ABSTRACT

Title of Document: RESTORATION ECOLOGY OF *POTAMOGETON PERFOLIATUS* IN MESOHALINE CHESAPEAKE BAY: THE NURSERY BED EFFECT

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Restoring once prominent species of submerged aquatic vegetation (SAV) back into Chesapeake Bay is crucial for overall restoration success. A resurgence of SAV has occurred since their dramatic declines in the 1960-70s and *Ruppia maritima* now dominates most of the shallows of the mesohaline regions of the Bay, with little re-growth of the once equally prominent *Potamogeton perfoliatus*. *P. perfoliatus* was transplanted into *R. maritima* beds of varying densities to test the "nursery" bed concept. GIS analysis of *R. maritima* density exerted the greatest influence on *P. perfoliatus* transplant success. In year two of the study, ~ 70% of the transplants had survived, with many *P. perfoliatus* satellite colonies forming within 400m of the original transplant sites. Experiments with plant segments show that fragmentation is the likely method of *P. perfoliatus* spread. These results indicate that restoration using nursery grounds is an effective method for re-establishment of this SAV species.

RESTORATION ECOLOGY OF *POTAMOGETON PERFOLIATUS* IN MESOHALINE CHESAPEAKE BAY: THE NURSERY BED EFFECT

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Thesis submitted to the Faculty of the Graduate School of the
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DEDICATION

I would like to dedicate this work to my parents, who have always supported me in every aspect of my life.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Laura Murray, for her advice and guidance throughout this process. I would also like to thank my committee, Dr. Tom Fisher and Dr. Walter Boynton for their leadership and assistance. I would like to extend a special thanks to Dr. Michael Kemp for his advice and suggestions throughout the course of my work. I greatly appreciate the help I received from Debbie Hinkle, Chris Markin, Jeremy Testa and Jess Davis in the lab and field. Dr. Bob Orth and Jenny Whiting supplied aerial photographs. This work was supported by the Center for Ocean Sciences Education Excellence, Maryland Sea Grant and Horn Point Laboratory. I am truly grateful to my family and friends for their constant support and understanding.

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INTRODUCTION

Ecology of submersed aquatic vegetation

Underwater macrophytes, or submersed aquatic vegetation (SAV), are monocot angiosperms that inhabit shallow coastal areas worldwide. Besides acting as important aquatic primary producers, they have numerous ecosystem functions including the ability to trap and stabilize sediments, reduce water column nutrients and provide a valuable habitat for juvenile fish and crabs and other marine and estuarine species (Anderson 1972; Kemp et al. 1984; Lubbers et al. 1990; Moore 2004). Light availability has been suggested to be the most important limiting factor for growth of SAV. Dennison et al. (1993) reported that the light attenuation coefficient (K_d,m^{-1}) through the water column of < 1.5-2.0 m^{-1} was required for survival and, in addition, total suspended solids should not exceed 15 mg l^{-1}. Kemp et al. (2004) confirmed these findings, stating that Chesapeake Bay macrophytes require between 13% (oligohaline regions) to 22% (mesohaline – polyhaline regions) surface irradiance for survival. Unfortunately, these conditions are often not met in coastal areas and estuaries with anthropogenic influences such as Chesapeake Bay.

Decline and recovery of submersed aquatic vegetation

A decline in the growth and survival of SAV has been documented in several areas worldwide (Short and Wyllie-Echeverria 1996; Short and Neckles 1999; Kendrick et al. 2002; Cardoso et al. 2004; Frederiksen et al. 2004). Causes range from climate change to anthropogenic affects. Accompanying such declines is a threat to the various species that rely on the protective cover of SAV beds (e.g. juvenile fish and crabs) as well

as, degrading water quality due to decreases in sediment and nutrient trapping (Ward et al. 1984).

Decreases in biomass and density of submersed aquatics can be attributed to several factors. Reductions in the amount of photosynthetically available radiation (PAR) in the water column results from increased sediment inputs and algal blooms (a result of elevated nutrient levels) which deprive plants of the necessary light to photosynthesize (Wetzel and Penhale 1983, Murray et al. 1999; Cardoso et al. 2004; Gallegos and Bergstrom 2005). In addition, other anthropogenic causes such as point and non-point source pollution, dredging and recreational activities can exacerbate these conditions and put additional stress on SAV beds (Short and Wyllie-Echeverria 1996).

Although once covering an estimated 250,000 ha of shoal area in Chesapeake Bay, underwater macrophytes have suffered a massive decline that began in the 1960s leaving most of the Bay's shallows unvegetated (Stevenson and Confer 1978; Orth and Moore 1983; Orth and Moore 1984; Kemp et al. 2005). By the mid 1980s, most of the remaining coverage was concentrated in the southern regions of the Bay (Orth and Moore 1983). Increased nutrient loading, resulting in enhanced algal and epiphytic growth, and increased turbidity, both of which reduce light availability to SAV, have been shown to be the primary causes of the decline (Kemp et al. 1983, Twilley et al 1985). However, an overall net increase of coverage has occurred in the past decade reaching a post-decline high of over 36,000 ha in 2002 (Orth et al. 2005). This growth has been attributed to a general improvement in water quality as a result of stricter regulations on nutrient inputs and the increasing use of best management practices in the surrounding watersheds (Boesch et al 2001; Orth et al. 2002), in conjunction with a series of low-flow years that

resulted in decreased diffuse nutrient loads in the Bay (Kemp et al. 2005). Furthermore, within the last 20 years there has been an increase in efforts toward restoration of SAV beds (Stevenson and Staver 1989; Kujawski and Thompson 2000; Goshorn 2006) and creation of new habitats to compensate for those lost (West et al. 2000).

In the mesohaline regions of Chesapeake Bay, and specifically the Choptank River on the Eastern Shore, low plant species diversity has accompanied the decline of SAV (Bayley et al. 1978, Orth et al 2005). Monotypic stands have now replaced once diverse vegetation communities. Three species were once equally prominent in the river: *Stuckenia pectinata* (formerly *Potamogeton pectinatus*), *Potamogeton perfoliatus* and *Ruppia maritima*; in the past these would thrive together within the same bed (Twilley et al. 1985). However, *R. maritima* is now the dominant species (~ 90% of the coverage) with little or no evidence of the others (Stevenson et al. 1993; Orth et al. 2005). *R. maritima* is known as a pioneer and "weedy" species noted for its colonizing abilities (Verhoeven 1980; Kautsky 1988; Stevenson et al. 1993) and therefore the resurgence of this single species is not surprising. In addition, *R. maritima* produces a resilient seed bank that can remain viable for several years, allowing the plant to return when conditions are suitable (Kautsky 1988).

The ephemeral nature of *R. maritima* is especially evident under poor water quality conditions. Its presence can act as an indicator of suitable environmental conditions (water quality and sediment composition) and, perhaps, as an indicator of suitable environment for the growth of other species of SAV. In addition to serving as an indicator species, established beds of *R. maritima* can alter their environment by reducing nutrients and turbidity, creating a microenvironment that further increases the quality of

the habitat, allowing other species to colonize (Kemp et al. 1983; Koch 2001; Moore 2004). *P. perfoliatus* is a highly competitive species with high production rates; however, it requires less disturbed (waves, wind) areas to be most productive (Kautsky 1988). Therefore, reintroducing this species into an existing *R. maritima* bed may help assure its successful restoration. Melton (2002) introduced two mesohaline SAV species (*P. perfoliatus* and *S. pectinata*) into existing *R. maritima* beds. His results indicate that transplants had the best success in bare areas within vegetated sites as opposed to non-vegetated sites.

Parameters affecting submersed aquatic vegetation abundance and health

In addition to light, other parameters play an important role in the survival of SAV. The amount and sources of nutrients for these aquatic plants can play a critical role in their survival. Submersed aquatics are able to take up nutrients from sediment porewaters as well as from the water column (Erftemeijer and Middelburg 1993; Clarke and Wharton 2000). Water column nutrients can fluctuate based on storm events and tidal inputs/outputs, whereas porewater nutrients remain relatively stable and can potentially offer a constant source of various nutrients (Barko et al. 1991). Of the two sources, Caffrey and Kemp (1992) showed that aquatic plants tend to take up more of their nutrients via roots rather than shoots. Sulfide, phosphate and ammonium represent a few of the porewater constituents that affect SAV growth. They can be useful in understanding plant growth, density and success (Udy and Dennison 1997; Johnson and Ostrofsky 2004).

The concentrations of nutrients found in the plant tissue can also serve as an indicator of plant health. The percentage of carbon (C), nitrogen (N) and phosphorus (P) and resulting C:N and N:P ratios in the plant tissue can be used as indices of plant nutrient limitation (Gerloff and Krombholz 1966; Atkinson and Smith 1983). Plant tissue C:N ratios have been implicated as an index of plant structural integrity (Kemp et al. 1984), and N:P ratios have been used to indicate possible nutrient limitation (Murray et al. 1993).

Not only are sediment nutrients a factor in the growth of SAV, the size of the sediment particles, or grain size, plays an important role as well. By examining the grain size of the sediment within various SAV beds a better understanding of how nutrients might move through the sediment and become available to the plants can be developed (Smart and Dick 1999). In general, SAV tends to survive better in more fine grain sediments, with a mixture of sand and mud (Kautsky 1988). Values of silt/clay that have been found in healthy SAV beds range from 0.4% to 72% (Koch 2001). Coarser sediment may be required for higher salinity species, allowing more oxygenation of the root zone and reducing sulfide concentrations (Koch 2001).

Propagation of submersed aquatic vegetation

Submerged aquatic vegetation is able to reproduce sexually (seeds) and asexually (vegetative propagules or fragments). Sexual reproduction through seed production and germination is the primary mechanism by which some species, such as *Zostera marina* and *R. maritima* spread (Verhoeven 1979; Ewanchuk and Williams 1996). However, asexual reproduction is generally considered the most important mechanism for dispersal

in many aquatic species such as *Hydrilla verticillata*, *Myriophyllum spicatum*, *P. perfoliatus* and *Syringodium isoetifolium* (Madsen et al 1988; Stevenson 1988; Kujawski and Thompson 1999; Rasheed 2004). One reason for the success of asexual reproduction is that seeds of *P. perfoliatus* and *P. crispus*, for example, can have low germination rates (Rogers and Breen 1980; Kujawski and Thompson 1999) or can fall close to the mother plant, as is the case in *Z. marina* (Orth et al. 1994), not allowing for widespread dispersal. Several studies have shown that plant fragments can serve as successful recruit mechanisms for plant dispersal in species like *M. spicatum* (Madsen et al 1988; Rybicki and Carter 1994), *P. pectinata* (Rybicki and Carter 1994), *Hydrilla verticillata* (Madsen and Smith 1999; Rybicki et al. 2001) and *Halodule wrightii* (Hall et al. 2006).

Aerial photography and GIS analysis of submersed aquatic vegetation

Aerial photography has long been used in studies of terrestrial systems to map landscape and land use change (Paine 1981; Fensham and Fairfax 2003; Plieninger 2006). However, it is becoming a common practice in the survey of aquatic systems as well and is being used worldwide as a tool to track changes in aquatic vegetation distribution and abundance (Kirkman 1996; Pasqualini et al. 1998; Lehmann 1998; Frederikson et al. 2003). Annual aerial mapping surveys of Chesapeake Bay SAV beds have been conducted since 1985 (Moore et al. 2000). These surveys provide an indication of the location of SAV in the entire Bay and allow for the monitoring of abundance from year to year. Photographs used in conjunction with a Geographic Information System (GIS) introduces a unique way of monitoring and evaluating specific SAV beds by facilitating bed area calculations, which integrate bed density, and other

geometric properties such as shape, perimeter and proximity (Schulte 2003). In contrast to traditional methods of biomass assessment (e.g. field sampling using quadrats and/or biomass cores) the use of aerial photographs allows for a whole bed assessment, which is much less labor intensive and provides a better overall view of bed structure over larger spatial scales.

While the ecology of submersed aquatic plants in fresh and marine systems is well documented, less work has been done in mesohaline areas. The dynamic estuarine environment is characterized by high interannual variability of such parameters as salinity, dissolved oxygen and turbidity, which can affect annual SAV growth and survival (Stevenson 1988). Field studies in these areas have been limited, restricting our knowledge of how mesohaline SAV species respond to their ever-changing environment. This limited knowledge of these changing regions makes the task of restoring SAV to mesohaline regions more challenging (Fonseca et al. 1988).

Preliminary data for my research was obtained from the results of a study conducted by Melton (2002). In his study, *P. perfoliatus* and *S. pectinata* were transplanted into existing *R. maritima* beds in the lower Choptank River with the objective to determine the effect of *R. maritima* bed patch density on transplant survival. Results indicated that transplants survive best when planted in bare patches within the *R. maritima* bed. In addition, Melton created a site suitability index for transplanting sites in the Choptank River. He found that Broad Creek, a tributary of the Choptank River, was highly suitable for transplanting and SAV restoration.

My thesis focuses on the restoration of *P. perfoliatus* in the Choptank River, testing the ability of *R. maritima* to serve as a nursery ground for SAV restoration. I addressed three main topics: *R. maritima* nursery bed affect on *P. perfoliatus* transplant growth, the success of the transplants in subsequent years, and the mechanism for the formation of *P. perfoliatus* colonies from the original transplants. First, the idea of using *R. maritima* as a nursery bed is tested with four main hypotheses: 1) Using *R. maritima* as a nursery ground for *P. perfoliatus* transplanting will increase transplant success; 2) As density of *R. maritima* increases, *P. perfoliatus* biomass will increase; 3) Nursery bed density will be the main factor contributing to *P. perfoliatus* growth and success; 4) If *P. perfoliatus* can become established, it will survive subsequent years and proliferate. To test these hypotheses, I correlated *R. maritima* biomass calculated from biomass coring and GIS analysis to *P. perfoliatus* growth. In addition, I assessed other physical parameters (sediment grain size, and sediment porewater) in the *R. maritima* beds in relation to transplant success.

Second, I focused on the survival and growth of the transplants the following the year, addressing two hypothesis: 1) If *P. perfoliatus* transplants were healthy at the end of 2004, they will have survived into 2005 and grown in area; 2) Successful transplants produce propagules that will colonize other areas of the nursery bed, forming satellite colonies. To test these hypotheses ground surveys by boat were used to pinpoint locations of *P. perfoliatus* expansion (satellite colonies) within the nursery bed. Aerial photographs were employed to evaluate densities of these areas, and to evaluate year-to-year success. Field measurements of recurring transplants were taken and compared to

first year values. Finally, I investigated propagation mechanisms in a mesocosm experiment in order to explain the spread of the transplants.

METHODS

Study Site Selection of R. maritima *nursery beds*

Ten study sites were located in Broad Creek, a tributary of the Choptank River in Chesapeake Bay (Fig. 1). Sites were chosen based on the presence or absence of *R. maritima* and subsequently classified, based on June biomass sampling (five biomass samples were taken at each site using a plexi-glass corer (0.0154 m^2) in June and October), as bare (non-vegetated, S. Hopkins), dense reproductive (Elbert's Cove East, S. Bridge, Deep Neck), sparse reproductive (Hambleton Island, S.S. Mulberry, N. Mulberry) or sparse non-reproductive (Elbert's Cove West, Neavitt, Cedar Point) (Table 1). Each density class was represented by three different sites, with the exception of the bare classification in which there was only one site (originally there had been two, but the other was destroyed in a storm shortly after planting). In addition to the *R. maritima* nursery bed sites, a *P. perfoliatus* bed in the Severn River, also a tributary of Chesapeake Bay, was chosen as a reference site.

Salinity at each site was measured four times throughout the experiment using an YSI model 85 and averaged. Mean low water (mlw) was calculated from water depths taken during each filed monitoring (four times) at each site and adjusted based on tidal heights and times (MD DNR, http://www.dnr.state.md.us/fisheries/access/tide_finder.html). Water column chlorophyll-*a* samples were taken in July in duplicate by filtering through a 0.7 μm Whatman GFF glass fiber filter. The samples were frozen until analysis (less than one

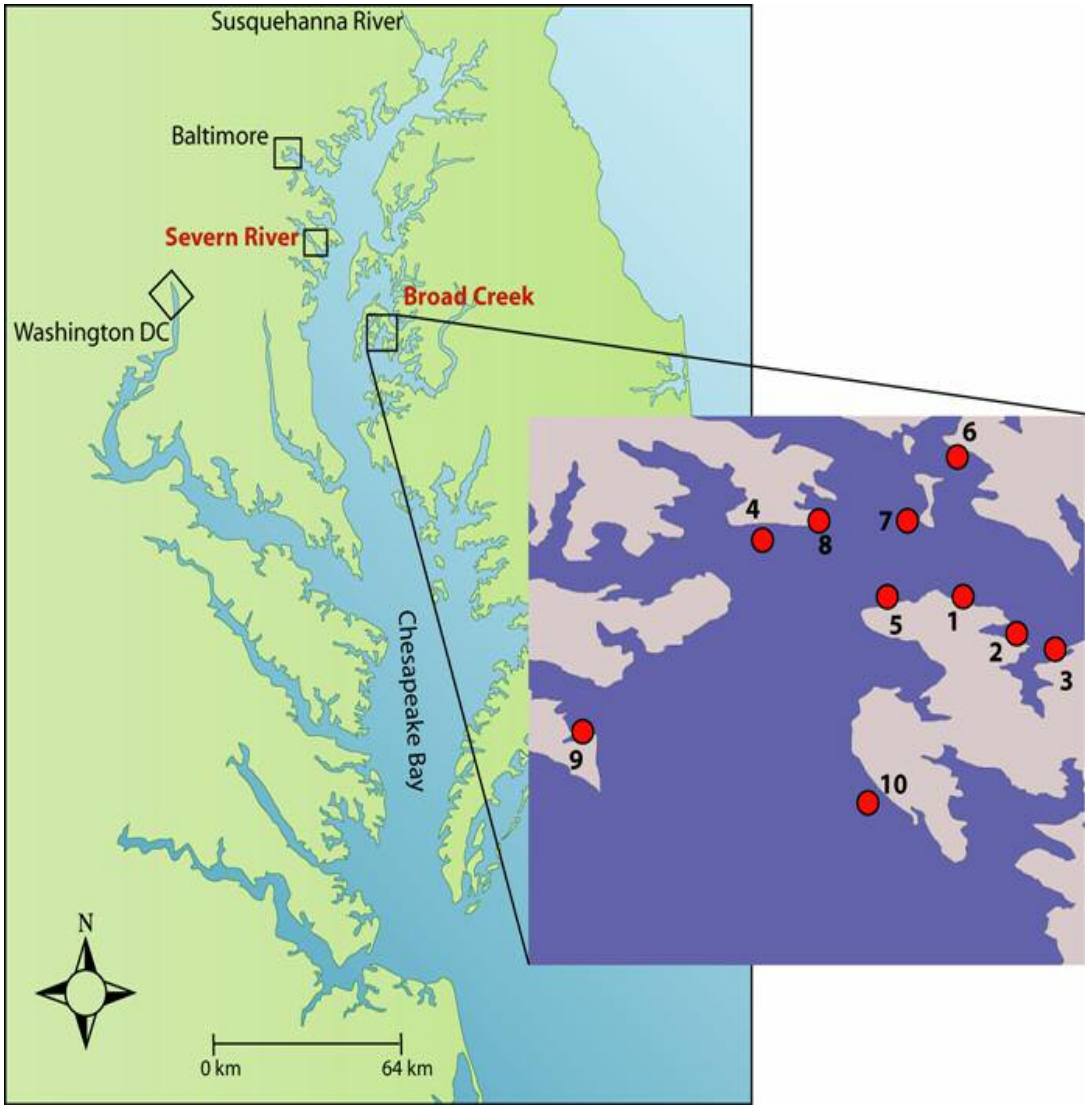


Figure 1. Chesapeake Bay, USA showing location of study sites, Severn River and Broad Creek in the Choptank River. Red dots indicate original ten study sites in Broad Creek: Cedar Point (1), Elbert's Cove West (2), Elbert's Cove East (3), S.S. Mulberry (4), Deep Neck (5),Hopkins Point (6), Hambleton Island (7), N. Mulberry (8), Neavitt (9) and S. Bridge Creek (10).

Table 1. Latitude and longitude and description, including June biomass and original density classification, of *R. maritima* nursery bed sites.

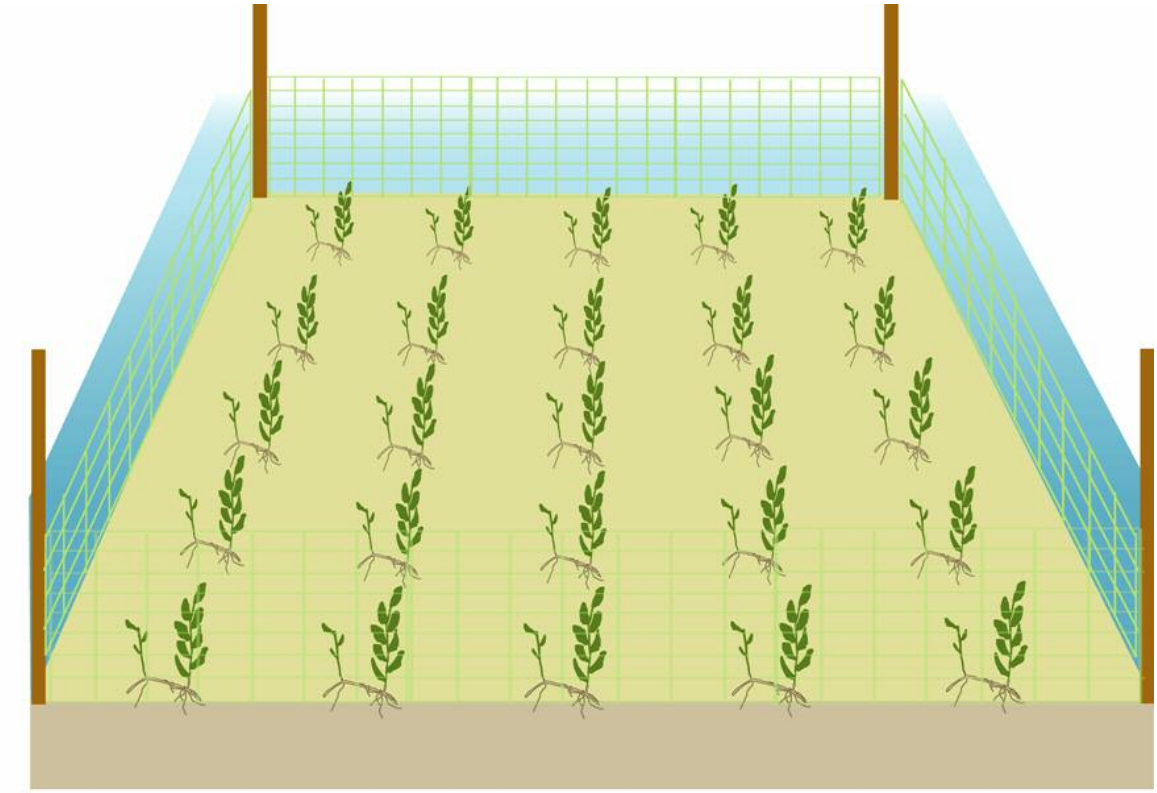
Site	latitude	longitude	*R. maritima* biomass (gdw m^{-2})	density class
Deep Neck	38°44.318	76°14.021	117.92	dense reproductive
EC* East	38°43.935	76°12.764	116.62	dense reproductive
S Bridge Crk	38°43.120	76°14.120	208.96	dense reproductive
Hambleton Is	38°45.061	76°13.912	32.73	sparse reproductive
N. Mulberry	38°45.046	76°14.579	79.87	sparse reproductive
S.S. Mulberry	38°44.902	76°14.910	32.60	sparse reproductive
Cedar	38°44.275	76°13.502	46.75	sparse vegetative
EC* West	38°43.992	76°13.085	26.49	sparse vegetative
Neavitt	38°43.074	76°16.391	25.97	sparse vegetative
Hopkins Pt	38°45.675	76°13.692	0.00	bare

*EC = Elbert's Cove

month). Chlorophyll-*a* was extracted using a 90% acetone solution, sonicated and measured with a Turner Designs 10-Au fluorometer (Parsons et al. 1984). Light through the water column (PAR) was measured in $\mu E\ m^{-2}\ s^{-1}$ at each site using a Li-Cor light sensor held at several depths (0 m to depth at 0.25 m intervals) at the beginning and end of the experiment. Light attenuation coefficients (k_d) was calculated using the Lambert-Beer equation, $I_z = I_o\ e^{-kz}$.

Propagation and planting of P. perfoliatus

P. perfoliatus was propagated from cuttings grown in the greenhouse facility at Horn Point Laboratory. Natural sediments were obtained from unvegetated SAV ponds located on the Horn Point property and placed into 36 x 27 x 10 cm trays. A layer of sand was placed on top to prevent sediment resuspension when placed in water. Cuttings from a naturally occurring *P. perfoliatus* bed in the Severn River were used as the starting plant material. Ten cm-long cuttings were planted in sediment-filled trays (density equaled 20 shoots per tray) following the procedure of Kujawski and Thompson (2000). Planted trays were placed in 2.4 x 0.61 x 0.61 m fiberglass greenhouse tanks filled with ambient Choptank River water (salinity of 10-12) and heated to maintain a temperature between 28-32^{o}C. This process was repeated until enough plants were produced for field planting (~ 70 trays). Each tray of *P. perfoliatus* was quartered to produce individual planting units of mature, rooted plants (~ 10 – 15 shoots per planting unit). Planting units were placed on 0.5m centers in 3m x 3m quadrats at the end of June 2004 (Fig 2). Bare patches within the *R. maritima* beds were selected for planting based on the results from previous research (Melton 2002). Where a bare area within the bed could not be found, a



Symbols courtesy of the Integration and Application Network (ian.umces.edu/symbols/), University of Maryland Center for Environmental Science.

Figure 2. Conceptual diagram of 9 m^2 transplant quadrat, including, *P. perfoliatus* planting units, corner stakes and fencing.

sparsely vegetated area was chosen and then weeded. Each quadrat was fenced to prevent disturbance from animals (e.g. mute swans and cow nose rays). GPS coordinates of each transplant area were taken using a Garmin GPS III Plus (15 m accuracy).

Plant tissue sampling, analysis and transplant monitoring

R. maritima biomass samples that were obtained from coring were separated into above-ground and below ground, live and dead plant parts, then dried and weighed and later used for CHN analysis. *P. perfoliatus* biomass samples were taken at the end of the study from the transplant areas as well as from the reference bed by cutting off 20 cm of length from four individual shoots (this was done to minimize impact to the transplant). These samples were used for CHN analysis by drying the plant material at 60 ^{o}C for 48 hours and then grinding. Samples were analyzed in a Control Equipment 440 Elemental Analyzer following the procedures in Lane (2000).

Transplant growth was evaluated in July, August, September and October 2004 at approximately 4-week intervals and measured via snorkeling by counting the number of live shoots in each planting unit. Coalescence of individual units began in September at which time a 0.25 x 0.25 m square was used to estimate density by triplicate random tossing of the square into the quadrat and recording the number of shoots. Average shoot density (number of shoots m^{-2}) was calculated for all counts. The length of three randomly selected shoots was measured at the transplant sites and used in the conversion to plant biomass from a previously determined length:weight regression for *P. perfoliatus* (Nagel 2006). As a result, *P. perfoliatus* biomass and density were perfectly correlated ($r^2 = 1$).

Density interpretation using aerial photographs

Black and white aerial photographs at a scale of 1:24,000 were obtained from the Virginia Institute of Marine Science for the growing seasons of 2004 and 2005. The photography was done by Air Photographics using a Wild RC-20 camera, with a 153 mm focal length Aviogon lens and Agfa Pan 200 film (Orth et al. 2004). Each of the *R. maritima* nursery beds were manually digitized with ArcMap version 9 resulting in vector polygons. Bed boundaries were visually interpreted as darker areas, and the deep edge of the bed and physical boundaries (i.e. landmasses) were used to constrain the polygon boundaries. In addition, since there were no clear boundaries between nursery beds, length boundaries were determined based on the tidal excursion in Broad Creek and the resulting distance water would move through the nursery bed on an ebb and flow tide. As stated previously, *R. maritima* affects the water as it passes through and therefore this water would have an influence on the transplant. After the polygons were created, the area of each bed was calculated using ArcMap.

A remote calculation of *R. maritima* density in each nursery bed was done using. ArcMap version 9 and aerial photographs. Dense, moderate and sparse areas within each bed were manually digitized in ArcMap and areas calculated. The specific densities (%CC) of these areas were determined using a crown-density scale (Moore et al. 2000). Overall bed density (%) was calculated with a weighted average using the percentage of each bed that was dense, moderate and sparse and the estimated densities of each of these areas.

Using methods outlined by Moore et al. (2000), total bed aboveground biomass (gdw) was calculated with the following formula:

$$\text{Monthly Biomass} = Mb * Cc * Ba$$

Where Mb = model monthly biomass for *R. maritima* (56.0 gdw m^{-2} as determined from Moore et al. 2000), Cc = photo-interpreted density class to ground cover conversion (%), and Ba = bed area (m^2). Biomass was calculated for the month of October based on available photographs.

Site sediment sampling and analysis

Surface sediment (1 – 5 cm) samples in the nursery beds and transplant areas were taken in triplicate at the beginning of the study for chlorophyll-*a* and CHN analysis following the same procedures as described above. Samples were obtained using a small plastic coring device (3 cm diameter). Sediment samples were taken in triplicate with the same plastic corer from random unvegetated patches within each nursery bed and transplant area for analysis of sediment grain size (Sweet et al. 1993). Samples were thoroughly mixed with 400ml of sodium hexametaphosphate ($(NaPO_3)_6$) dispersant solution (5.5g/L) and poured through a 63 μm sieve into a 1L graduated cylinder. Dispersant was added to the graduated cylinder to give a total volume of 1L and thoroughly mixed for 10s to evenly distribute the sediment. Twenty seconds after mixing ceased a 20ml aliquot was taken at a depth of 20 cm, pipetted into a 50 ml beaker and placed in the drying oven. After the sample in the graduated cylinder settled for 2h 3min., another 20ml aliquot was taken at a depth of 10 cm, pipetted into a 50 ml beaker and placed in the drying oven. These represented the silt/clay and clay fraction of the

sample, respectively. The portion remaining in the sieve (gravel/sand fraction) was rinsed with DI water into a 50 ml beaker and placed in the drying oven.

Dialysis porewater samplers (peepers) were used in July at each site to obtain sediment pore water concentrations of sulfide, phosphate and ammonium. Peepers were made out of grey PVC and measured 25 x 3.0 x 2.5 cm with five 10 ml sampling ports (2.0 cm diameter) that, when pushed down flush to the sediment surface, obtained samples at depths of 1.5 cm, 4.5 cm, 7.5 cm, 11.5 cm and 16.5 cm. Each port was filled with DI water (sparged with N_2 gas to remove dissolved oxygen) and covered directly with a 0.2 μm polycarbonate membrane overlain with a 125 μm mesh screening for strength and to keep particulate matter from clogging the membrane. One peeper was placed in each of the *P. perfoliatus* transplant sites and in the adjacent *R. maritima* beds. Additional peepers were placed in the Severn River *P. perfoliatus* reference bed. All peepers were retrieved after 10 d of equilibration. Using a syringe, the entire water sample was extracted from each port. One ml for each PO_4 and H_2S and 0.2 ml for NH_4 was placed in separate plastic vials; DI water was added so each sample equaled 5 ml. In addition, the H_2S was fixed with 0.8 ml of diamine to prevent oxidation. The remaining sample was saved and archived by freezing. All samples were frozen, then thawed and analyzed spectrophotometrically following the procedures of Cline (1969) and Parsons et al. (1984).

Analysis of transplant success and satellite colony formation

During the summer of 2005, all nursery bed sites were revisited to check for *P. perfoliatus* transplant survival and success. Latitude and longitude of transplant locations were used to help locate transplants within the nursery bed. When a transplant site was re-located, GPS coordinates were recorded, perimeter measured and density determined using a 0.25 m^2 square quadrat (as described above).

In addition to locating the original transplants, "satellite colonies" of *P. perfoliatus* were found at two sites. These satellites are small patches of *P. perfoliatus* that are separate from, but in close proximity to (within 400m), the original transplants. The position of these satellites was taken and the area estimated. To determine if satellites propagated from the transplants, extensive ground surveys were conducted in the tidal tributaries of Broad Creek. No other *P. perfoliatus* stands were found, indicating that the likely parent source for the satellites were from our restoration efforts.

The coordinates of the original transplants and the satellites were imported into ArcMap GIS and then added to the map containing the vector polygons of the *R. maritima* nursery beds. Approximate distances of the satellites from the transplants were estimated from the GIS analysis. Density of *R. maritima* surrounding the satellites was calculated.

Propagation from fragmentation

A greenhouse mesocosm experiment was designed to investigate the possible mechanism for satellite colony formation. Six greenhouse tanks were filled with natural sediment to a depth of ~ 4.0 cm. Three tanks were left unvegetated and three were

planted with *S. pectinata* and *R. maritima* to a density of ~ 45% to mimic the density of the nursery bed where satellites were found. Tanks were filled with ambient Choptank River water and maintained at a salinity between 8 – 9. Water was circulated via submersible pump (Maxi-jet 400, 206 gph) with the timing adjusted to mimic tidal flow (e.g. 4 hrs on, 2 hrs off). Thirty 10 cm fragments of *P. perfoliatus* for each experimental tank were cut from the apical end of mature plants and labeled (numbered from 1 – 30) using a small piece of labeling tape attached to a short piece of thread and tied onto each cutting (preliminary tests were done to ensure that labeling would not affect movement, horizontally or vertically, of individual cuttings). Position of cuttings in the tank (sunk, floating, midlevel and/or tangled) were observed once a day for the first week and every other day for the remainder of the experiment (three additional weeks). Weekly measurements were performed in which the length of the cutting was measured, as well as, any new branches, shoots, rhizomes and roots (for floating cuttings). Cuttings that had sunk were only touched during this time so as not to disturb the rooting process. Tanks were skimmed with an aquarium net each day and floating cuttings cleaned of any fouling by gently rubbing between thumb and forefinger. Walls of the tanks were cleaned of algae once a week and filters cleaned twice a week.

Statistical analysis

Analysis of variance (ANOVA, α=0.05) was performed on transplant biomass data and fragment growth data using SAS 9.1. Homogeneity of variance was checked using Levene's test (α=0.05). Graphical representation of the data showed some parameters to be non-normal; log transformations of those data were implemented. A

CLUSTER analysis using Ward's minimum variance method was performed using SAS 9.1. This analysis was performed with variables that affected *P. perfoliatus* transplant success. Single linear regression analysis was preformed using Microsoft Excel 2003 and multiple linear regression analysis in SAS 9.1.

RESULTS

R. maritima affect on *P. perfoliatus* growth

Water characteristics of all *R. maritima* nursery beds fell within the range of acceptable values for SAV growth and health (Table 2). All k_d values were below the 2 m threshold (Dennison et al. 1993) and average salinity remained within range for *P. perfoliatus* growth, 6.76 – 9.10 (Stevenson and Staver 1989). Final *R. maritima* biomass in October as determine by the biomass coring method and final *P. perfoliatus* biomass for each site is reported in table 2. Information for S. Bridge Creek is not presented in the table; this site was removed from analysis due to severe site destruction during a storm. Water column nutrients were within range for healthy SAV growth (Dennison et al. 1993) and ranged from 0.22 – 2.54μM for NO_3, 3.0 – 11.6μM for NH_4 and 0.06 – 0.78μM for PO_4 as reported for Broad Creek by Nagel (2006).

R. maritima biomass assessed by the coring method was used to determine original nursery bed density classifications (Table 1). In the past, the coring method has been a widely used method for biomass determination of SAV. A comparison of the *R. maritima* density classifications to the monthly average growth of *P. perfoliatus* shows a variable response (Fig. 3). Overall growth was highest in the dense *R. maritima* nursery

Table 2. Average (± SE) water quality parameters over the growing season for *R. maritima* beds in Broad Creek including mean low water depth (mlw), light attenuation (kd m^{-1}), salinity and chlorophyll *a* concentration. Final *R. maritima* October biomass (as per coring method) and *P. perfoliatus* biomass are reported in grams dry weight (gdw)

Site	**mlw depth(m)**	**kd m^{-1}**	**Salinity**	**H_2O chl *a* (ug/L)**	***R. maritima* Biomass (gdw m^{-2})**	***P. perfoliatus* Biomass (gdw m^{-2})**
Cedar	0.32	0.55	6.76	5.76 ± 0.46	36.9	15.02
EC* West	0.41	0.82	7.10	6.22 ± 0.09	75.3	25.11
EC* East	0.55	1.54	7.43	7.98 ± 0.25	62.2	17.17
S.S. Mulberry	0.70	1.03	8.83	7.42 ± 0.16	97.5	12.23
Deep Neck	0.74	0.71	7.55	6.57 ± 0.46	24.8	13.41
S. Hopkins	0.56	1.15	7.43	7.31 ± 0.34	0.00	6.545
Hambleton Is.	0.34	0.85	7.76	8.23 ± 0.63	25.3	29.93
N. Mulberry	0.41	0.73	8.10	9.18 ± 0.27	96.1	6.54
Neavitt	0.64	1.17	9.10	14.98 ± 0.70	21.3	2.68

* EC= Elbert's Cove
nd = no data

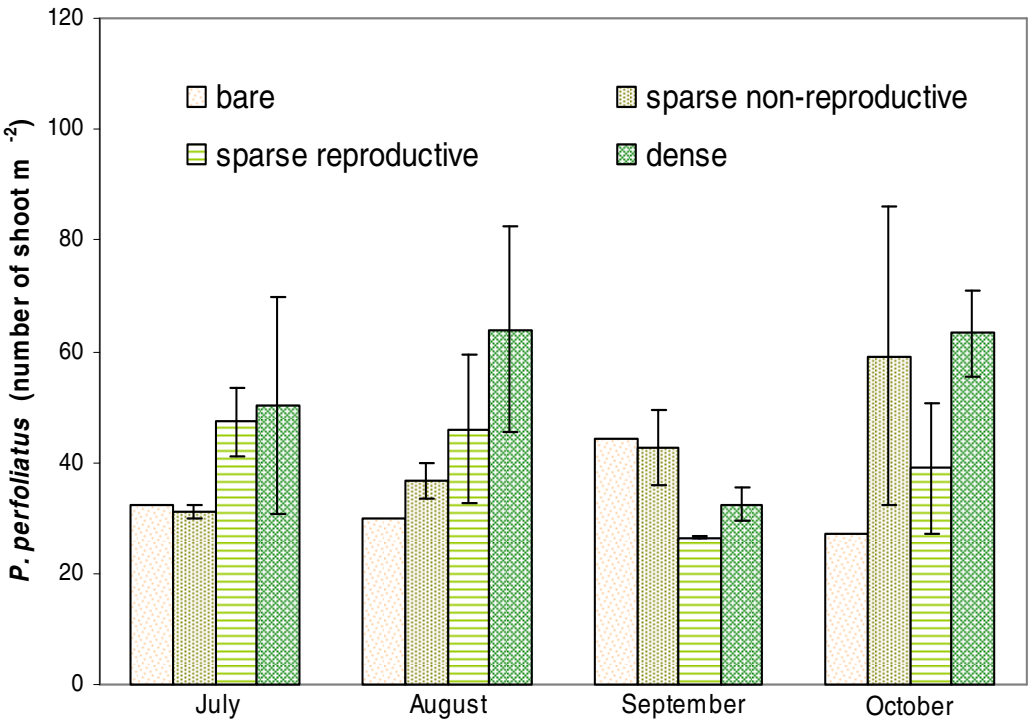


Figure 3. Monthly mean (± SE) shoot number of *P. perfoliatus* in transplants in varying densities of *R. maritima* nursery beds as determined by biomass coring. ANOVA showed no significant differences ($\alpha = 0.05$) between or among treatments.

beds, although ANOVA showed no significant differences between or among treatments ($\alpha = 0.05$). Declines in September are attributed to poor water visibility and associated errors in measurements. The final *R. maritima* biomass, as determined by the coring method (Table 2), displayed little correlation with final *P. perfoliatus* transplant biomass ($r^2 = 0.04$ $p = 0.315$).

Manual digitization (using GIS) of nursery beds on aerial photographs included an outline of vegetation using deep edge boundaries and tidal excursion boundaries (Fig. 4). GPS coordinates of *P. perfoliatus* transplants were imported and superimposed on the aerial photographs. The digitized area (reported as a percent of total bed area) of each density category (sparse, moderate, and dense) of *R. maritima* that occurred within the beds resulted in distinct polygons within the original overall bed polygon (example Fig. 5). The resulting areas of these density polygons (%), total bed density (%) and resulting biomass (gdw and gdw m^{-2}) are presented in table 3. Bed densities at the study sites ranged from 75% (dense) to 27% (sparse) with intra-bed density ranging from 85% to 25%. Bed biomass ranged from a low of 15.39 gdw m^{-2} at Neavitt to a high of 42.11 gdw m^{-2} at Elbert's Cove West.

When the Hambleton Island site (site 7) is examined more closely, we find that outside factors may have affected the *R. maritima* biomass at this location. It is believed that mute swans (an invasive species to the area) may have dramatically decreased the *R. maritima* biomass of this site. During one site visit, >50 swans were seen in and around the bed. A study by Hindman (unpublished data) showed that mute swans could reduce SAV biomass in a bed by 57.4% in one year. Allowing for this correction in biomass at

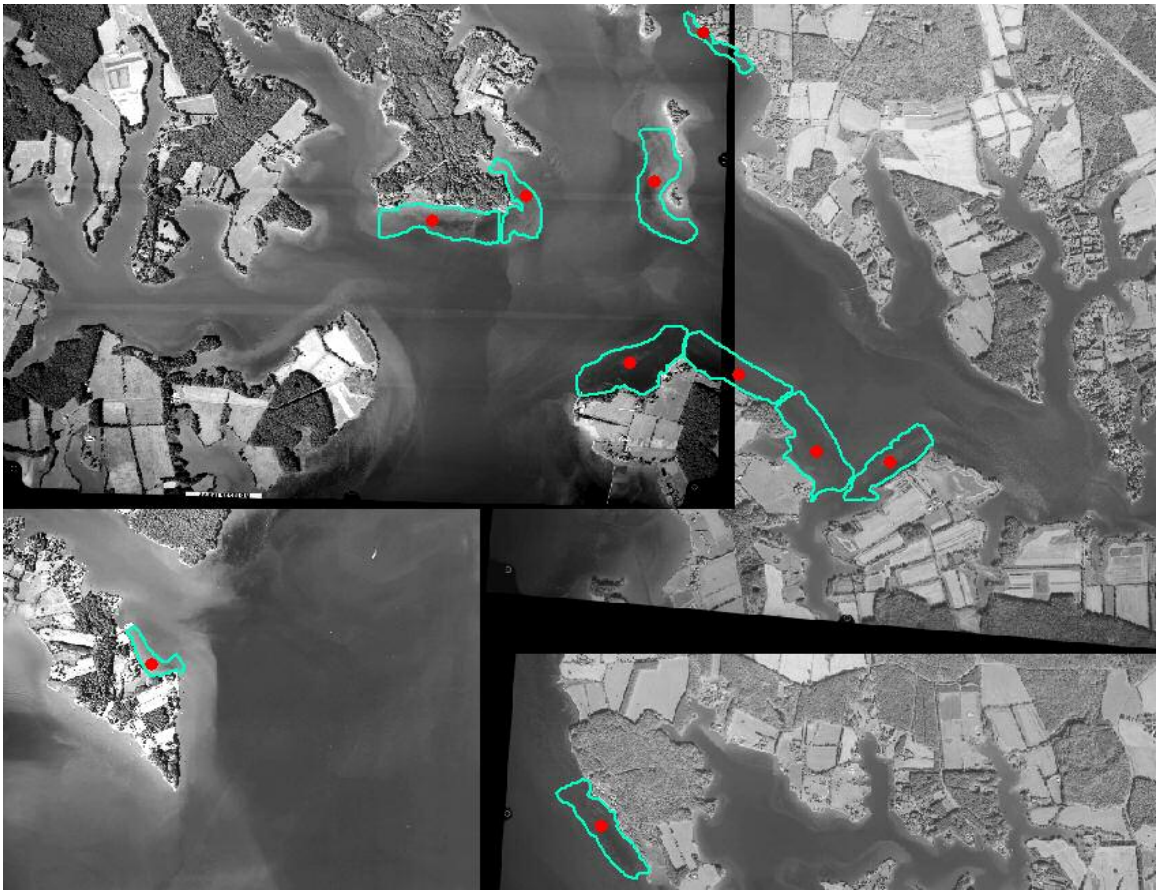


Figure 4. Aerial photograph with digitized *R. maritima* beds shown in green and *P. perfoliatus* transplants (red dots)

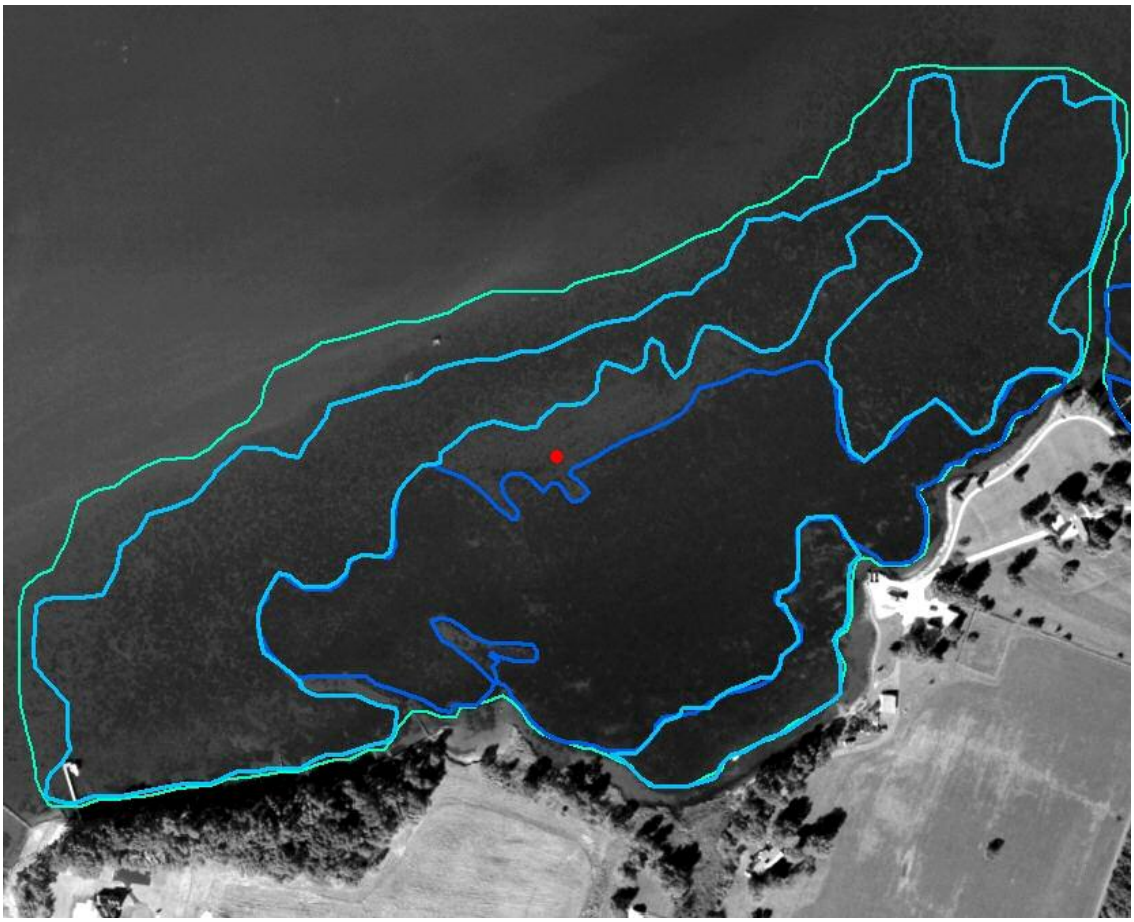


Figure 5. Example of a *R. maritima* bed that has been digitized according to density. Dense areas are outlined by the dark blue line and moderate areas are outlined by the light blue line (remaining area is considered sparse). The light green line is the bed boundary; red dot is the transplant. Areas of each digitized section were calculated, their exact density determined, and then used as part of a weighted average to find the average whole bed density.

Table 3. Total nursery bed area and percent dense, moderate, sparse density of *R. maritima*. Density classification ("how dense", CC %) ranges are: 100 – 70, dense; 70 – 40, moderate; < 40, sparse. Nursery beds are ranked by density. Weighted averages resulted in the average whole bed density. Total bed biomass is calculated using bed density, bed area, and the model monthly biomass of 56.0 gdw m^{-2} for *R. maritima*.

Nursery Bed and (rank)	Total area (m^2)	% Dense	CC %	% Moderate	CC%	% Sparse	CC%	Bed density (%)	Total bed biomass (gdw)	biomass (gdw m^{-2})
Cedar Pt (1)	171076	67.34	85	32.66	55	0	0	75.20	7204568	42.11
EC* West (2)	222590	56.02	85	34.83	60	9.15	35	71.72	8939672	40.16
EC* East (3)	138017	56.81	75	43.19	50	0	0	64.20	4962244	35.95
SS Mulberry(4)	184083	40.15	85	39.26	55	20.59	35	62.93	6486830	35.24
Deep Neck (5)	231625	31.27	85	46.18	60	22.54	35	62.18	8065666	34.82
S Hopkins (6)	45895	33.09	75	29.57	55	37.34	30	52.28	1343731	29.28
Hambleton Is (7)	196086	20.62	75	35.20	45	44.19	25	42.35	4650162	23.71
N Mulberry (8)	106324	9.64	75	29.81	45	60.54	35	41.84	2491143	23.43
Neavitt (9)	54324	0	0	9.95	50	90.05	25	27.49	836234	15.39

* EC = Elbert's Cove
CC % = density classification

the Hambleton Island site brings *R. maritima* biomass up to 41.6 gdw m^{-2}. *P. perfoliatus* biomass would not have been affected by grazing since fencing was surrounding the quadrat, effectively keeping out swans and other large grazers and thus explaining its persistently high biomass. With Hambleton Island removed, there is a highly significant ($r^2 = 0.72$, $p = 0.004$) linear correlation and an even higher nonlinear correlation ($r^2 = 0.89$, $p < 0.001$) between density weighted nursery bed biomass and final transplanted *P. perfoliatus* biomass (Fig. 6). This correlation remains the same when considering nursery bed density (%) and *P. perfoliatus* biomass. However, *R. maritima* bed area is not as strongly correlated to *P. perfoliatus* biomass ($r^2 = 0.62$, $p = 0.01$).

In order to compare the accuracy of the coring method verses the GIS analysis model for determining nursery bed biomass, the residuals (observed, coring – predicted, model) were plotted with the observed (coring) biomass (Fig 7). There is a strong positive correlation, indicating the coring method's increased deviation from the model at higher biomass, suggesting the coring method's limited ability to pick up variances in biomass and density.

Sediment characteristics

Sediment characteristics of *R. maritima* nursery beds are presented in table 4. Sediment chl *a* ranged from a low of 37.04 at Neavitt to a high of 114.11 at SS Mulberry and had no correlation with *P. perfoliatus* biomass. In addition, both sediment %N and %P displayed no relationship with either transplant or nursery bed biomass. *P. perfoliatus* biomass did decrease with increasing percent C in the sediment and was marginally significant as it approached $\alpha = 0.05$ ($r^2 = 0.26$, $p = 0.082$). Furthermore, the

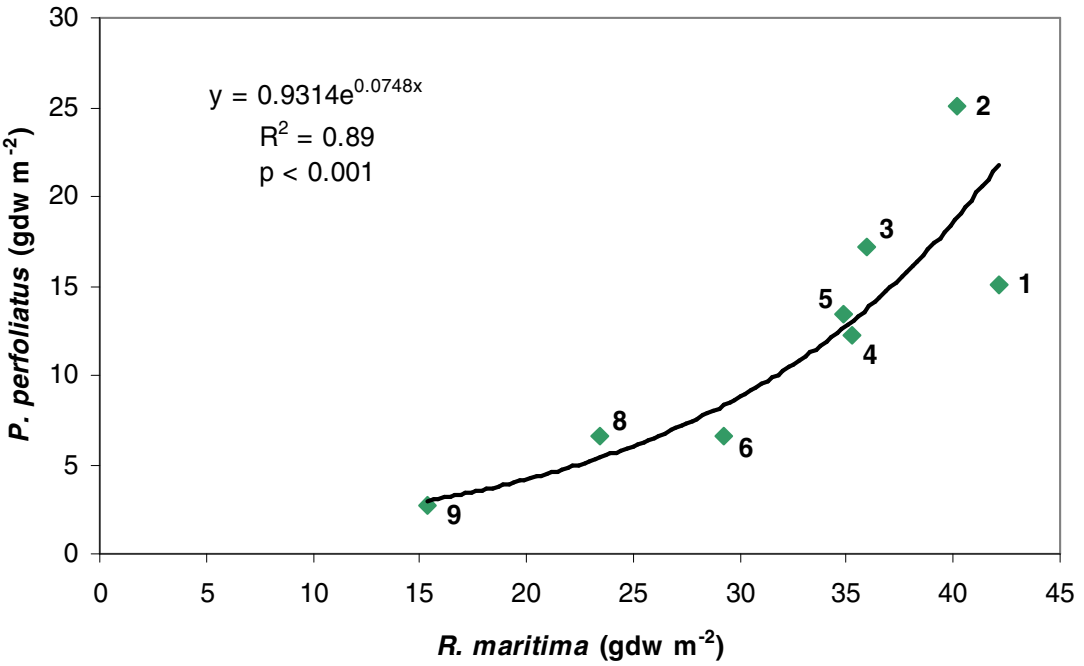


Figure 6. Correlation of *Ruppia maritima* nursery bed biomass, as determined from GIS analysis, and final *P. perfoliatus* transplant biomass. Black trend line and statistics include all sites (green points); red trend line and statistics reflect adjusted Hambleton Island (7) biomass (red point) due to mute swan grazing. Numbers correspond to biomass rankings, table 2.

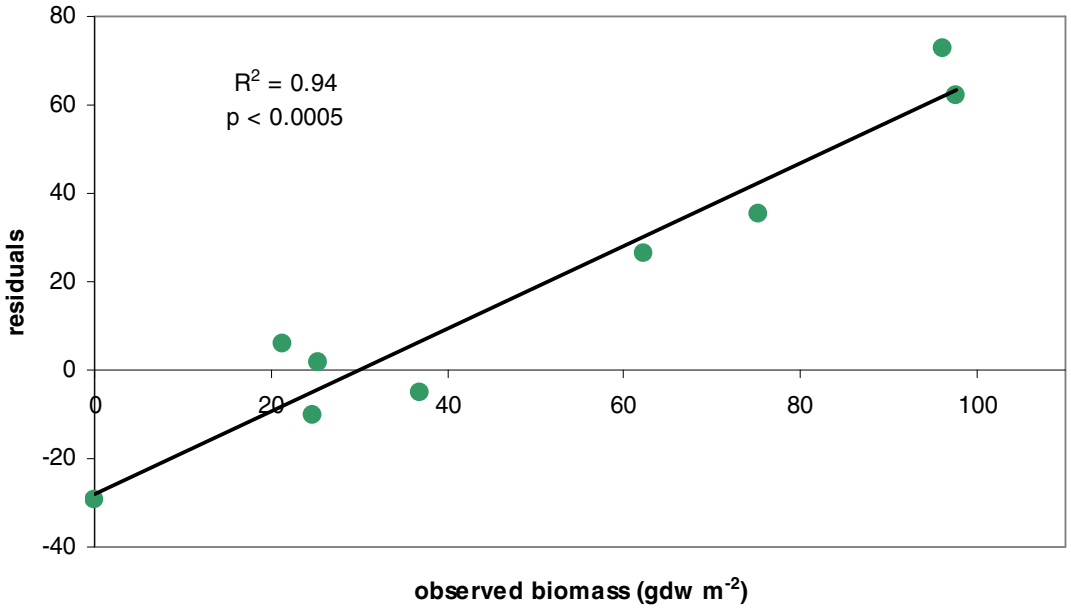


Figure 7. Correlation of observed *R. maritima* biomass using coring method and residuals (coring biomass – model biomass).

Table 4. Average (± SE) sediment characteristics for *R. maritima* beds, including chlorophyll *a*, percent carbon (%C), nitrogen (%N) and phosphorus (%P) and percent silt/clay.

	Sediment chl *a* ($mg\ m^{-2}$)	**% C**	**% N**	**% P**	**% silt/clay**
Cedar (1)	43.32 ± 3.74	0.19 ± 0.059	0.03 ± 0.004	0.01 ± 0.001	8.95 ± 0.44
EC* West (2)	43.14 ± 19.76	0.27 ± 0.020	0.05 ± 0.004	0.01 ± 0.001	9.83 ± 1.37
EC* East (3)	67.27 ± 12.84	0.10 ± 0.025	0.02 ± 0.009	0.01 ± 0.001	4.09 ± 3.87
S.S. Mulberry (4)	114.11 ± 9.12	0.14 ± 0.012	0.03 ± 0.005	0.01 ± 0.001	3.57 ± 2.65
Deep Neck (5)	37.73 ± 4.82	0.22 ± 0.041	0.03 ± 0.006	0.01 ± 0.001	13.33 ± 3.40
S. Hopkins (6)	47.30 ± 6.59	0.52 ± 0.017	0.06 ± 0.003	0.01 ± 0.001	9.28 ± 2.53
Hambleton Is. (7)	63.01 ± 15.30	0.12 ± 0.011	0.03 ± 0.002	0.01 ± 0.001	2.86 ± 1.99
N. Mulberry (8)	55.22 ± 8.45	0.20 ± 0.007	0.04 ± 0.003	0.01 ± 0.001	14.56 ± 6.82
Neavitt (9)	37.04 ± 14.30	0.33 ± 0.020	0.04 ± 0.005	0.01 ± 0.001	13.53 ± 4.57

* EC=
Elbert's Cove

amount of silt/clay in the sediment had a significant negative affect on *P. perfoliatus* biomass ($r^2 = 0.52$, $p = 0.014$), decreasing as the sediment grain size decreased. Percent silt/clay and percent C in the sediment were highly correlated ($r^2 = 0.71$). There was no correlation between sediment grain size and porewater nutrients.

Plant tissue nutrients and porewater

Average N and P leaf and stem tissue nutrients and elemental ratios for *R. maritima* and *P. perfoliatus* at each site are presented in table 5. No standard deviations are reported for *P. perfoliatus* since only one "sample" (4 shoots each) from each transplant was taken in order to reduce impact on growth and survival. Percent N ranged from 2.27 to 2.96 for *R. maritima* and from 1.74 to 2.82 for *P. perfoliatus*. Percent P ranged from 0.23 to 0.41 for *R. maritima* and from 0.14 to 0.46 for *P. perfoliatus*. There was no relationship between plant tissue nutrients (%P, %N, or N:P ratio) and biomass of either species.

Average porewater constituents (sulfide, H_2S; ammonia, NH_4; phosphate, PO_4) are reported for the rooting layer as an average of the top sampling ports (0 – 7.5 cm) (Table 5). There was a positive relationship between nursery bed porewater N:P ratios and *P. perfoliatus* biomass that was marginally significant as it approached $\alpha = 0.05$ ($r^2 = 0.25$, $p = 0.086$). However, the N:P ratios in the plant tissues of both *P. perfoliatus* and *R. maritima* did not change in relation to increasing porewater N:P ratios.

Both *R. maritima* and *P. perfoliatus* above ground tissue nitrogen decreased with increasing NH_4 porewater concentrations ($r^2 = 0.60$ and 0.43, respectively). Porewater

Table 5. Plant tissue nitrogen and phosphorus and resulting N:P ratios (mean ± SE) for above ground biomass and sediment porewater sulfide, ammonia and phosphate (mean ± SE) for the rooting layer (top 7.5cm, three values) for *R. maritima* nursery beds and *P. perfoliatus* transplants.

	plant	**tissue**	**nutrients**	**sediment**	**porewater**	**constituents**	
Site	**% N**	**% P**	**N:P**	**H_2S (µM)**	**NH_4 (µM)**	**PO_4 (µM)**	**N:P**
R. maritima							
Cedar (1)	2.27 ± 0.03	0.27 ± 0.03	15.91 ± 1.54	157.64 ± 131.65	327.89 ± 141.29	11.82 ± 1.38	23.40
EC* West (2)	2.54 ± 0.11	0.36 ± 0.02	12.92 ± 0.67	1859.15 ± 187.83	264.58 ± 51.35	32.75 ± 2.77	11.49
EC* East (3)	2.64 ± 0.08	0.31 ± 0.02	15.66 ± 0.32	88.30 ± 17.46	900.52 ± 208.03	45.86 ± 4.35	18.86
S.S. Mulberry (4)	2.69 ± 0.14	0.23 ± 0.02	21.12 ± 0.50	548.21 ± 410.26	220.17 ± 88.54	22.44 ± 4.04	14.45
Deep Neck (5)	2.82 ± 0.15	0.37 ± 0.01	13.85 ± 0.27	54.17 ± 19.01	243.79 ± 23.77	31.53 ± 2.74	7.36
S. Hopkins (6)	nd	nd	nd	46.59 ± 3.79	158.75 ± 38.59	19.63 ± 6.16	7.39
Hambleton Is. (7)	2.71 ± 0.04	0.40 ± 0.04	12.74 ± 1.56	850.48 ± 406.60	286.31 ± 242.38	25.79 ± 1.44	15.51
N. Mulberry (8)	2.72 ± 0.11	0.29 ± 0.03	17.47 ± 1.05	820.69 ± 561.50	478.13 ± 64.67	82.64 ± 1.22	5.52
Neavitt (9)	2.96 ± 0.11	0.41 ± 0.41	13.37 ± 1.08	0.00 ± 0.00	68.98 ± 24.29	12.03 ± 2.78	7.31
P. perfoliatus							
Cedar (1)	2.34	0.25	17.02	65.55 ± 12.39	108.67 ± 30.41	5.88 ± 0.58	15.90
EC* West (2)	2.55	0.31	15.02	1300.10 ± 360.89	138.90 ± 22.02	29.05 ± 6.20	4.19
EC* East (3)	1.74	0.14	21.94	50.92 ± 10.34	511.21 ± 120.38	25.07 ± 2.11	16.68
S.S. Mulberry (4)	2.85	0.32	16.26	85.05 ± 14.05	109.61 ± 15.12	9.16 ± 0.58	12.17
Deep Neck (5)	2.49	0.30	15.27	45.50 ± 10.15	167.25 ± 59.53	21.18 ± 5.92	8.24
S. Hopkins (6)	2.82	0.46	11.27	34.13 ± 0.94	382.70 ± 97.23	15.00 ± 1.08	17.41
Hambleton Is. (7)	2.48	0.27	16.64	49.30 ± 6.66	179.54 ± 20.85	16.71 ± 1.43	10.20
N. Mulberry (8)	2.71	0.31	16.02	133.26 ±83.77	137.01 ± 6.61	11.98 ± 1.04	11.42
Neavitt (9)	2.17	0.29	13.51	239.98 ± 213.72	222.06 ± 56.51	28.28 ± 3.23	9.75

* EC= Elbert's Cove

PO_4 concentrations had no affect on plant tissue phosphorus of either species. In fact, tissue %P remained relatively constant throughout the range of PO_4 concentrations. *P. perfoliatus* biomass decreased as porewater sulfide concentrations increased and was marginally significant as it approached $\alpha = 0.05$ ($r^2 = 0.34$, $p = 0.064$). The exception was at the Elbert's Cove West site, where high *P. perfoliatus* biomass occurred despite high H_2S concentrations. The Grubb's outlier test indicated that this site was an anomaly. However, SAV has been shown to survive in areas of high sulfide if other parameters for growth are met. (Wicks 2005).

Depth profiles of porewater H_2S, NH_4 and PO_4 concentrations are shown in figure 8 and illustrate the plants' affect on sediment. Sulfide concentrations in the Severn River *P. perfoliatus* reference bed were significantly lower than both the *P. perfoliatus* transplant and *R. maritima* nursery bed concentrations. The NH_4 porewater profiles show a similar pattern, with significant differences between *P. perfoliatus* and *R. maritima* below the rooting zone (>8 cm). However, the PO_4 profiles show no significant difference in concentration throughout depth, although averages follow the same pattern as the sulfide profiles.

Cluster analysis

Using the parameters of *R. maritima* biomass, *P. perfoliatus* biomass, sediment % silt/clay, sediment %C, sediment porewater N:P ratios and sediment porewater H_2S, a Ward's cluster analysis was performed. This analysis was performed to see how sites might group together and to determine the degrees of similarity between sites. The

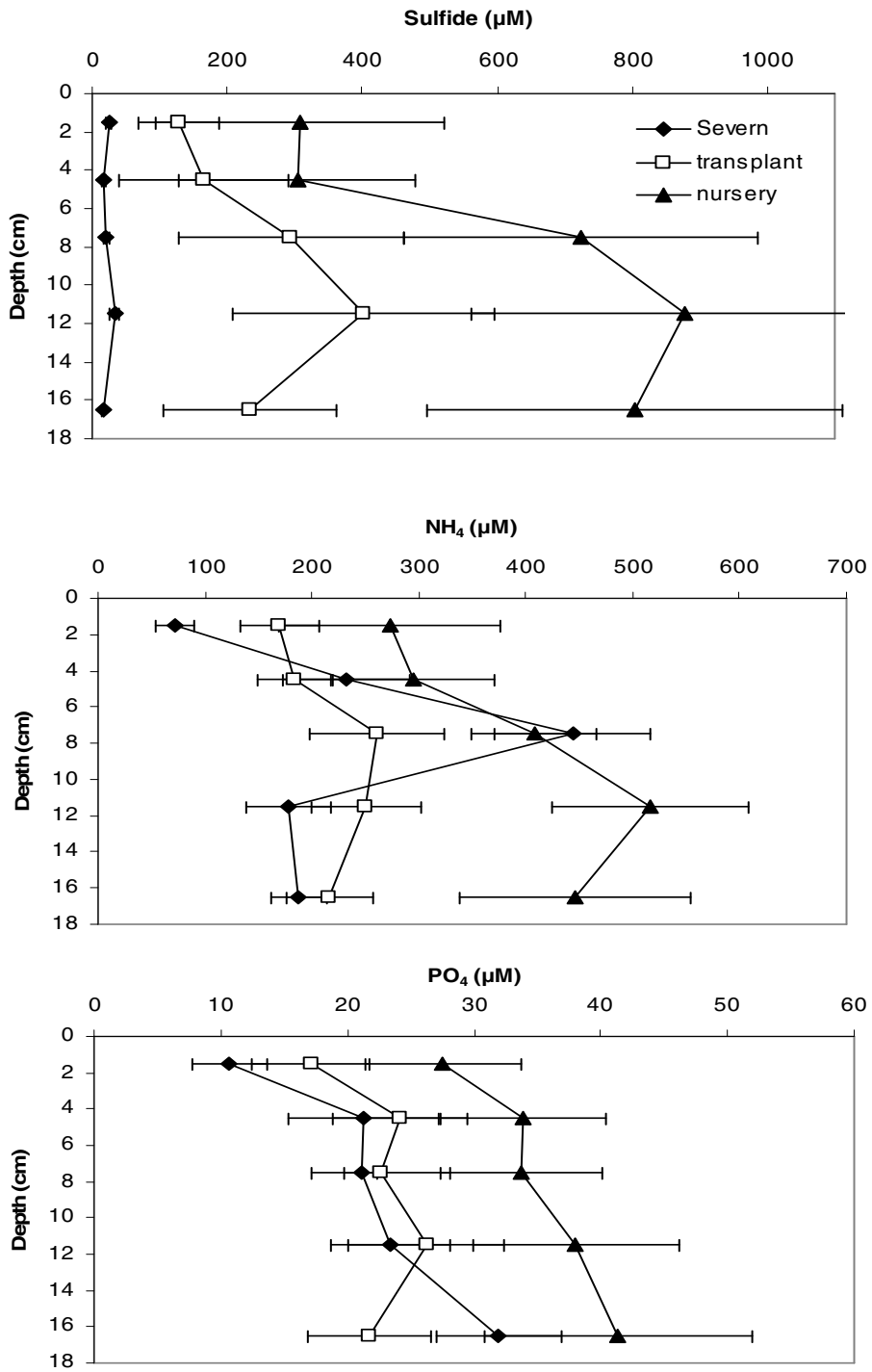


Figure 8. Sediment depth porewater profiles for H_2S, NH_4 and PO_4 in the *R. maritima* nursery bed, *P. perfoliatus* transplant and naturally occurring *P. perfoliatus* bed in Severn River. Symbols are the mean (± SE) of all sites in Broad Creek for *R. maritima* and *P. perfoliatus* and triplicate samples in Severn River *P. perfoliatus* bed.

analysis resulted in *R. maritima* nursery beds dividing into two distinct clusters (Fig. 9a-c). Several analyses were done using various combinations of the influential parameters. These two groupings separated out sites with high *R. maritima* biomass and more favorable sediment conditions from those with lower *R. maritima* biomass and less favorable sediment conditions. Most sites remained in the same group despite these variations with the exception of two sites, Hambleton Island (7) and Elbert's Cove West (2). Elbert's Cove West placement varied based on *P. perfoliatus* inclusion (Fig. 9a). A possible explanation for this inconsistency is this site had the second highest *P. perfoliatus* biomass yet sediment characteristics were in the moderate range compared to the rest of the sites in this study. The placement of Hambleton Is. varied based on the inclusion/exclusion of *R. maritima* biomass (Fig. 9b), which is not surprising recalling that this is the site that was likely affected by swan grazing. Therefore, lack of *R. maritima* biomass at this site is not an accurate indicator of unsuitable sediment conditions.

The groupings from the cluster analysis are presented in figure 10, where the ranges of values for the sediment characteristics are depicted for each site. Values are divided into four ranges and color coded, green being the most optimal, red the least. Ranges are specifically for this study and are based on the natural grouping of values in the original data.

A statistical summary of the results from the multiple linear regressions are presented in table 6. From this analysis it is clear that both *R. maritima* bed biomass and density had the most significant correlation with transplant growth and thus are the most dominant factors.

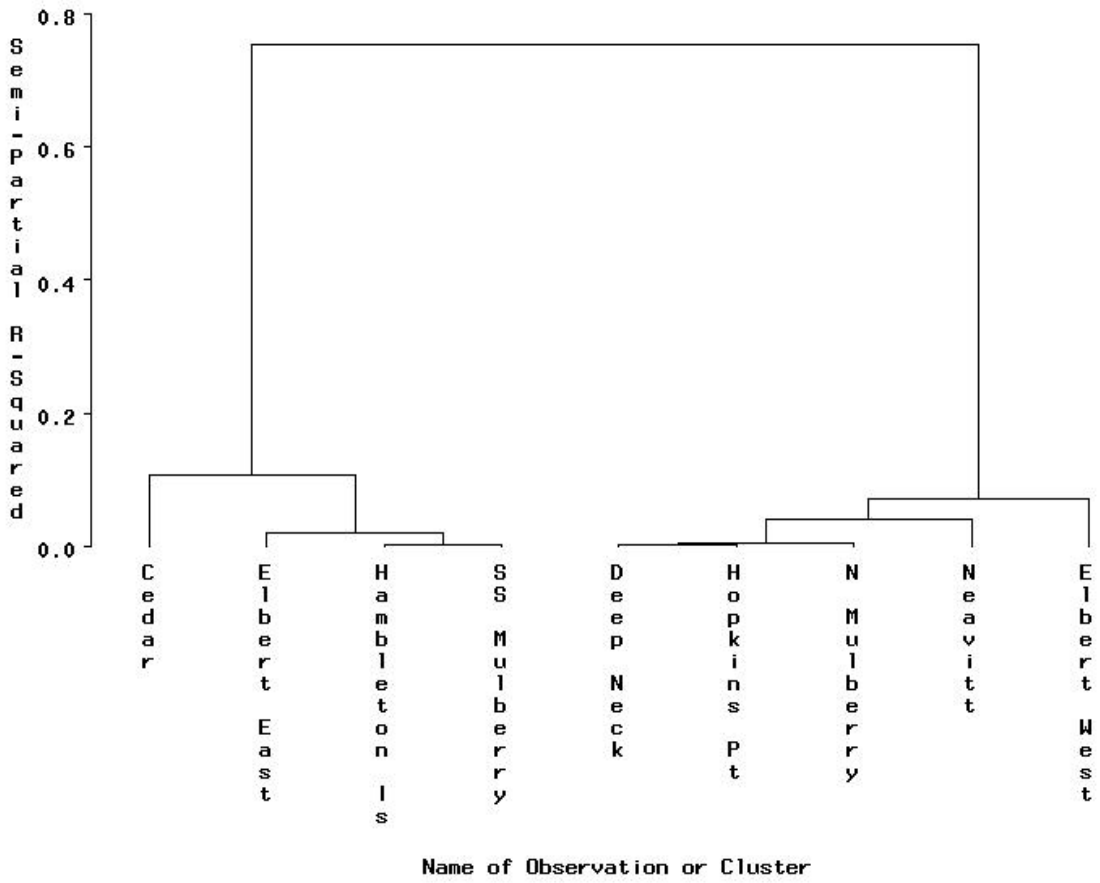


Figure 9a. Ward's Cluster analysis using parameters of sediment % silt/clay, sediment %C, sediment porewater N:P ratios and sediment porewater H_2S. *R. maritima* and *P. perfoliatus* biomass were excluded. Partial r-squared indicates level of similarity.

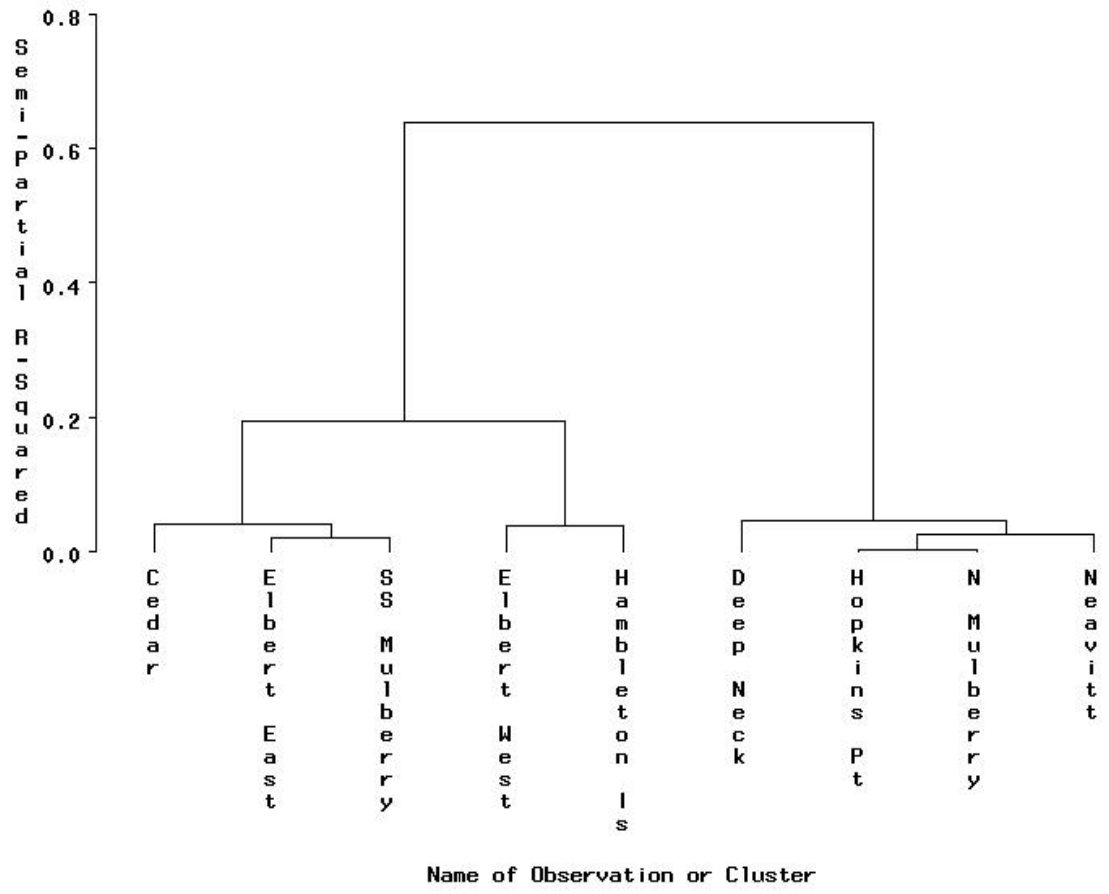


Figure 9b. Ward's Cluster analysis using parameters of *P. perfoliatus* biomass, sediment % silt/clay, sediment %C, sediment porewater N:P ratios and sediment porewater H_2S. *R. maritima* biomass was excluded. Partial r-squared indicates level of similarity.

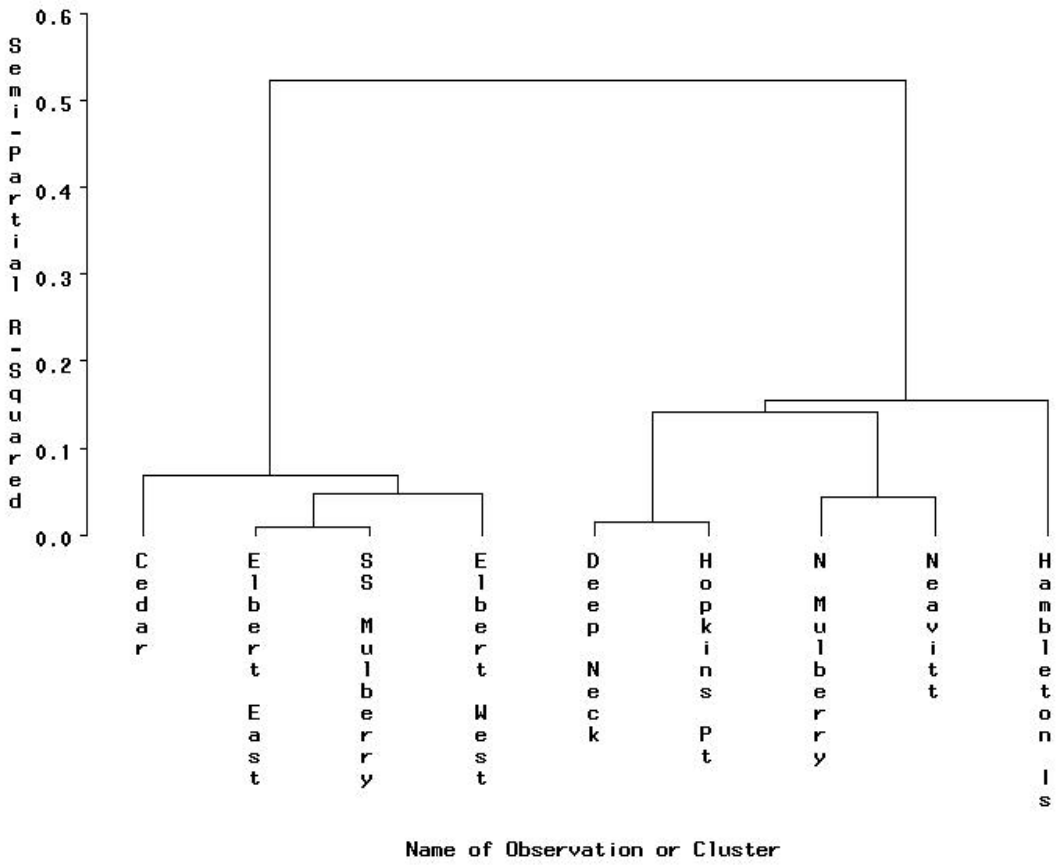


Figure 9c. Ward's Cluster analysis using parameters of *R. maritima* biomass, *P. perfoliatus* biomass, sediment % silt/clay, sediment %C, sediment porewater N:P ratios and sediment porewater H_2S. Partial r-squared indicates level of similarity.

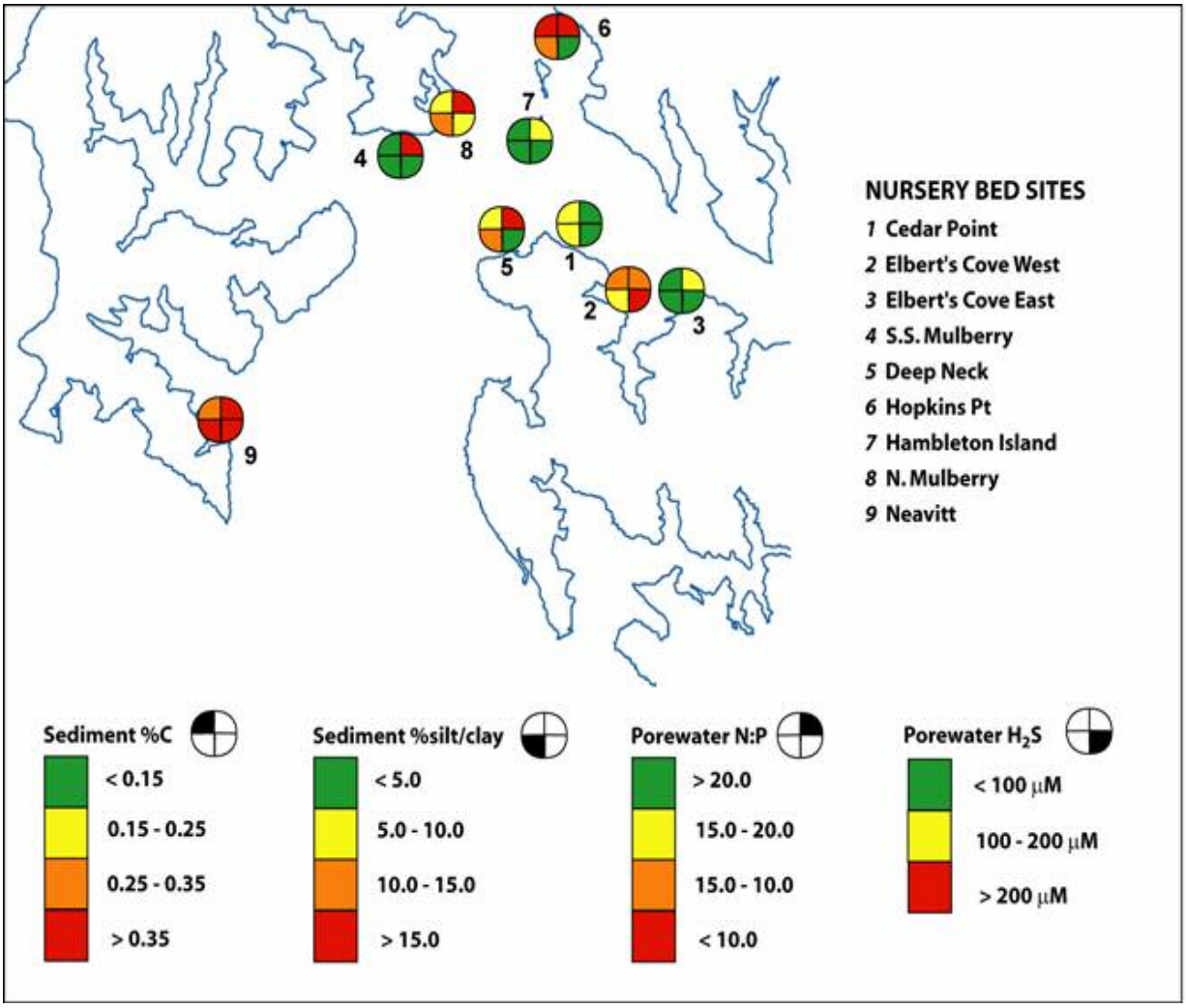


Figure 10. Values, ranging from optimal (green) to suboptimal (red), for four sediment characteristics in *R. maritima* nursery beds. Numbers next to bed names refer to the *R. maritima* biomass rankings of the sites used throughout this study.

Table 6. Results from a multiple linear regression of parameters with potential to affect *P. perfoliatus* transplant growth. Reported p-value is for a one-tailed test. Significance is determined as not significant (NS, $p \geq 0.1$), moderately significant (MS, $0.1 > p \geq 0.01$) or highly significant (HS, $p < 0.01$).

parameter vs *P. perfoliatus* biomass	**r^2**	**p value**	**significance**
R. maritima biomass (coring)	0.04	0.315	NS
R. maritima biomass (GIS)	0.72	0.004	HS
R. maritima density (GIS)	0.72	0.004	HS
R. maritima bed area (GIS)	0.62	0.010	MS
sediment %silt/clay	0.52	0.014	MS
sediment porewater N:P ratios	0.25	0.086	MS
sediment %C	0.26	0.082	MS
sediment %N	0.08	0.238	NS
porewater H_2S	0.34	0.064	MS
porewater PO_4	0.02	0.370	NS
porewater NH_4	0.01	0.385	NS

Transplant success

In the second year of this study (2005), six of the original nine *P. perfoliatus* transplant sites were located (Cedar Pt (1), Elbert's Cove West (2), Elbert's Cove East (3), Deep Neck (5), S. Hopkins (6) and Hambleton Island (8)). The area of each of the located transplants was equal to (9 m^2) or greater than (up to 15.75 m^2) the original planted area (Table 7). For four of the six transplants sites, *P. perfoliatus* shoot number m^{-2} and shoot length are reported (Table 7). Not only did transplants survive and increase in area, the density (shoots m^{-2}) of *P. perfoliatus* in the four measured transplants increased from 2004 to 2005 (Fig. 11).

Satellite colony formation

During 2005 field surveys, satellite colonies of *P. perfoliatus* were found in two of the original nursery beds, Elbert's Cove East (12 satellites) and Cedar Pt. (six satellites) (Fig 12, green dots). These two sites were also the location of *P. perfoliatus* plantings in 2001 (a total of 11.25m^2 at each site). These satellite colonies are areas of *P. perfoliatus* that are in close proximity to the original transplants (both 2001 and 2004) but at a distance as to not be considered part of the original. By analyzing these colonies in GIS ArcMap, the distance from the original transplants to the satellite was found to be between ~10m – 400m. Further analysis of the bed also revealed that all the satellites occurred within a moderate density of the nursery bed (between 45 – 55% coverage). The satellites ranged in area from 0.25 m^2 to 250 m^2 with the total areas of 63 and 320 m^2 in Elbert's Cove East and Cedar Pt, respectively (Table 7). While total area of the original transplanted sites of *P. perfoliatus* increased by 23% from 2004 to 2005 (Fig. 13), adding satellite area increased the total area of *P. perfoliatus* by 621%. When the

Table 7. Total area (m^2), shoot length (cm) and shoot number (m^{-2}) mean (± SE) for year two of the study.

Location	Transplant area (m^2)	shoot length (cm)	shoots (m^{-2})	Number of satellites	Total satellite area (m^2)
Deep Neck	14.43	135 ± 1	120 ± 20	0	0
Cedar Pt	11.25	117.67 ± 12	1200 ± 180	7	320
EC West	9	109.25 ± 2.53	1620 ± 102.53	0	0
EC East	9	nd	nd	12	62.75
S Hopkins	9	170	360	0	0
Hambleton Is	15.75	nd	nd	0	0

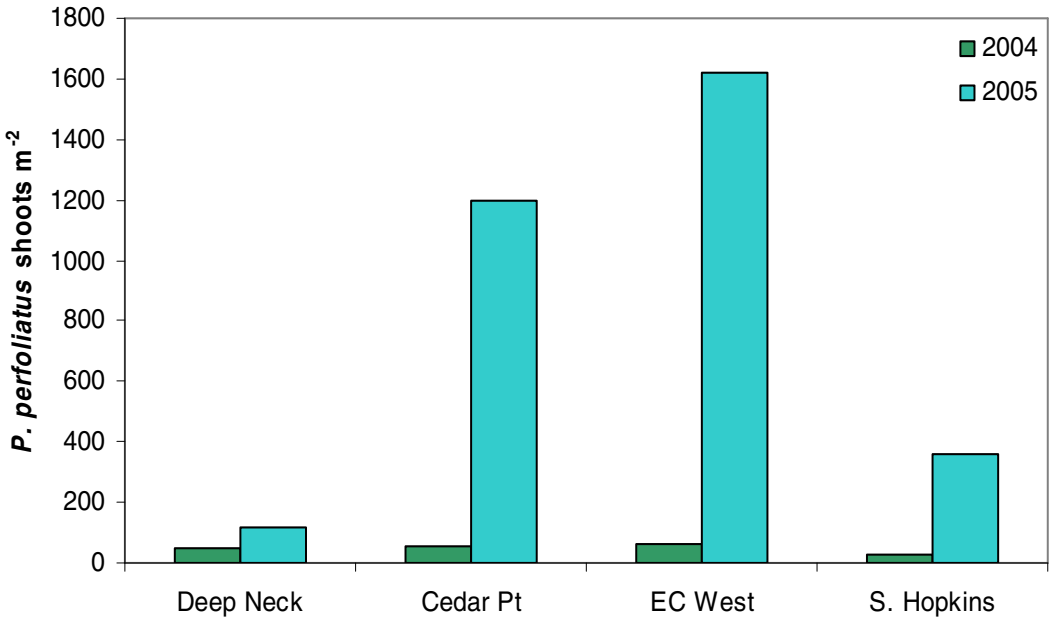


Figure 11. Change in shoot density (number of shoots m^{-2}) of *P. perfoliatus* in transplants from 2004 (green bars) to 2005 (aqua bars) for the four sites measured in Broad Creek.

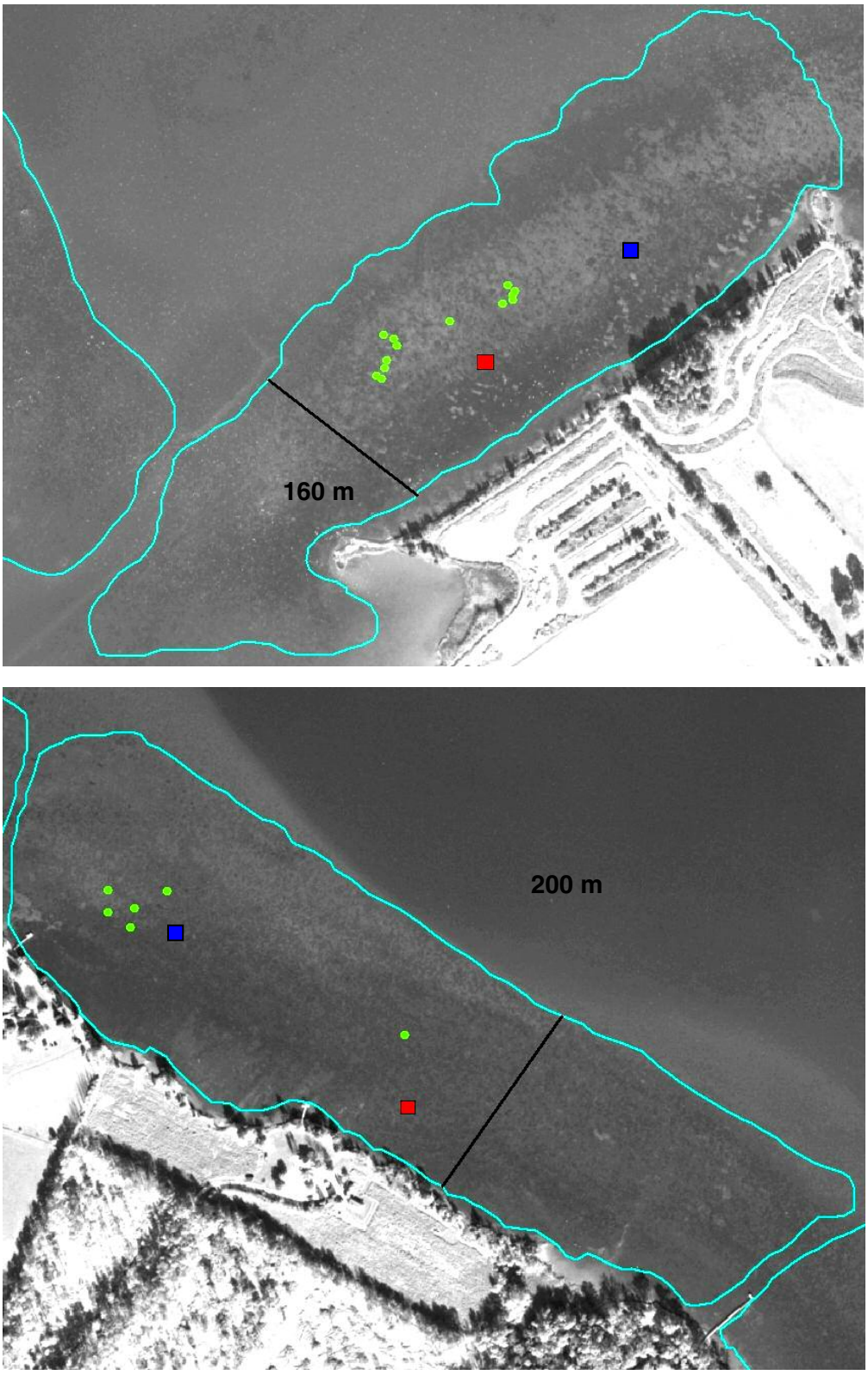


Figure 12. 2005 aerial photograph of Elbert's Cove East (top) and Cedar Point (bottom) *R. maritima* nursery beds. Green dots represent locations of satellites, red square represents original transplant. Blue square represents location of 2001 transplants (11.25 m^2 per site in 2001).

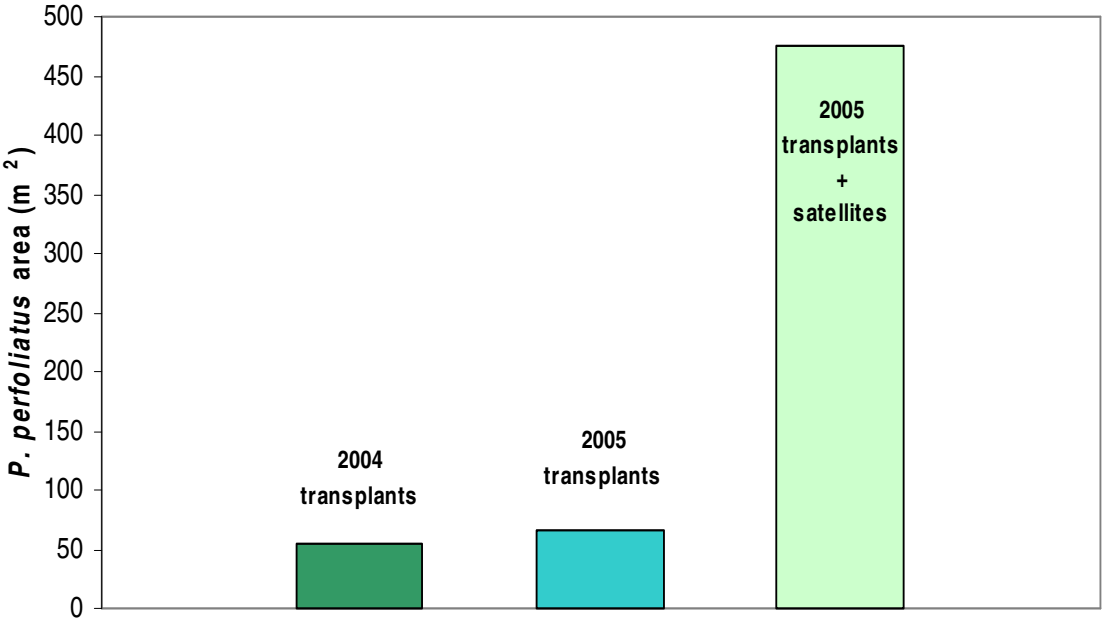


Figure 13. A comparison of the total area of *P. perfoliatus* transplanted in 2004 to the total area of *P. perfoliatus* transplants found in 2005 and the combined area of transplants plus satellites.

area of 2001 transplants is taken into account as well, this increase in *P. perfoliatus* translates to ~9.5 m^2 of satellites for every 1 m^2 of *P. perfoliatus* planted.

Propagation by fragmentation

Results from the fragmentation experiment indicate that few *P. perfoliatus* fragments sank by day one (8.9% ± 3.18), but by the fifth day, over 50% of the fragments sank in all tanks and by day 15, virtually all cuttings sank (Fig. 14). Although there was some variation from day to day, generally once a fragment sank it did not resurface. There was no statistical difference in sinking between the tanks planted with *S. pectinata* (plant) and those with only sediment (bare). A diel experiment, with observations at sunrise and mid-afternoon, was conducted to determine if the release of gases associated with photosynthesis and respiration affected sinking and floating of fragments. No trend was found in any of the data from that trial.

Fragments that had sunk began to root in the second week of the experiment. By week four, over 45% of the fragments in plant tanks and 60% in bare tanks were rooted (Fig. 14). Again, there was no statistical difference between the rooting in plant vs. bare tanks. Of the total number of fragments (96) that rooted, 95% had new shoots growing by the end of the experiment. It is interesting to note that over 75% of the original rooted fragments were dead by day 28, after new shoots had begun to grow. Each rooted fragment produced an average of 2.27 new shoots, with an average length of 15.3 cm (Fig. 15). Unrooted fragments produced significantly less ($\alpha = 0.05$) new shoots compared to rooted fragments and grew an average of less than 1 cm over the course of

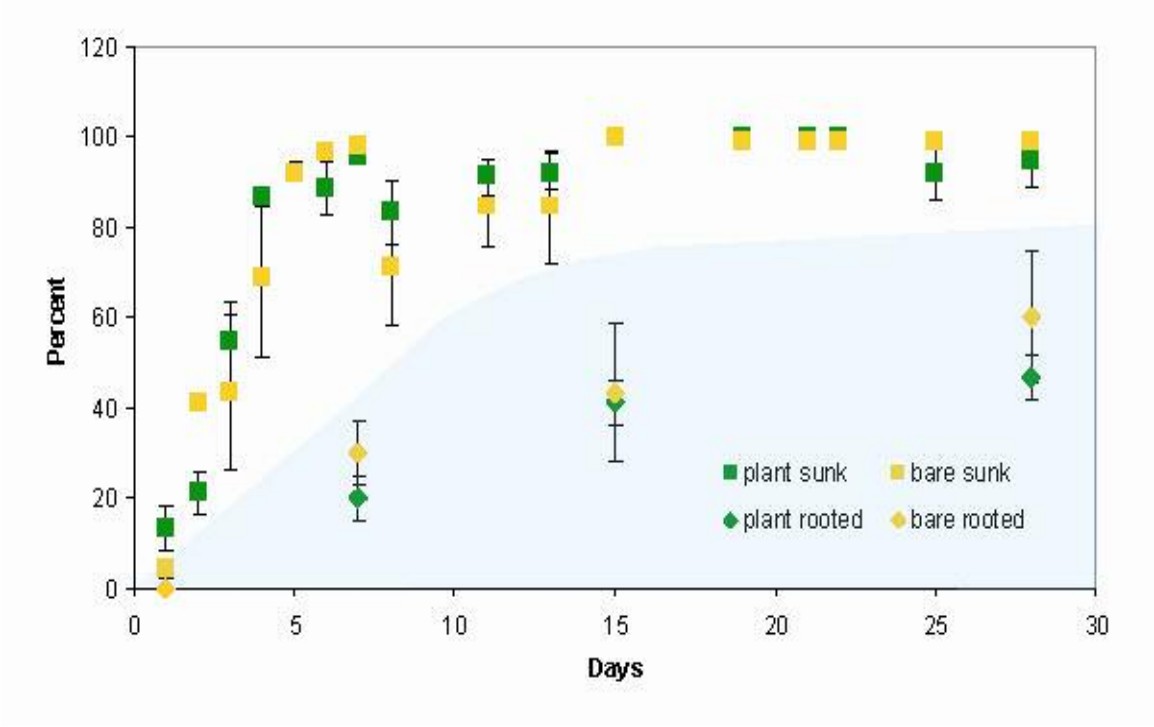


Figure 14. Mean percent (± SE) of fragments that sank over time (days) in tanks with *S. pectinata* ("plant", green squares)) and tanks with only sediment ("bare", yellow squares); and fragments that rooted in plant (green diamonds) and bare (yellow diamonds) tanks.

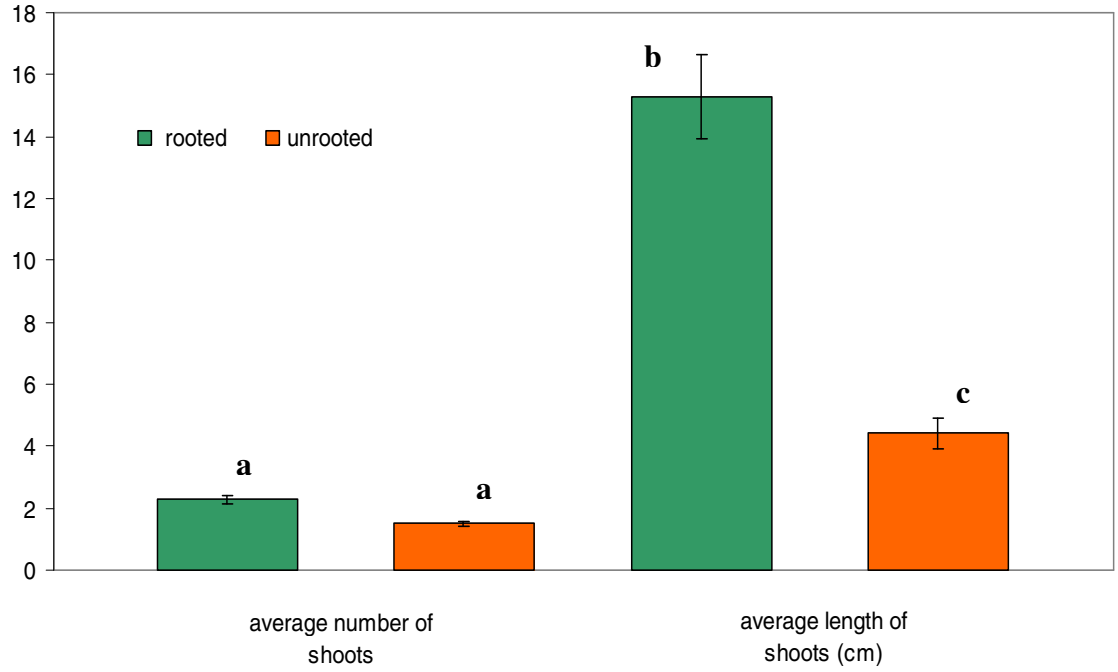


Figure 15. Mean (± SE) number of new shoots coming from original fragments, rooted and unrooted, and the average length of the new shoots. Bars with the same letters represent no significant difference at $\alpha = 0.05$.

the experiment. In addition, 67.9% and 65.5% of the total unrooted fragments (bare and plant tanks combined) had new branches and new rhizomes, respectively.

DISCUSSION

Affect of R. maritima *density on transplant success*

Two different approaches were used to evaluate *R. maritima* bed biomass and density, the traditional coring method and the newer GIS analysis. The resulting biomass of *R. maritima* using the original coring method showed no correlation with transplant success (Fig. 3). Although there appeared to be a slight trend in some of the data (sparse non-reproductive), there was no significant difference between or among any of the treatments. The influence of density and biomass of the nursery beds on transplants was only revealed when GIS techniques were employed in conjunction with the use of the model by Moore et al. (2000). The use of aerial photographs in combination with GIS techniques allowed for a better whole bed assessment of the biomass and density of *R. maritima* beds. As opposed to the coring method, in which sampling was concentrated in one small area (<30m from transplant), the GIS technique was able to take into account biomass and density of the entire bed. When this was done, *R. maritima* density was highly correlated with transplant success (Fig. 6).

Comparisons between the coring method and the model (GIS analysis) showed that at higher biomass, the coring method deviated more from the model than at lower biomass (Fig. 7). The coring method assumed a fairly uniform bed and therefore, by only sampling in a discrete area, inconsistencies in the bed biomass/density were missed. This error in sampling appears to be magnified at higher biomass. It is because of this error

that the GIS analysis was deemed a more accurate biomass and density assessment, since it takes into account the entirety of the bed. As a result, all analyses done with GIS derived *R. maritima* biomass and density showed a strong correlation with *P. perfoliatus* transplant growth.

The biomass, as well as density, of the *R. maritima* nursery beds was found to be the greatest determinate of *P. perfoliatus* transplant success, with a strong correlation (r^2 = 0.89, Fig 6) between *R. maritima* biomass and *P. perfoliatus* biomass. Previously, *R. maritima* beds in Broad Creek with large differences in percent cover were found to have distinct biological differences in terms of plant health (Schulte 2003). Denser beds suppressed the growth of epiphytic algae and transferred nutrients from the water column to the sediment. Overall, *R. maritima* plants were healthier at higher densities than at lower, patchier densities. It can easily be inferred, then, that the healthier *R. maritima* plants found at higher densities will make a more suitable nursery bed than sparse, less healthy areas of *R. maritima*.

In this study, the affect of nursery bed area on the transplant was not as strong as the affect of biomass/density. This illustrates the importance of bed density, not just bed size, in transplant success. As mentioned previously, SAV beds are able to modify their environment by reducing TSS and high nutrient concentrations (Kemp et al. 1983; Koch 2001; Moore 2004). This ability is directly related to bed biomass and density. Moore (2004) reported that at least 25 – 50% of the bottom would have to be vegetated for significant reductions in turbidity to take place. Therefore, a smaller, dense bed would be able to impact water quality more than a larger, sparse bed and, in turn, create a more suitable nursery ground for transplants. In relation to the findings of Schulte (2003),

Bartleson (2004) found that large, dense *R. maritima* beds had the greatest affect on nutrient concentrations in the water due to increased nutrient uptake. In addition, he reported that small beds had little effect on water quality. It is no surprise that these healthier, dense beds of *R. maritima* create the most suitable habitat for SAV growth and therefore represent the most optimal nursery ground for *P. perfoliatus*. The enhanced success of *P. perfoliatus* transplants with increasing *R. maritima* density in this study supports the hypothesis of the importance of nursery bed density.

Affect of sediment characteristics on transplant success

In general, most sediment characteristics did not show any strong relationship to transplant biomass. Sediment and plant nutrient values were within range for healthy SAV growth. Plant tissue nutrients were above critical concentrations for plant growth and survival of 1.3% and 0.13% for N and P respectively (Gerloff and Krombholz 1966; Atkinson and Smith 1983). Clarke and Wharton reported sediment %N values in healthy SAV beds between 0.02 and 0.52%; in this study the values ranged from 0.02 to 0.06%. Sediment %P was below the 0.02% reported by Erftemeijer and Middelburg (1993) and Kamp-Nielsen et al. (2002). Rooting depth porewater H_2S, NH_4 and PO_4 concentrations were consistent with previously reported values in SAV beds (Terrados et al. 1999; Eldridge and Morse 2000). Chesapeake Bay SAV prefers silt/clay in the range of 6 – 10% (Koch 2001). In this study, the most optimal sites contained less than 5% silt/clay and the least optimal >15%. The top four sites (in terms of *R. maritima* biomass) fell within, or below that range. In addition, porewater N:P ratios fell within

previously reported ranges (Kamp-Nielson et al. 2002; Mellors et al. 2005) as did sediment %C (Clarke and Wharton 2001).

Lack of correlation between *P. perfoliatus* plant tissue and sediment nutrient concentrations indicate that these areas are not nutrient limited and these parameters did not affect transplant success directly. However, four sediment characteristics, % silt/clay, %C, porewater N:P ratios and porewater H_2S were moderately correlated ($0.10 > p > 0.01$) and could represent secondary affects on *P. perfoliatus* transplant success (Table 6). Although sediment parameters did not have a strong affect on *P. perfoliatus* biomass, the cluster analysis indicates that that higher *R. maritima* biomass accompanies the more optimal sediment conditions (Fig. 10). The clustering of sites with high *R. maritima* biomass and most optimal sediment conditions were separated from sites with lower *R. maritima* biomass and less optimal sediment conditions. It can be inferred that the sediment conditions affect *R. maritima* biomass which in turn affects transplant success. One deviation from the sediment characteristics – *R. maritima* biomass relationship was at Elbert's Cove West (2). At this site, sediment characteristics were only in the moderate range for this study, however, *R. maritima* biomass was high. In addition, *P. perfoliatus* biomass was also high. A possible reason for this discrepancy could be lack of permanent *R. maritima* biomass at this location from year to year. However, data from aerial surveys indicates that this bed has been persistent since 1994 (Orth et al. 2005), with the exception of 2000 when no SAV was found at any of the sites. However, density at this particular site may have varied from year to year. Although SAV is present, at low densities the affect on the environment will not be as great (Bartleson 2004, Moore 2004). Therefore, if the *R. maritima* biomass at Elbert's Cove West had

been consistently low up until 2004, sediment characteristics would be in the moderate range for SAV growth. The correlation of *P. perfoliatus* biomass to *R. maritima* biomass, and not to the sediment characteristics, supports the hypothesis that transplant success is more dependent on nursery bed biomass and density.

Transplant success and satellite colony formation

Almost 70% of the original *P. perfoliatus* transplants survived into the following year (Table 7). These six transplants were located in the most optimal areas in terms of *R. maritima* biomass and sediment characteristics (Fig. 10). Both N. Mulberry (8) and Neavitt (9) had poor combinations of sediment characteristics as well as low *R. maritima* biomass. The combination of these two influential parameters is the likely explanation for the unsuccessful transplants at these two sites. However, the original transplant was also not found at the SS Mulberry site (4), a seemingly ideal place. When ground surveys were conducted in year two, this site was found with high *Stuckenia pectinata* biomass (*S. pectinata* was transplanted into this area in 2001 (Melton 2002)). Engelhardt (2002) reported that *S. pectinata* is a dominant competitor in mixed cultures and in addition, decreases the biomass of those species that are equally or more productive in monocultures. Furthermore, experimental ponds on the Horn Point Lab property that were planted with both *S. pectinata* and *P. perfoliatus* (Twilley et al. 1985) are now dominated by *S. pectinata*. It can therefore be argued that the absence of the *P. perfoliatus* transplant at SS Mulberry may be attributed to competition between these two SAV species.

Not only did transplants survive within the "better" of the nursery sites, they were able to spread in size and form 19 new satellite colonies between two sites, Cedar Pt (1) and Elbert's Cove East (3). Similar rates of transplant survival have been reported in marine environments (Davis and Short 1997; Campbell and Paling 2003). In contrast to the site with *S. pectinata*, the formation of the satellite colonies indicates that *P. perfoliatus* is not being out-competed by *R. maritima*. In fact, success was so great that transplants increased the total area of *P. perfoliatus* by ~ 600% (Fig. 13). It is important to remember, however, that in addition to *P. perfoliatus* transplanted in 2004 at Cedar Pt and Elbert's Cove East, in 2001, 11.25 m^2 of *P. perfoliatus* was transplanted at each of those sites as well. There is no way to determine if the satellite colonies at these two sites propagated from the 2004 or 2001 transplants or whether one year is enough to allow for satellite colony formation. Nevertheless, the existence of the satellite colonies confirms the transplants' ability to spread into the nursery bed.

While this study had high transplant survival, other restoration projects in the mesohaline area of Chesapeake Bay have been met with limited success. These restoration efforts were focused on restoring native grasses in bare, once vegetated areas. Several transplant areas did not survive past the first year with the majority not surviving past five years (IAN 2005). Success may have been greater if nursery beds were used as part of the restoration design. Fonseca et al. (1988) stated that without current and wave reduction at the transplant site, transplanting will not produce long-term increases in SAV abundance. The presence of established SAV beds can reduce this waning affect. Hammerstrom et al. (1998) recognized the importance of existing vegetation as a habitat modifier and nursery ground and suggested the use of *R. maritima* to enhance the

restoration success of *Halodule wrightii* in Galveston Bay, Texas. The survival and spread of *P. perfoliatus* transplants with the use of nursery beds in our study is promising for successful restoration of this SAV species.

One possible mechanism for the spreading of *P. perfoliatus* into satellite colonies is propagation through fragmentation. The fragment experiment confirmed that fragments of *P. perfoliatus* are able to sink and re-root, forming new areas of vegetation. Hall et al (2006) performed a similar with study *Halodule wrightii* and *Halophila johnsonii* but using only bare sediment tanks. Their results showed successful rooting of fragments as well, but at a lower percentage than this experiment. In this study there was no significant difference in the sinking or rooting of fragments in plant or bare tanks (Fig. 14). Therefore, if the fragments are able to stay in a suitable area, they are able to root and grow. This experiment demonstrates the importance of existing plants (an SAV bed) that will trap fragments in a suitable area as opposed to fragments floating away to a less apt environment. As a result, the nursery bed not only enhances the success of transplants, it facilitates the spread of *P. perfoliatus* into the bed by "trapping" fragments.

It is important to note that while the presence of plants is vital for trapping fragments, the existence of bare areas within the bed are crucial for allowing the fragments to root. Since all satellites were found within moderate densities of the nursery bed, where fragments are likely to encounter bare patches of sediment on which to settle, it is probable that fragments settle on bare or sparsely vegetated areas within the bed. Based on the direction of tidal flow in Broad Creek, it seems that fragments were trapped by *R. maritima* during ebb tide. Judging from the locations of the satellite colonies (Fig 13) fragments float channel-ward, away from the transplant though sparse areas, and then

become trapped in the denser *R. maritima*. During the next flood tide fragments are likely released from the plants and allowed to float back into the sparse area, sink and settle. The current speed in Broad Creek outside the grass bed is 20 cm s^{-1} (0.4 kts) (http://tidesandcurrents.noaa.gov). Therefore, if a fragment is not trapped in the nursery bed, during an ebb tide it can be carried 800 m away from the nursery bed, most likely into the channel and out of the river.

Satellites were found at the two of the more optimal sites, Cedar Pt and Elbert's Cove East (Figs. 10 and 12). It is unclear, however, why other "good" sites did not have satellite colony formation. One of the seemingly favorable sites was Hambleton Is. This site was affected by swan grazing and therefore had decreased *R. maritima* biomass. The lack of biomass and the subsequent lack of satellites may confirm the importance of existing plants as traps for fragments enabling them to stay in appropriate areas for growth. However, lack of satellites could also be a result of fragments being eaten by swans.

CONCLUSION

The restoration of submerged aquatic vegetation in various areas has been met with mixed results and has focused mainly on restoration in bare, once-vegetated areas (Davis and Short 1997; Hammerstrom et al. 1998; Qui et al. 2001; Cambell and Paling 2003) or same-species restoration within a bed (Fonseca et al. 1988; Zimmerman et al. 1995). This study showed that using an established SAV bed as a nursery ground is a successful method for restoring additional species into mesohaline Chesapeake Bay. Certain bed characteristics play a role in the success and survival of transplants and here *R. maritima*

biomass/density was the primary forcing function in determining *P. perfoliatus* survival with sediment characteristics representing secondary factors. In addition, the success and propagation of the transplants were tightly tied to *R. maritima* biomass and density, and, in part, to suitable sediment characteristics. Figure 16 summarizes these interactions, showing that more optimal sediment conditions are associated with higher *R. maritima* biomass which, in turn, affects transplant success. By combining %silt/clay, %C, N:P porewater ratios and porewater H_2S into one variable representing all four characteristics, there is a strong relationship between that variable and *R. maritima* biomass.

Results from this study can be important in planning future SAV restoration projects in Chesapeake Bay. By selecting SAV beds that are currently supporting moderate to high densities of vegetation the likelihood of transplant success is greatly increased. The presence of SAV in a particular area is also an indicator of suitable sediment and water quality conditions, as shown by this study, and therefore eliminates the need for costly and time consuming water quality and sediment analysis prior to planting. Multi-species restoration projects aim to restore whole ecosystem functioning through the reintroduction of native species. The use of nursery beds allows for successful reestablishment of stable SAV species, as well as, facilitates the growth and spread of these new species.

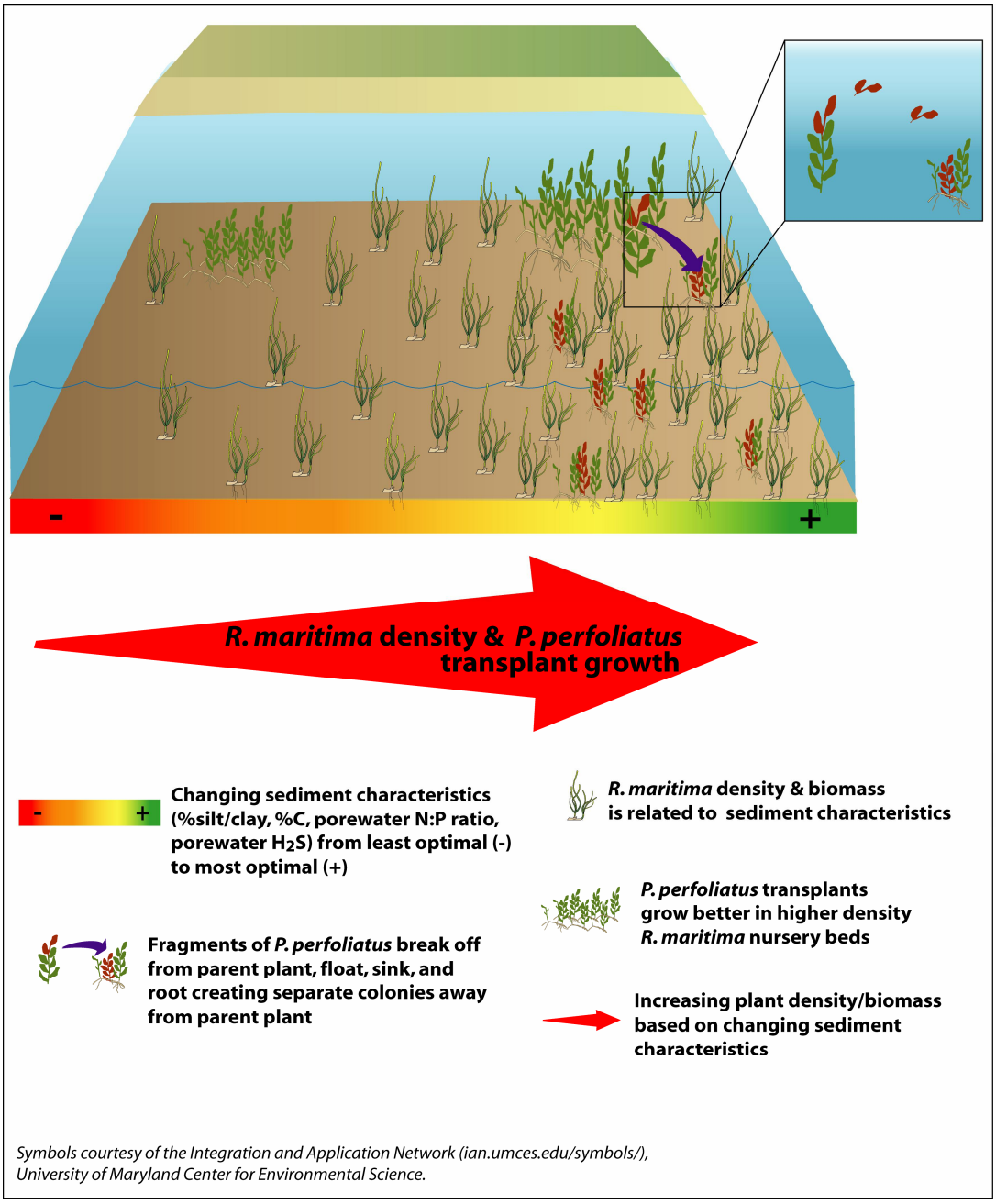


Figure 16. Conceptual diagram of the interactions between *R. maritima* density, sediment characteristics (%silt/clay, %C, porewater N:P ratios, porewater H_2S) and *P. perfoliatus* biomass.

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