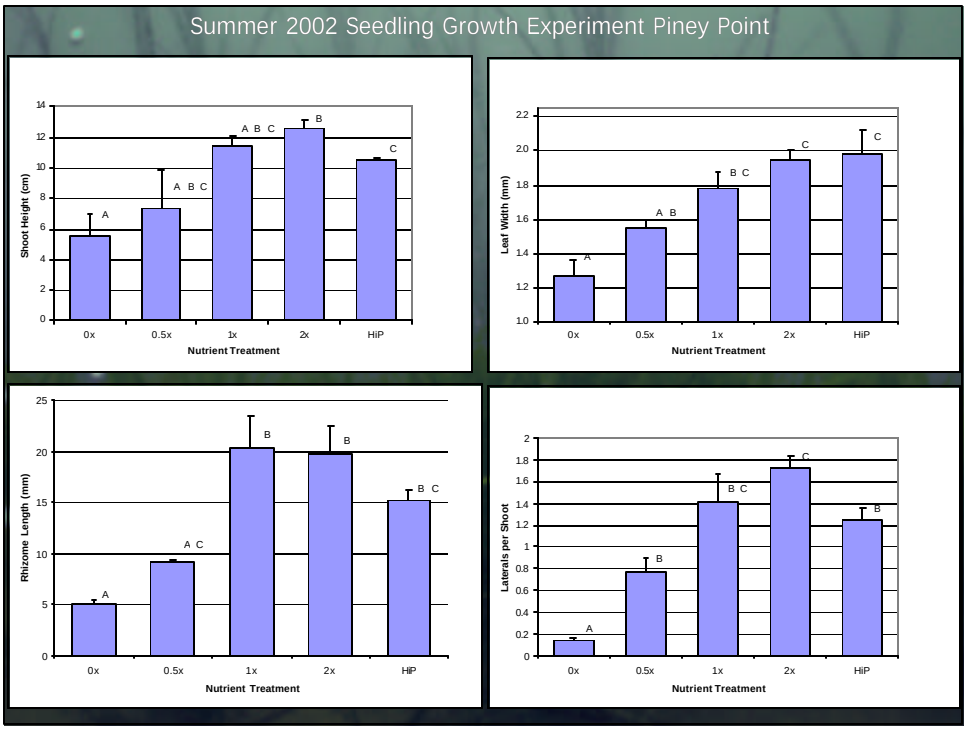
Fall 2001 Seedling Growth Experiment SMCM





Summer 2002 Seedling
Growth Experiment
Piney Point


Summer 2002 Seedling Growth Experiment
Piney Point
Seed Germination Experiment at Piney Point
Germination (# of seedlings starting with 150)
Planted June 14
Planted June 21
Planted June 28
Planted July 16
Days after Planting
Growth Rate
Maximum Height (cm)
no fertilizer
0.5 g 14:14:14 Osmocote
1g 14:14:14 Osmocote
2 g 14:14:14 Osmocote
0.95 g 19:6:6 Osmocote
Weeks after Planting



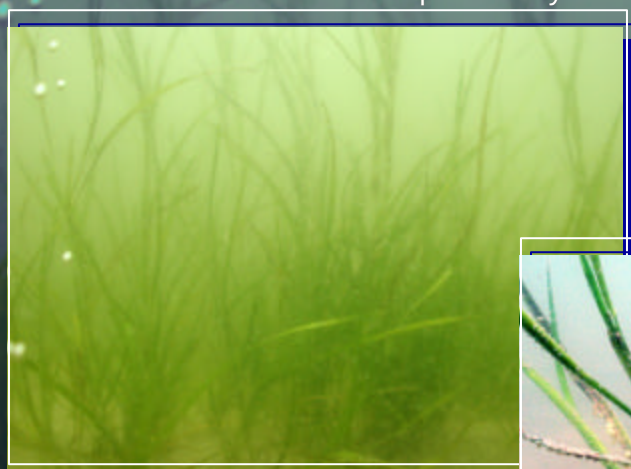
Conclusions

- Eelgrass for restoration projects can be either propagated vegetatively or grown from seed in land-based culture systems
- Vegetative propagation does not require the collection of field material after the initial culture stock is established
- Use of seeds lowers culture costs as the system is in operation for approximately 6 months
- Low germination rates this year, possibly due to cooler temperatures and lower salinities

Cost-Effectiveness

1. Investment in culture facilities
   a) Tanks, pumps and chillers (or heat pumps)
   b) Lighting
2. Costs associated with running the system
   a) Electrical costs
   b) Maintaining pumps, chillers and lights
3. Culture costs
   a) Collecting/processing vegetative shoots and/or seeds
   b) Collecting sediments
   c) Planting shoots and/or seeds in tanks
   d) Cleaning tanks and plants
   e) Harvesting plants and preparing for outplanting

Research funded by the Wilson Bridge Mitigation Program and the Chesapeake Bay Trust



Eelgrass Restoration in Chesapeake Bay:

Are seeds the way to go?

Robert J. Orth

Virginia Institute of Marine Science
College of William and Mary

www.vims.edu/bio/sav

OR

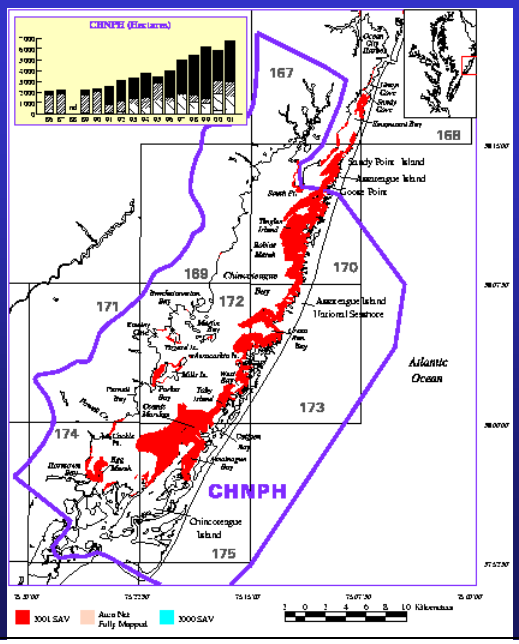
‘Strategy to Accelerate Protection and Restoration of SAV in Chesapeake Bay’

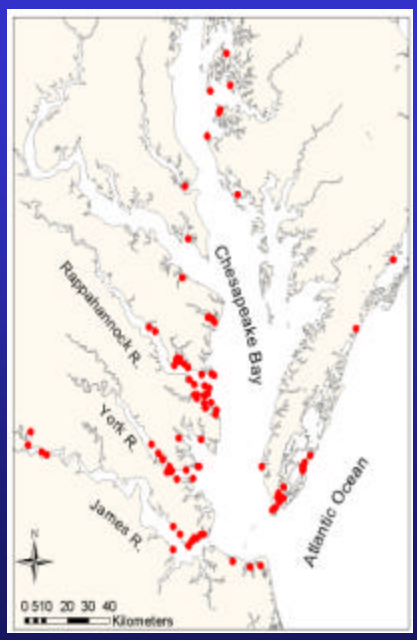
By Dec. 2008, plant at least 1000 acres at multiple sites!!

Seagrass Transplants – Variety of Techniques to Plant Adult Plants

Labor intensive
Tedious
Potential donor bed impacts
Small areas planted

RECOVERY OF SEAGRASS TO CHINCOTEAGUE BAY 1986-2001





Transplant Sites
1979-2002

- Nearly 90 sites planted

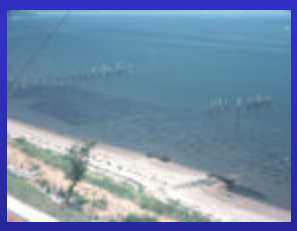
SPECIES USED

- Eelgrass (*Zostera marina*) ******
- Wild celery (*Vallisneria americana*)
- Sago Pondweed (*Stuckenia pectinata*)
- Elodea (*Elodea canadensis*)
- Coontail (*Ceratophyllum demersum*)

VIMS - BUNDLE TRANSPLANTS
0.5, 1.0 and 2.0 m centers in 1982 and 1983

1984

1985

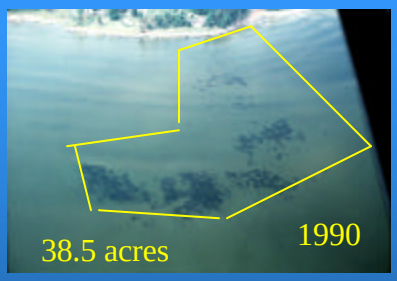


1987

1988

1990-2002

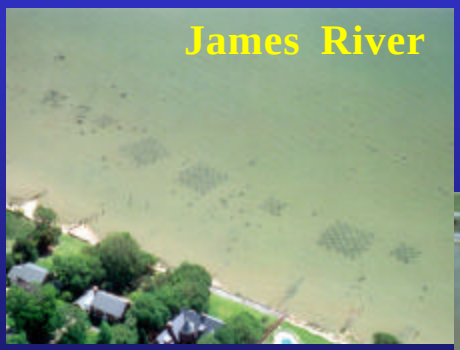
PIANKATANK RIVER
(transplanted 1984-1989– adult plants and seeds)



1995: all gone

We MUST collect WQ data to understand failures

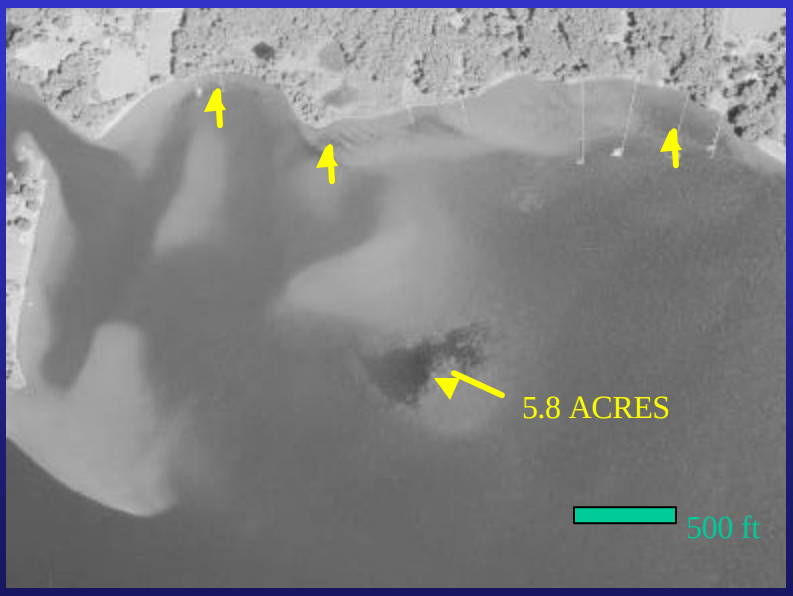
VIMS SINGLE SHOOT TRANSPLANTS
Planted fall 1996 – Aerial photos taken June 1997



13,440 shoots, 192 - 4 m^2 plots
3 patch sizes
$4m^2$, $100m^2$, $400m^2$

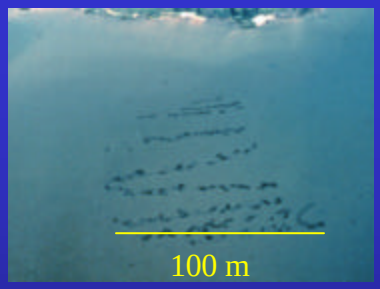
Monitor Merrimac - James River (1997,1998)

PIANKATANK RIVER – HEALY CREEK – JUNE 2002



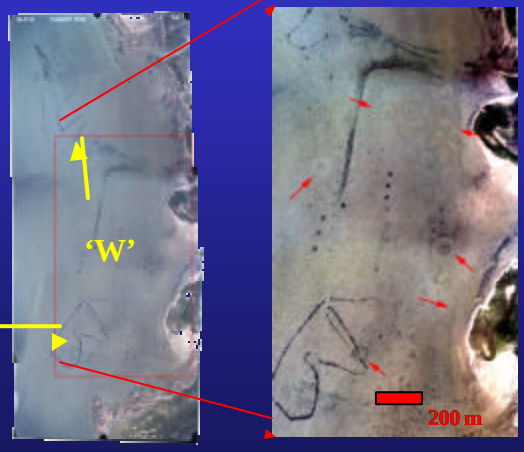
YORK RIVER 1989

Seeds broadcast from port and starboard side of boat in fall, 1988



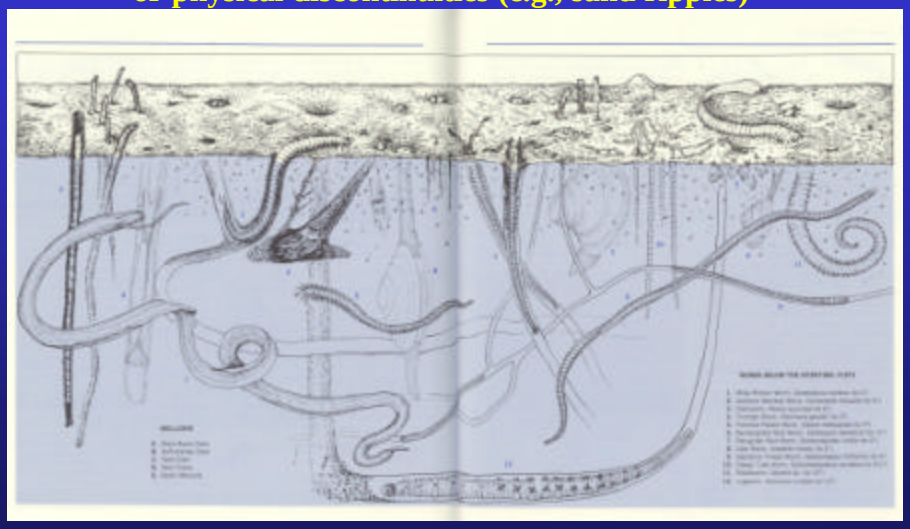
YORK RIVER
1989

SOUTH BAY
2001
(seeds broadcast Fall 1999)



Orth, Moore and Luckenbach (1994) Ecology 75:1927-1939

Seeds retained where they settle because of topographic complexities of sediment surface due to bioturbation or physical discontinuities (e.g., sand ripples)



Luckenbach and Orth (1999) Aquatic Botany 62:235-247

SEED COLLECTION

LATE MAY – MID-JUNE

2001
6.6 million seeds in 204 collecting hours = 32,500 seeds/hour
2002
2.5 million seeds in 246 collecting hours = 10,000 seeds/hour
2003
5.2 million seeds in 310 collecting hours = 16,800 seeds/hour
----
Broadcast in August to October prior to seed germination in mid Nov.

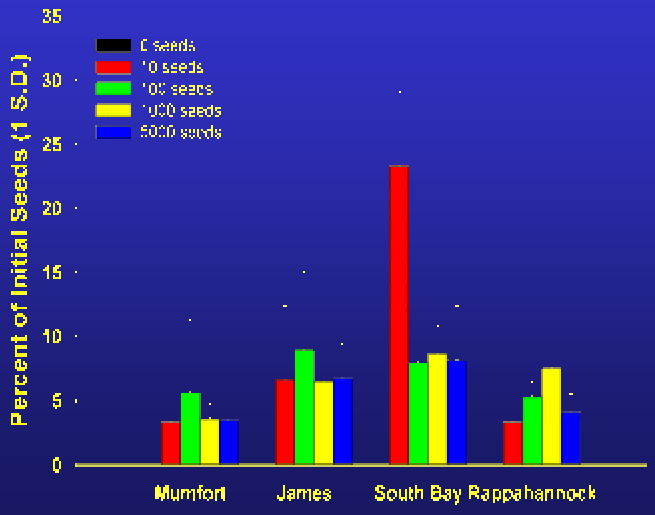
SEEDS- 1989 to present

PRO

- **Low donor bed impact**
- **Can collect 'heaps' of seeds!**
- **Easy for volunteers to collect and disperse**

- **CON**
  - **Only 5-15% of seeds germinate and survive**

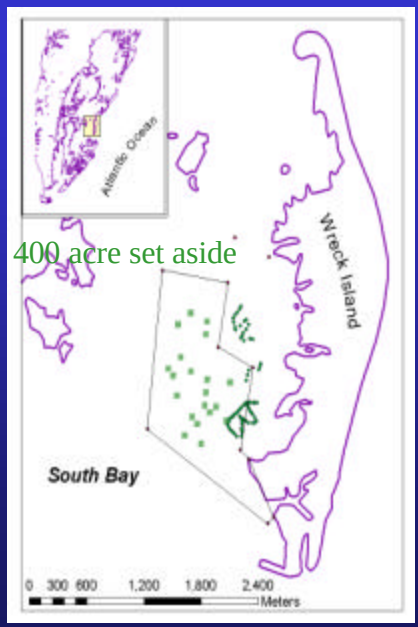
Seedling Abundance vs. Initial Seed Density



Orth, Fishman, Harwell and Marion (2003) Mar. Ecol. Prog. Ser. 250:71-79.

Seaside Heritage Program

Funded by Virginia's NOAA Coastal Program



SEED DISPERSAL

2001 - 42 acres @ 100K and 200K per acre
2002 - 32 acres @ 50K and 100K per acre

100K = 25 seeds/m²

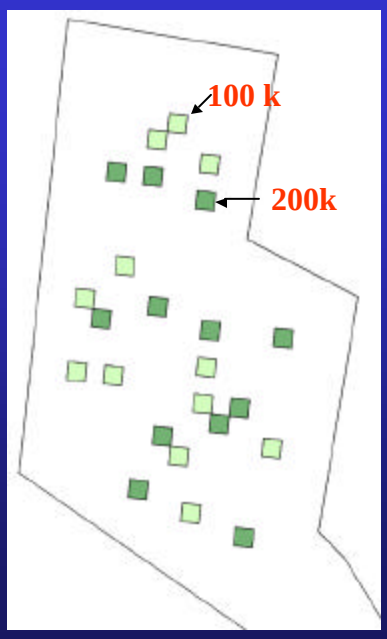
******TIME TO SET UP PLOT AND BROADCAST SEEDS = 1 HOUR FOR THREE PEOPLE******

SOUTH BAY EELGRASS IN DIFFERENT TREATMENTS - 2002

1998 – small test plot

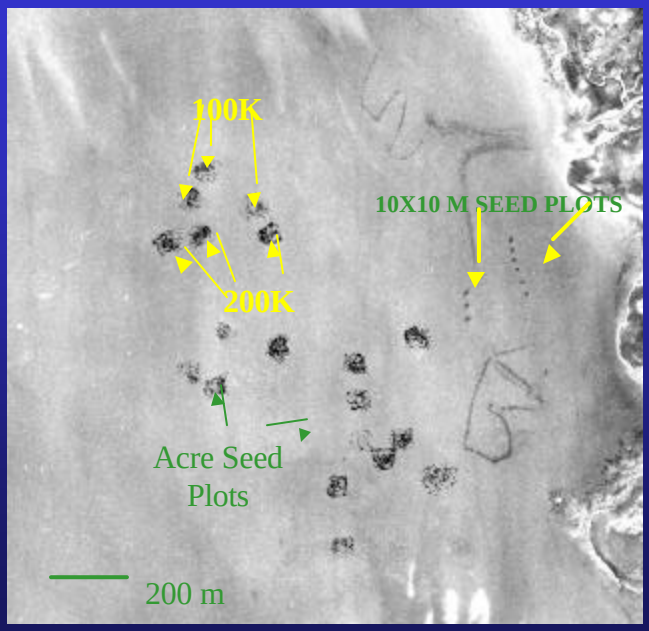
1999 – seed broadcast

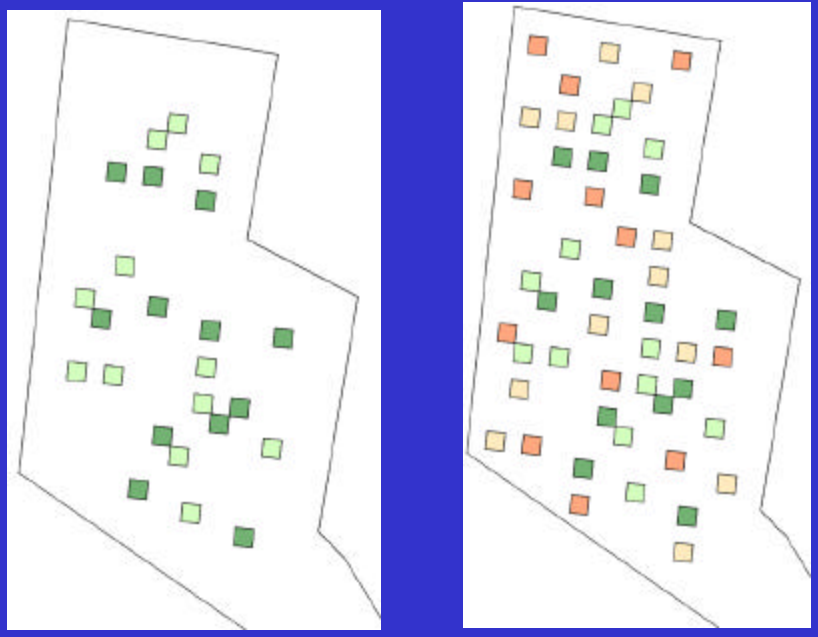
2000 – 10x10 m seed plot



400 acre set aside and
Location of 1 acre seed plots planted at seed densities of 100k and 200k per acre

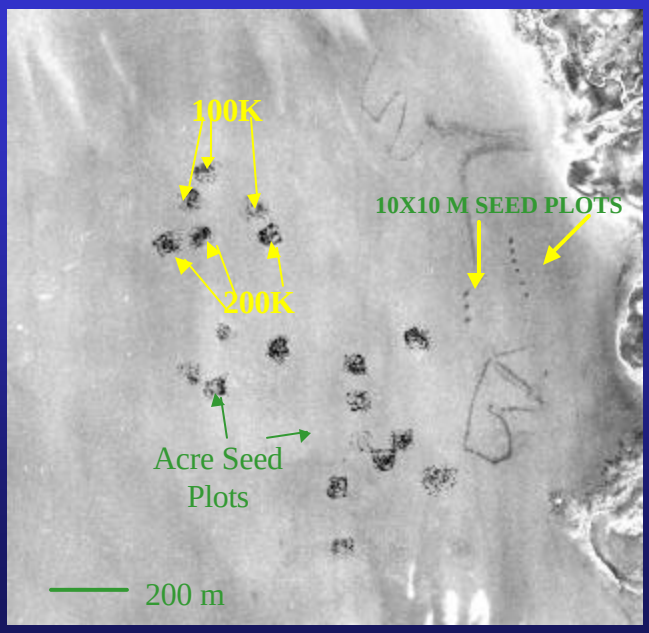
SOUTH BAY – JULY 2002 (Seeds broadcast fall, 2001)



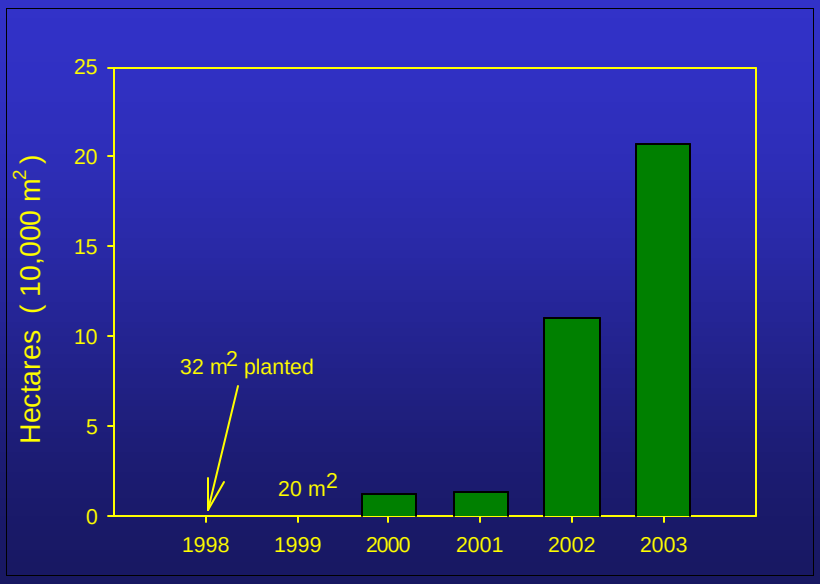


SOUTH BAY – JUNE 2003 (Seeds broadcast fall, 2001)

SOUTH BAY – JULY 2002 (Seeds broadcast fall, 2001)



SEAGRASS COVERAGE IN SOUTH BAY



TRANSPLANT METHODS*

METHOD	# Plants or Seeds/ PU	TIME* (min)
ADULT PLANTS		
Woven Mats	15	30.0 PU^{-1}
Turf	~40	6.4 PU^{-1}
Cores	~15	5.7 PU^{-1}
Bundles	5-12	4.9 PU^{-1}
Single Shoots	1	0.4 PU^{-1}
SEEDS		
Burlap/Wire	550 m^{-2}	32.8 m^{-2}
Peat Pots	10 seeds	3.8 PU^{-1}
Seed Bags	10 seeds	3.3 PU^{-1}
Broadcast	12-50 m^{-2}	0.3 m^{-2}

*Includes:
Collection
Preparation
Planting

TIME PER SUCCESSFUL PLANTING UNIT AT 24 WEEKS* AVERAGED FOR BOTH SITES

- **Machine 40.6 sec**
- **Manual 22.4 sec**
- **Seed 4.5 sec**

* only includes time to plant

TAKE HOME LESSONS

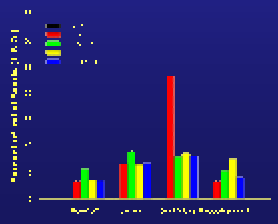
- Seed production – narrow window but generally large numbers produced for many species
- Reproductive shoots with seeds easy to harvest and store

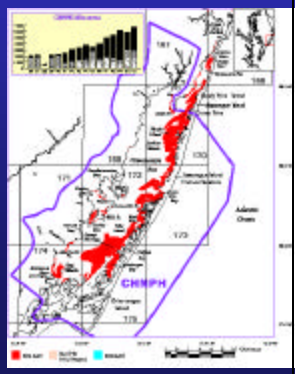
TAKE HOME LESSONS

- Low donor bed impact
- Easier than using adult plants
- Genetic issues
- MUST conduct basic experiments

TAKE HOME LESSONS

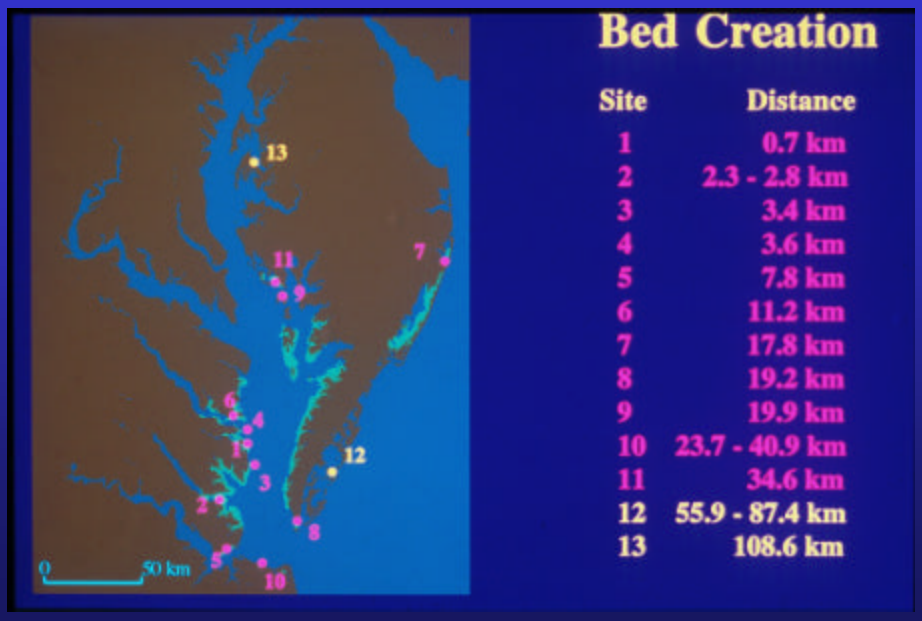
- WATER QUALITY!!!!
- Natural variation can dwarf human efforts
- Large increases in seagrass populations most likely due to seed input not vegetative spread





FUNDING AGENCIES

- **Virginia Saltwater Recreational Fishing License Fund**
- **Virginia Coastal Resource Management Program (NOAA)**
- **Special State Initiatives to VIMS**

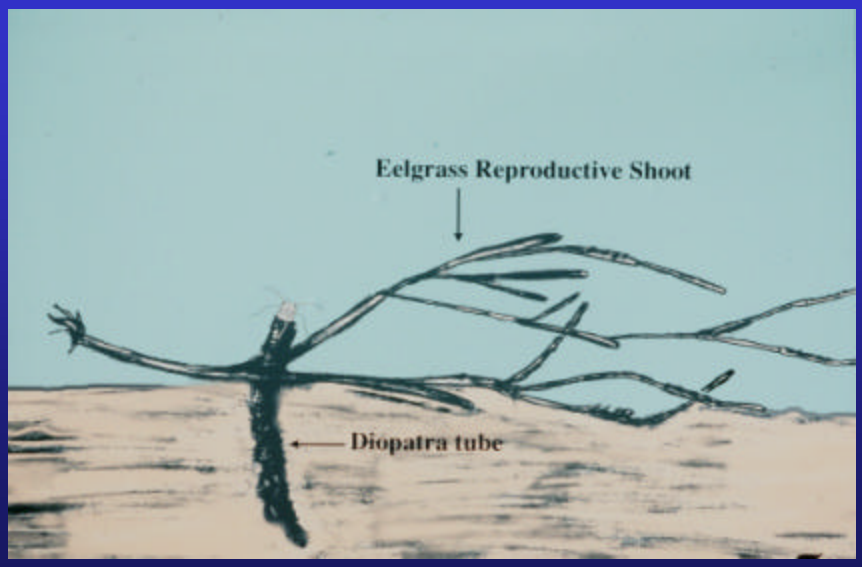


Bed Creation

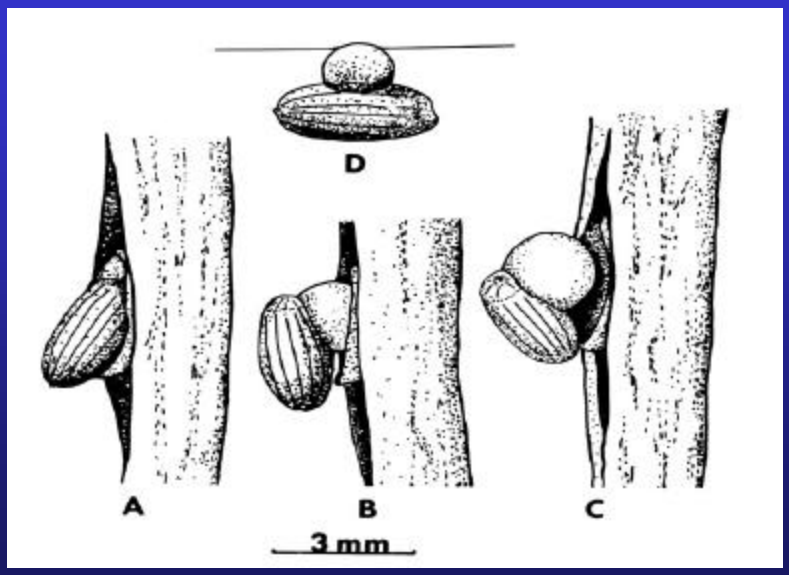
Site	Distance
1	0.7 km
2	2.3 - 2.8 km
3	3.4 km
4	3.6 km
5	7.8 km
6	11.2 km
7	17.8 km
8	19.2 km
9	19.9 km
10	23.7 - 40.9 km
11	34.6 km
12	55.9 - 87.4 km
13	108.6 km

Harwell and Orth (2002) Ecology 83:3319-3330

Influence of Diopatra on reproductive shoots by entraining then in tube caps

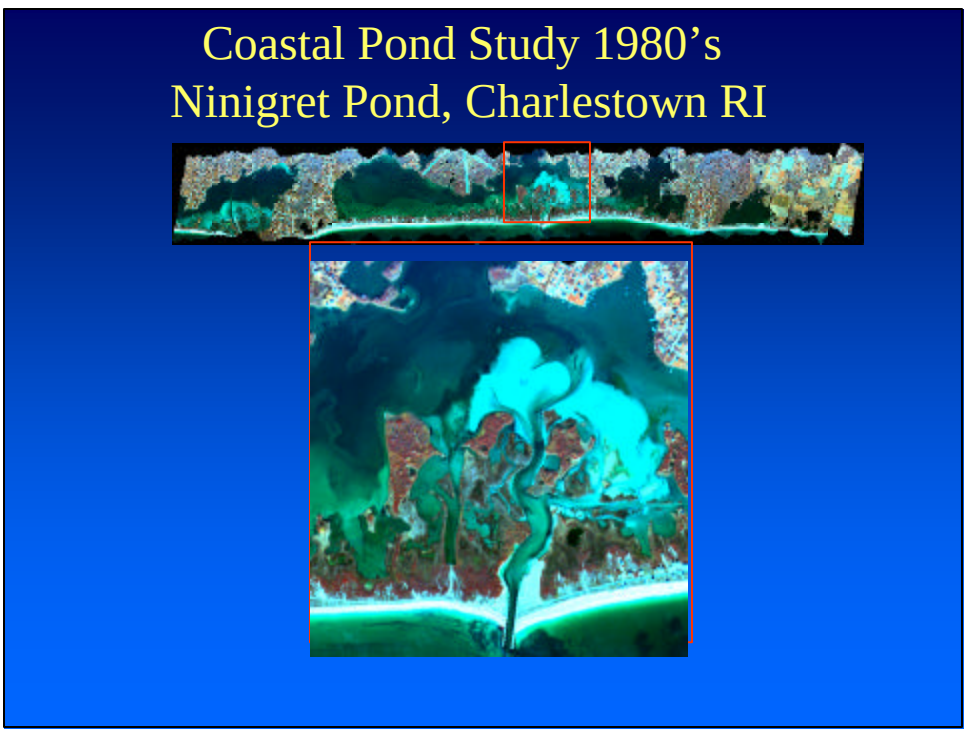


Harwell and Orth (2001) Aquatic Bot 70 1-7



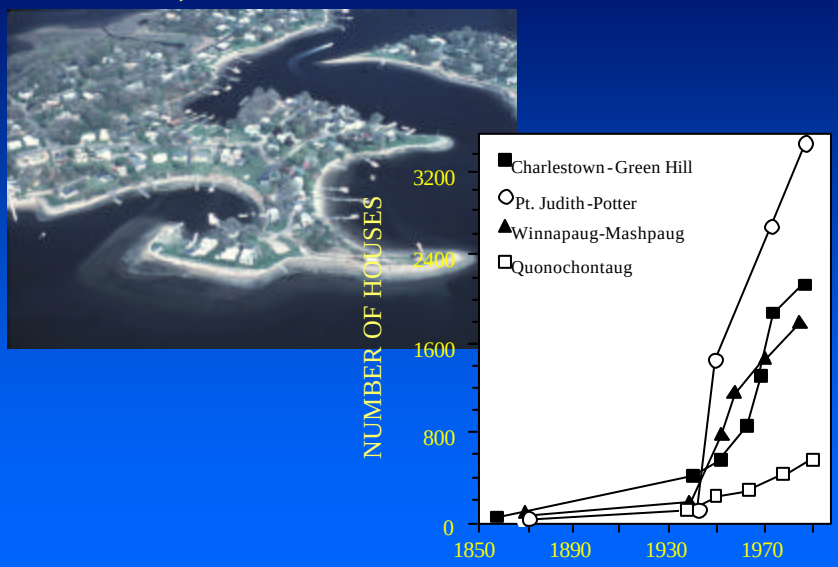
from Churchill, et al., 1985

Seagrass Ecology and the Use of
Eelgrass Seeds for Restoration
Stephen Granger, Scott Nixon,
Mike Traber and Betty Buckley
University of Rhode Island, GSO
METRIC 1 2 3 4 5 6

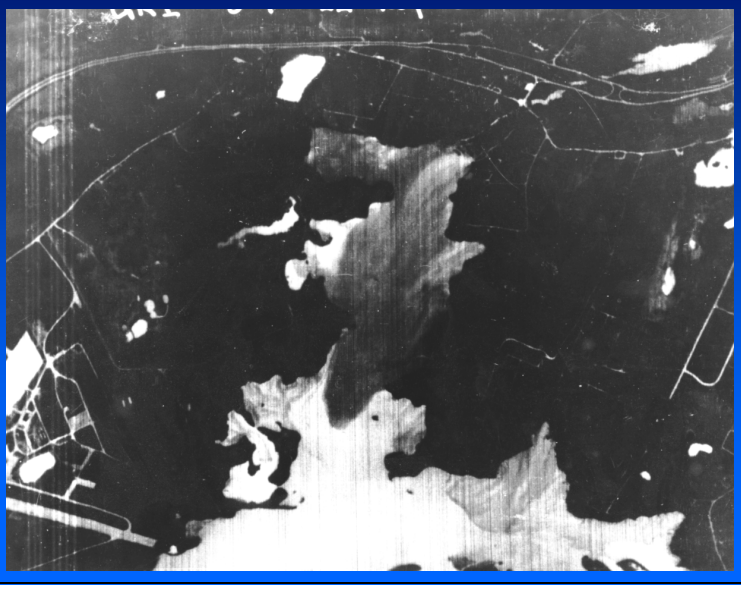

Coastal Pond Study 1980's
Ninigret Pond, Charlestown RI

Residential Development

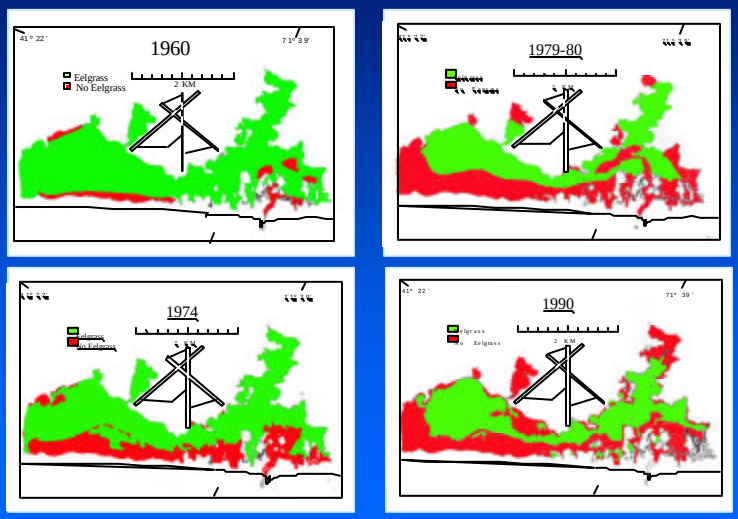
Point Judith Pond, Rhode Island



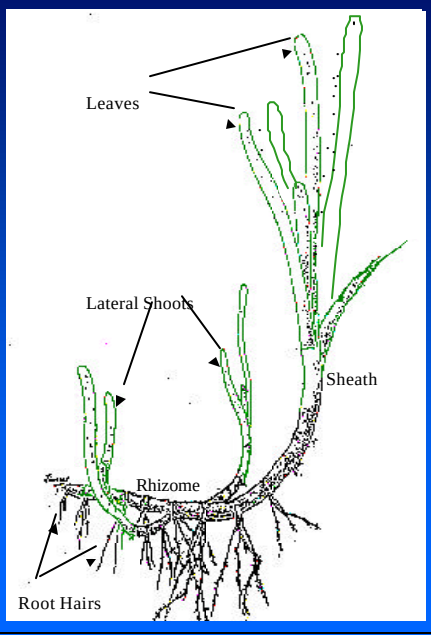
Groundwater Infiltration



Ninigret Pond, Rhode Island 1960-1990



Zostera marina L.: Plant Morphology

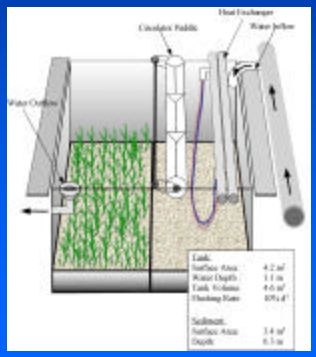


Coastal Lagoon Mesocosms at the URI Graduate School of Oceanography

1990

First Experiments

in Enclosures



Chronic Nutrient Enrichment in Coastal Embayments

- Elongation of seagrass canopy with nitrogen enrichment; independent of water clarity.
- Higher above-ground/below-ground ratio.
- Greater time required to create a new leaf.

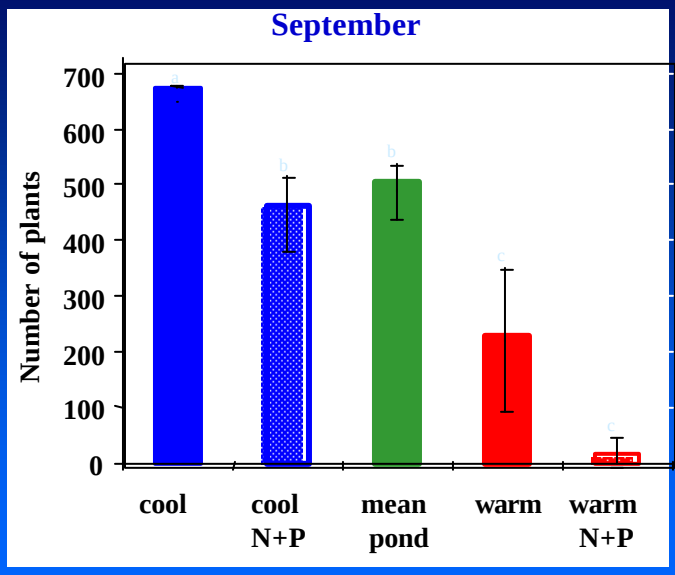
SUMMARY TABLE

Treatment	Shoot/Root Ratio	n	ANOVA grouping
Control	6.7 (0.9)	12	A
Low	8.2 (3.6)	12	A
Low + filter feeders	13.9 (2.0)	12	B
High + filter feeders	11.2 (3.3)	5	B

Treatments withANOVA grouping A are significantly different than group B (95% confidence).

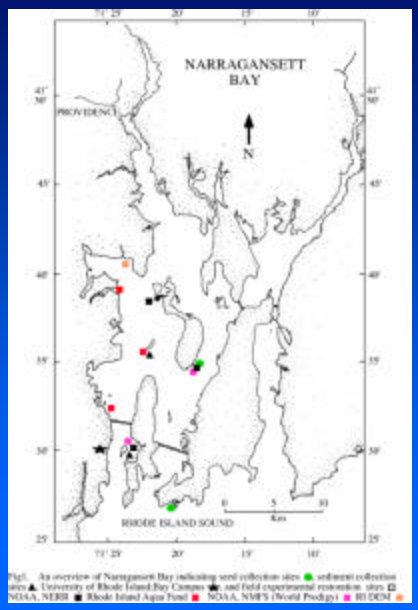
Shoot/Root ratios were determined from the g. dry weight of above ground biomass divided by the g. dry weight of th first two rhizome nodes with root hairs (see photo to left). Standard deviations are shown as ( ).

Number of Eelgrass Plants



Treatments with different letters are significantly different at the 95% confidence level

Mid-1990's and Several Attempts at Seagrass Restoration



Common Restoration Techniques

Staple

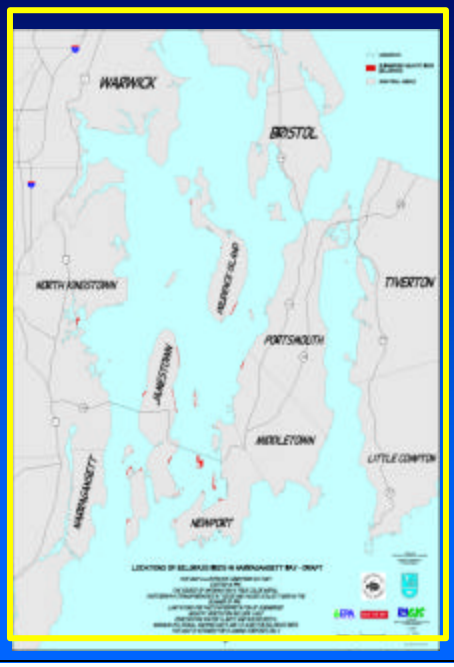
TERF

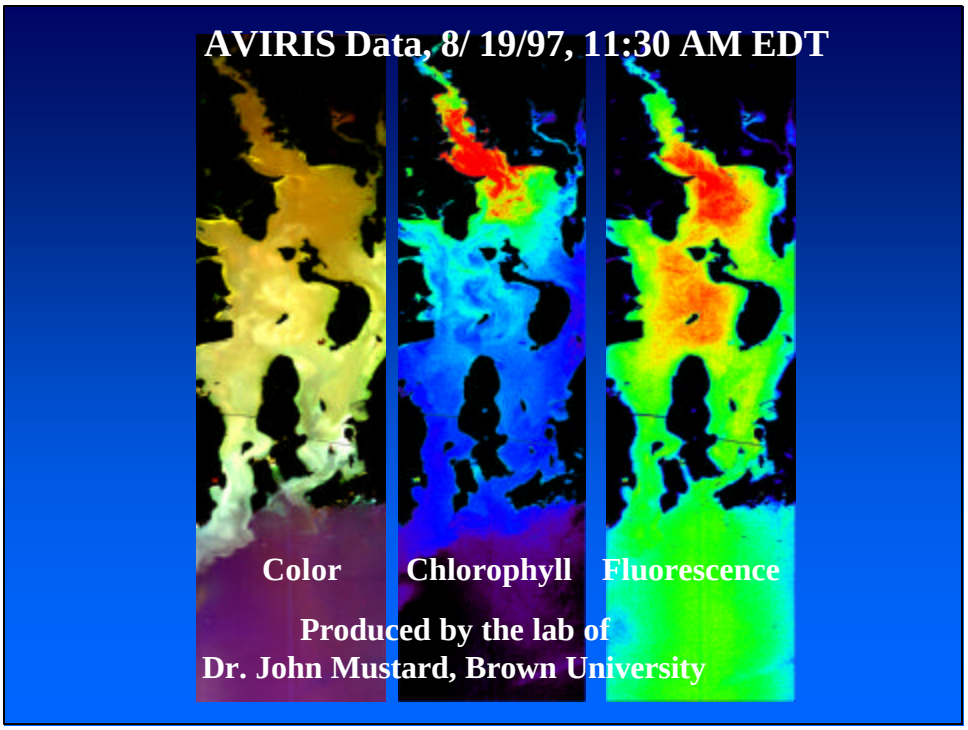
Plugs / Peat Pot

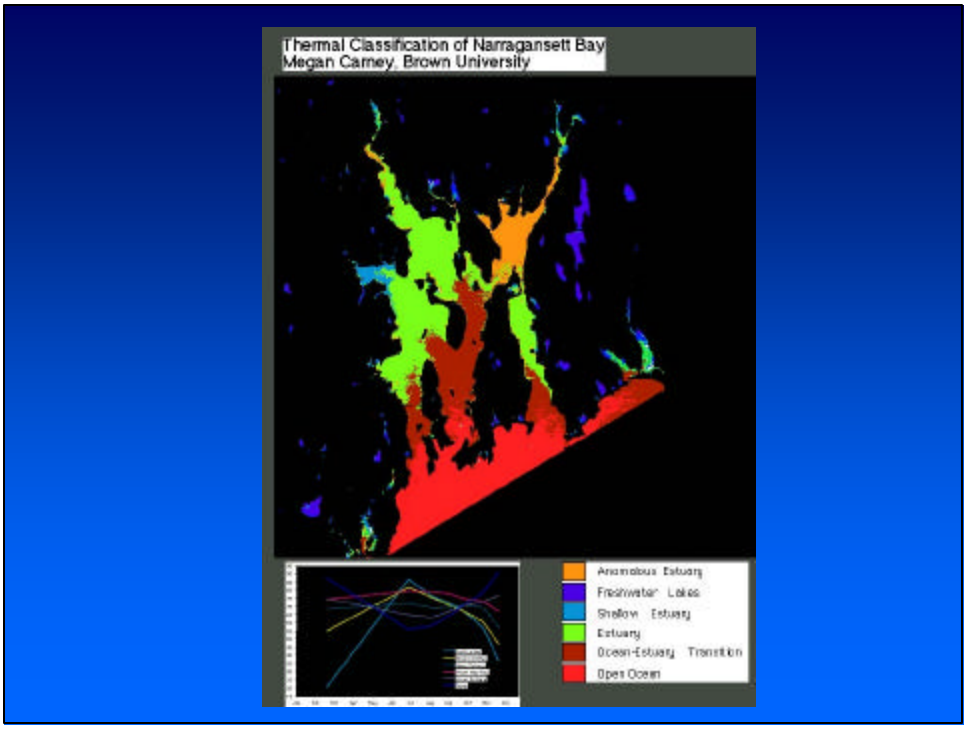
Seeds

Present Day Seagrass Distribution

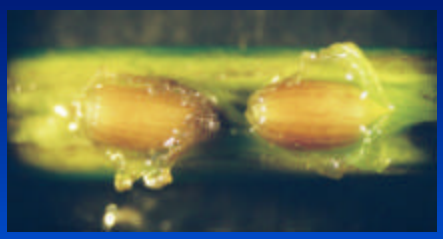
1995
Aerial Photo-
Interpretation




AVIRIS Data, 8/ 19/97, 11:30 AM EDT
Color
Chlorophyll
Fluorescence
Produced by the lab of
Dr. John Mustard, Brown University


Thermal Classification of Narragansett Bay
Megan Carney, Brown University
Anomalous Estuary
Freshwater Lakes
Shallow Estuary
Estuary
Ocean-Estuary Transition
Open Ocean

NOAA CICEET and NERRS Funded Research; Why Use Seeds ?



- **Less labor intensive to collect and distribute**
- **Less destructive to the donor site**
- **Increased genetic diversity at restored site**
- **Can be held for a period time before planting**

Seed Collection Process

Flowering plant Collection

Plants are held while seeds release

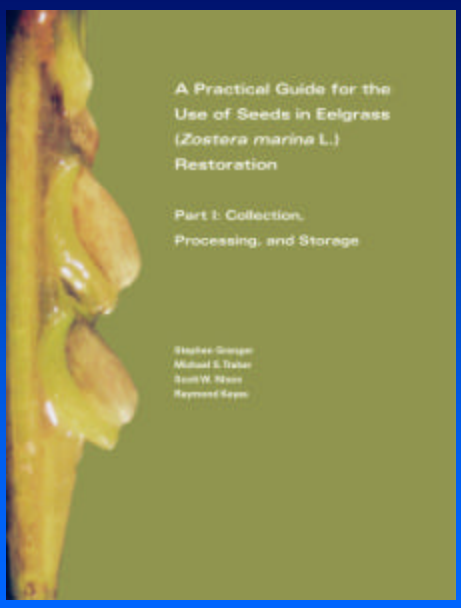
Vegetative material are removed

Tank Wash Down

Seed Recovery

Seed Holding

Seed Collection Process

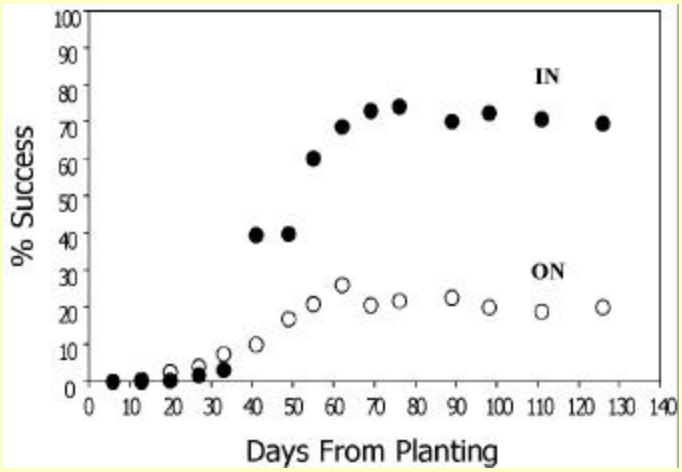


Available at
Rhode Island
Sea Grant
Publications Office

Seed Planting Strategies

- Planting <u>In</u> vs. scattering <u>On</u> the sediment
- What is the optimal density for seeding?
- How will the sediment type effect seedling growth?

Seed Planting Strategies



Increasing seed density by applying an outer layer of clay while maintaining high moisture content.

Seeds Distribution Techniques

Hand Casting

Or

Mechanized

Experiments on Seeding Density & Sediment Type

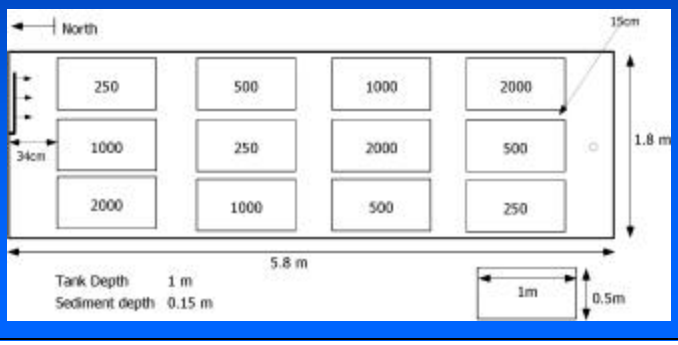
High Organic

Low Organic

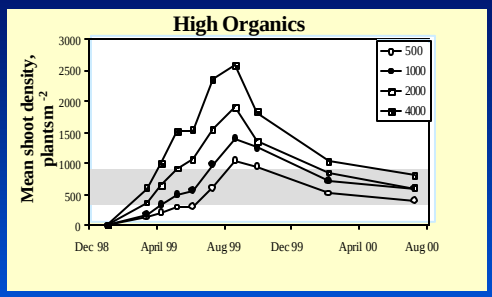
Sediment Characteristics

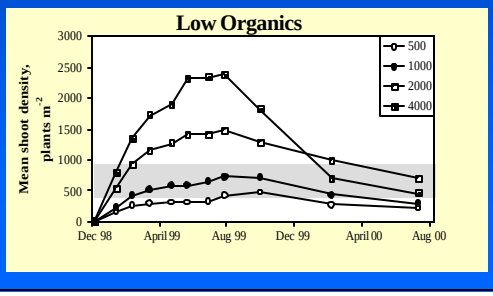
	High Organic Narragansett Bay	Low Organic Rhode Island Sound
Location		
Latitude	41° 30'	41° 21'
Longitude	71° 24'	71° 32'
% Organic Content		
0-2cm	1.7	0.56
2-6cm	1.63	0.51
Redox Layer		
Depth, cm	1.3	2.5

Experimental Design

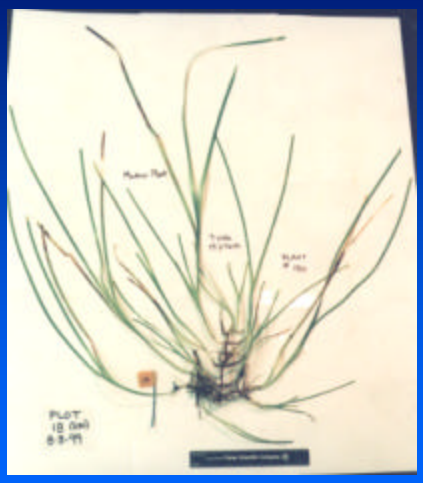


Shoot Density Time-line

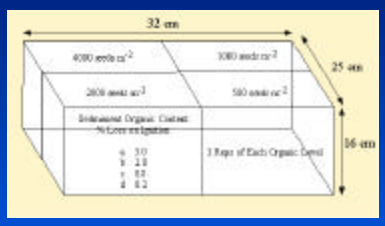


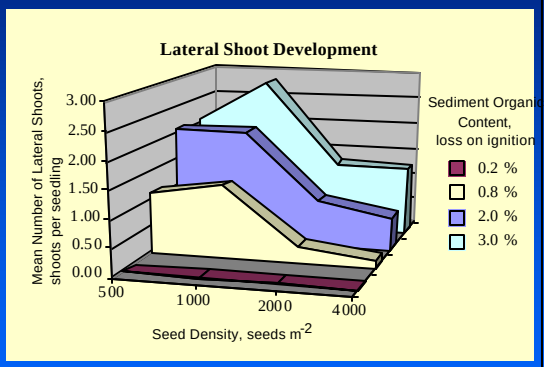


Lateral Shoot and Node Production



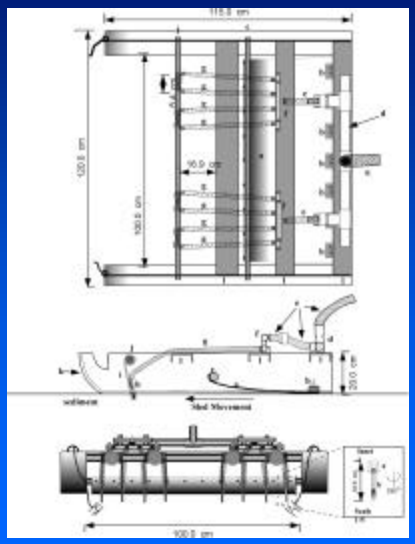
Sediment Organic-Seeding Density Experiment



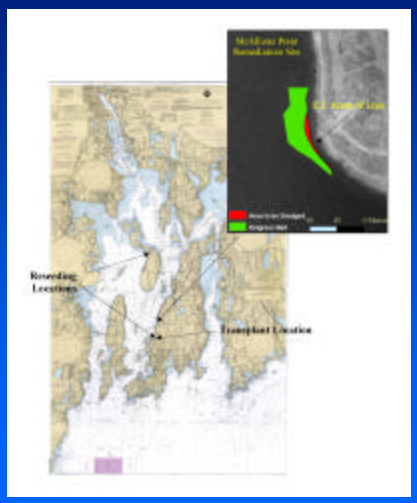


Gel-Injection Seeding

Gel-Injection Seeding



McAllister Point Remediation and Restoration



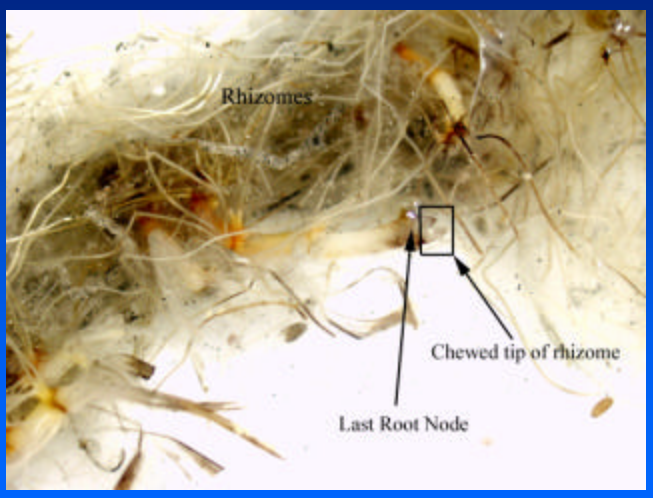
2001-2002 Seeding

Gel Injection Seeding
Prudence Island, RI
October 2001

Funded by

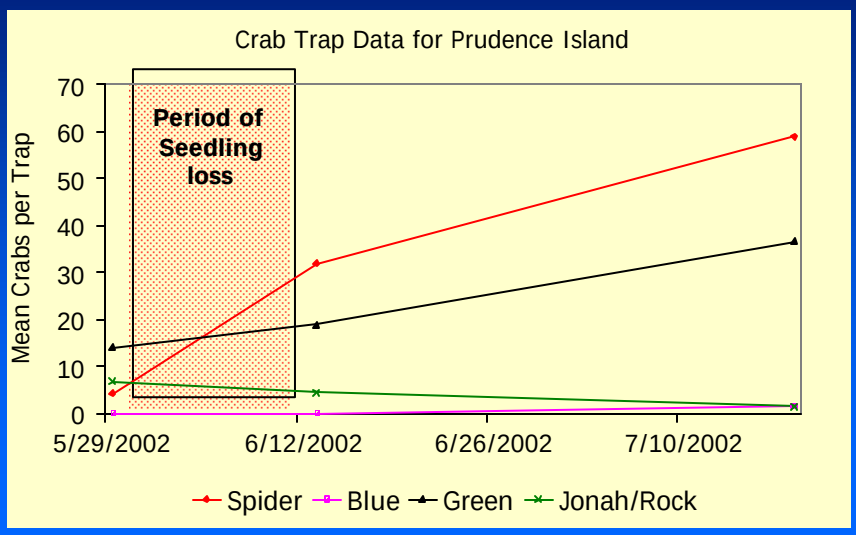
Potential Hurdles, Bioturbation

Damage to Seedling observed, June 2002



Potential Hurdles, Bioturbation

Damage to Seedling observed, June 2002



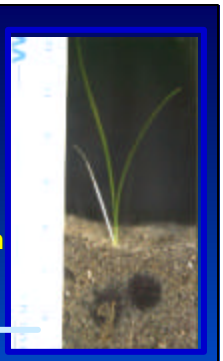
Potential Hurdles, Bioturbation

Damage to Seedling observed, June 2002

Present Research Efforts Funded by SeaGrant

- Investigating alternative suspension media to reduce or enhance sediment respiration. Adjusting the redox layer to optimize seed germination.
- Testing more heat tolerant seedlings propagated from seed stocks collected in Chesapeake Bay.
- Planting strategies to overwhelm seedling loss from grazing.
- Fall meeting of geneticists and plant propagators/breeders to consider the implications of interbreeding between Narragansett Bay and Chesapeake Bay populations.

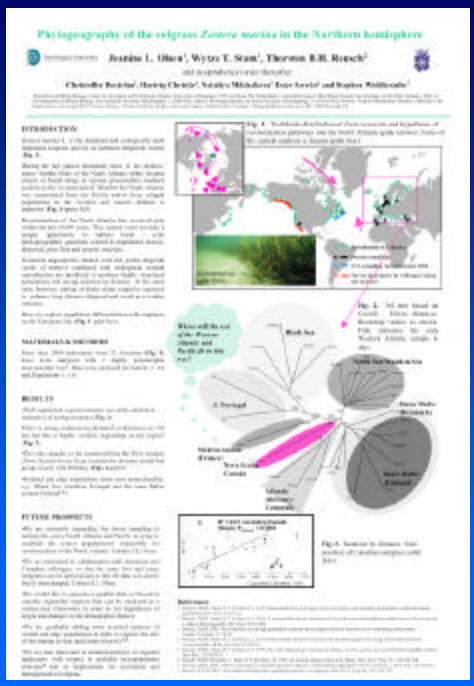
Gel-Injection Seeding



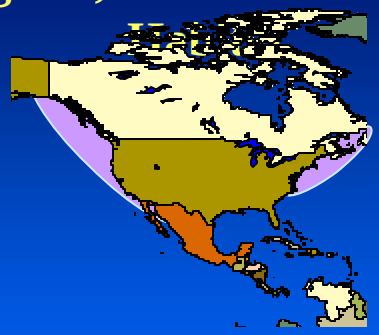
Gelling Agents Tested

Gel Type	% Germination Mean	(Stdev)	n
Sodium Bentonite (Clay)	59**	(11.7)	3
Cab-O-Sil (Silica Based Thickener)	53**	(6.9)	3
Agri-gel, (Organic gel used in terrestrial seeding)	39	(9.4)	3
Knox Gelatin (Food Gelling agent, Pig Skin)	12	(4.8)	3
No Gel, Hand planting	33	(4.8)	3

** Significantly higher, ANOV(P<0.05)



North American Eelgrass, *Zostera marina* L.



Eelgrass thrives in a broad range of environmental conditions from coarse sands and gravel in exposed locations to fine grained mud in quiescent embayments.

The North Atlantic *Zostera marina* population was nearly decimated during the 1930's by a virulent outbreak of a marine slime mold. Since the 1960's *Zostera marina* has successfully repopulated much of its former habitat.

Conclusions

- Planting seeds below the surface increases germination.
- Increasing seeding density had a negative effect on lateral shoot development.
- Increasing sediment organic content had a positive effect on lateral shoot development.
- All seeding densities came to a similar shoot density by the end of year 2, indicating a carrying capacity might be achieved.
- Gel-injection seeding looks promising but still in its infancy.

Acknowledgments

- Mike Traber, URI GSO
- Scott Nixon, URI GSO
- Malia Swartz, RI Seagrant
- Chris Deacutis, RI DEM
- Susan Adamovicz, RI DEM

Buoy-Deployed Seeding: A New Approach to Restoring Seagrass Using Seed

Chris Pickerell, Stephen Schott, and Sandy Wyllie-Echeverria

SAV Propagation Workshop
Maritime Institute, Baltimore, MD
September 3-4, 2003

Peconic Estuary, Long Island, New York

- **Average salinity: ~27ppt**
- **Mean tidal range: 0.75m**
- **Depth range for eelgrass (*Zostera marina*): 1-5m**
- **Existing eelgrass : 1551 acres**
- **Historic eelgrass: ~6240 acres**
- **~75% lost since 1930**

Eelgrass Restoration Efforts to Date

- Work began in 1996-1997 using the staple method (Fonseca, et al., 1982) and harvested adult shoots.
- TERFS (Burdick & Short, 2002) was adopted in 2000 utilizing floating and beach-cast shoots.
- Broadcast seeding (Orth, personal communication) began in 2001 after visiting VIMS.
- Development of the Buoy Deployed Seeding System (BuDSS) began soon after broadcast seeding (2001).

Seed Collection, Processing and Storage

(Churchill and Riner, 1978; Orth, et. al. 1994 and Granger et. al., 2002)

Our Goal

To design a planting method that closely mimics the natural ability of floating and rafting reproductive shoots of *Zostera* to disperse seeds long distances from a donor meadow. In so doing we would eliminate the need for flower storage and handling and the labor associated with it as well as provide a greater opportunity for the public to get involved with the process of seagrass restoration.

Basic Requirements

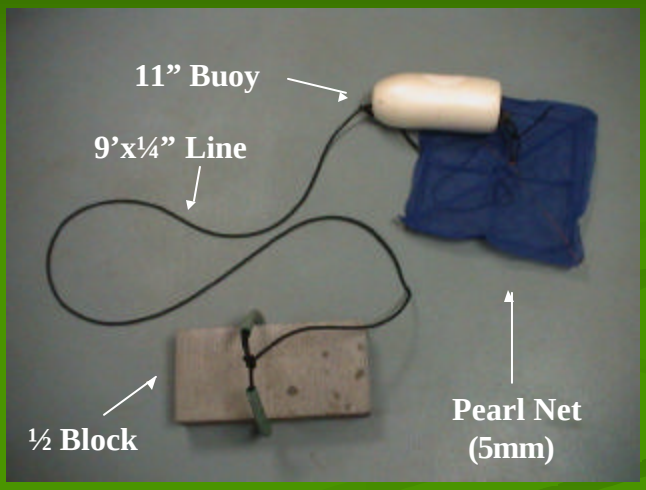
- Some means of holding reproductive shoots.
- Floatation to hold the shoots near the surface of the water to maximize spread.
- Anchor and line to hold the shoots over a defined area.

Prototype I

Design Considerations

- Reliable
- Inexpensive
- Easy to construct and deploy.
- Sturdy enough to be reused over multiple seasons.
- Adaptable to different planting densities, depths and energy environments.
- Use off-the-shelf components.

Prototype II



The Buoy Deployed Seeding System (BuDSS)

2002 BuDSS Deployment Locations

- Red Cedar Bluff: sandy, open bay
- Southold Bay: sandy, open bay
- Jessups Cove: muddy, shallow cove
- Sag Harbor Upper Cove: muddy, cove
- Sag Harbor Causeway: sand/mud, cove

Sag Harbor Causeway

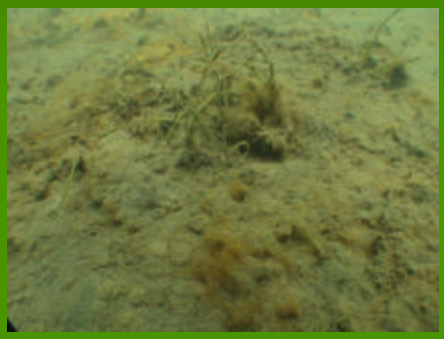
Restoration Site

- This site supported eelgrass as recently as 1994.
- Broadcast seeding "successful" in 2001.
- Depth: 1.3m
- Tidal Range: 0.75m
- Sediment Type : 0% gravel/96% sand/4% clay with 6% Organic Matter

Sag Harbor Causeway
Restoration Site Deployment

- Our goal was to plant 2 - 0.10acre (0.04 hectare) plots at density of 200 seeds/m^2.
- Each buoy arc covered 29m^2 and was stocked with flowers that were expected to yield 5,800 seeds.
- 15 buoys were set in a 3x5 grid with 15ft OC spacing.
- Collected and deployed flowers on June 26, 2002.
- Conducted side-by-side broadcast seeding in September.

Sag Harbor Causeway
Restoration Site Monitoring



April 2003

June 2003

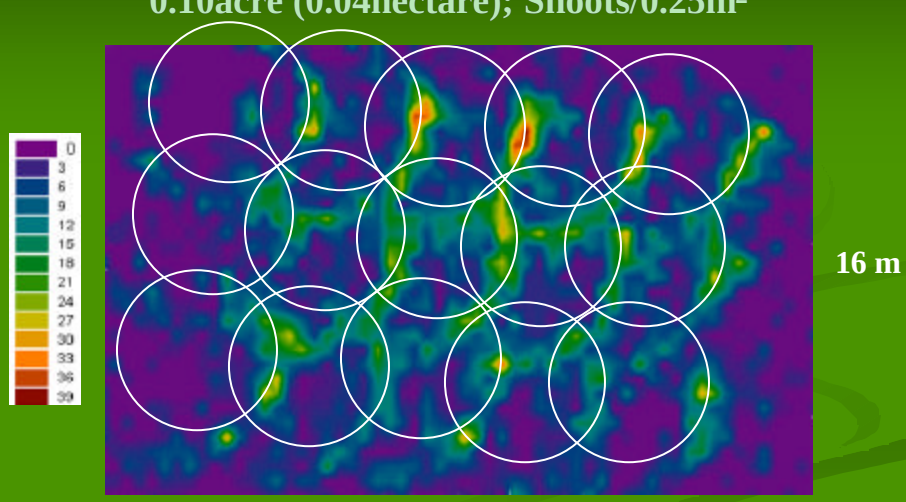
Results

- Seedling distribution closely corresponded to the arc of each buoy indicating minimal transport following release.
- Counts within plots (June) indicated at least 4% recruitment from predicted* seed fall.
- A mean of 2.8 laterals per genet were observed for all plots (BuDSS and Broadcast).
- There was a consistent, but different seedling distribution signature for the BuDSS and broadcast plots.

*Predicted seed fall was less than actual in subsequent tests.

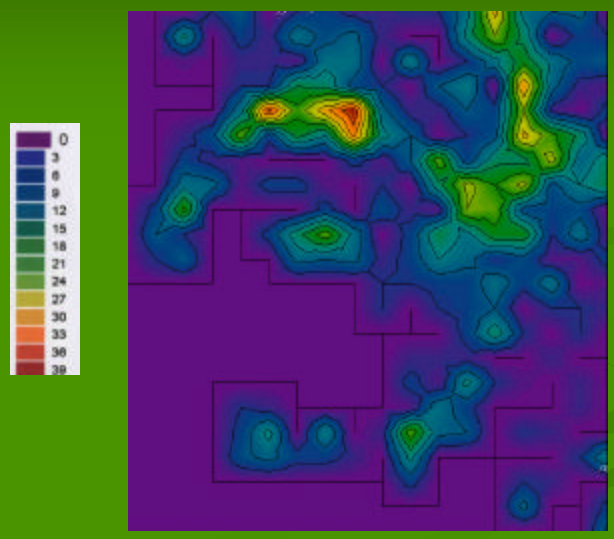
Seedling Density Contour Plot

0.10acre (0.04hectare); Shoots/0.25m^2



Single Buoy Arc

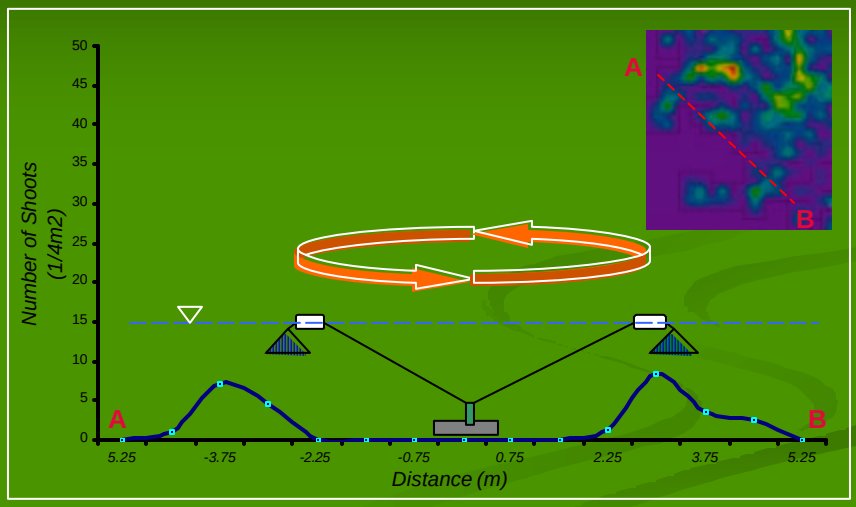
(Shoots/0.25m^2)



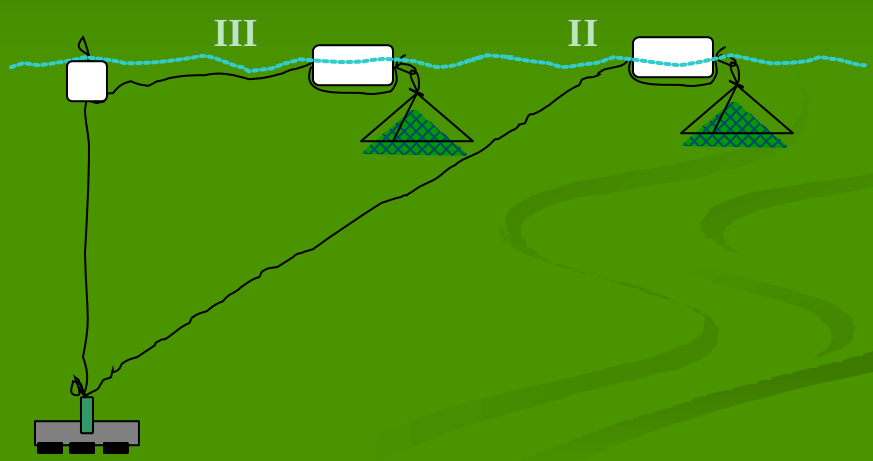
Lessons Learned

1. Seedling recruitment below each buoy was predictable, but not as evenly distributed as desired.

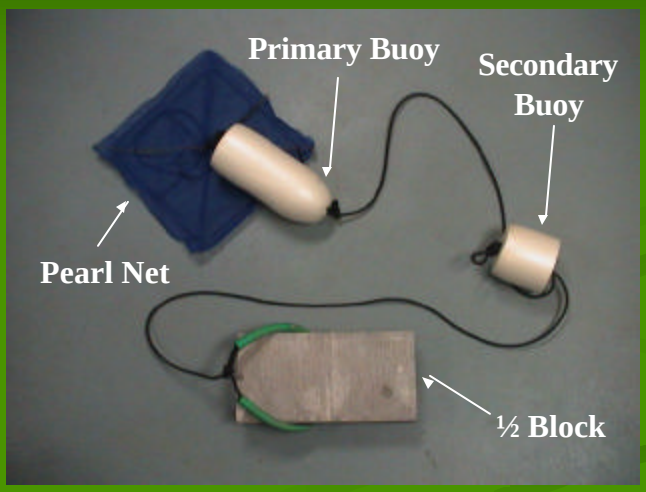
Typical Seedling Distribution Around a Single Buoy



Development of Prototype III



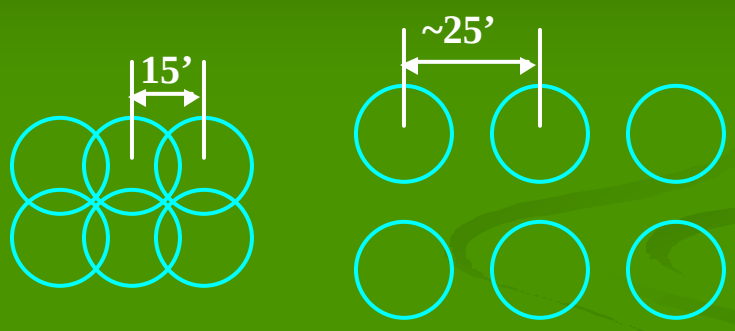
Prototype III



Lessons Learned

2. It would be possible to plant a larger area with the same number of buoys with a greater OC spacing between buoys.

Modified Buoy Spacing



2002 deployment

2003 deployment

Lessons Learned

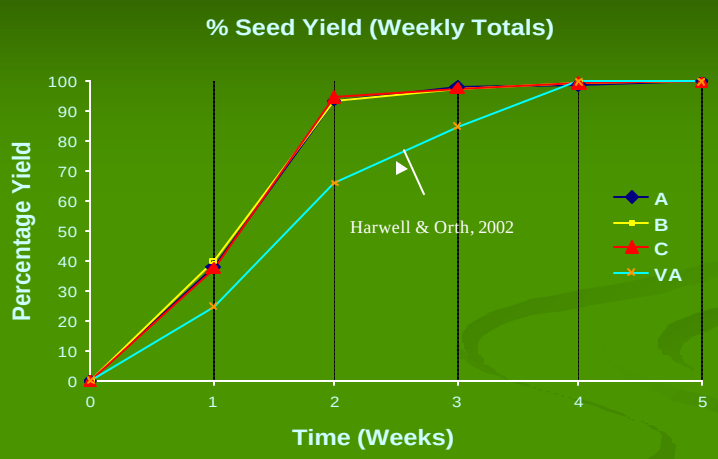
3. Our seed(ling) yield was not as high a expected based on preliminary counts of Stage IV seeds (DeCock, 1980) in spathes.

Seed Release Estimates

(How many and when?)

- Weekly counts from nets (Noyack Cr.)
- Data from the literature (Virginia; Harwell and Orth 2002.)
- Daily counts from nets (Mulford Pt.) Data not presented.

Seed Yield of Harvested Flowers



- Predicted total yield = ~5000 seeds per net, based on estimates of ~50 seeds/reproductive shoot and a stocking rate of 100 shoots/net
- Observed total yield (mean) = 2353 seeds per net

Costs

- Seed Collection (20 diver hrs./acre)
  - A well trained diver at a productive site can collect ~300 reproductive shoots/hour; enough to stock 3 nets. A 15 buoy deployment (0.25 acre at the wider OC spacing) would require 5 diver hours.
- Materials ($400/acre)
  - Each buoy/net/anchor combination costs $6.50.
  - Total materials cost for a 0.25 acre planting would be ~ $100.
- Deployment ($0-?)
  - Depending on the location of the planting site relative to collection site and whether a boat is used during seed collection, there may be no additional cost associated with deployment.
- Monitoring
  - Monitoring costs vary considerably with need. Cost would involve dive time, boat and/or travel time.

Advantages and Disadvantages

- ADVANTAGES:
  - Practical:
    - Minimal handling of flowers and seeds required
    - No need for storage and handling facility and the energy and labor necessary to maintain it
    - Visible to the public
  - Theoretical:
    - Mimic's natural phenological schedule
    - May reduce predation by staggering seed dispersal over time
    - May yield a more even distribution of seeds given the combination of time and natural forces at work
- DISADVANTAGES:
  - Practical
    - Visible to the public (could be an attractive nuisance)
    - Navigation issues
  - Theoretical
    - Mimic's natural phenological schedule (seed predators still active)
    - May allow more time for predation, export, or over burial

What's Next?

- Further refine method to improve seedling distribution and buoy spacing.
- Develop a modified version for high-energy and deeper water environments.
- Test with additional species.

Acknowledgements

- New York State Department of State, Long Island Community Foundation and The National Fish and Wildlife Foundation for funding this ongoing work.
- The Peconic Estuary Program
- The Towns of Southold and Southampton
- Bob Orth, VIMS
- Steve Granger, URI
- Jerry Churchill, Adelphi University
- Jon Semlear, Bayman and Southampton Town Trustee
- Mallory Delany for preparation of the Power Point presentation.
- Kim Petersen, Matt Parsons, and Mallory Delany for their assistance in the field.

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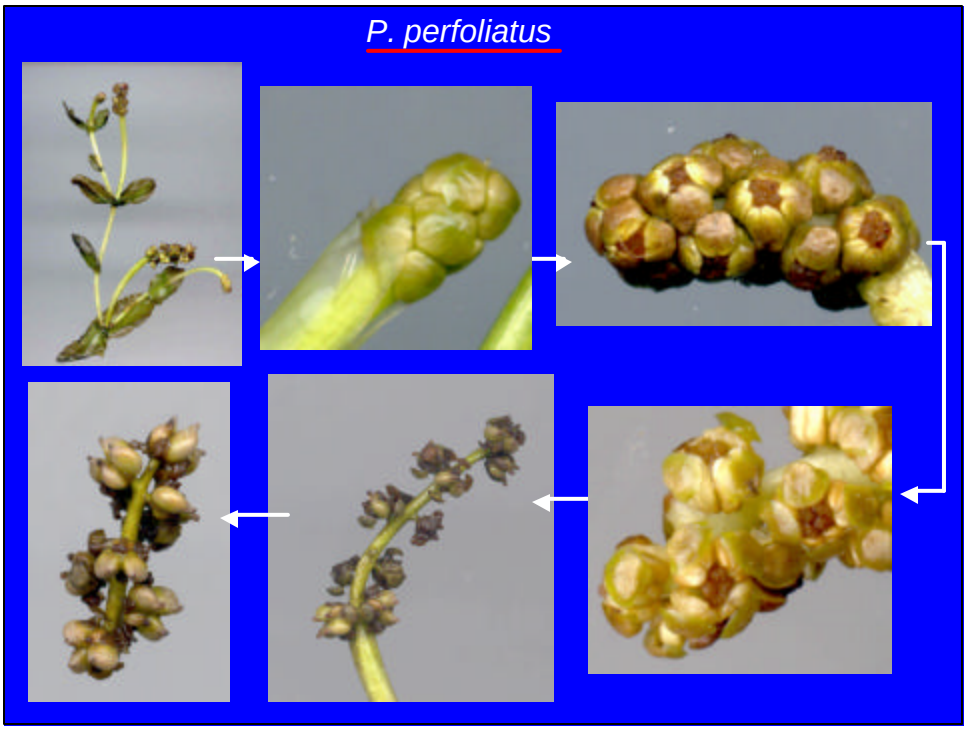
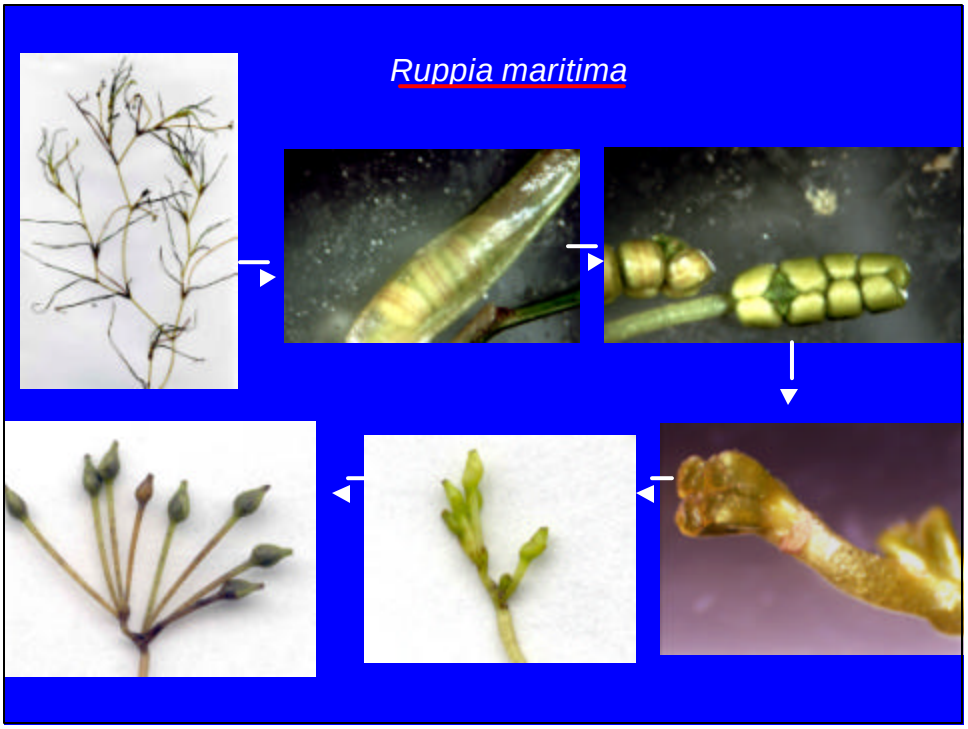
Orth, R.J., M. Luckenbach, and K.A. Moore. 1994. Seed dispersal in a marine macrophyte: implications for colonization and restoration. Ecology **75**:1927-1939.

Reproductive Potential of Natural Populations of *Ruppia maritima* and *Potamogeton perfoliatus* by Seed in the Mid-Chesapeake Bay

M. Stephen Ailstock
Kelly W. Caffey
Jay Kunkle
Andrew E. Watts
Christopher L. Wharton

Reproductive Potential	*Potamogeton perfoliatus*	*Ruppia maritima*
1) Plants/unit area	Highly variable	Highly variable
2) Stems/plant	Highly variable	Highly variable
3) Inflorescences/ stem		
4) Flowers/ inflorescence		
5) Carpels/flower		
6) Ovules/carpel		
Seeds/ inflorescence		
Seeds/stem		


P. perfoliatus


Ruppia maritima

Reproductive Potential	*Potamogeton perfoliatus*	*Ruppia maritima*
1) Plants/unit area	Highly variable	Highly variable
2) Stems/plant	Highly variable	Highly variable
3) Inflorescences/ stem	2.4	2.6
4) Flowers/ Inflorescence	5-12 (9)	2
5) Carpels/flower	4	4
6) Ovules/carpel	1	1
Seeds/ inflorescence	20-48	8
Seeds/stem	48-115	20.8

Redhead – Location Eastern Bay

*Average ranges of three, 1/4lb. Samples					
Date:	Immature Inflorescence	Mature Inflorescence	Inflorescence with Immature Seed	Inflorescence with Mature Seed	Potential Seeds per lb. (Inflorescences x36x4)
07/29/03	10-48	18-54	3-28	32-81	9072-30384
08/07/03	20-40	30-40	14-19	60-93	17856-27648
08/14/03	5-8	7-15	5-15	42-130	8496-24192
^Averages of three, 1/4lb. Samples					
Date:	Immature Inflorescence	Mature Inflorescence	Inflorescence with Immature Seed	Inflorescence with Mature Seed	Potential Seeds per lb. (Inflorescences x36x4)
07/29/03	25	32	12	52	17424
08/07/03	31	35	16	72	22176
08/14/03	7	10	10	77	14976
**Counts per 25 individual stems					
Date:	Immature Inflorescence	Mature Inflorescence	Inflorescence with Immature Seed	Inflorescence with Mature Seed	Seeds/stem (Inflorescences x36/25)
07/29/03	17	20	6	19	89
08/07/03	18	15	2	39	107
08/14/03	8	4	1	31	63

Ruppia – Location Taylor's Island

*Ranges of three, 1/4lb. Samples.

Date:	Immature Inflorescence	Potential Seed Production	Mature Inflorescence	Potential Seed Production	Immature Seed	Mature Seed	Total Potential Seeds for one, 1/4lb.	Potential Seeds per lb. (Seedsx4)
07/28/03	0-6	0-48	0-2	0-16	460-669	78-100	538-833	2152-3332
08/01/03	7-11	56-88	1-5	8-40	291-619	84-138	439-885	1756-3540
08/05/03	3-7	24-56	1-2	8-16	134-234	49-65	215-371	860-1484
08/20/03	0	0-64	0	0	0-9	14-17	14-90	56-360

^^ Averages of three, 1/4lb. Samples.

Date:	Immature Inflorescence	Potential Seed Production	Mature Inflorescence	Potential Seed Production	Immature Seed	Mature Seed	Total Potential Seeds for one, 1/4lb.	Potential Seeds per lb. (Seedsx4)
07/28/03	3.66	29.28	1.33	10.64	535.33	86.66	661.91	2647.64
08/01/03	8.66	34.64	2.66	21.28	405.66	107.66	569.24	2276.96
08/05/03	5.33	42.64	1.66	13.28	188.33	54.33	298.58	1194.32
08/20/03	0	0	0	0	5.66	15	20.66	82.64

**Counts per 25 individual stems

Date:	Immature Inflorescence	Potential Seed Production	Mature Inflorescence	Potential Seed Production	Inflorescence with immature Seed	Inflorescence with Mature Seed	Total Potential Seeds for 25 stems	Potential Seeds/stem (Inflor. x8/25)
07/28/03	2	16	1	8	48	11	496	19.8
08/01/03	5	40	1	8	57	15	624	24.9
08/05/03	4	32	2	16	57	14	616	24.6

Factors Affecting Reproductive Potential

1) **Plant vigor** - Photosynthesis - Ambient environment

2) **Plant growth** - Physical damage - Bioturbation

3) **Flowering** - Plant vigor - Plant growth - Stage of growth

4) **Floral abortions** - Miscarriages

5) **Seed set** - Pollination - Plant density - Habitat stability

6) **Seed maturation** - Plant vigor - Plant growth - Habitat stability

7) **Seed dispersal** - Water currents - Waterfowl

8) **Overwintering success** - Habitat stability - Bioturbation

Effects of Waterfowl Classes on Factors Affecting Reproductive Potential

Factor	Resident Waterfowl	Migrating Waterfowl
1) Plant vigor	Direct continuous	Indirect sporatic (overwintering structures)
2) Plant growth	Direct continuous	None - Favorable (Apicial dominance)
3) Flowering	Direct continuous	None
4) Floral abortion	N.A.	N.A.
5) Seed set	N.A.	N.A.
6) Seed maturation	Direct continuous	None
7) Seed dispersal	None	Significant
8) Overwintering success	?	?

Effects of Mute Swans on the Reproductive Potential of *Potamogeton perfoliatus* and *Ruppia maritima* (30 days)

R. maritima

1,550 seeds/lb x 2.2 lb/kg x *3.8 kg/day/swan x 30 days = 388,740 potential seeds/swan

P. perfoliatus

18,192 seeds/lb x 2.2 lb/kg x *3.8kg/day/swan x30 days = 4,562,553/seeds/swan

*Willey and Halla 1972

This work was supported by the U.S. Army Engineer Research and Development Center, Vicksburg, MS with special thanks to Deborah Shafer and Mark Mendelsohn, ACOE

Propagation and Production of SAV transplant stock for ecosystem restoration

Michael Smart

USAE ERDC LAERF

Lewisville, Texas

US Army Corps of Engineers

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Why have we lost our SAV?

Man-made systems (reservoirs)

Natural systems

- Disturbance
  - Eutrophication, watershed development, storms, etc
- Displaced by nonindigenous species
  - hydrilla, Eurasian watermilfoil
- Nonindigenous animals
  - Common carp, nutria, grass carp, Canada geese
- Management actions
  - Dredging, herbicides

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If we restore environmental conditions (water quality) will SAV recover?

Dependence on seed bank / propagule bank

Obstacles:

- **Biotic disturbance**
  - Nonindigenous species
- **Physical disturbance**
  - Wind, waves, erosion, loss of substrate
- **Water level fluctuations**
  - Common in multipurpose reservoirs

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If replanting is required, what propagules should we use?

Seed?

- **Availability**
- **Viability**
- **Germination cues**
- **Storage**
- **Slow establishment**

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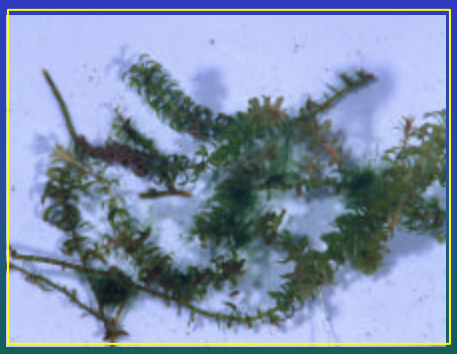
If replanting is required, what propagules should we use?

Fragments?

- Buoyancy
- Limited reserves
- Nonindigenous species



fragments

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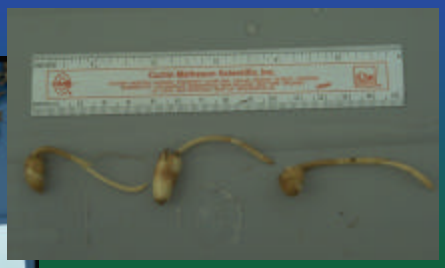
If replanting is required, what propagules should we use?

Tubers / winterbuds?

- Availability, buoyancy, handling



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If replanting is required, what propagules should we use?

Bare-root transplants?

- Availability, buoyancy, handling, nonindigenous species

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If replanting is required, what propagules should we use?

Nursery-grown plants

- Robust, mature plant; transportable with well-developed shoots and roots contained in a sediment matrix that is readily incorporated into the bottom substrate

Quickest, hardiest, most likely to succeed

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So, where do you get nursery-grown SAV transplants?

Commercial growers?

- Limited availability

"Grasses in classes", etc?

- Limited availability

Grow your own?

- You control species selection, timing, etc.

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So, how do you grow SAV transplants?

Based on techniques developed in the early 1980's

- Smart and Barko, 1985. Laboratory culture of submersed freshwater macrophytes on natural sediments. Aquatic Botany 21:251-263.

Depends on the use of natural sediment as source of N and P.

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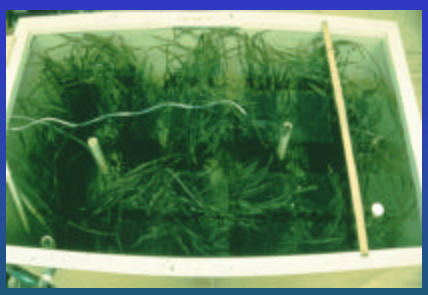
Plant Growth Requirements

- Light
- Temperature
- Nutrition
  - Sediment / Water
- Photosynthetic carbon source



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Plant Growth Requirements: Light

- Greater than 50% of full sunlight detrimental
  - 33% or 50% neutral-density shade fabric
- Clear water (no phytoplankton blooms)
- Greater than 12:12 photoperiod advantageous
- Difficult to provide adequate artificial light on large scale

Most economical and efficient production during spring, summer, and fall in outdoor facilities

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Plant Growth Requirements: Temperature

Optimum for many species near 28C

- **Range: 25-30C**
- **Protect from hard freeze in winter**

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Plant Growth Requirements: Nutrition

Sediment requirements

- **Rooted SAV derives much of its N and most of its P from sediment**
- **P in water grows algae**

The *sediment* should have a high fertility and an ability to retain P

fine-textured, mineral (not organic) sediment

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Plant Growth Requirements: Nutrition

Water requirements

- Rooted SAV derives much of its N and most of its P from sediment
- P in water grows algae

The *sediment* should have a high fertility and an ability to retain P

alum-treated water is clear and P-free

tap water must de-chlorinated

a 1-2 cm layer of aquarium gravel over the sediment can help reduce P release

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Plant Growth Requirements: Nutrition

Water requirements (cont'd)

- Many species of SAV have a high requirement for K in the water column

may need to occasionally monitor K concentration and add as needed

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Plant Growth Requirements: Photosynthetic carbon source

Water requirements

- The concentration of free CO_2 in most freshwaters is low, particularly at pH levels above 8.3
- Many species of SAV utilize and benefit from bicarbonate
- Many species have a requirement for Ca in solution

While aeration can help replenish CO_2 taken up in photosynthesis, this does not eliminate the need for bicarbonate and Ca. pH should be monitored and alkalinity should be checked occasionally. If alkalinity declines, Ca may need to be added as well.

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Plant Propagule Production Requirements: Containers

Must be easily transported

- Plastic nursery pots, 3 to 4” diameter
- Weakly-rooted species might benefit from peat liners
- Held in trays to prevent tipping

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Plant Propagule Production Requirements: Tanks

Containers must be easily accessible

- Constructed of wood and lined
- Concrete or fiberglass raceways
- Rubbermaid tanks
- 50 – 100 cm water depth
- Flat bottom
- Water supply

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Plant Propagule Production Maintenance Requirements

Weed control

- Monocultures are easier

  One tank – one species

  If fragments can spread, they will

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Plant Propagule Production Maintenance Requirements

Pest control

- Watch for insect damage and deal with it early
- Snails can be a problem occasionally
- *Gambusia* (mosquito fish)

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Plant Propagule Production Maintenance Requirements

Algae control

- Prevention is easier than control

Water exchange (with alum-treated water)

Filtration (sand or DE filters)

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Plant Propagule Production Maintenance Requirements

Water quality maintenance

- Rapidly growing plants profoundly alter water chemistry

Partial water exchanges to maintain alkalinity, Ca, and K

Filtration if needed for turbidity

Aeration (air lifts) for mixing, gas exchange

Consider CO_2 augmentation for high production systems

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Plant Propagule Production Maintenance Requirements

Sediment nutrient depletion

- Rapidly growing plants can quickly deplete sediment N

Fertilize sediments with NH_4 prior to planting

Add N to sediments as needed

Add N sparingly to water (<1 mg N/L) - use caution

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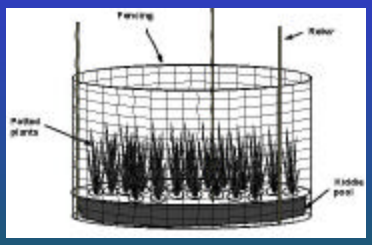
In-lake Plant Propagule Production: An Alternative to an Off-site Facility

- **Use sediment from the site**
- **Kiddy pools or "floats"**
- **Protect from disturbance**
  - ***Pre-conditions plants to WQ conditions at restoration site***
  - ***Minimizes transportation and labor***



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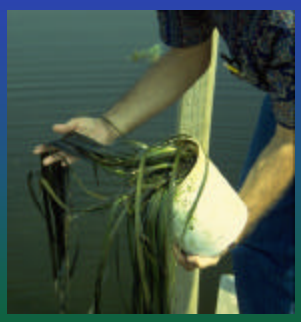
Plant Propagule Production

The end result



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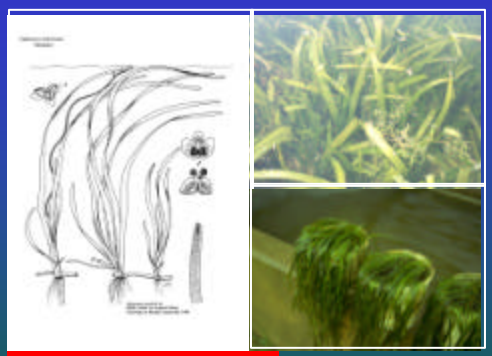
Handbook for Ecosystem Restoration

- **Growth characteristics**
  Growth form, reproduction, perennation
- **Range**
- **Use and habitat**
- **Culture**
  Propagule, light, container, substrate, fertilization, depth, comments



Vallisneria americana

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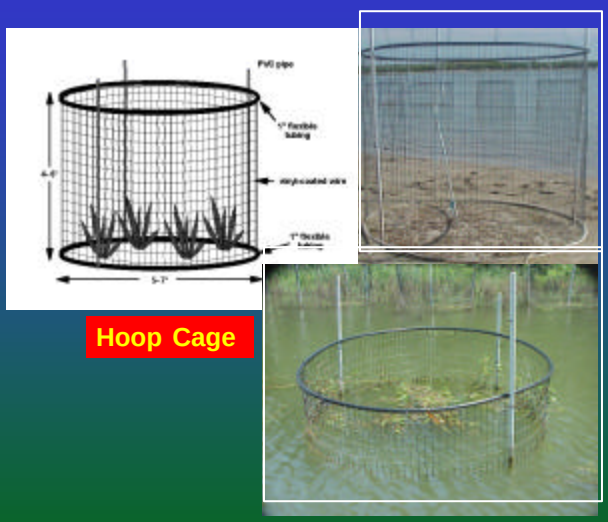
Handbook for Ecosystem Restoration

- **Field planting**
  - Propagule
  - Season
  - Substrate
  - Water depth
  - Exclosure type
  - Comments



Hoop Cage

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Propagation and Production of SAV

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Applications and Limitations of Micropropagation for the Production of Underwater Grasses

M. Stephen Ailstock
C. Michael Norman
Kathleen J. Durham

Micropropagation – the manipulation of small quantities of axenic plant material ranging from simple cells to stem pieces under conditions favorable to the formation of new plants.

Related Terms – Tissue culture – Cell culture – Axenic culture

Examples of Agronomic Plants Propagated by Micropropagation

Boston Fern	Rhododendron	Strawberries
African Violets	Mountain Laurel	Potatoes
Tulips – Lilies	Apples	Perennial Corn

Advantages of Micropropagation

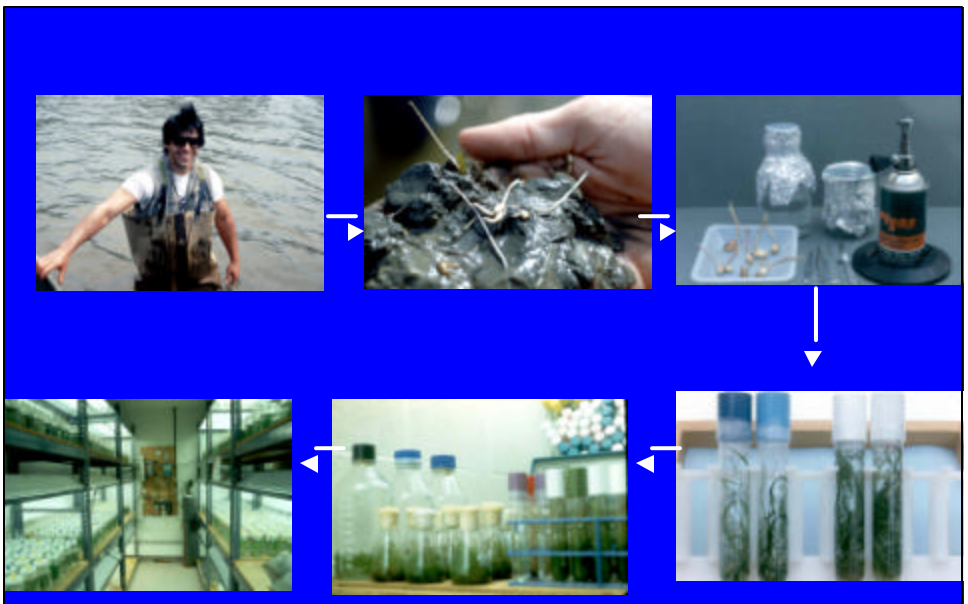
1) No seasonal constraints
2) Large numbers of plants produced
3) Inexpensive
4) Plants are axenic and disease free (specific techniques)
5) Plants are clones

Disadvantages of Micropropagation

1) Plants are clones
2) Some specialized training requirements
3) What to do with all the plants produced
4) Transitioning to field sites

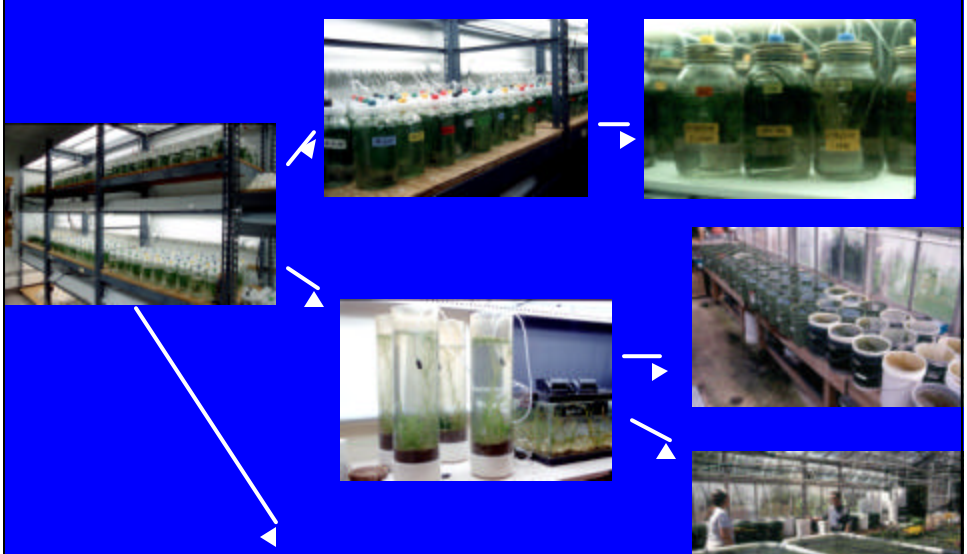
Procedural Requirements for Developing a Micropropagation System

1) **Species Selection** – Desirable ecotypes – Value – Demand – Applications
2) **Explant Choices** – Sterile – Semi-sterile – Meristems
3) **Disinfestation of Explants** – Bacteria – Fungi – Algae
4) **Development of Propagation Media** – Minerals – Carbohydrates – Plant growth regulators
5) **Media Refinement**
6) **Development of Growth Media** – Minerals
7) **Development of a Transition Protocol** – Lab – Greenhouse - Field



Application of Micropropagation to Submersed Aquatic Plants

- Physiological studies of plant growth and development
- Contaminant dose/response studies – chemical ecology
- Bioassays of sediment and water
- Education/demonstration projects
- Plant production for field establishment



Transplant Applications

(continued next page)

Transplant Applications

Costs for Basic Propagation Facility

1) Laboratory

•Autoclave	$6,000
•Laminar Flow Hood	$5,000
•Culture Room	$9,000

2) Propagation Cost/1000 Multi-stemmed Transplants

•Media	$ 22
•Culture Tubes	$ 48
•Labor	$ 160
	$ 230

3) Preparation for Field Establishment

•Containers	$ 30
•Labor	$ 160
	$ 190

Total Production Costs	$ 420/1000	$0.42/plant

Challenges for using Micropropagation for Production of Submersed Aquatic Plants

- Limited species – Little success with seagrasses
- Sporadic demand for quantities of plants
- Short planning horizons for field applications
- Ill-defined project objectives
- Significant gaps in basic plant physiology

This work was supported by the Maryland Port Administration with special thanks to Mr. Nathaniel Brown

Bay Grasses in Classes Overview

- Students learn the importance of SAV while growing different species in their classroom.
- Participate in restoration effort
- Create plant stock for restoration activities

  Since 1998-

  ~ 28,000 students participated

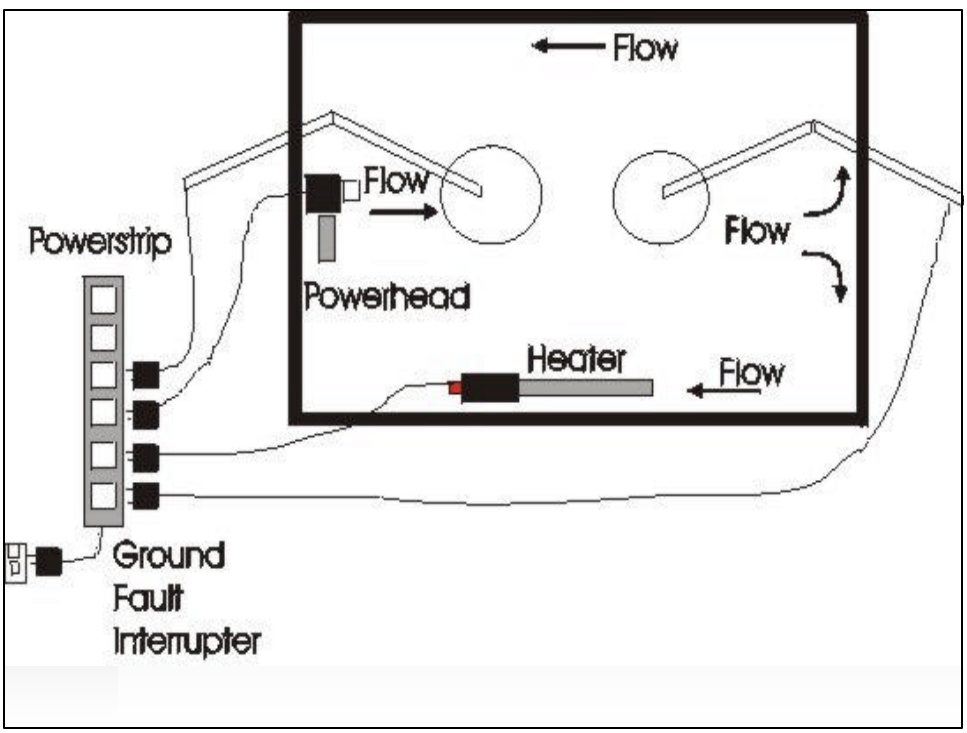
  ~ 2,300 m^2 of wild celery and sago pondweed planted at 8 sites

Susquehanna River
Piney Run Reservoir
Baltimore
Rocky Point Park
Chester River
Washington D.C.
Corsica River
Choptank River
Centreville
Marshyhope Creek
Patuxent River
Nanticoke River
Pocomoke River
Potomac River
Solomon's Island

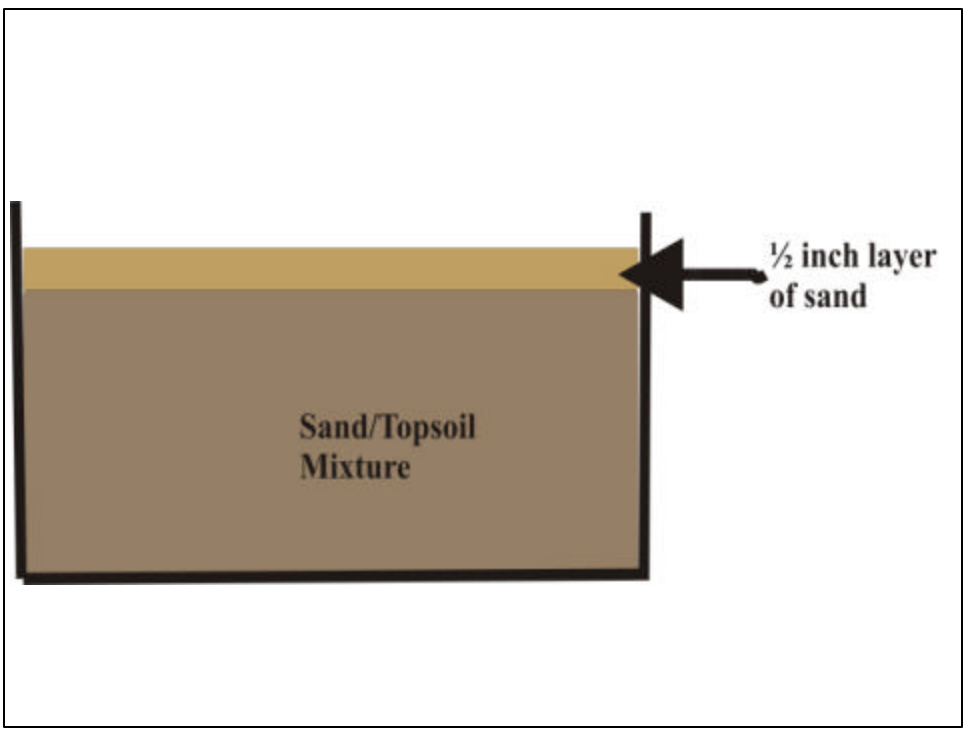
Materials: Total List for 2 growth chambers

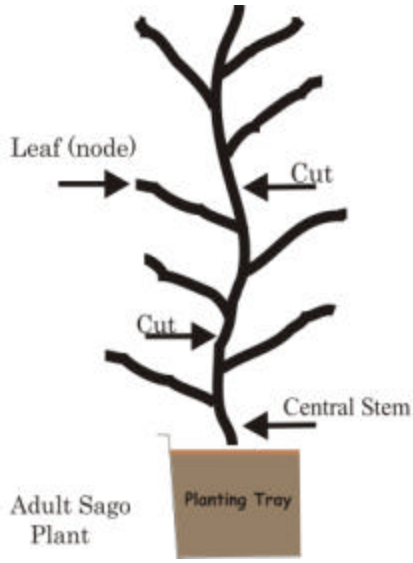
- 2 - growth chambers
- 2 - sponge filters
- 2 - powerheads
- 4 - incandescent light bulbs (60 watt)
- 4 - light shrouds (swing arm desk lamp)
- 2 - power strips with surge protectors
- 2 - ground fault interrupters (GFI)
- 2 - thermometers
- 2 - submersible aquarium heaters
- 1 - pH test kit
- 1 - nitrate test kit
- 6 - planting trays
- 1 - foam sheet
- 1 - bag of topsoil (40 pounds, lower organic content than potting soil)
- 1 – bag sand

Grasses in Classes


Flow
Flow
Powerstrip
Powerhead
Flow
Heater
Flow
Ground
Fault
Interrupter

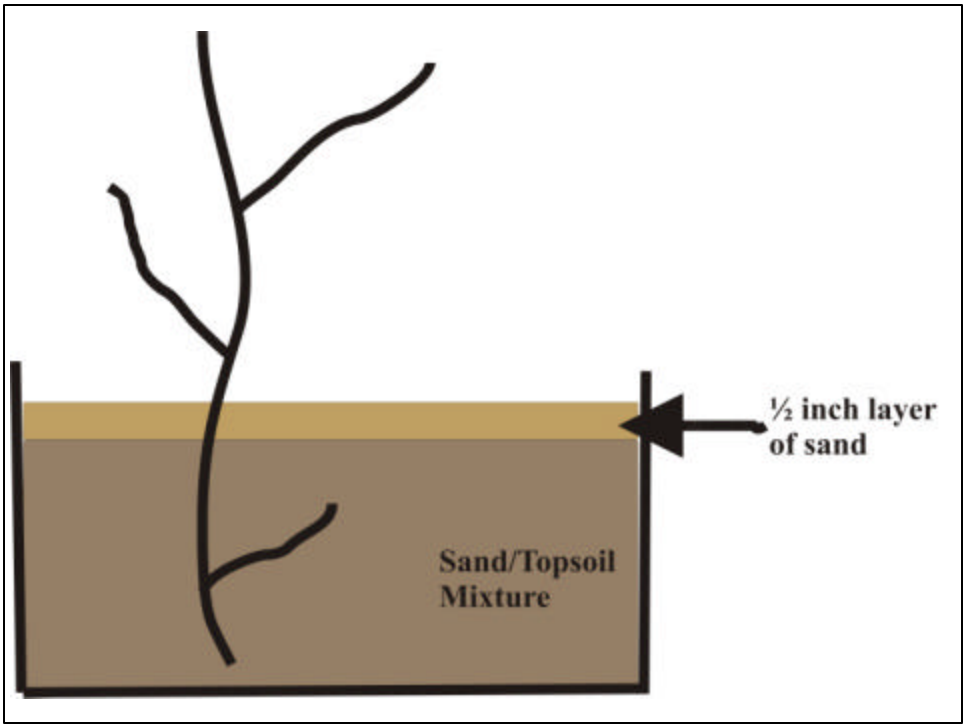
NATURE'S PRIDE
Family of Fine Landscape Products
TOP SOIL
Net Wt. 40 Lbs. (18.1 Kg)
QUIKRETE
ALL-PURPOSE
SAND


½ inch layer of sand
Sand/Topsoil Mixture


Leaf (node)
Cut
Cut
Central Stem
Adult Sago Plant
Planting Tray

Planting Tray

Sago Plant
After Cutting


½ inch layer
of sand
Sand/Topsoil
Mixture

Tips for Micropropagation

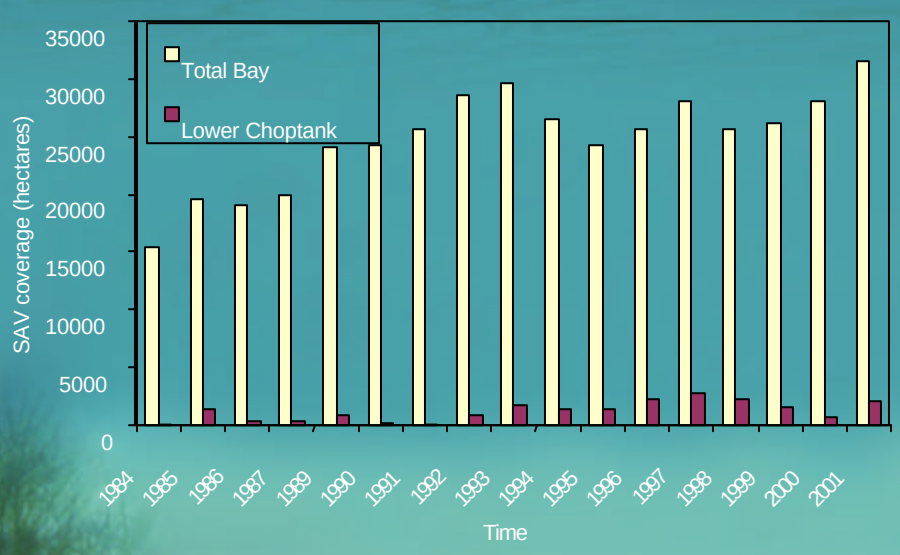
- 84 degrees- lower temps grow too slow, but higher temps create algae problems
- Keep it short- as the plants get too long, they will brown and lose leaves
- Keep tanks about chest high
- Plants will keep in refrigerator after micropropagation for weeks

Use of colonizing species of submersed aquatic vegetation as nurse crops in restoration projects

Laura Murray and John Melton

University of Maryland
Center for Environmental Science
Horn Point Laboratory

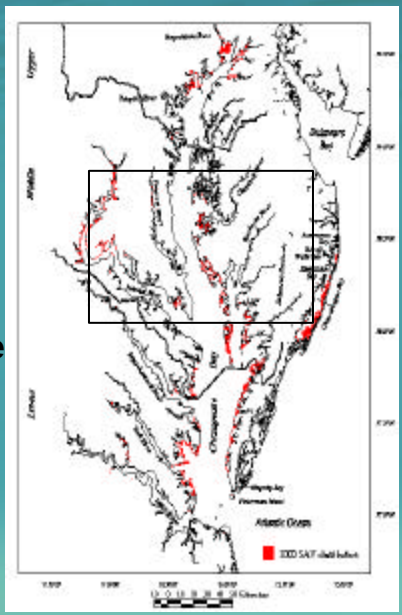
Recent Coverage of SAV in Chesapeake Bay and the Lower Choptank River



Orth et. al 1984-2001

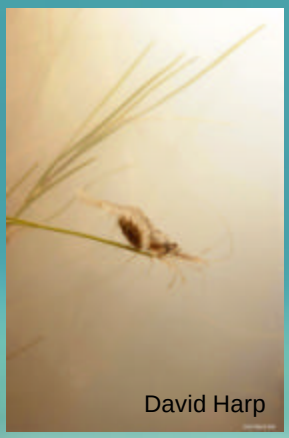
Historically 16 species of SAV were commonly found in the Chesapeake Bay or nearby rivers.

In the mesohaline regions, there is low species diversity when compared to pre-decline years.
(Stevenson and Confer, 1978)



www.vims.edu

Approximately 90% of the SAV coverage in the Choptank River is one species, *Ruppia maritima* (Orth et. al, 1984-2001).



David Harp

R. maritima has been characterized as a colonizing species, reproducing mainly by seeds

Other, more stable species of SAV, are have not re-established

Potamogeton pectinatus

Potamogeton perfoliatus

SAV beds can modify their environment

Trap suspended materials
Clean water
Increase sediment nutrients and reduce water column nutrients

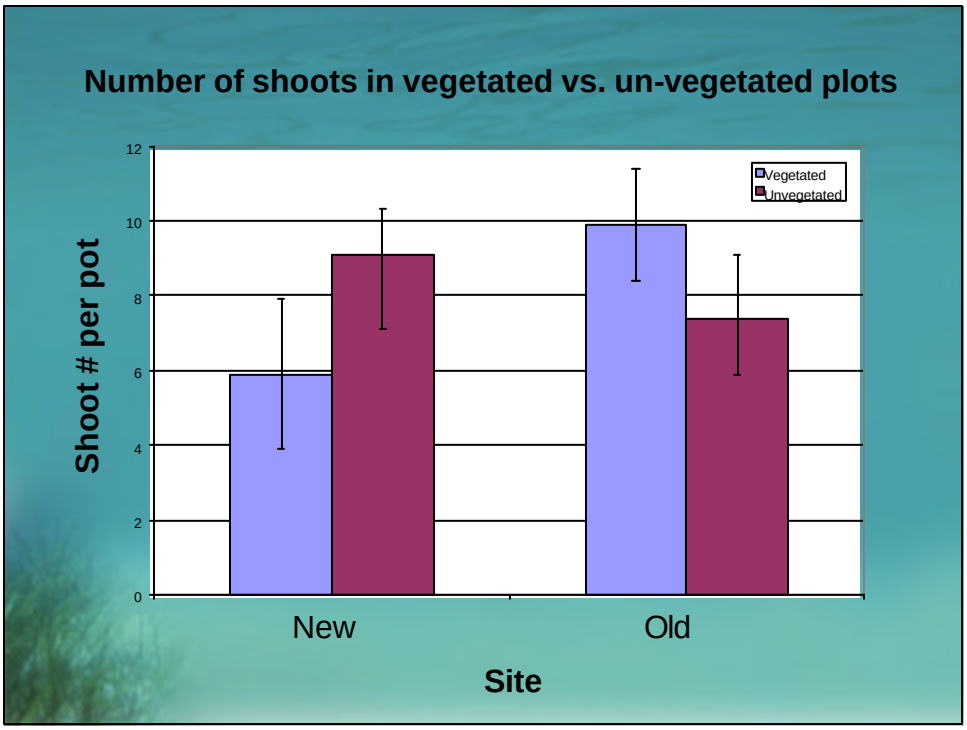
Planting other SAV species in *R. maritima* beds can:

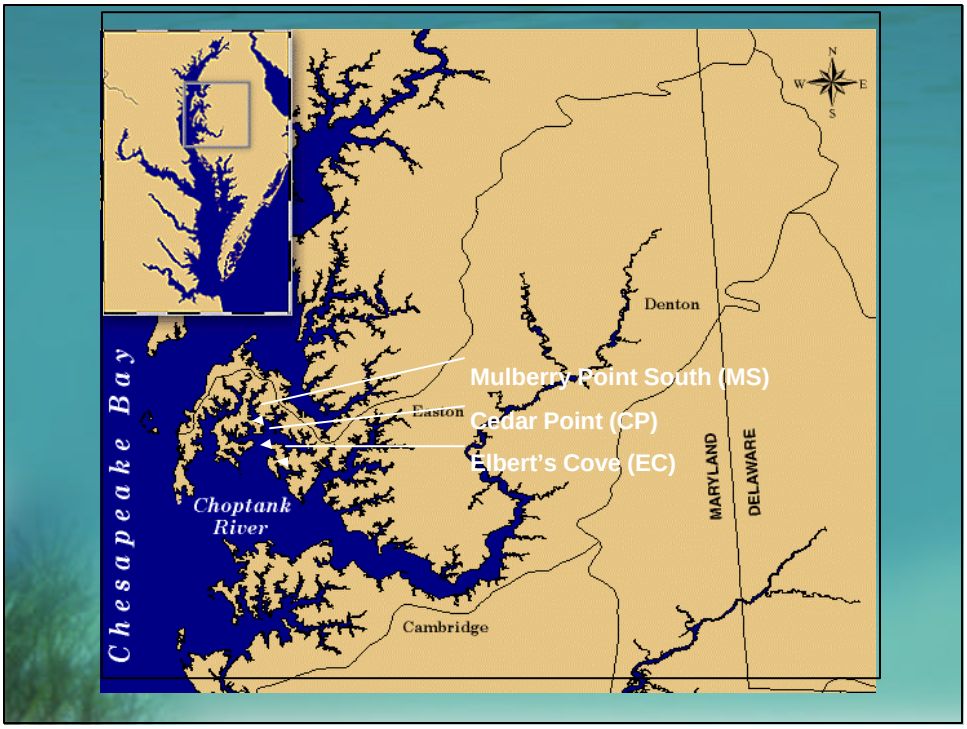
- **Increase species diversity**
- **Provide more stable SAV beds**

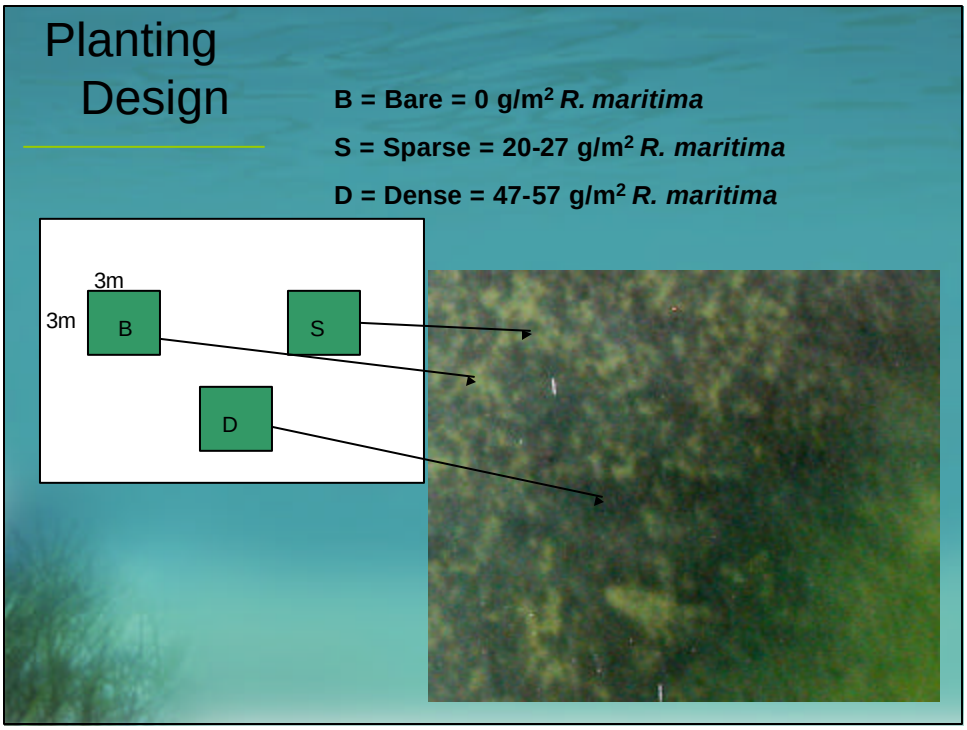
Existing *R. maritima* beds can serve as nurse crops for other species of SAV

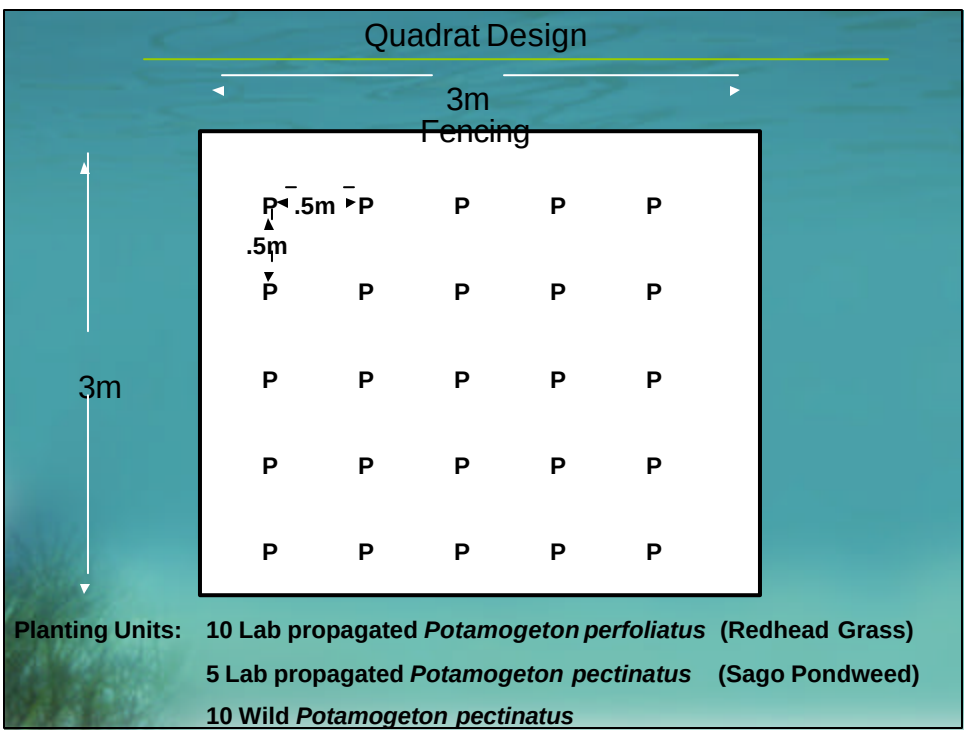
Recent experiment supports hypothesis:

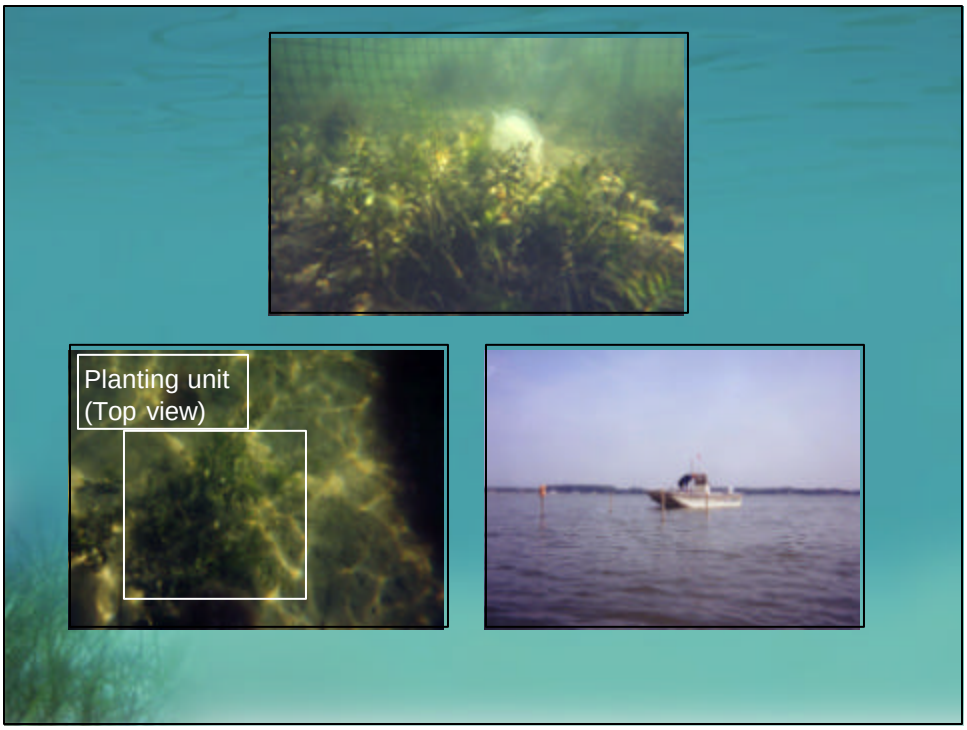
***Potamogeton perfoliatus* planted in sediments collected from "old" and "new" *R. maritima* beds and from adjacent un-vegetated areas.**

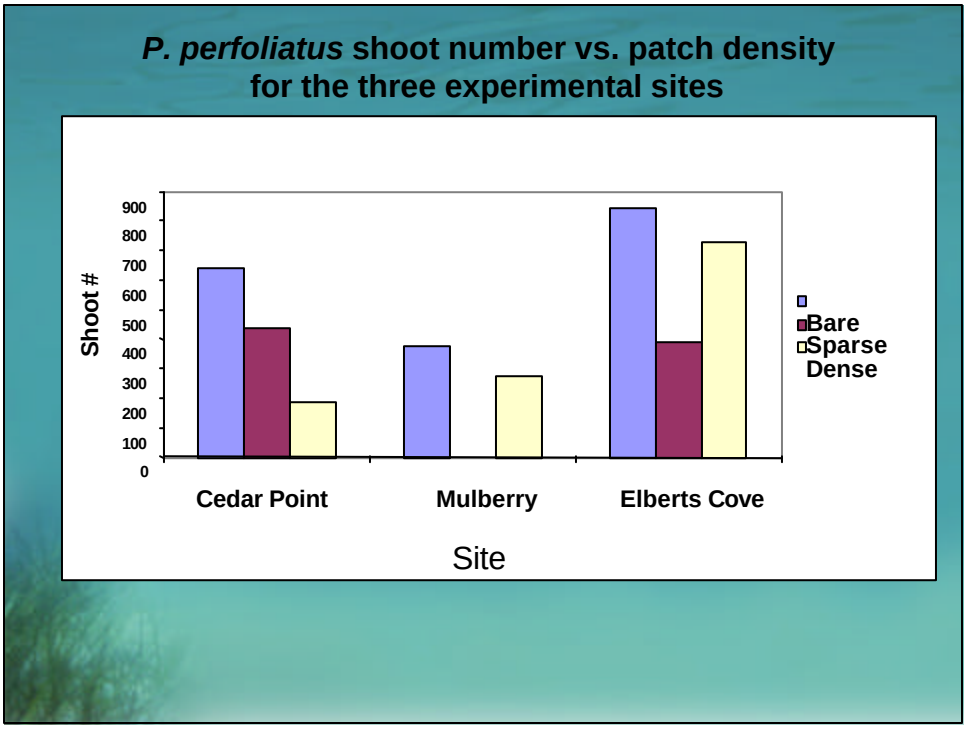

Number of shoots in vegetated vs. un-vegetated plots
Shoot # per pot
0
2
4
6
8
10
12
Vegetated
Unvegetated
New
Old
Site

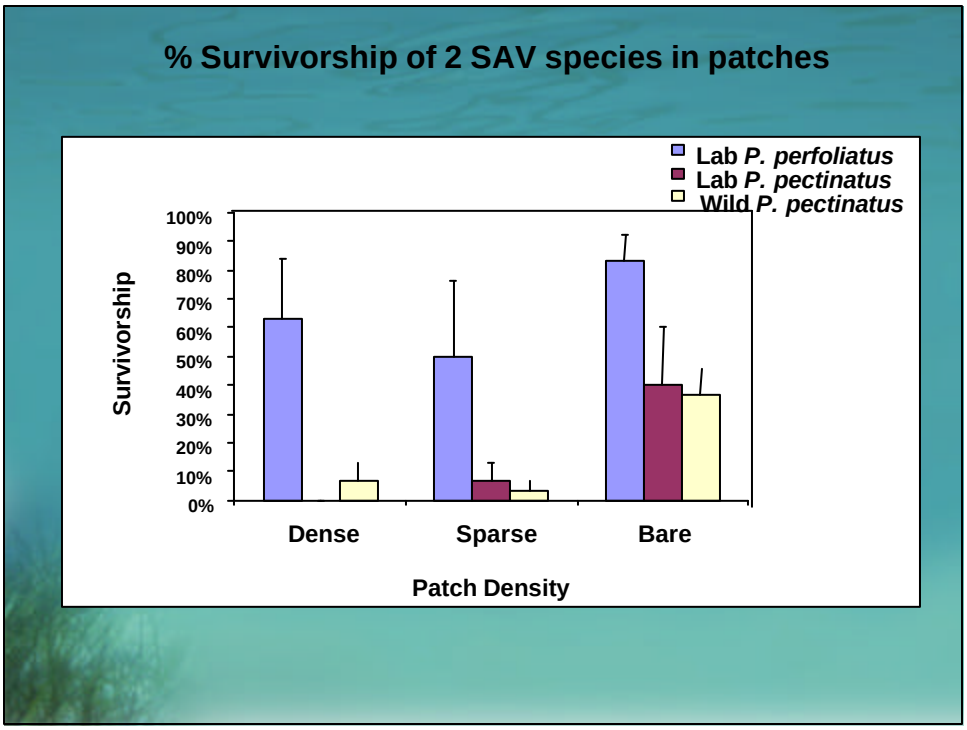

Chesapeake Bay
Choptank River
Easton
Cambridge
Denton
MARYLAND
DELAWARE
Mulberry Point South (MS)
Cedar Point (CP)
Elbert's Cove (EC)
N
W
E
S

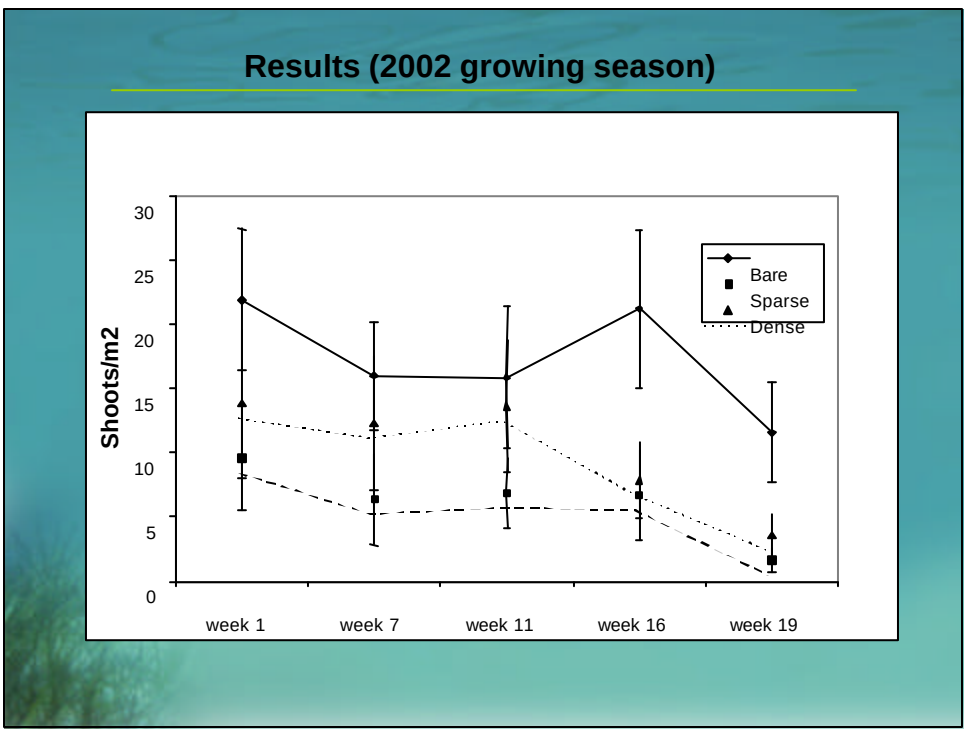

Planting Design
B = Bare = 0 g/m² R. maritima
S = Sparse = 20-27 g/m² R. maritima
D = Dense = 47-57 g/m² R. maritima
3m
3m
B
S
D


Quadrat Design
3m
Fencing
.5m
.5m
3m
P
Planting Units: 10 Lab propagated Potamogeton perfoliatus (Redhead Grass)
5 Lab propagated Potamogeton pectinatus (Sago Pondweed)
10 Wild Potamogeton pectinatus


Planting unit
(Top view)


P. perfoliatus shoot number vs. patch density for the three experimental sites
Shoot #
900
800
700
600
500
400
300
200
100
0
Cedar Point
Mulberry
Elberts Cove
Site
Bare
Sparse
Dense


% Survivorship of 2 SAV species in patches
Lab P. perfoliatus
Lab P. pectinatus
Wild P. pectinatus
Survivorship
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Dense
Sparse
Bare
Patch Density


Results (2002 growing season)
Shoots/m2
30
25
20
15
10
5
0
week 1
week 7
week 11
week 16
week 19
Bare
Sparse
Dense

Conclusions:

***R. maritima* beds can serve as "nurse crops" for restoration of other SAV species, especially older beds**

Restoration of SAV in bare patches within existing beds may have higher rates of success

Survival of *P. perfoliatus* was higher than for *P. pectinatus*

Founder Colonies for Restoration of Aquatic Plant Communities in Unvegetated Freshwater Ecosystems

Michael Smart

US Army Engineer Research and Development Center

Lewisville Aquatic Ecosystem Research Facility

Lewisville, Texas

Obstacles to natural establishment (and impediments to restoration)

- **Lack of propagules**
- **Adverse environmental conditions**
- **Biotic disturbance and herbivory**
- **Physical disturbance (excess energy)**

The fact is, a lot more has changed than just water quality!

"Rogues Gallery"

Our SAV did not co-evolve with these guys!

This is what I see in freshwater systems: Herbivory is the overriding factor

Herbivory is the overriding factor in freshwater systems

Herbivory is the overriding factor in freshwater systems

It's not just about the water quality

- In many freshwater ecosystems we have made substantial improvements in water quality, yet these improvements are not always accompanied by an increase in SAV.
- Many of these systems remain in what we would call an "*unvegetated state*".
- Is it that we are lacking the necessary plant propagules, or is it something else?

Onondaga Lake, NY (the "most polluted lake in the US")

Even in Onondaga Lake, "America's dirtiest lake", we have made substantial progress in cleaning up the water. Of course SAV recovery has been minimal.

Have we just not improved the water quality enough? Or is there something else?

Even here, it's not just the water quality

In a multiagency effort aimed at restoring Onondaga Lake, we found that we could, in fact, restore SAV -- provided that we protected the transplants from both waves and herbivores.

In some cases, we even had recovery of species that we had not planted! These must have come from the seedbank.

Had we not installed the wave breaks and exclosures we wouldn't have known.

Seedbank Assessment

The lesson here is that we do *not* always know *why* the plants are not there.

Before we go about "restoring" SAV (or making decisions regarding restoration) we should at least assess the sediment seedbank.

Seedbank assessment:

Lake Okeechobee, FL

Test Plantings

We should also routinely conduct test plantings of a variety of species. (in FW settings)

Test plantings should include robust transplants both inside and outside of exclosures.

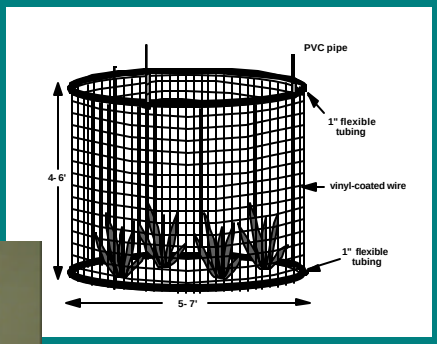
Unplanted exclosures could test the ability of SAV to recover from the seedbank (if any).

***Heteranthera* in Lake Waco, TX**

Test Plantings

Test plantings should utilize exclosures of proven design and constructed of durable materials.

Exclosure integrity should be verified monthly during the growing season

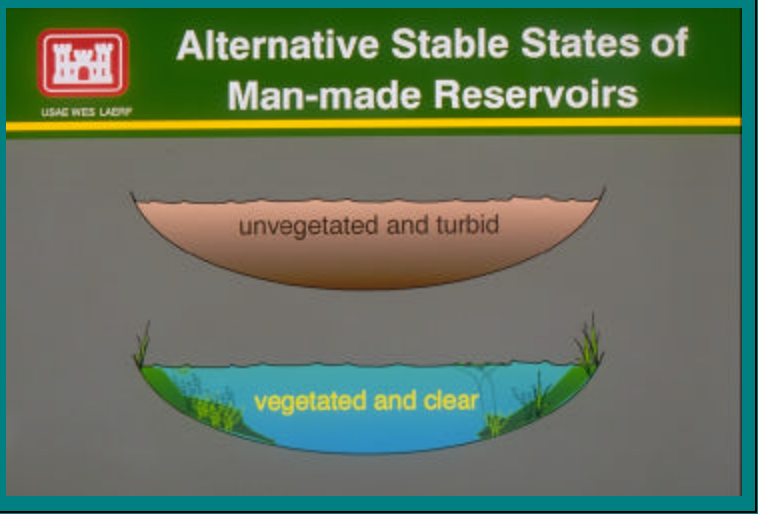


"Hoop" cage design

"Maybe you can't get there from here"

At least not with just water quality improvements.

In Europe they frequently employ drawdown, dredging, and fish eradication to achieve SAV restoration.



The overriding importance of herbivory (in fresh water) …

(in my opinion) precludes the use of extensive planting of *unprotected* seed, seedlings, or bare-root plants.

So, given that you *will* have to provide herbivore protection …

large-scale planting efforts are *not* the answer!

(No matter what the Congressman says.)

What we want are large-scale *results*.

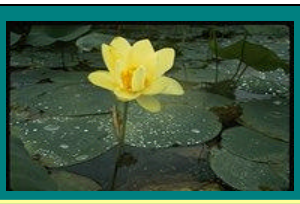
"Founder Colony" Approach

Introduction of *mature transplants*, *protected from herbivory*, into *selected favorable sites to* ensure establishment and sustainability of founder colonies, resulting in:

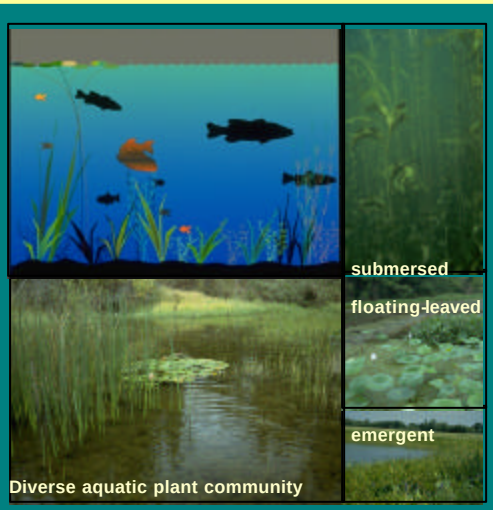
- modification and improvement of environmental conditions
  - sediment stabilization, water column filtration, and nutrient uptake, resulting in improved water clarity, improving the light climate
- development of a seedbank/tuberbank for recovery following adverse conditions
- continual production of propagules to ensure spread when conditions become suitable

Mature transplants
(nursery grown)

Plants - Diversity is good!



Plant a diversity of species and growth forms to maximize habitat diversity and resilience



Establishing aquatic plant communities in Texas lakes

A cooperative effort with Texas Parks and Wildlife to develop, test, and refine aquatic plant establishment methodology in selected reservoirs representing a diversity of environmental conditions

- Lakes:
  - **Jacksonville, Conroe, Cooper, Grapevine, Waco, Coleman, Choke Canyon**
- Exclosures:
  - **none, small, large**
- Plants:
  - **21 species (emergent, floating-leaved, submersed)**

Herbivore exclosures

'Tomato' Cage

Constructed from 2" by 4" mesh galvanized welded wire, this exclosure protects a single plant within a 2 to 3 ft diameter circular cage.

Herbivore exclosures

Fenced Plot

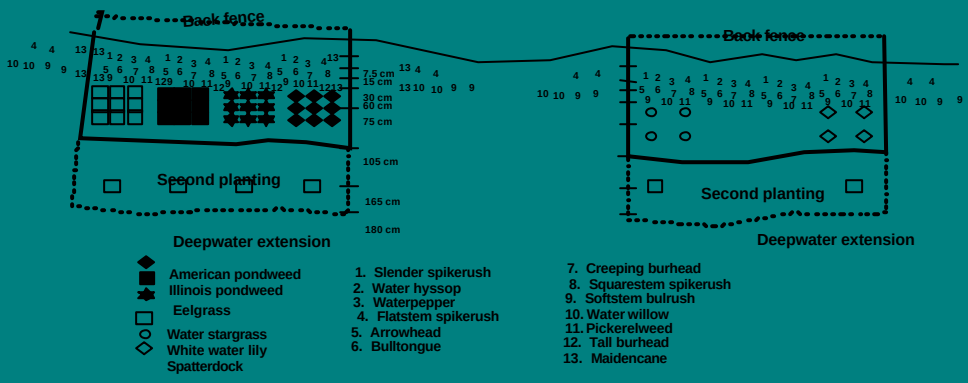
A rectangular pen, constructed from 2" by 4" mesh galvanized welded wire, at a depth of 3.5 ft, this exclosure protects several submersed plants.

Herbivore exclosures

Shoreline Fence

Constructed from 2” by 4” mesh galvanized welded wire placed along the 3 ft depth contour and extending back to the shoreline, this exclosure protects many plants of a variety of growth forms.

1999 Plantings: Shoreline Fences

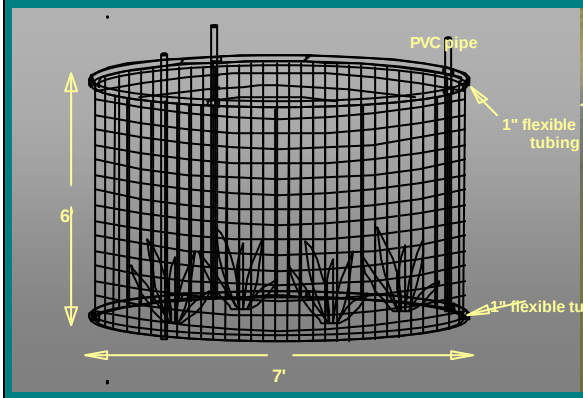


Herbivore exclosures

Fenced Cove

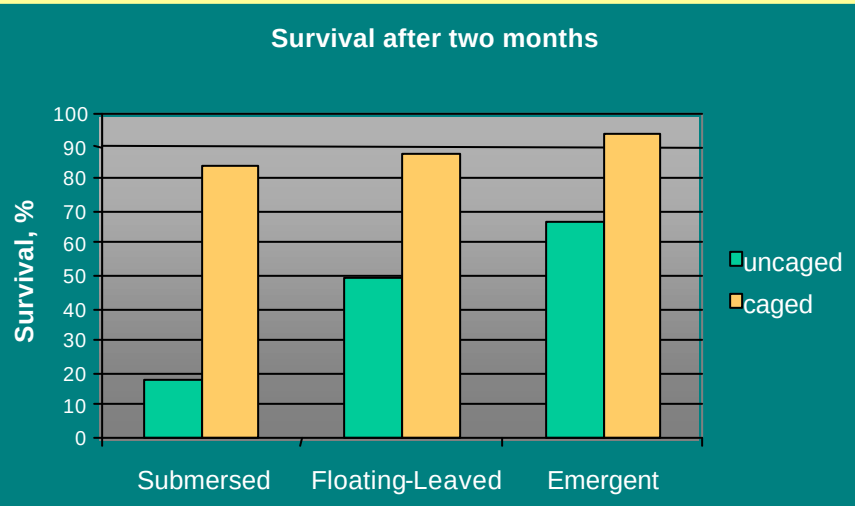
Constructed from 2” by 4” mesh galvanized welded wire placed across the mouth of a shallow cove, this exclosure protects many plants of a variety of growth forms.

Hoop cages for ‘chasing’ water levels

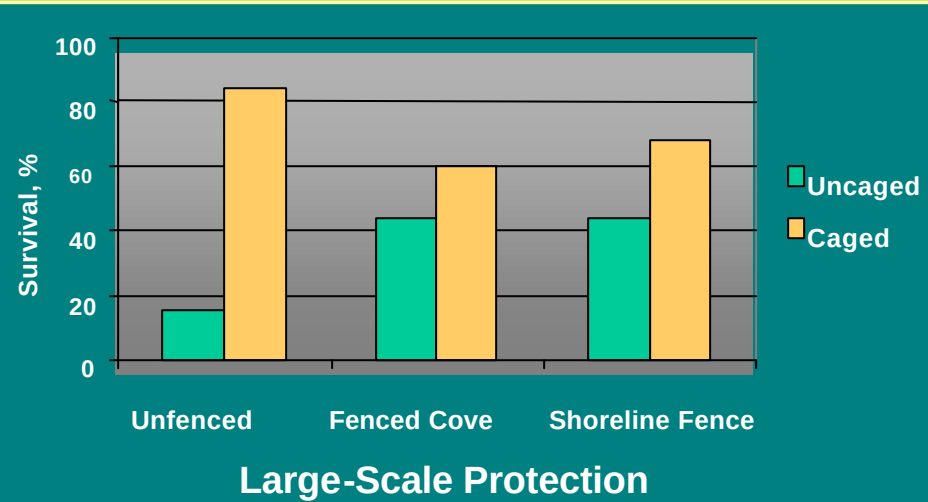


Plant at 2 and 4 ft depths and plant additional cages on 2-ft intervals as water levels drop

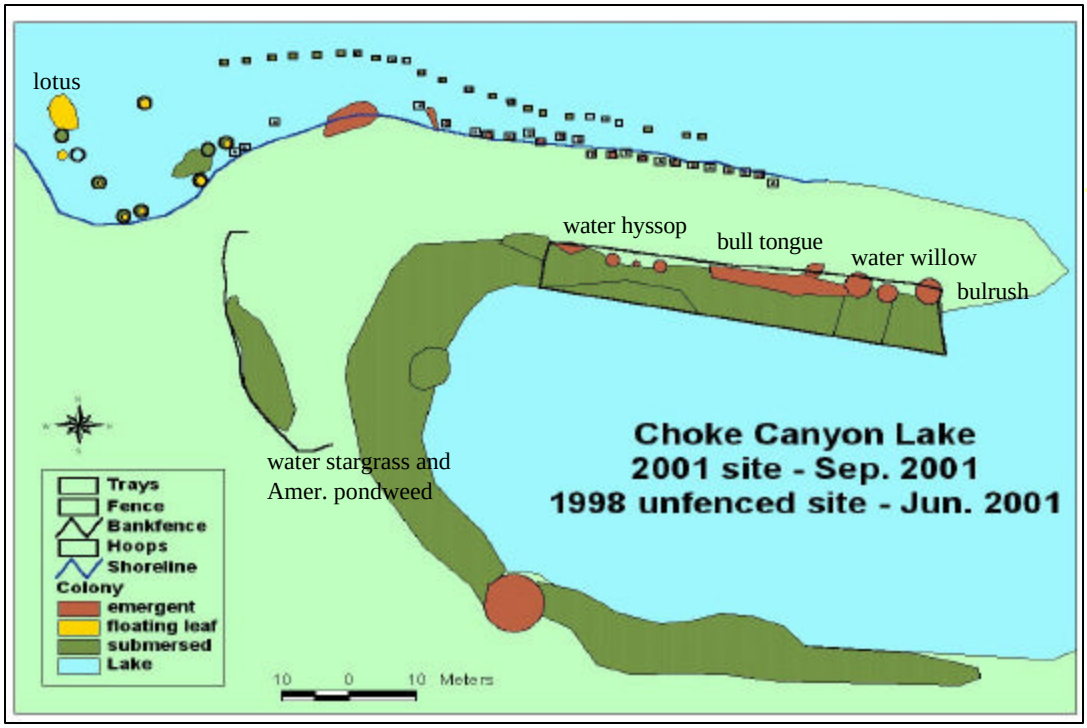
Effects of herbivory/biotic disturbance on survival of different growth forms

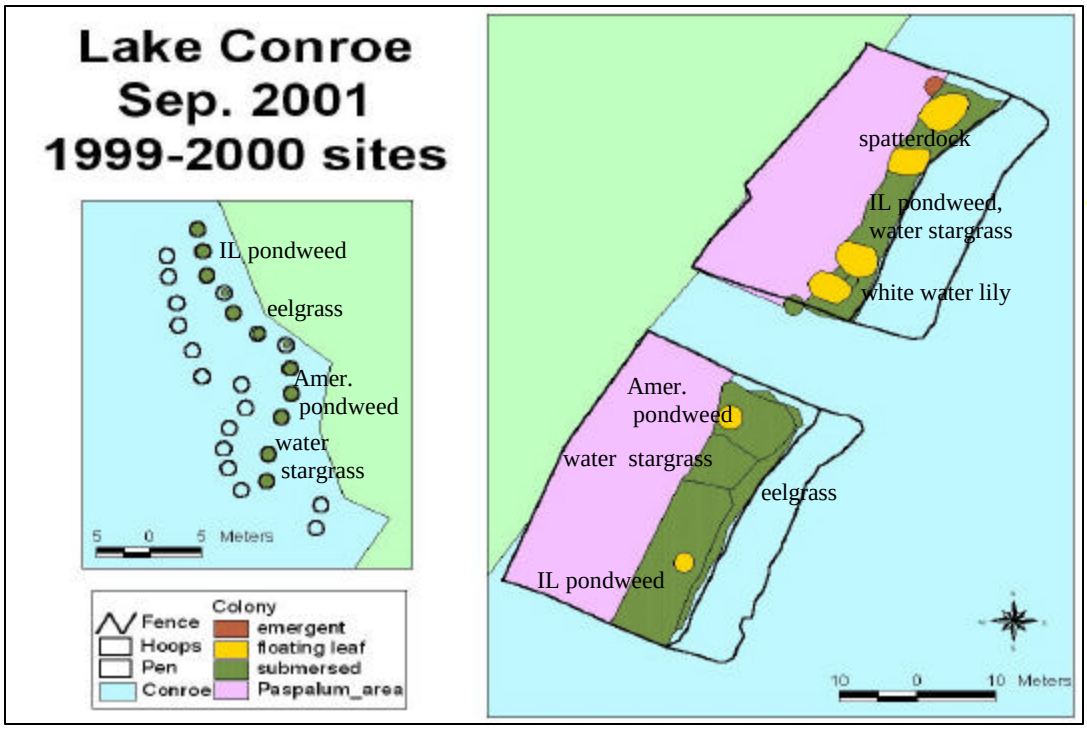


Survival of caged and uncaged SAV, with and without large-scale protection

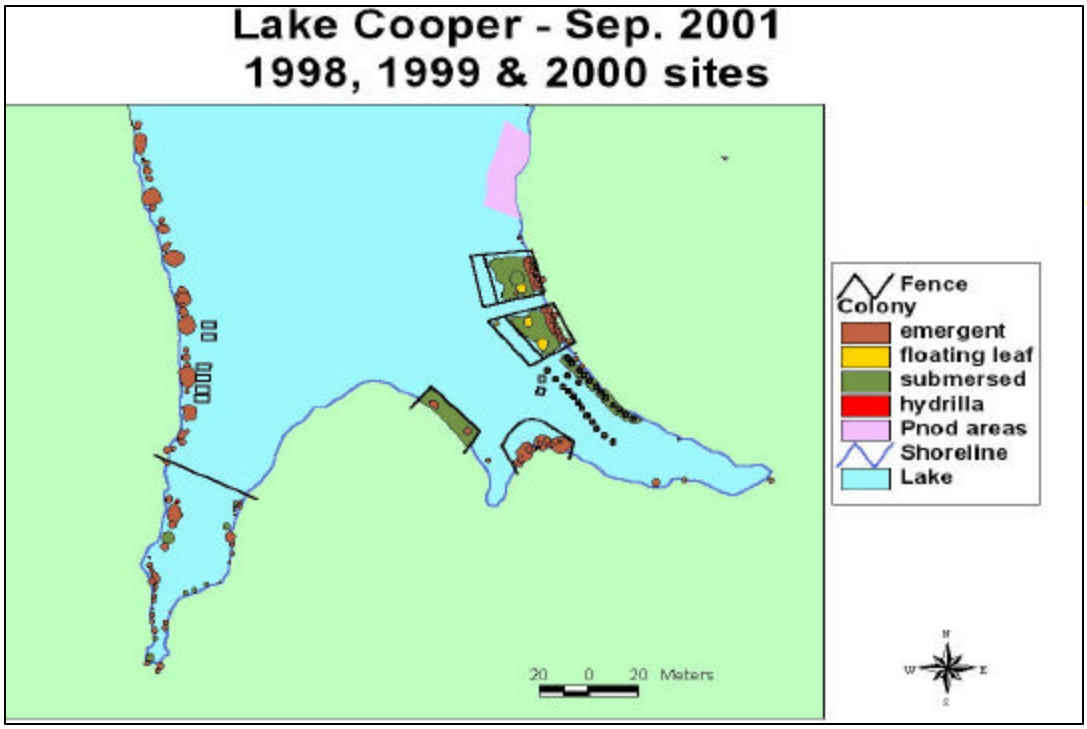


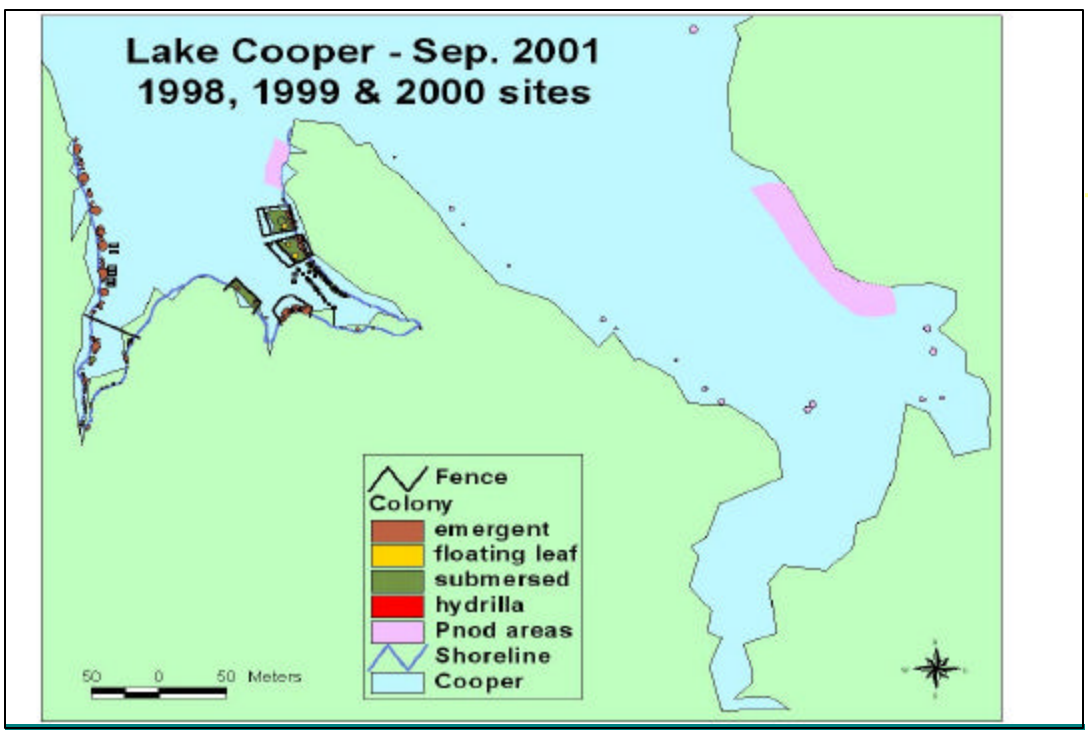
Large-scale, longer-term results: GPS mapping of plant colonies

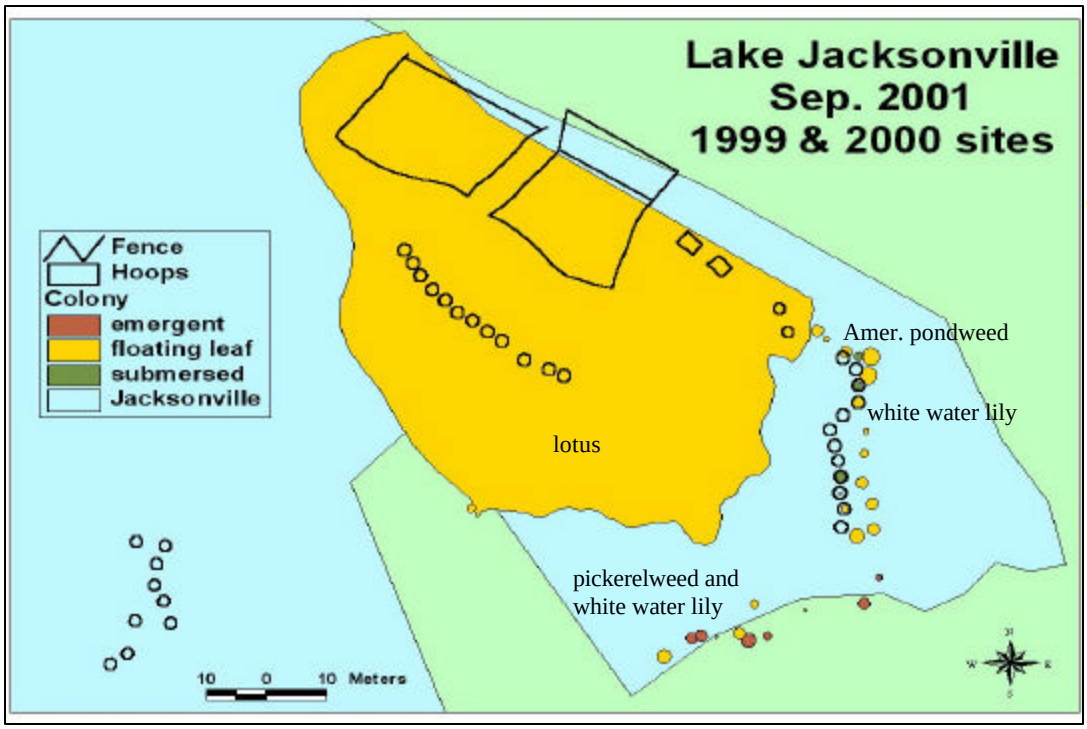


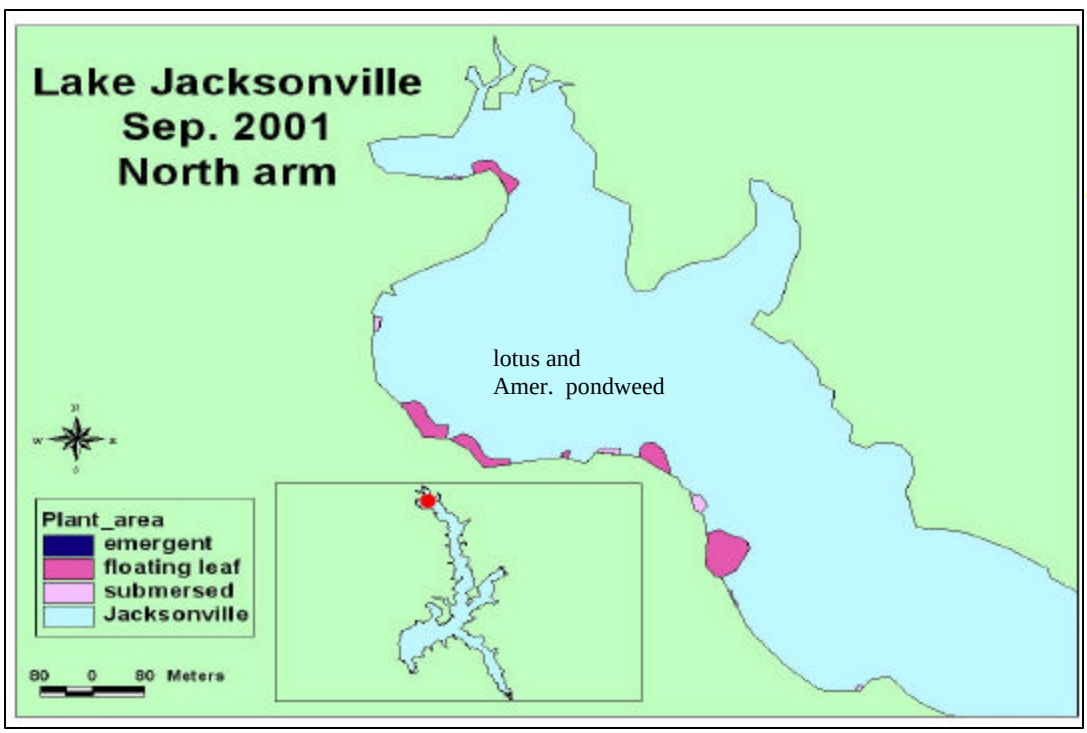

Lake Conroe
Sep. 2001
1999-2000 sites
IL pondweed
eelgrass
Amer. pondweed
water stargrass
5 0 5 Meters
Fence
Hoops
Pen
Conroe
Colony
emergent
floating leaf
submersed
Paspalum_area
spatterdock
IL pondweed, water stargrass
white water lily
Amer. pondweed
water stargrass
eelgrass
IL pondweed
10 0 10 Meters

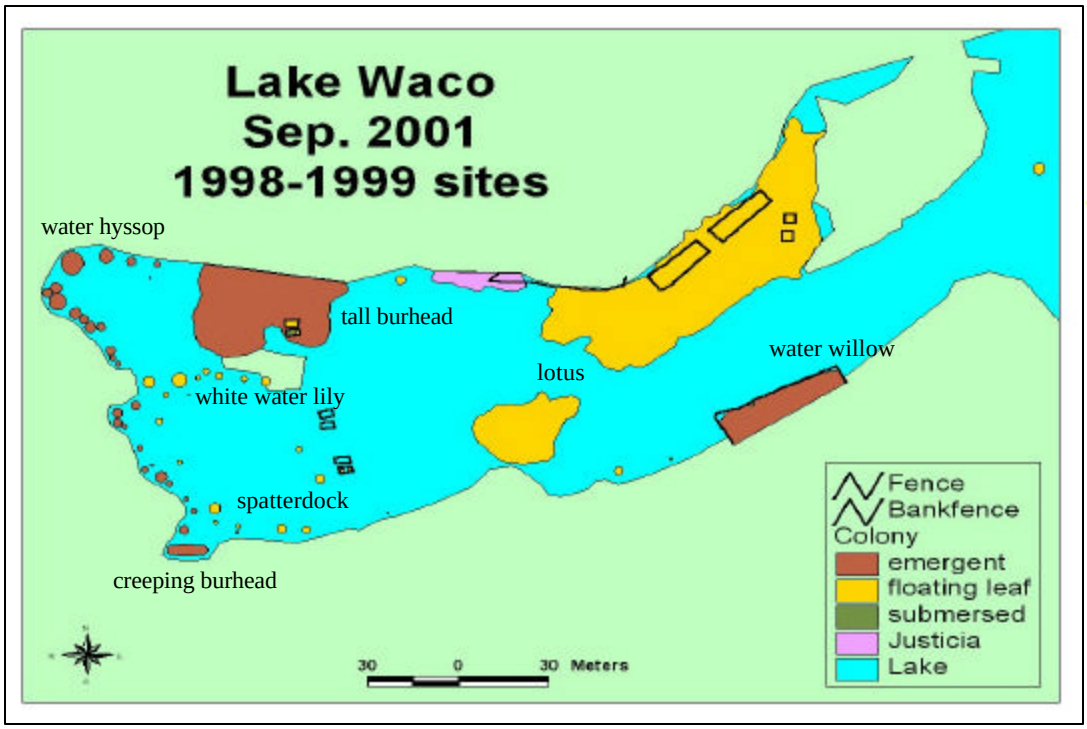
Lake Conroe - 2002


Lake Cooper - Sep. 2001
1998, 1999 & 2000 sites
Fence
Colony
emergent
floating leaf
submersed
hydrilla
Pnod areas
Shoreline
Lake
20 0 20 Meters


Lake Cooper - Sep. 2001
1998, 1999 & 2000 sites
Fence
Colony
emergent
floating leaf
submersed
hydrilla
Pnod areas
Shoreline
Cooper
50 0 50 Meters


Lake Jacksonville
Sep. 2001
1999 & 2000 sites
Fence
Hoops
Colony
emergent
floating leaf
submersed
Jacksonville
Amer. pondweed
white water lily
lotus
pickerelweed and
white water lily
10 0 10 Meters


Lake Jacksonville
Sep. 2001
North arm
lotus and
Amer. pondweed
Plant_area
emergent
floating leaf
submersed
Jacksonville
80 0 80 Meters



Conclusions and Recommendations

- Protect plantings with herbivore exclosures
- Plant a diversity of growth forms and species
- Mature, robust transplants can handle adverse water quality conditions
- Establish founder colonies in multiple locations to ensure propagule supply
- Fonder colonies will help to improve improve water quality
- Founder colonies will produce the millions of propagules that will be needed to vegetate the "1000 acres"

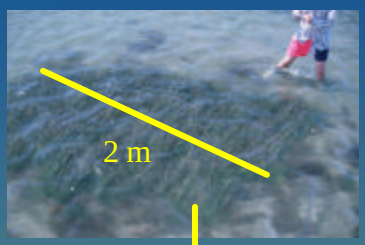
Bob's question: "Can we afford to do transplants?"

Answer: In fresh water, can we afford not to?

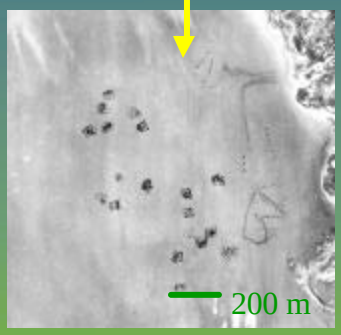
Eelgrass Restoration in Chesapeake Bay:

The emerging issues with large-scale restoration using seeds

Robert J. Orth
Virginia Institute of Marine Science
College of William and Mary
www.vims.edu/biosav



? ?



‘Strategy to Accelerate Protection and Restoration of SAV in Chesapeake Bay’

By Dec. 2008, plant at least 1000 acres at multiple sites!!

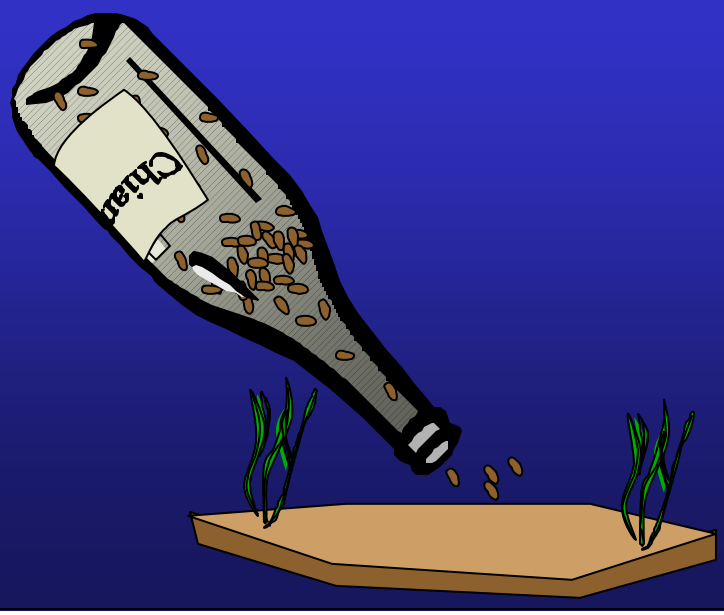
WHAT DO WE KNOW?

- Seeds available for harvesting in a 3 week window
- 10-20% of shoots are reproductive (although there are exceptions)
- Reproductive shoot densities: up to 370 m^{-2} (1.5 million $acre^{-1}$ but spatial and temporal patchiness is the norm)
- Viable seeds per reproductive shoot – 20-150 (depends on length) (225 million seeds $acre^{-1}$)

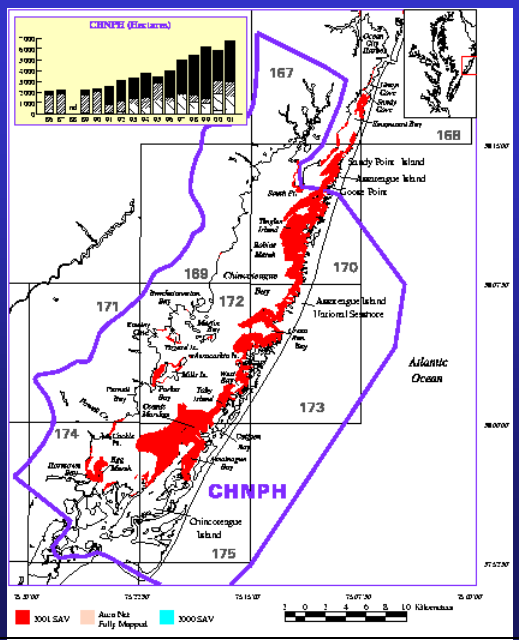
WHAT DO WE KNOW?

- Broadcast seeds remain close to where they settle on sediment surface
- Seed germination in mid-November related to temperature and anoxia in sediment
- Low initial rate of seedling establishment (5-10%)

WHERE ARE THE BOTTLENECKS?



RECOVERY OF SEAGRASS TO CHINCOTEAGUE BAY 1986-2001



WHERE ARE THE BOTTLENECKS?

Hand harvest labor intensive and only a few million seeds collected

SEED COLLECTION
LATE MAY – MID-JUNE

2001
6.6 million seeds in 204 collecting hours = 32,500 seeds/hour

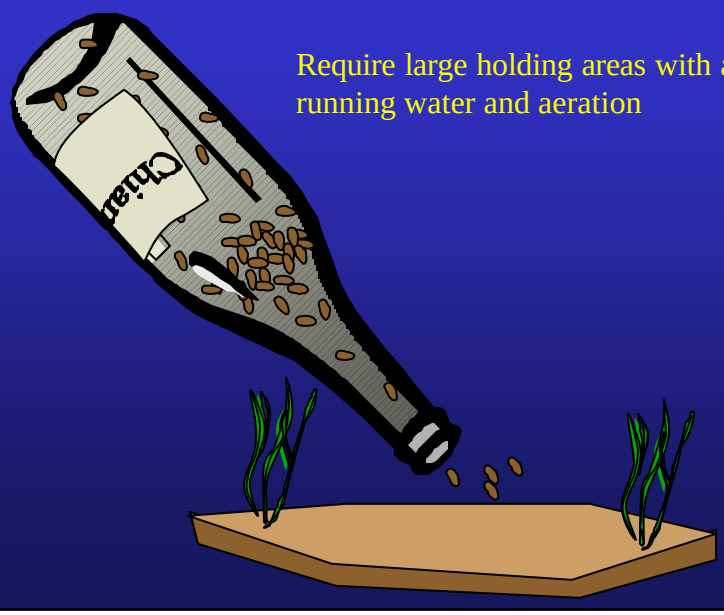
2002
2.5 million seeds in 246 collecting hours = 10,000 seeds/hour

2003
5.2 million seeds in 310 collecting hours = 16,800 seeds/hour

SOLUTIONS??

- Mass harvest reproductive shoots at period of peak seed release to insure collecting most number of viable seeds

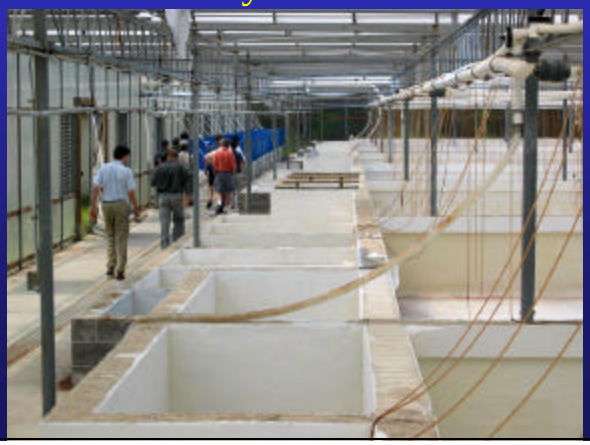
WHERE ARE THE BOTTLENECKS?



Require large holding areas with adequate running water and aeration

SOLUTIONS??

- Build or use existing facilities that have the holding capacity, e.g. Piney Point Aquaculture facility



WHERE ARE THE BOTTLENECKS?

Problems of mass storage of plant material
i.e., Seed mortality

SOLUTIONS??

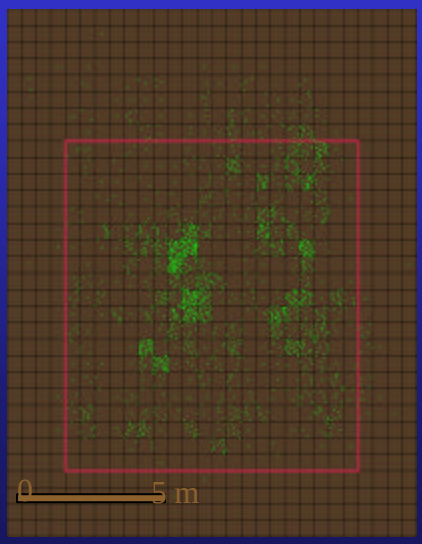
- Conduct experiments on effects of temperature and dissolved oxygen, as well as seed scarification

Is seed distribution a bottleneck?

seeds
V25

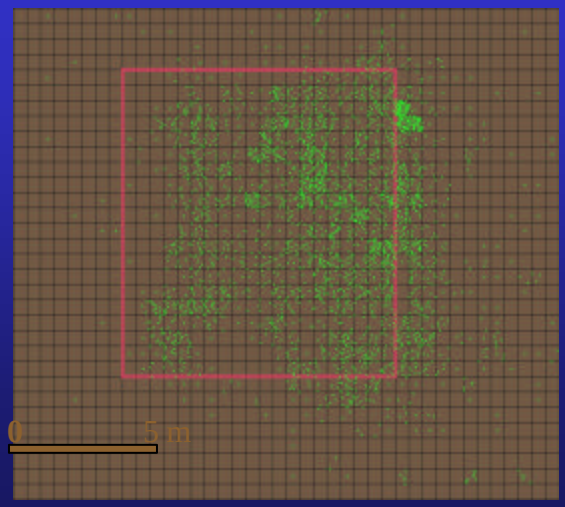
Rappahannock River

- 50,000 seeds broadcast in 100 m^2
- 2333 seedlings total (4% of all seeds broadcast)
- 2173 seedlings in plot (93% of total seedlings)

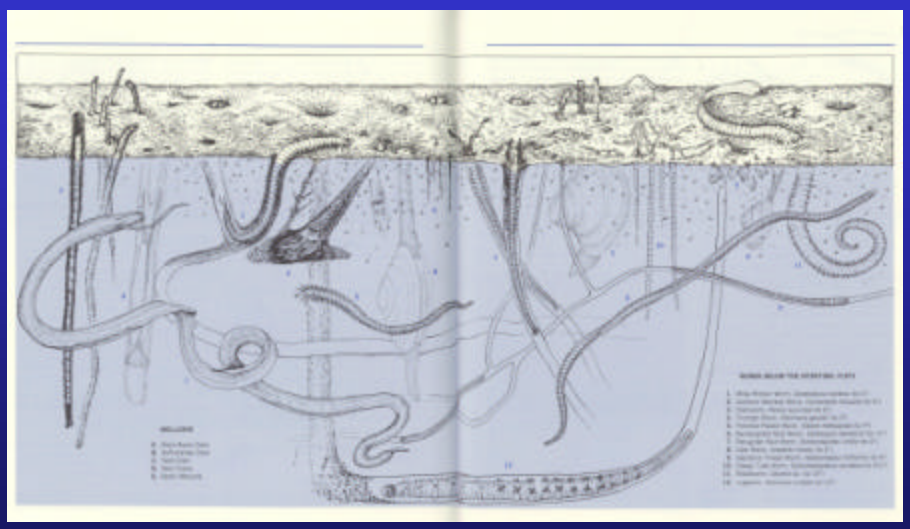


South Bay

- 50,000 seeds broadcast in 100 m^2
- 3237 seedlings total (6.5% of all seeds broadcast)
- 2295 seedlings in plot (71% of total seedlings)



Seeds retained close to where they settle due to topographic complexities of sediment surface (bioturbation, sand ripples)



Luckenbach and Orth (1999) Aquatic Botany 62:235-247

Why the meter-scale patchiness?

1) operator error

- correctable with broadcasting technology

2) patchy distribution of surface roughness
3) post-broadcast redistribution by waves

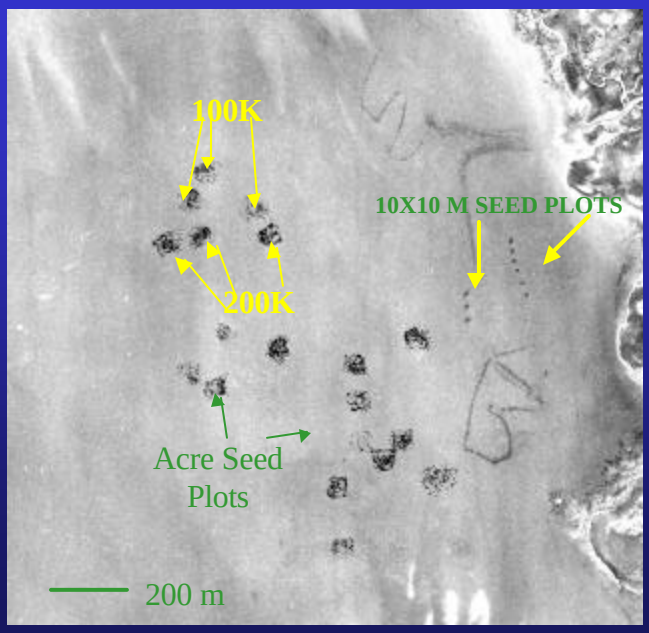
} facts of life

Does evenness matter to the PLANTS?

- At the highest densities (500-1000 seeds/m^2), shoot competition due to cm-scale clumping is observed
- Restoration applications utilize much lower densities (12-48 seeds/m^2)
- Uneven distribution on the scale of meters unlikely to affect plant growth (similar to natural patchy pattern)

→ Not a bottleneck, in terms of restricting plant growth

SOUTH BAY – JULY 2002 (Seeds broadcast fall, 2001)



Does evenness matter to the PLANTERS?

Monitoring methods may be sensitive to evenness:

- frequency counts
- % cover of random samples estimated by divers
- remote sensing – total pixel counts

→ Match distribution method to monitoring method

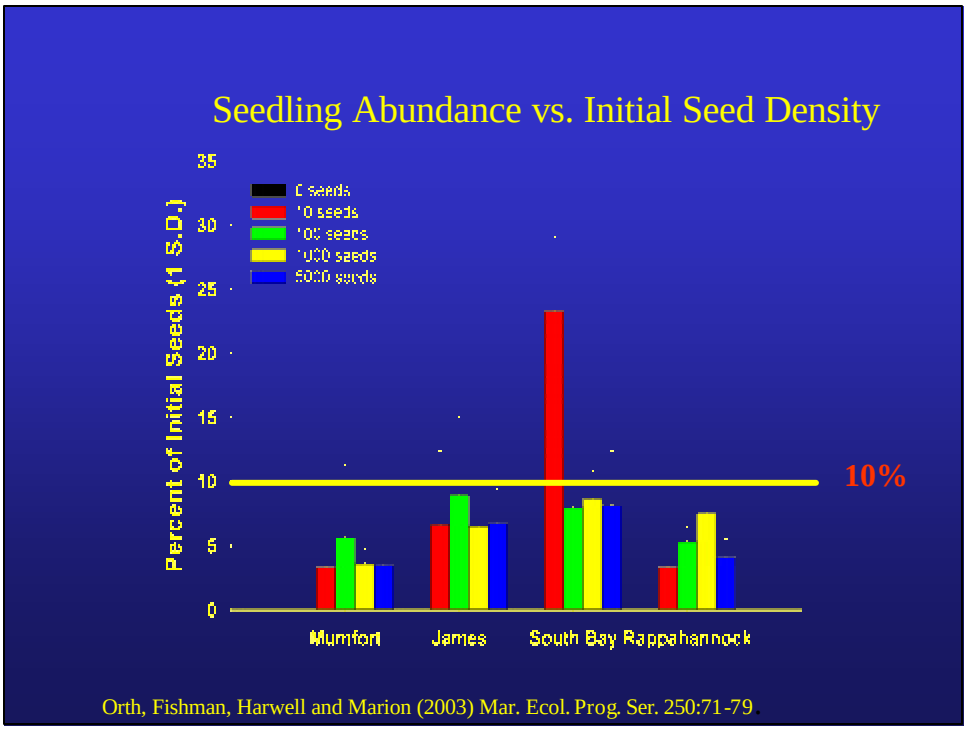
WHERE ARE THE BOTTLENECKS?

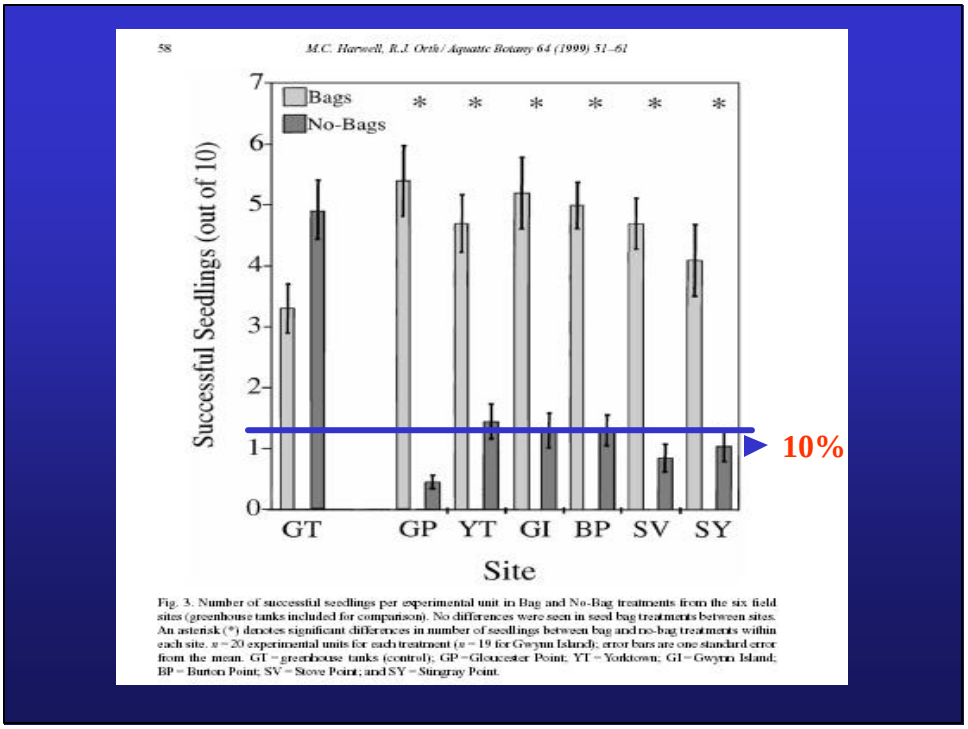
Low seedling success: 5-10%

100 Meter^2 Seed Plot Results

Site	No of quadrats	No of measured cells	Total # seedlings	% of 50,000 seeds	% seedlings inside plot
James	70	1120	6921.4	13.8	92.5
Rappahannock	49	784	2333.4	4.7	93.1
South Bay Offshore	63	1008	3237.2	6.5	70.9
South Bay Inshore	56	896	2127.4	4.3	79.3
Magothy Bay	49	784	5146.6	10.3	92.2
Lynnhaven	49	784	2351.9	4.7	85.7

Orth, Fishman, Harwell and Marion (2003) Mar. Ecol. Prog. Ser. 250:71-79

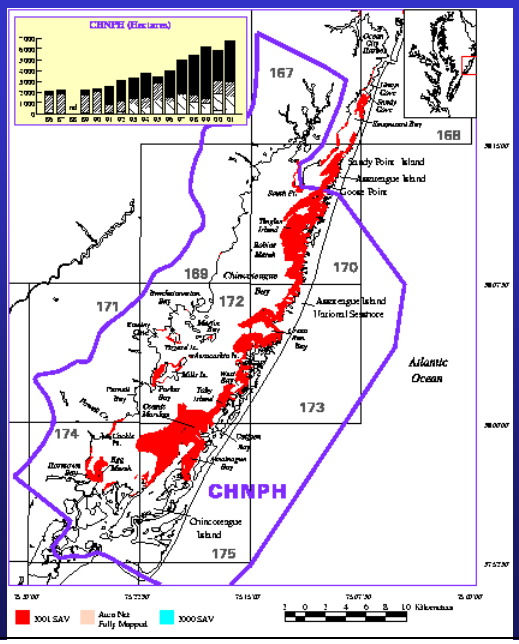

Seedling Abundance vs. Initial Seed Density
Percent of Initial Seeds (1 S.D.)
35
30
25
20
15
10
5
0
0 seeds
10 seeds
100 seeds
1000 seeds
5000 seeds
10%
Mumfort
James
South Bay
Rappahannock
Orth, Fishman, Harwell and Marion (2003) Mar. Ecol. Prog. Ser. 250:71-79


58
M.C. Harwell, R.J. Orth / Aquatic Botany 64 (1999) 51–61
Bags
No-Bags
Successful Seedlings (out of 10)
7
6
5
4
3
2
1
0
10%
GT
GP
YT
GI
BP
SV
SY
Site
Fig. 3. Number of successful seedlings per experimental unit in Bag and No-Bag treatments from the six field sites (greenhouse tanks included for comparison). No differences were seen in seed bag treatments between sites. An asterisk (*) denotes significant differences in number of seedlings between bag and no-bag treatments within each site. n = 20 experimental units for each treatment (n = 19 for Gwynn Island); error bars are one standard error from the mean. GT = greenhouse tanks (control); GP = Gloucester Point; YT = Yorktown; GI = Gwynn Island; BP = Burton Point; SV = Stove Point; and SY = Stingray Point.

SOLUTIONS??

- Test methods of protecting seeds:
  - decrease predation
  - create more hospitable environment for seed germination
- Assess time compared to broadcasting for seedling success

RECOVERY OF SEAGRASS TO CHINCOTEAGUE BAY 1986-2001



The Adaptation and Application of Modern Agricultural Production Practices to SAV Restoration

- Tony Mazzaccaro Ph.D.
- Arthur L. Allen Ph.D.
- Eric B. May Ph.D.
- University of Maryland Eastern Shore, Dept. of Natural Sciences, Living Marine Resources Cooperative Science Center

Basic Needs for Successful, Large Scale SAV Restoration

- 1. A Large, Cost effective supply of Seed
- and Seedlings

- 2. Efficient Mechanical Means to Plant
- Them

Secondary Needs

- 1. Selective Breeding to Produce Superior
- Performing Cultivars
- a. Higher Seed Germination Rates
- b. More Robust, faster growing Plants
- c. Increased Tolerance to Selected
- Environmental Conditions
- d. Increased Seed Production, etc.

2. Judicious Restoration Site Selection

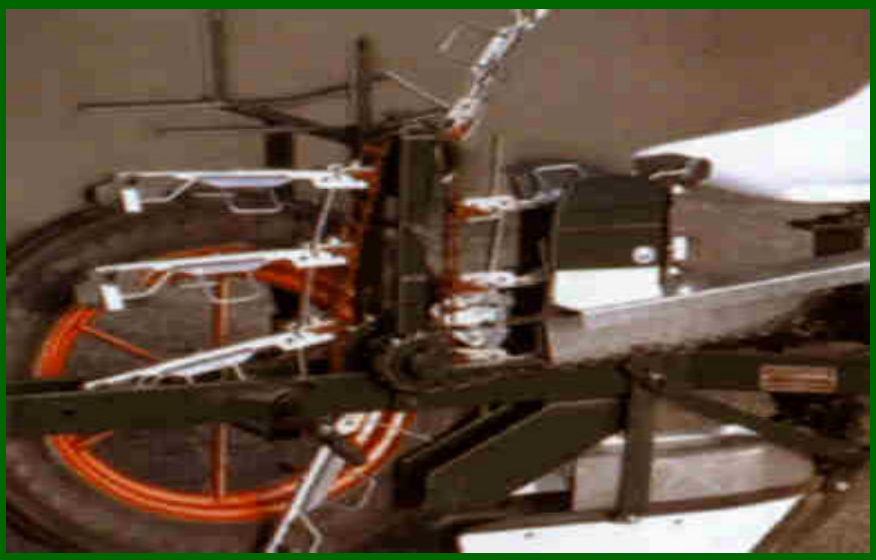
Basic Transplanting Machine

Advanced Model With possible Drive Wheel

Multiple Row Configuration

The Planting Arms on Disk Drive

Planting Arms on Chain Drive



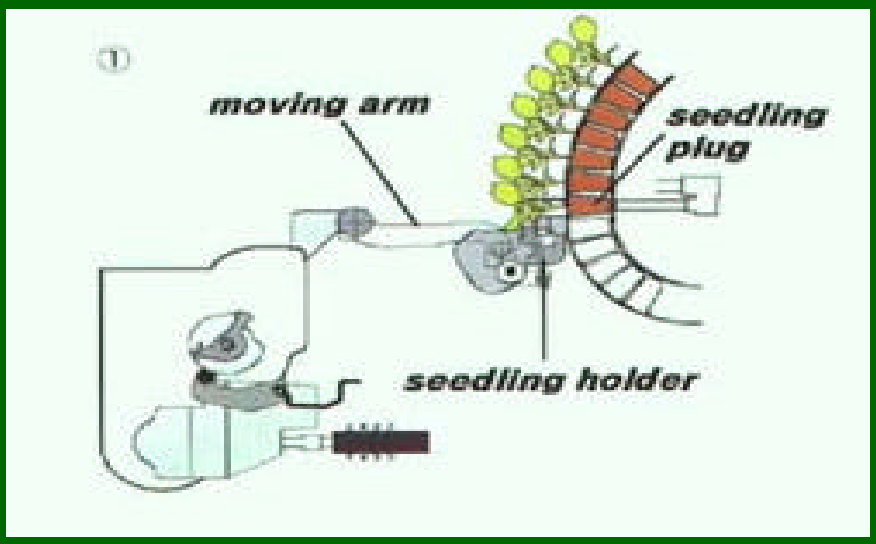
Various Planting Arms

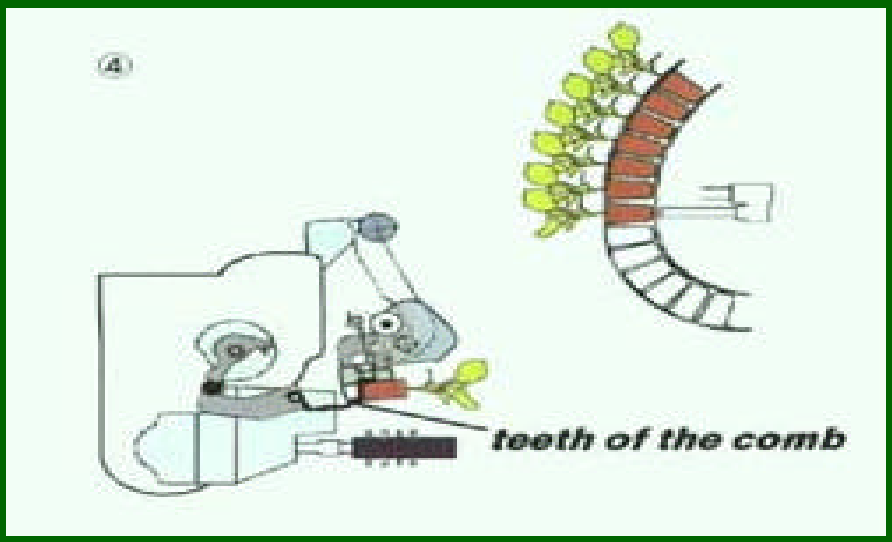
Small Acreage Rice Planter

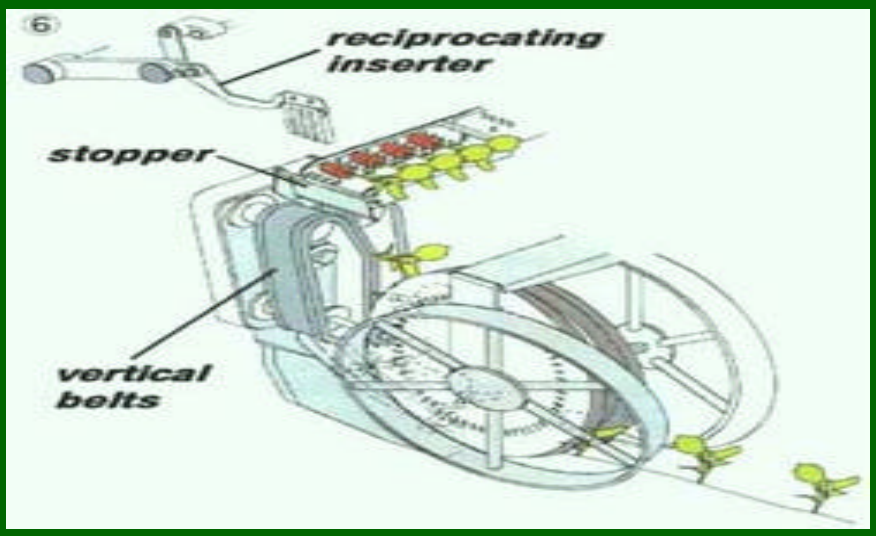
Rice Planter

Minoru Flexible Flats

The Minoru System




4
teeth of the comb


6
reciprocating inserter
stopper
vertical belts

Minoru Tractor pulled Planter

Minoru System Two Row Planter

Automatic Tray Filler and Seed Planter

Tray Planter 4,000 Plants Per Hour

Cutting Planting Machine, 20,000 plants per hour

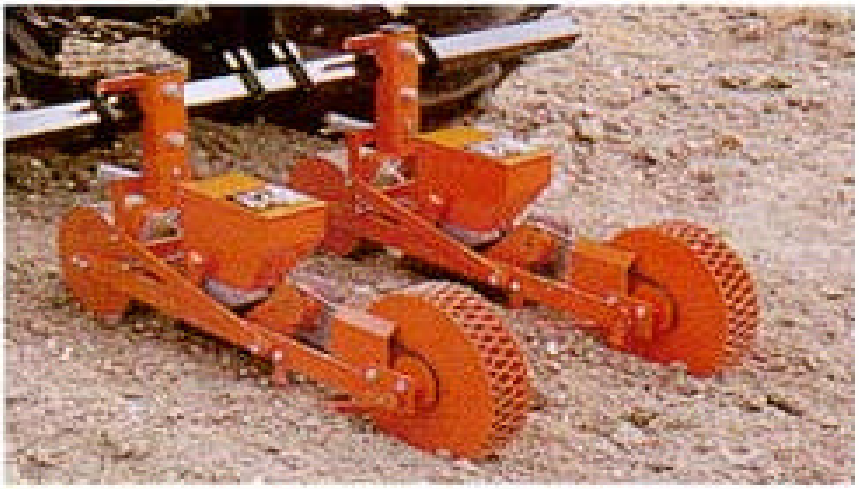
Cuttings planted

Cuttings in Greenhouse

Seed Drill

Seed Drill

Stanhay Planter



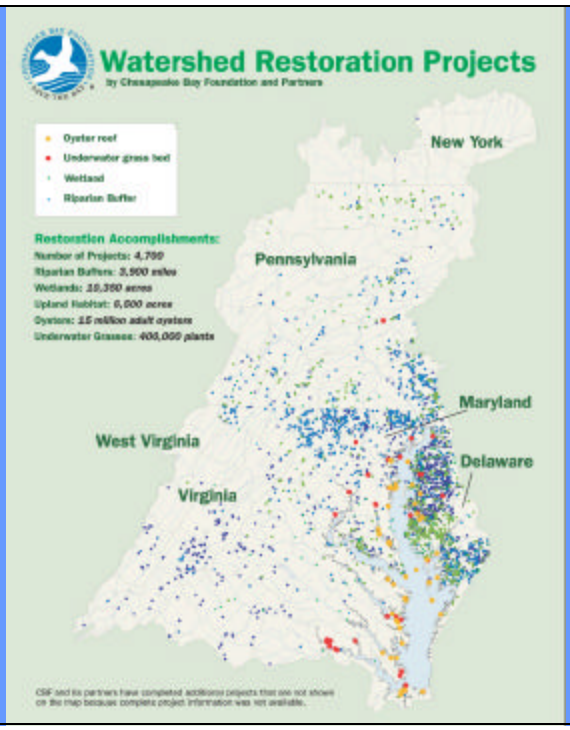
Stanhay Planter

Onion Mower

Large Scale Underwater Grass Restoration: Experiences of the Chesapeake Bay Foundation

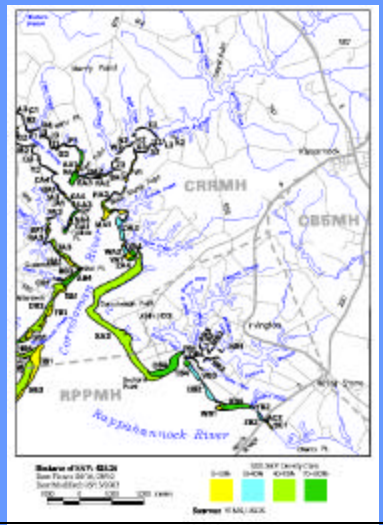
CBF's Underwater Grass Restoration Priorities:

- ❖ Improve water quality by reducing nitrogen inputs into the Bay and it's tributaries
- ❖ Engage an active constituency in hands-on restoration and other water quality improvement goals
- ❖ Examine and test new planting technologies



Large Scale Test Planting: October 2001

Site Selection: Sites in Rappahannock and James Rivers chosen based on at least two years of successful test plots (CBF and/or VIMS)



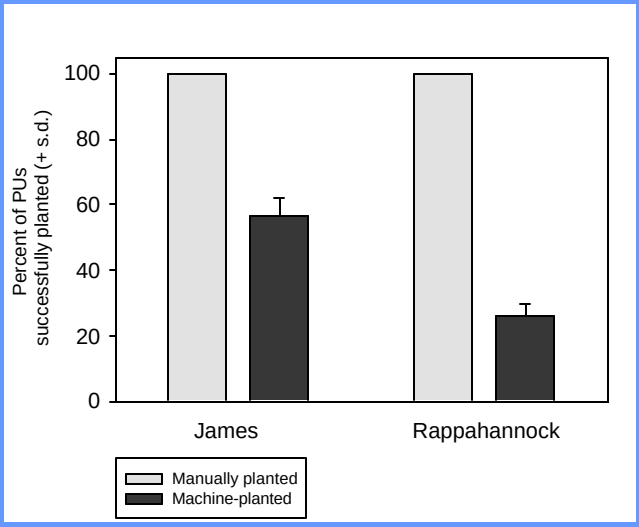
Eelgrass collected from donor beds in York River; Volunteers employed to collect plants and assemble for boat planting

Clip attachment used on two-wheeled pontoon planting boat (Seagrass Recovery, Inc.)

One acre plots planted at each site by CBF; adjacent test plot to compare hand versus machine planting coordinated by VIMS

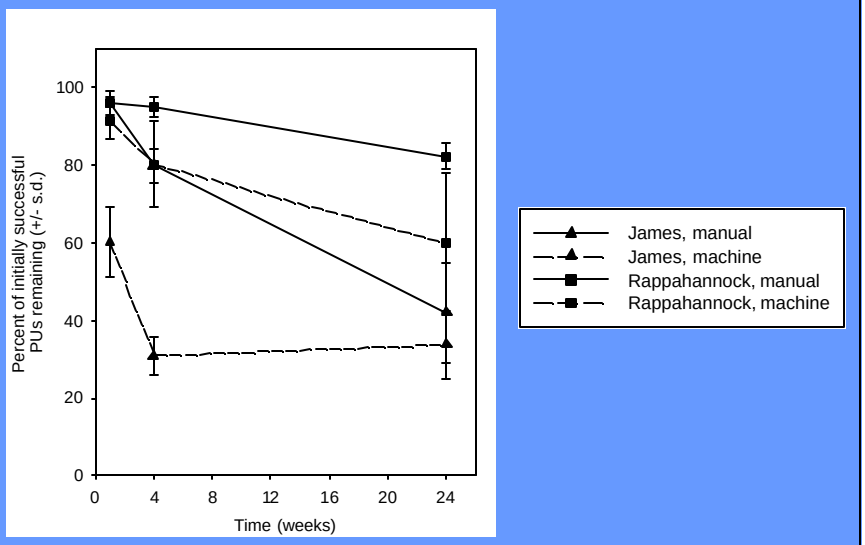
Results from Test Plots- Rappahannock and James Rivers

Percent of Successful Planting Units (VIMS Data)



Results from Test Plots- Rappahannock and James Rivers

Survival of Successfully Planted Planting Units (VIMS Data)



One Acre Plots in Rappahannock and James Rivers

(10-15,000 plants in each acre plot; planted bare root in bundles of 2-5 plants)

<u>James River:</u>

Nov 2001- 40% survival

May 2002- 30% survival

October 2002- 30% survival

June 2003- 30% survival

<u>Rappahannock River:</u>

Nov 2001- 65% survival

May 2002- 45% survival

October 2002- 40% survival

June 2003- 40% survival

Conclusions from 2001 Large Scale Planting

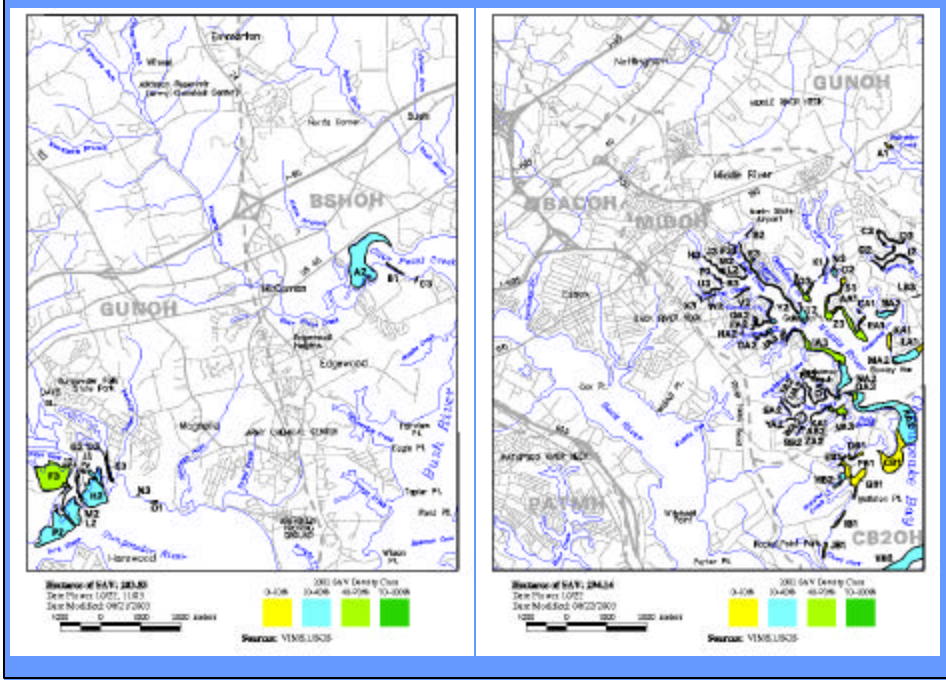
- Mechanical planting was not as efficient as hand planting
- Great loss of eelgrass when attaching to clip on wheel, but "floaters" were collected and planted
- Labor intensive collection and preparation process
- No large source of eelgrass plants without field collection
- More time required to fine tune mechanisms
- Increase planting efficiency – Different planting mechanism
- Test freshwater species – Wild Celery
- Avoid harvesting existing plants - Use plants grown in peat pellets according to protocol developed by Seagrass Recovery, Inc.

July 2003 Large Scale Test Planting

Funding provided by RAE and partners include NOAA CB office and MD NERRS

Site Selection:

- Otter Point Creek (Bush River) and Rocky Point (Middle River) both had at least 2-3 years of successful test plots
- Two different sediment types (muck and hard sand)
- Both easily accessible for subsequent monitoring as well as plenty of bottom for ½ acre plots as well as test rows



Plant Sources:

- ❖ Seedlings: wild celery grown in peat pots (5,500 total)
- ❖ Bare Root plants assembled in peat pots (12,500 total)
- ❖ Peat Pots with wild celery seeds (1,800 total)
- ❖ ½ acre plots planted with boat at each site
- ❖ 12 test rows (each row consisted of 2 hand planted and 2 machine planted rows) at each site

Conclusions from 2003 Large Scale Planting

- Study results not available yet, but planting efficiency appeared greater than 2001.
- Ability to grow material for mechanical planting was substantial improvement but it is still labor intensive propagation and preparation process
- Need biodegradable alternative to metal base for peat pellets
- Peat pellets with bare root appeared most effective
- Different sediment types require adjustments to mechanisms which in small scale projects can be a significant amount of time
- Bottom debris common in freshwater areas presents challenges to mechanical planting
- If successful, mechanical planting should be pursued further