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An Improved Method for Processing Large Quantities of Seeds of Mesohaline Submerged Aquatic Plants

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PROBLEM: Seeds offer an efficient and cost-effective method for providing new plants for large-scale plantings and hence are used for the production of all major domesticated crop plants. Similarly, seed propagation offers the most cost-effective approach for restoring large, genetically diverse, self-maintaining populations of submersed aquatic plants. The successful use of seeds in submerged aquatic plant restoration has been limited because methods for the harvesting, processing, and sowing of seeds have been limited to a single species (e.g. *Zostera marina).* Techniques that have been successfully applied to this species are harvesting and transferring seed-bearing stems (Pickerell et al. 2005) and harvesting, isolating, and sowing seeds directly into locations proposed for restoration (Orth et al. 2003; Granger et al. 2002). Recently, protocols have been established for the harvest, processing, storage, and germination testing of other submersed aquatic plant species (Ailstock and Shafer 2006). A limiting factor for the efficient production of the large numbers of seeds contemplated by large-scale restoration has been the processing of large quantities of seed-bearing stems to isolate the seeds (Ailstock et al. 2010).

PURPOSE: Previous pilot studies (Ailstock and Shafer 2004) established the seed reproductive potential of two species of submersed aquatic angiosperms, *Ruppia maritima* (widgeon grass) and *Potamogeton perfoliatus* (redhead grass) that predominate in the mesohaline reaches of the mid-Chesapeake Bay. Additional work resulted in protocols for the large-scale collection, processing, storage, and viability testing of large numbers of seeds of these same species (Ailstock et al. 2010). A limiting factor in these protocols was the use of mechanical agitation using an old-fashioned wringer washing machine to dislodge the seeds from the harvested seed-bearing stems (Figure 1) (Ailstock and Shafer 2006). The small volume of the washing machine limits the amount of stem material that can be processed at any one time and the configuration of the drain system precludes modifications that can improve seed capture and retention. This technical note outlines a system that has been successfully used to isolate seeds of submerged aquatic plants that is free of the constraints imposed by the use of the wringer washing machine system (Figure 1). In this improved method, large tanks having a vigorous aeration system and refined screening have been used to isolate seeds from the

Figure 1. Wringer washing machine setup for processing small batches of seed-bearing plant stems.

seed-bearing stems of *Ruppia maritima* (widgeon grass), *Potamogeton perfoliatus* (redhead grass), *Stukenia pectinata* (sago pondweed), and *Zannichellia palustris* (horned pondweed).

BACKGROUND: Optimal seed formation for several estuarine submerged aquatic vegetation (SAV) species in the mid-Chesapeake Bay occurs in June, July, and August. This period coincides with the formation of tropical depressions that can significantly elevate site energetics. Since the stems naturally tend to weaken and detach during and immediately after peak seed formation, the appearance of storms can eliminate an otherwise promising collection site literally overnight. Thus, multiple collection sites, some in protected coves, should be identified in advance of seed harvesting. Collecting throughout the reproductive cycle optimizes genetic diversity and ensures that phenotypes that mature both early and late are captured in the collection process. Although such collecting will reduce the efficiency of seed harvest, those seeds that are collected will include a range of other phenotypes, which is important for improving establishment under the variable conditions of the mesohaline environment. There are two options for seed collection. The first utilizes seeds retained in detached stems that accumulate as wrack along shorelines following anthesis (Ailstock and Shafer 2004). This material offers the advantage of a seed source that would otherwise be unavailable for natural reproduction. One disadvantage of wrack is that most seeds have been dispersed; therefore, per-unit seed yields are lower than that of material harvested during active growth. A second disadvantage is that the availability of wrack is heavily influenced by weather. Sites along the west-facing shorelines of protected coves can yield abundant wrack in a year where prevailing west winds are light. The same location may yield little if any wrack when storms disperse the broken stems to open-water environments.

A more reliable method for harvesting seeds is to collect seeds directly from actively growing beds prior to stem detachment. For all four species, *R. maritima*, *P. perfoliatus*, *S. pectinata,* and *Z. palustris*, approximately 75 to 85 percent of the floral structures are produced in the upper third of the stems regardless of water depth (Ailstock, unpublished data). Therefore, the topmost one-third of the seed-bearing photosynthetic stems can be hand-harvested and placed in porous aquaculture baskets. This technique leaves the majority of the photosynthetic stems in place and appears to have negligible effect on the subsoil roots and rhizomes. When hand harvesting is used, many reproductive stems remain intact, thereby minimizing potential adverse effects on the population by leaving considerable amounts of seed in the collection area.

Although collection efficiency will vary with stem densities of the selected populations, many sites allow for the collection of a packed 10-gal (40-L) basket in 20 minutes with modest effort. Once the baskets are dewatered by gravity, the yield is approximately 20 lb (9 kg) of seed-bearing stems for *R. maritima*, *P. perfoliatus*, *S. pectinata,* and *Z. palustris*. The baskets are then transported to the processing facility and the collected material is placed in large plastic aquaculture tanks. The stem material should be spread in the tanks to a depth of not more than 1 ft (0.3 m) and monitored for temperature as a safeguard against excessive temperatures caused by composting of the heavily hydrated organic mixture that can effectively pasteurize and kill the seeds prior to further processing (Ailstock and Shafer 2006).

SEED PROCESSING: Reproductive structures in the collected stem material occur in various stages of development ranging from immature flowers to seed stalks from which mature seeds have already detached. Seed processing is a method of isolating the mature seeds from the stems and other less-developed reproductive structures (Benech-Arnold and Sanchez 2004, Raghavan 2000). An old-fashioned wringer washing machine (Speed Queen, Lehman Hardware & Appliances, Inc., Kidron, Ohio) had been used to provide the agitation needed to detach and collect seeds in quantity from the collected material. However, the small tub size limited processing to one-fourth to one-half basket of wrack material. A new system was developed that can accommodate up to four baskets of material at a time (Figure 2). This system uses forced air to create turbulence, which dislodges the seeds from the harvested stems. With slight modification, this system can be enlarged by using larger tanks and piping systems.

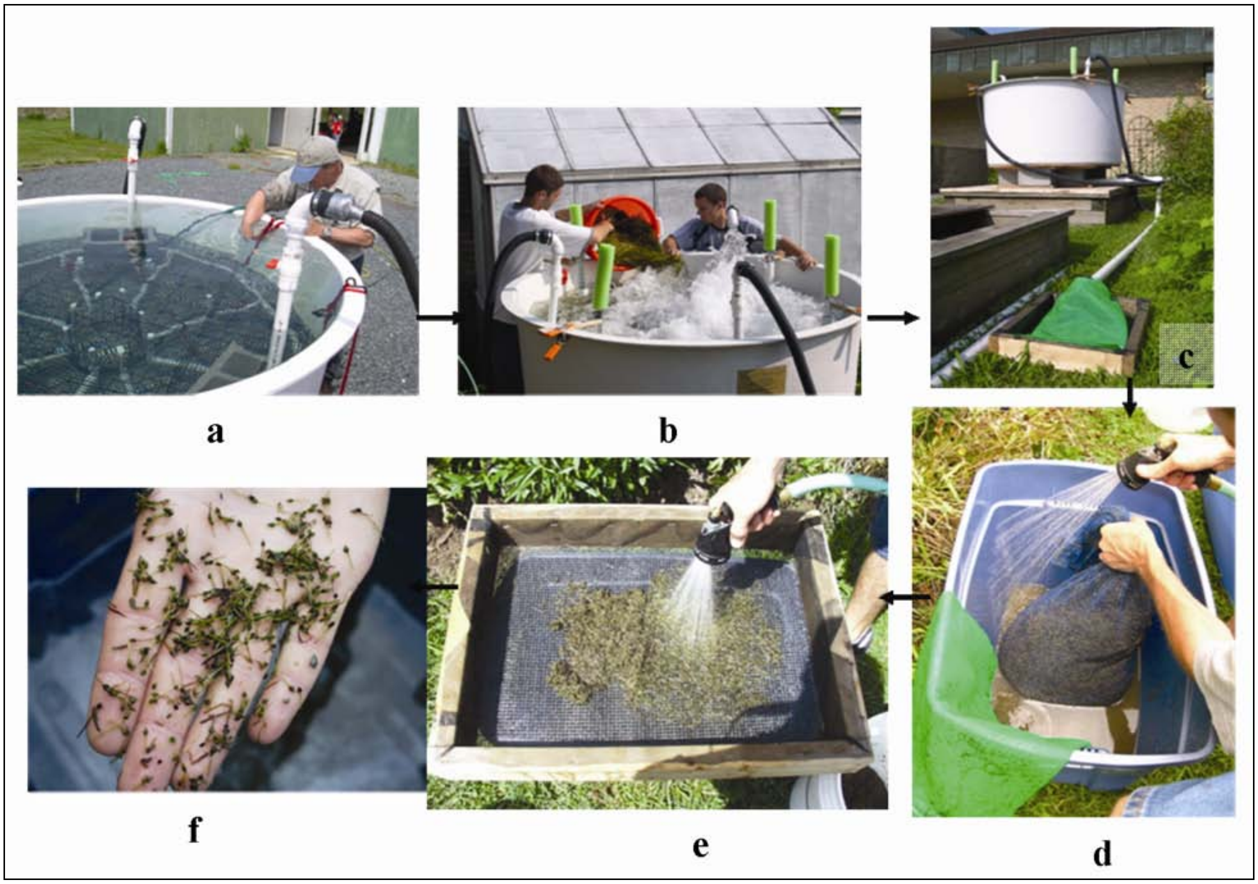

Figure 2. Seed processing procedure using turbulator. 2a. Turbulator tank prepared for seed processing. 2b. Harvested wrack material is added to allow separation of seed from stems. 2c. After processing, seeds that have been detached and settle to the bottom are captured in a mesh bag as tank is drained. 2d. Seeds and small debris captured while draining tank. 2e. Screening seeds to remove remaining vegetative debris. 2f. representative seeds of *R. maritima* after initial screening. Seed stalks are removed by further processing.

In the revised system (dubbed the "turbulator"), several components are important for efficient processing. The eight inner and eight outer interior air ports are each controlled by separate shutoff valves so that air flows can be adjusted to maximize turbulence in the water column. Without this fine-tuning, the water tends to oscillate in the tank with a gentle sloshing motion that fails to dislodge seeds. The wire and plastic mesh liner is used to minimize the settling of stem debris around the drain and is sealed around the culture tank perimeter with a gasket made of foam “swim noodles.” Copper tubing is used to create a skeleton that supports the wire mesh and PVC piping and to attach the pulls that are used for removing the aeration system from the culture tank for routine maintenance and cleaning. The system as used employs three 5.0-h.p. commercial wet/dry shop vacuums to create the air flows necessary to power the system. Figure 3 is a schematic of the current turbulator with the component parts listed in Table 1.

A number of factors including species, time of collection, and reproductive development influence the time needed to detach seeds from the harvested stems. The general process begins by storing baskets of harvested wrack in shallow bins for 2-5 days at cool temperatures (20-23 °C) to facilitate aging and after-ripening of seeds. The temperature of this material should be monitored and mixed when temperatures rise 2 °C or more above ambient temperatures (Ailstock and Shafer 2006, Ailstock et al. 2010). The harvested wrack is then placed in the turbulator and aerated for 15-30 minutes. At this time the wrack is removed and replaced with 2-3 baskets of new wrack material. The processed wrack is retained for additional storage in shallow bins to continue the after-ripening process for an additional 2-5 days at which time the wrack is processed a second time. Often this second processing yields more seeds than the initial processing (Ailstock et al 2010.); however, these seeds tend to be diluted by a greater degree of vegetative debris produced by partial decay of the leaves.

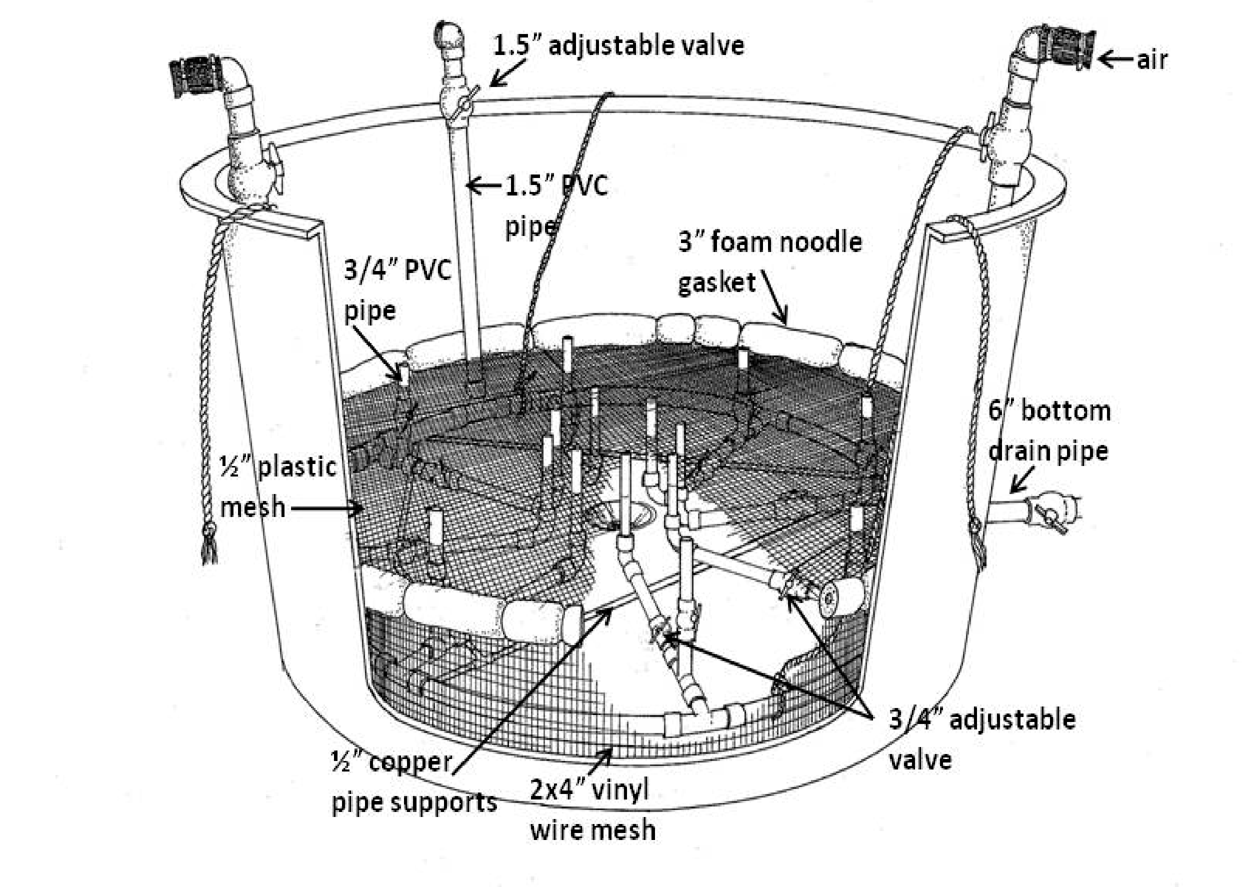

Figure 3. Schematic of turbulator design showing sizes and major component parts.

Table 1. Tools and components for constructing a "turbulator" to isolate underwater plant seeds from harvested wrack.

Quantity	Size	Description
Culture Tank		
1	72"W x 39"H	tank w/6" drain
PVC Pipe		
12'	1.5"	schedule 40 pipe
10'	1"	schedule 40 pipe
18'	.75"	schedule 40 pipe
20'	1.5"	Flexible PVC sch 40
PVC Fittings		
3	1.5"	90 elbow – female to street
3	1.5"	adjustable valve
11	1.5"	Tee
8	1"	Tee
8	1"	90 elbow
8	1"	adjustable valve
8	1.5" to 1"	reduction fitting
16	1" to .75"	reduction fitting
8	.75"	adjustable valve
Copper Pipe and Fittings		
20'	0.5"	copper pipe
2	0.5"	Tee
1	0.5"	45 degree fitting
2	0.5"	end caps
1	0.5"	90 elbow
Wire Mesh		
110 sq. ft.	2" x 4"	vinyl coated wire mesh
110 sq. ft.	0.5" x 0.5"	plastic mesh
Other items		
3	1.5"	rubber coupling
20'		pool noodle
10	3"	hose clamps
1	large can	PVC primer
1	large can	PVC glue
1	1.5 – 2"	PVC cutter
4	5'	Wooden replacement
4		metal straps
4	4"	carriage bolts w/nut
4	4"	hose clamps
4	1"w x 6"	90 – bracket (flat)
3	5.0 hp	Shop vac
3	addl kit	shop vac hoses
Tools Needed		
100	6"	zip ties
200	medium	pig rings
4	8"	spring clamps
PVC cutter	hack saw	Razor knife
Wire cutters	googles	copper cutter
drill	bits	bolt cutters
regular screw driver	metal file	can flux
propane torch w/can	extension cords	pig ring crimp tool
roll solder		

After repeating this process of removing and replacing wrack 4-5 times (total of 8-15 baskets aerated), the aeration assembly is removed from the tank for cleaning and inspection. The tank is then drained from the bottom through a 2-in. drain pipe to which two plastic mesh bags are attached. The inner bag has a mesh size of 3 mm and the outer bag a mesh size of 1.5 mm. These bags retain the detached seeds and the fine particulates of leaf and stem debris that were not retained by the ½-in. vinyl plastic mesh of the aeration system. The collected seeds and debris are removed from the bags and washed over a Series 5 wire and plastic screens that range in size from 14 mm to 1.6 mm. These screens are made by attaching the various size meshes to 49- to 64-cm wooden frames. This fine screening is needed to completely separate the seeds from the miscellaneous vegetative debris in preparation for seed storage and testing (described in Ailstock et al. 2010).

POTENTIAL FOR FURTHER USE: Seeds have proven to be the most cost-effective means of producing large numbers of genetically diverse plants. In order to exploit seeds for the large-scale restoration of underwater flowering plants it will be necessary to identify the environmental conditions favorable to seedling establishment as well as the in situ barriers against successful establishment. Both areas of inquiry require the use of uniform seed stocks whose performance under controlled conditions is known. The techniques presented in this technical note describe a way of isolating large numbers of seeds from four mesohaline species native to the Chesapeake Bay and are applicable to other underwater plants that produce large numbers of seeds. The seeds of these plants are now being used to identify the conditions and habitats necessary for reliable restoration of these species.

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