ABSTRACT

Title of Dissertation: A BIODEGRADABLE POT TECHNIQUE, AND EMERGY ANALYSIS TO IMPROVE RESTORATION OUTCOMES OF POTAMOGETON PERFOLIATUS L.

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Potamogeton perfoliatus (L.) (*P. perfoliatus*), is a species of submersed aquatic vegetation (SAV) in mesohaline Chesapeake Bay that provides important ecosystem services but has been in decline. Efforts to restore its populations have met with mixed success. While the challenges to healthy SAV growth such as inadequate light for photosynthesis, poor water quality, and site disturbance have been well documented, studies using microcosms have failed to specifically examine other factors such as propagule type and seed storage duration, source population, plant growth response to a fully characterized substrate, and planting techniques, for the duration of an entire simulated growing season. Also, no studies have conducted an environmental cost accounting to assess the sustainability of a given restoration approach. This research investigated the growth and reproductive responses of *P. perfoliatus* propagules to various substrates and planting techniques; and conducted an emergy analysis case study, a type of environmental cost accounting, to compare two restoration techniques. *P. perfoliatus* net primary productivity and reproductive potential was highest when grown in sediment cores taken from SAV beds (~1.0gDW/m^2/day, 18% stems with

inflorescences), with peat/oyster shell being the next most desirable substrate choice for propagation (~0.86 gDW/m^2/day, 4% stems with inflorescences). Seeds grown in biodegradable pots grew no differently than seeds grown in control polyethylene pots, or seeds planted by hand onto the bare sediment surface of the microcosm, (although hand-planting required multiple attempts to keep buoyant, germinated seeds in place). Seeds grown from harvests four years apart also showed no differences in yield (~0.56 gDW/m^2/day). Biodegradable pots lost on average 60 percent of their mass over 12 weeks, and degraded more in brackish vs. fresh SAV bed sediments in the field. Emergy analysis indicated that planting seed-filled biodegradable pots resulted in 97% more area (m^2) SAV bed restored than hand transplanting sods, and was more ecologically sustainable. These results indicate that appropriate substrates for propagation and restoration sites, and the ability to securely place propagules in the sediment, may be critical to *P. perfoliatus* establishment and success, thereby enhancing SAV habitat in Chesapeake Bay.

A BIODEGRADABLE POT TECHNIQUE, AND EMERGY ANALYSIS TO IMPROVE RESTORATION OF POTAMOGETON PERFOLIATUS L.

By

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Dissertation submitted to the Faculty of the Graduate School of

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PREFACE:

This dissertation contains an introduction chapter, three research chapters, a concluding chapter, and seven supporting appendices. Each research chapter is presented in a longer manuscript format that will be shortened when submitted for publication, and an abstract added. Therefore, background and methods may be repeated, and tables and figures appear at the end of each chapter. A single reference section occurs at the end of the dissertation for literature cited throughout. Copyright clearance has been obtained as required.

DEDICATION:

In memory of my father (1948-2010)

In memory of Debbie Morrin-Nordlund: (1962-2014)

ACKNOWLEDGEMENTS

I thank Dr. Patrick Kangas as an advisor and mentor and for the amazing opportunity to work in my major field of passion and interest – the world of vegetation ecology, environmental systems, research and design - in this case as it applies to submersed aquatic vegetation restoration. I would like to thank committee member Dr. Joseph Sullivan for his support and resources, e.g. the use of his lab to process samples and to create general chaos, and his greenhouse space to conduct microcosm experiments during the latter part of my research. I thank Dr. Court Stevenson and Dr. Jeffrey Cornwell and their staff at Horn Point Lab for their assistance and tutelage on field ecology and various sampling methodologies, and general thoughtful support as mentors. I would also like to thank Dr. Andrew Baldwin, whose insightful comments and corrections on my proposal lead to a better project, and improved writing and statistical analysis.

This dissertation project was supported by two significant grants. The first, from USDA-NRCS, was initiated through the assistance of the Norm Berg National Plant Materials Center Manager John Englert, now Plant Materials Program Leader of USDA-NRCS, and Ginger Murphy, now of the Foreign Agricultural Service. They encouraged a grant project based out of the Plant Materials Center that would stimulate improvements in Submersed Aquatic Vegetation (SAV) Restoration by "thinking out of the box – perhaps using seed tapes or something." The next grant involved a related and serendipitous circumstance. While the research on SAV plant propagation, substrates, and seed tape materials for grant no. 1 was underway, one day, a determined and

enthusiastic person by the name of Norris “Chirp” Shannahan dropped into the Plant Materials Center front office. The staff listened to his pitch: Chirp said he had an idea to develop “tree bombs” – saplings wrapped in plastic that would then be dropped onto steep, inaccessible slopes after forest fires to reforest barren hillsides. This was no ordinary plastic, explained Chirp, it was PHA (polyhydroxyalkanoate) plastic developed by Dr. Oliver Peoples (of the Metabolix Corporation in Cambridge, MA). The PMC staff exchanged wide-eyed glances – and immediately realized this stuff was the ticket. From Chirp’s excellent idea of biodegradable containers for tree restoration – the staff knew this was the path to the first ever, biodegradable, aquatic, turion and seed pot! After Chirp and I worked on several design iterations using the biodegradable plastic, we eventually settled on using injection-mold grade plastic to form a pot. Consequently we, (my advisor Patrick Kangas, materials and injection mold engineer Chirp Shannahan, corporate sponsor Alan Berry, and I), wrote a proposal that resulted in the second grant – an award from MIPS – a Maryland Industrial Partnerships Grant. I was also fortunate to receive a summer research fellowship through the University of Maryland Graduate School. This dissertation research would not have been within my reach without the support of research assistantships and funding of this magnitude. Funding in the last four and a half years through the USGS Pathways Program, teaching assistantships at the University of MD, and assistance from my family that enabled me to take complete leave from work to finish the final chapter of this research, provided the final encouragement, to get the job done.

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excellent talents of the late Debbie Morrin-Nordlund, who, before her untimely death, provided administrative, and general, critical guidance for how to be an architect of degree completion. She is sorely missed beyond words, and we appreciate the talents and efforts of those who continue on to fulfill her role in the MEES Program. I would also send much thanks to Jamsheed Djamshidi, Gary Seibel and the many other helpful staff at the Project Development Center at the Environmental Science and Technology Department at UMD-College Park who assisted with the production of field equipment such as probes and tanks that I needed to design from scratch, as well as the considerable help of summer interns Elena van Roggen, Anastasia Borovikov, Beri Shifaw, Joe Cardoso, Jim Bonifant, Spiro Dimakas, and Ruben Schwartz. The Greenhouse Staff at UMD – Shawn Faulkner and Sydney Wallace and the Norm Berg National Plant Materials Center (PMC) staff: John Englert, Shawn Belt, RJ Ugiansky, and Dan Dusty were key in providing facilities support infrastructure and keeping the greenhouse facilities running smoothly. Shawn Belt gets tremendous credit for being encouraging and professional enough to suggest that I visit PMC in the first place. I must also thank numerous other faculty and graduate students at UMD for providing guidance, stimulating discussions, and support along the way. The Jack and Barbara Hanna Family and the Ron and Jeannie Yerby family graciously provided their Bay-side properties for oligohaline and mesohaline field sites, respectively. Billy Moulden and the community at Sherwood Forest on the Severn River, the Chesapeake Bay Environmental Center at Kent Narrows, and the management at the Holiday Inn Express at Grasonville-Kent Island, provided important sources for turions and SAV bed sediment cores. I very much appreciate the U-MD College Park as a top notch research institution, and The Graduate

School in particular (Thank you Ms. Toyin Oguntolu et al.!!) for enthusiastically supporting graduate student research. I greatly benefitted from discussions and ideas about current and critical future research that was not able to be a part of this effort with Walter Schmidt (USDA-ARS), and also the considerable support of Greg McCarty (USDA-ARS) and Dean Hively (USGS), and their staff who provided research facilities, equipment, and samples processing. Dave Ruppert and Dong Liang provided helpful assistance and feedback on the analysis of data. I cannot thank enough those friends who were there for me when I needed them most providing assistance, encouragement and guidance – among those I should specifically mention are Carren Oler, Karen Prestegaard and Emma Shirley. Throughout this effort, my son has been a source of inspiration, field help, a steady supporter, and an impressive, hardworking human being – thanks Bryce! I'd like to thank my Mom and Dad, who have given me their love and support, and instilled in me curiosity and enthusiasm for studying the natural world. Last of all, and perhaps most importantly, I learned that I had to believe in myself, and my ideas, in order to accomplish the goals I set out to achieve.

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CHAPTER 1

Introduction to restoration of *P. perfoliatus*

INTRODUCTION

Background

Potamogeton perfoliatus L. (also known as redhead grass) is a species of submersed aquatic vegetation (SAV) important to ecosystem services in Chesapeake Bay and globally (Perry and Uhler 1988, Meyer et al. 2013, Wolfer and Straile 2004, Ozimek et al. 1976). When healthy, *P. perfoliatus* and other SAV beds stabilize sediments, reduce shoreline erosion, and provide valuable habitat and food sources for a variety of fish, benthic macroinvertebrates, crustaceans and waterfowl (Lubbers et al. 1990). They provide protection from predators, as well as attracting epiphytes and zooplankton upon which other species graze, thereby providing an important link in the food web (Heck et al. 2003, Costanza et al. 1997, Duarte 2000). Globally, *P. perfoliatus* is found in salinities ranging from fresh to 18 parts salinity, with a geographical range north of Mexico to Manitoba in the eastern half of North America, to Eurasia, North Africa and Australia (Ogden 1943). In middle and upper Chesapeake Bay, it is a seasonal, perennial, monoecious monocot, and was historically found in many tributaries and embayments (Dennison et al. 1993, Brush and Hilgartner 2000). Fossil remains documenting origins of *P. perfoliatus* and other SAV species have been dated back to the Cretaceous, approximately 145-66 million years BP (Berry 1930).

Within the last 70 years, benthic communities, including submersed aquatic vegetation (SAV) populations, and *P. perfoliatus* in particular, have become increasingly degraded or disappeared globally and from many areas of Chesapeake Bay, due to disease, invasive species, anthropogenic pollution such as toxic contaminants and

herbicides, nutrient enrichment, and sediment erosion (Kemp et al. 1983, Stevenson and Confer 1978). Cultural eutrophication such as phosphorus, nitrogen and total suspended solids cause turbidity and algal blooms that shade submersed plants and consume oxygen during decomposition, creating an environment unsuitable for benthic vegetation (Hauxwell and Valiela 2004, Burkholder et al. 2007, Bostrom et al. 2006, McGlathery et al. 2007, Krause-Jensen et al. 2011, Tyler et al. 2003). Although improving in some areas, portions of Chesapeake Bay are still experiencing the ill effects of pollution and poor water quality that initially reduced SAV acreages decades ago (Kemp et al. 2004, Waycott et al. 2009, Short and Wyllie-Echeverria 1996).

Identification of research gaps

In general, excesses of nitrogen and organic matter are viewed as major threats to coastal habitat function (Nixon 1995, Howarth and Marino 2006, Schindler 2006, Krause-Jensen et al. 2008). Efforts to restore *P. perfoliatus* to habitats that have undergone exposure to eutrophic conditions have not been very successful (Meyer et al. 2013, Bergstrom 2006, S. Ailstock, personal conversation). Restoration of aquatic habitats is not an easy task due to their complexity (Zedler 1987, Kusler 1990, D'Avanzo 1987, Bayraktarov et al. 2016). SAV restoration projects encounter more difficulties when there are knowledge gaps regarding species and habitat needs, particularly when the level of human effort required to restore the system (Fonseca 2011), or the hierarchical energy signature (Kangas 2004, Odum 1996, Allen and Star 1982), are not well understood.

Performance criteria are used for seagrass restoration projects, i.e. vegetation coverage, light requirement targets and hydrodynamics (Fonseca et al. 2002, 1988,

Dennison et al. 1993), but rarely include specifics regarding substrate (Koch 2001, van Breedveld 1975). Freshwater wetland, saltmarsh, forest, and stream restoration projects routinely identify "reference" sites against which to compare restoration success, and may include target variables such as redox, pH, substrate composition, and channel geomorphology (Palmer et al. 1997, Stolt et al. 2000). Only a few studies have been conducted relating substrate and *P. perfoliatus* responses to nutrients. They indicate a significant relationship between substrate % organic carbon (% organic matter) and yield (Misra 1938, Haslam 1978, Meyer et al. 2013). This specific information provides a valuable basis of comparison between habitats and populations, but also has great potential to inform the composition of horticultural mixes for propagation, and site selection criteria for restorations. The lack of selection, and subsequent detailed information about, reference sites may be one reason there have been considerable challenges with *P. perfoliatus* restoration.

Another research gap for *P. perfoliatus* is the lack of studies on genetic diversity and relative fitness within and between populations, which is important but costly (Lloyd et al. 2011, 2012). There appear to be no published reports of experimentation with common garden experiments or restoration genetics for different populations of *P. perfoliatus*. More genetic studies have been published for *Zostera marina* (e.g. Jueterbock et al. 2016, Williams and Davis 1996), and for *Vallisneria americana* Michx. (Lloyd et al. 2011, Marsden et al. 2013, Engelhardt et al. 2014). For vegetative reproduction, *P. perfoliatus* turion production has been estimated by Wolfer and Straile (2004) in Lake Constance, Switzerland, but particular note was not made of substrate, turion size, or any subsequent differences in responses to sediment composition. Xie and

Yu (2011) reported effects of nutrient levels on *Potamogeton crispus* turion production. They determined that increased nutrient availability produced smaller turions, however, their experiment did not use turions from different populations to track any distinctions in subsequent growth.

For sexual reproduction and restoration, methods of harvest and storage of *P. perfoliatus* seeds have been described, however most methods report reduced seed germination and viability after one year (Ailstock et al. 2010a). As a result, seeds of most SAV species are discarded after one year. Documentation for longer term seed dormancy and viability regarding *P. perfoliatus* has been largely anecdotal (Muenscher 1938).

Toxic contaminants associated with materials such as plastics pollution are becoming an increasing concern in estuarine and coastal habitats (Cole et al. 2011, Mani et al. 2015). Studies have focused on the ability of *P. perfoliatus* to uptake heavy metals (Matache et al. 2013), but few report on interactions between fossil fuel-based or bio-based plastics and terrestrial and aquatic plants. Thorhaug and Austin (1976) document a study using polyethylene plastic to wrap propagules for restoration. More emphasis was placed on seedling success, and less on substrate/plastic/plant interactions. Van Breedveld (1975) wrapped Thalassia sp. in polyethylene bags for restoration transplanting. Plant survival after 546 days was 60%, however growth of root/rhizome or shoots was barely noticeable compared with non-plastic wrapped shoots. Restoration approaches for *P. perfoliatus* have consisted primarily of planting shoots, sods, and broadcasting seeds (Shafer and Bergstrom 2006, Bergstrom 2006). More recent developments and innovations in restoration technologies for mesohaline SAV restoration are not apparent in the literature.

Cost estimates for coastal restoration are estimated at USD160 m^{-2}, with those of seagrass restoration estimated to be even higher. SAV habitats are reported to be among the most difficult to restore, with the lowest survival rates (Bayraktarov et al. 2016). Specific information on *P. perfoliatus* restoration outcomes and costs are few. Only Schafer and Bergstrom (2008), and Bergstrom (2006), have shared costs of *P. perfoliatus* and other mesohaline species in Chesapeake Bay. More comprehensive budget information for restoration of *Zostera marina* was reported and reviewed by Bush et al. (2010), but large, onetime costs were not shared. In addition to dollar cost, it is helpful to be able to model and compare overall sustainability of restoration methods. While studies using environmental cost accounting, or embodied energy evaluation (emergy), have been used for marine spatial planning that included seagrasses (Picone et al. 2017, Franzese et al. 2017), no studies have used environmental cost accounting to evaluate or compare SAV restoration methodologies.

Theoretical motivation, chapter summaries, and hypotheses

In order to work towards, in the words of Eugene Odum (1984), "bridging the gap between the laboratory and the real world in environmental science," this research used microcosm experiments to understand research gaps of *P. perfoliatus.* Microcosms and mescosms can play an important role in understanding and simplifying relationships that are not easily observable in nature. In the mesohaline Chesapeake Bay, where light penetration is rarely more than one to two meters, and conditions are quite variable, these tools can facilitate an understanding of how species function under various parameters (Short 1987). When those relationships have been explored, the challenges of the estuarine environment can be better understood and managed through field experiments.

One of the most compelling reasons behind restoration of degraded or destroyed SAV habitat is to ensure a more robust recovery through the addition of the appropriate species propagules (Dennison 2009). The goal of this research was to explore various facets of the life history of *Potamogeton perfoliatus* (L.) (Chapter 2), to test a newly developed restoration method using a biodegradable pot filled with seeds (Chapter 3), and to conduct economic and environmental cost accounting (emergy analysis) to compare the biodegradable pot with hand transplanting of sods (Chapter 4), in order to improve conservation and restoration of this species.

The microcosm experiment in Chapter 2 was devised to better understand turion growth responses to two SAV bed sediments and four horticultural substrates. The turions and SAV bed sediment cores were collected from same two sites, and the horticultural substrates consisted of sand (low nutrients), soil sand (high nutrients), oyster shell, and oyster shell/peat (intermediate/refractory nutrients). Substrate parameters have been very generally explored in numerous studies and described as "muddy" or "sandy". High sediment organic content for many SAV species has been documented to be problematic (Misra 1938, Wicks et al. 2009). This chapter looked at a suite of variables to determine whether there were any important distinctions to be made between growth of two different populations of turions and sediment/substrate conditions. These substrate parameters included particle size, redox and pH in shallow and deep portions of substrate, biomass, flowering, stem density, stem length, inflorescences, and micro and macronutrients. No other studies have characterized SAV bed sediment or horticultural substrates used for restoration to this extent, and it was of value to observe variable turion response to treatments over a simulated growing season.

Three hypotheses were considered in Chapter 2:

▪ Turions from two populations collected from the same two sites as the sediment cores would grow best on their own bed sediments;

▪ SAV bed sediment cores would exhibit features such as lower redox values (Eh) and pH, compared with other substrate treatments and be moderate/intermediate in levels of available nutrients (such as C and N). Sand would be lowest in quantity of nutrients, and soil/sand would be highest in labile %TN, P, %OC and K. Oyster shell/peat and oyster shell would be intermediate in nutrient availability;

▪ SAV bed sediment cores collected from two different *P. perfoliatus* beds, and oyster shell/peat would likely support greater turion growth than would horticultural substrates of sand, soil/sand, and oyster shell alone.

The goal of Chapter 3 was to test seed growth when planted in biodegradable pots made from polyhydroxyalkanoate (PHA) plastic, and the degradation performance of the pots in microcosms and in the field. The first microcosm experiment (MEI) compared seed growth in the PHA pots to growth in control pots made from fossil fuel-based polyethylene (PE). The second microcosm experiment (MEII) compared growth of seeds from two different harvest years (< 1 year old and 4.5 years old) in the PHA pots with growth on bare microcosm sediment. In both microcosm experiments, biodegradation was observed in unplanted and planted treatments. In the field experiment, biodegradation was observed at two different sites at two different salinities and sediment types in the upper tidal fresh Chesapeake Bay and the mesohaline portion of Chesapeake Bay.

Chapter Three consisted of the following hypotheses:

▪ Seeds grown in PHA pots would yield higher biomass than when grown in control PE pots (MEI);

▪ PHA pots would degrade more rapidly than the less degradable PE pots (MEI);

▪ Pot degradation would be greater in planted than unplanted pots (MEI);

▪ Lower redox conditions (as a proxy for anaerobic microbial activity) in deeper portions of the sediment would correspond with greater PHA pot biodegradation than shallower areas (with more positive redox) (MEI);

▪ Seeds from either harvest year would grow better in inoculated PHA pots than on bare microcosm sediment (MEII);

▪ Recently harvested seeds (< 1 yr old) would grow better than seeds harvested and germinated 4.5 years prior to the experiment (MEII);

▪ Differences in redox measurements would coincide with less degradation in the top portion of the spindle (more positive Eh), and greater diameter loss in the deeper portion of the spindle (MEII, similar to MEI);

▪ Pot degradation would be greater in planted than unplanted pots (MEII);

▪ PHA pot degradation would be greater at the mesohaline site than in the tidal fresh site due to loamier sediment content, differences in temperature, and higher salinity at the mesohaline site (Field Experiment).

The goal of Chapter 4 was a case study modelled to compare two different *P. perfoliatus* restoration methods. One method was a frequently used restoration protocol of propagating sods of *P. perfoliatus* (squares of turf grown from turions) and transplanting by hand at the restoration site. The other method used the newly designed PHA pots filled with seeds, which were then transplanted at the restoration site. The two

methods were compared by assessing budgetary differences on a SAV bed restored m^{-2} basis. Environmental cost accounting (emergy analysis) was conducted to determine relative sustainability of the two methods, and was also based on an SAV bed restored m^{-2} basis.

Chapter 4 considered the following hypotheses:

▪ The net emergy yield, and the sustainability indices based on emergy analysis of the PHA pot and seed restoration technique, would be an improvement over the more traditional hand transplanting of sods technique;

▪ The PHA pot method will produce more square meters of restored SAV bed, resulting in a greater cost savings on a USD$ m^{-2} basis.

Chapter 5 provides a brief summary of individual chapter findings, and provides a list of the conclusions and recommendations that tie together how this research contributed to the restoration ecology of *P. perfoliatus*.

CHAPTER 2:

Potamogeton perfoliatus L. growth responses to SAV bed sediments and horticultural substrates

INTRODUCTION

Submersed aquatic vegetation (SAV), and the sediments in which they grow, have been identified to be among the most productive and increasingly important habitats for carbon sequestration (Macreadie et al. 2015, Raven and Beardall 2014, Greiner et al. 2013), in addition to many other critical ecosystem services (Heck et al. 2003, Costanza et al. 1997, Duarte 2000). Eutrophication and other perturbations have reduced SAV coverage and species assemblages worldwide (Duarte et al. 2008), and this has in turn placed SAV-dependent species at high risk (Hughes et al. 2009, Jacobsen and Friberg 1995, Kenow and Rusch 1996). Global carbon release due to SAV habitat destruction and sediment erosion is estimated at 299 Pg annually (Fourqurean et al. 2012). In mesohaline Chesapeake Bay, SAV beds cover approximately 20,000 hectares, less than half of what scientists, managers, and policy makers estimate will comprise a "healthy Bay" target (Orth et al. 2017). *Potamogeton perfoliatus* L., or redhead grass, a perennial species once predominant both in monoculture and mixed beds with other species, has undergone one of the most marked reductions of any species in Middle Chesapeake Bay (Orth et al. 2017, Orth et al. 2015).

Aerial (Orth et al. 2015, 2016), and in situ (Hengst et al. 2010) studies have indicated that the habitat previously shared by both *P. perfoliatus* and *Ruppia maritima* L. (another mesohaline species), has now become dominated by *Ruppia.* This facultative, perennial/annual species is characterized as being better adapted to fluctuations in water column nutrients, temperature, and seed burial in highly mobile, fine-grained substrates (Burkholder et al. 1994, Cho et al. 2009, Strazisar et al. 2016, Ailstock et al. 2010a, Orth et al. 2015, Orth et al. 2017). In other research, *Ruppia* has been grown successfully

without any substrate (Setchell 1924, Seeliger et al. 1984, Thursby 1984). This may be an indication that habitats and associated sediments where *P. perfoliatus* previously grew have undergone a fundamental change that no longer adequately supports this species (Kautsky 1988, Krause-Jensen et al. 2011, McGlathery 2001, Mesters 1995, Scheffer et al. 2001). In addition to habitat degradation, this loss of species diversity is a concern because it indicates reduced system resiliency (Tilman et al. 2006, Folke et al. 2004).

Similar to other SAV species, *P. perfoliatus* can occur in both monotypic and multispecies beds and patches (Wolfer and Straile 2004, Hutchinson 1975, Meyer et al. 2013, Ozimek et al. 1976). A significant debate in conservation biology has focused on the pros and cons of single species, multiple species, and general habitat conservation (Towns and Williams 2013). However, most agree that understanding the specific habitat requirements of at least one species that is not a "lost cause" but in decline (i.e. a number of healthy populations still persist), is a preferred point at which to attempt to conserve and restore community resiliency (Lindenmayer et al. 2007, Towns and Williams 2013). For *P. perfoliatus,* and many other SAV species, it is still not well understood how sediment conditions affect growth and persistence of these populations (Shields and Moore 2016, Short 1987, Fraser et al. 2016, Meyer et al. 2013). Furthering the knowledge of substrate-SAV interactions is a key factor in predicting, (and implementing) restoration success (Wicks et al. 2009).

Historical evidence of *P. perfoliatus* indicates that it was found in many of the shallow brackish waters in Chesapeake Bay and its tributaries well before the colonial period (Brush and Hilgartner 2000). *P. perfoliatus* was initially found in the upper reaches of most of the mesohaline to fresh tributaries, but over time it was unable to

persist under the siltation and erosional forces of colonial era deforestation, and slowly migrated downstream, with SAV seed banks (i.e. SAV populations) peaking around 1700 (Brush and Hilgartner 2000). Reports have been somewhat qualitative and conflicting about how much nutrient enrichment excludes *P. perfoliatus* from a given habitat. Haslam (1978) reported that *P. perfoliatus* is generally absent from eutrophic waters with enriched sediments, while Meyer et al. (2013) document *P. perfoliatus* as being a eutrophic species along with *Myriophyllum spicatum* L.

Natural history accounts describe occurrences of *P. perfoliatus* in conjunction with alkaline habitats (Godfrey and Wooten 1979, Haslam 1978). In Chesapeake Bay, place names such as Limehouse Cove (on the South River), and Chalk Point (on the Patuxent), where historic, and in most cases no longer extant, populations of *P. perfoliatus* have been documented, indicate the potential affinity for geologic or even anthropologic sources of alkalinity (Phillip and Brown 1965, Stankelis et al. 2003). Linnaeus (1753) identified *P. perfoliatus* in his taxonomic description as being associated with fluvial, lacustrine and argillic habitats, argillic possibly also implying the presence of calcaero-argillic sediments that provide an alkaline environment.

P. perfoliatus is able to uptake and use bicarbonate as an alternative carbon source for photosynthesis (Prins and Elzenga 1989, Allen and Spence 1981). This carbon concentrating mechanism, while not unique among aquatic plants, likely has a significant basis in its evolutionary ecology and may play a factor in its success in specific habitats containing alkaline substrates (Stevenson 1988, Stepien 2015). Another advantage may be an ability to facultatively sequester C from the sediments via lacunal connections to the roots, but this has not yet been investigated for *P. perfoliatus* (Nielsen and Borum

2008, Raven et al. 1988, Wium-Andersen 1971). Alkaline, or calcium-rich habitats, may in some way affect morphology and adaptive plasticity of *P. perfoliatus* through heterophylly (Pearsall and Hanby 1925), enabling this species to grow at greater depths (Stevenson and Confer 1978, Sculthorpe 1967). In addition, a calcium-rich environment may provide various other nutritional benefits to the plant from $CaCO_3$-P precipitation on the surface of leaves (Corman et al. 2016).

Researchers have made considerable progress to understand and describe *P. perfoliatus* ecology, and much of it appears to relate either directly or tangentially to sediment quality or sediment processes. Germination biology is highly affected by sediment grain size and burial (Ailstock et al. 2010a, 2010b, Ailstock and Shafer 2004, Ailstock et al. 2011, Koch et al. 2010). Microbial ecology is both plant-organismal and substrate-related and is critical to plant health and nutrient uptake (Crump and Koch 2008). Tissue elemental concentrations, while rarely proportional to ranges found in sediment, may serve as important indicators about differences between species, physiological needs, and nutrient supply (Short 1987, Li et al. 2013, Larcher 2003, Meyer et al. 2013, Talevska 2004, Schuette and Alder 1927, Wolfer and Straile 2004). Light availability is related in part to suspended sediments, and therefore it is helpful to understand surrounding watershed scale processes, soils, and geology, in addition to localized sediment dynamics and other disturbances (Palinkas and Koch 2012, Dennison et al. 1993, Goldsborough and Kemp 1988). Light availability and substrates also play a role in turion production (Ailstock et al. 1991, Goldsborough and Kemp 1988). Grain size, general textural class, and broad descriptions of SAV bed sediments provide a foundation for ongoing efforts to understand the ideal range of edaphic requirements for

primary productivity (Stevenson 1988, Stevenson and Confer 1978, Ozimek et al. 1976, Haslam 1978, Koch 2001, Denny 1980, Arnold et al. 2000, Moyle 1945, Meyer et al. 2013). Finally, propagation/restoration techniques rely on ranges of sediment conditions at the transplant site that will support growth. Horticultural substrates used for propagation ideally approximate bed sediments to the extent that they encourage healthy plant growth (Ailstock et al. 2011, Kujawski and Thompson 2000, Bergstrom 2006, Shafer and Bergstrom 2008, Hengst et al. 2010). However, the specific, elemental nature of the nutrients of the substrates, nor the sediments for transplanting, have not been fully elaborated upon for *P. perfoliatus* or many other SAV species (Short 1987).

In many terrestrial plants, the elements preferentially incorporated into foliage are in the order N, P, Ca, Mg, and S (Larcher 2003). However, these values vary for both terrestrial and aquatic species. Talevska (2004) found that N, K, and Ca were the primary nutrients by weight in *P. perfoliatus* leaves during early season, whereas during peak seasonal biomass, the order changed, and followed generally K, N, Ca, and Na. In both cases nutrient order was then followed by Mg, Fe, P, and Mn. A similar order of concentrations of nutrients were also confirmed for other Potamogeton species in Wisconsin (Schuette and Alder 1927). These studies indicate that *P. perfoliatus*, like many other plant species, readily moves nutrients to various tissues as its physiology requires, and that the tissue content is variable during different portions of the growth cycle (Talevska 2004, Taiz and Zeiger 2006, Larcher 2003). However in these studies, the sediments in which these plants grew were not similarly evaluated or characterized. It is generally accepted that N, P, Fe, Mn, and other micronutrients are taken up into the plant via the roots, while K, Ca, Mg, Na, SO_4, and Cl^- are likely obtained either via the

water column or through the sediment solution (White and Broadley 2003, Barko et al. 1986).

Texture and type of sediment have an influence on redox potential, pH, and microbial activity in reduced conditions (Fraser et al. 2016, Husson 2013, Cronk and Fennessy 2001). Organic matter accumulating in sediments plays an important role in plant nutrition (Capone 1983, Lopez et al. 1995, Evrard et al. 2005, Kilminster et al. 2006). As Eh (in mV) decreases with reduced oxygen in the sediment, (approximately +350 mV), a cascade of reductions of other ions significant to plants follows (Ponnamperuma 1984, Laanbroek 1990). The general order is reduction of Nitrate NO_3^- (to NH_4^+, N_2O and N_2 at about +250 mV), Mn^{4+} (to Mn^{2+} at about +225 mV), Fe^{3+} (to Fe^{2+} at about +120 mV), SO_4^{2-} (to S_2^{2-} HS^-, H_2S at about -75mV to -150mV), and lastly carbon dioxide CO_2 (to CH_4 at about -250 mV to -350 mV). Essential plant nutrients such as Ca, K, Mg, and P, though not reduced themselves, are all cations that are more available to plants in reduced conditions and at various pH levels (Brady and Weil 2002, Taiz and Zeiger 2006). For example, below a pH of 9.2 and an Eh of less than 350-400mV, NH_4^+ dominates (Husson 2013, Marschner 1995). Ammonium is a preferred form of nitrogen for many aquatic plants (Meyer et al. 2013, Caffrey and Kemp 1992), and has an effect on rhizospheric pH and subsequent ability of plants to assimilate other cations and anions (Hinsinger et al. 2003, Marschner 1995). However, analytes that can be toxic at higher levels, such as copper (Cu), zinc (Zn), and manganese (Mn), are also more available (Laanbroek 1990).

In cases where SAV and other aquatic plants are propagated for restoration, researchers have used various sediment/substrate mixes that establish plant growth for

transplanting (Statton et al. 2013, Kujawski and Thompson 2000). Smart and Barko (1985) found that natural sediments were ideal for use in the laboratory for microcosm experiments as they provided a source of nitrogen, phosphorous, and micronutrients for healthy plant growth yet avoided algal blooms associated with the higher nutrient content of artificial culture and solutions. Other efforts to develop propagation and restoration mixes for native submersed and coastal aquatic plants have included uniquely horticultural substrates (Kujawski and Thompson 2000, Ailstock et al. 1991), mixtures of natural sediments, sediments mixed with horticultural materials, or use of inoculant and organic material to increase fertility (Barko and Smart 1986, Cook et al. 2011, Jiang et al. 2008, Shields and Moore 2016). Ecological engineering structures have also been built at restoration sites that encourage accretion of organic materials by the natural energy of the system itself (Sofawi et al. 2017). The major disconnect between horticultural and SAV bed sediments is that natural sediments (and their organic additions), are formed over very different scales of space and time (Demas and Rabenhorst 2001, Erich et al. 2010), whereas horticultural substrates are usually combined mixtures of various substrates that are likely to have more labile materials more appropriate for terrestrial plant growth (sensu Smart and Barko 1985, Barko and Smart 1986).

Given the importance of alkaline environments for *P. perfoliatus* and some other SAV species, variations of shell mixed with sand, soil, and organic additions have been tested in propagation systems. A peat/oyster shell substrate was found to be almost three times more effective than sand to increase axenic root and shoot growth in *Stuckenia pectinata* (L.) Börner, another mesohaline species in Chesapeake Bay and in the same family as *P. perfoliatus* (Ailstock et al. 1991). In a seven week experiment, Kujawski and

Thompson (2000) grew *P. perfoliatus* stem cuttings in various combinations in soil, sand, shell, and slow-release fertilizer (placed just below the surface of the substrate). They found significant densities of new shoots, longer shoots, and longer rhizomes, but the lowest root growth (by half), compared with any other treatment combination of those three without fertilizer. Topsoil (without fertilizer) demonstrated the most consistent overall biomass for stem density, shoot, root, and rhizome length. Biomass was 50% less than the fertilized topsoil treatment, but root growth was twice as much as the fertilized treatment. Plant growth in sand alone supported root growth but, even in combination with other unfertilized substrates, appeared to hinder length of shoots and rhizomes. In seagrasses, it has been found that sand from siliceous sources generally limits N availability and uptake, and consequently plant growth (Alcoverro et al. 1997, Short 1987, Cambridge and Kendrick 2009). In horticulture, robust root growth often results in larger perennial underground structures (e.g. bulbs, turions), which may result in more and larger flowers (Corr and Widmer 1991), but this has not been investigated in SAV species.

An abundance of organic matter often creates an environment too rich in oxygen demand, and therefore may limit plant growth for various SAV species (van Wijck et al. 1992, Barko and Smart 1986). Barko and Smart (1986) used homogenized lake sediment to investigate how low percent sediment organic matter leads to better plant growth, higher stem density and longer stem lengths for hydrilla and myriophyllum, and found that sediments high in sand content or alternatively high organic carbon both inhibit growth. Zhu et al. (2014) found that higher sediment organic content and increased substrate fertility altered plant plasticity in *Myriophyllum spicatum* L. It made the plants

more likely to fragment and uproot; thereby reducing the possibility for plant regeneration or restoration – particularly in those plants without already established root systems. Productivity in the field for *P. perfoliatus* in riverine areas with sandy, gravelly, compact bottoms, was poorer than when growing in substrates higher in silt or mud (Ozimek et al. 1976).

Microcosm studies only rarely use intact cores of sediments when measuring various substrate parameters (Kilminster et al. 2006). The coarse (>2mm) fraction of the sediment, or evidence of bioturbation, also relevant components of the character of the sediment and to nutrient cycling (Brady and Weil 2002, Meysman et al. 2006, Jones et al. 1994, Benelli et al. 2017) may be mentioned (Schuette and Alder 1927, Zhang et al. 2015c, Thangaradjou and Kannan 2007), but less frequently measured and reported (Vinithkumar et al. 1999). Instead, field sediments are often sieved, homogenized, sterilized, or otherwise disturbed when placed in a laboratory microcosm setting. This may ensure consistency across treatments so that other variables of interest can be detected such as species interactions (Shields and Moore 2016, Sharpe and Baldwin 2012), genotypic effects (West et al. 2013, Engelhardt et al. 2014), photosynthetic response (Goldsborough and Kemp 1988, Chambers 1987, Li et al. 2013), water column quality (Burkholder et al. 1994), or uniform, consistent responses to additions of organic matter or nutrients (Short 1987, Barko and Smart 1986).

However, disturbance of sediments can fundamentally change the biochemistry of the soil and water column by altering metabolism (Liebert 1997, Tang et al. 2011, Tiedje et al. 1989) and substrate quality (Abadie et al. 2016), subsequently limiting plant productivity (Holmer et al. 2003, Marba et al. 2006). Laakso and Setälä (1999), and

Azevedo et al. (2017), found that altered substrate created changes in microbial and faunal diversity, as well as the presence of nutrients – factors that have been found to affect primary production in agricultural systems. Studies on plant responses to sediments may focus only on C, N, P and K, without looking at other elements, as they are typically considered as either limiting factors in primary productivity or harmful products of cultural eutrophication (Caffrey and Kemp 1992, Jackson et al. 2017).

Fewer studies link some combination of sediment texture, Eh, pH, microbial /fungal communities, and nutrients with primary productivity (Jackson and Vallaire 2009, 2007, Meyer et al. 2013, Donnelly and Hebert 1999), and at present are more common in agriculture, terrestrial restoration, and mariculture (Meena et al. 2017, Yildrim et al. 2016, Asmelash et al. 2016, Xie et al. 2017). For example, aquacultural practices have identified sediment requirements for the edible Chinese water chestnut (*Eleocharis dulcis*). Plants require a sandy loam substrate (pH 6.5-7.2) to which added mulch can be beneficial. Sprouting conditions are 13.6 deg. C, with a base fertilizer planting N:P:K ratio of 1.00:0.50:1.75 (Kleinhenz et al. 2001, Michaels 2017). While SAV planting practices are generally not this specific, this type of information may be helpful to guide future optimal propagation or restoration of aquatic submersed plants in Chesapeake Bay and elsewhere.

Length of studies to measure primary productivity response to sediment treatments may be less than 4-6 weeks, which does not typically allow for observation of flowering of *P. perfoliatus* (Ailstock and Shafer 2004). Thus an understanding of the effects of most sediment treatments on both sexual and vegetative reproduction has not been possible. Barko and Smart (1986) were interested primarily in sediment-plant

interactions but perhaps not evidence of floral development, and thus the experiment was five weeks. Shields and Moore (2016) observed plant species interactions and the effects of sediment types and salinity on various relative growth rates and other parameters for a period of eleven weeks, however, the study does not mention production of inflorescences. Homogenized, natural sediment was used, and nitrogen, phosphorus, and organic matter were measured, but not sediment texture. Neckles et al. (1993) focused on epiphyte community response to nutrients in the water column, and so a homogenized, sandy substrate with low organic content was not further described. Many studies use terms such as "sand" or "mud" to denote how much organic material is present in a given mixed, natural substrate, but an elaboration of other components of the sediment may not be of interest (van Zuidam et al. 2014, Rickett 1922, Zhang et al. 2015c).

These examples illustrate that each microcosm study has its own set of objectives, thus a more detailed analysis of sediment used, whether in the form of intact cores from the field, homogenized field sediment, or completely artificial substrates, may not be deemed important, but in so doing, critical information may be lost. Using peat/oyster shell substrate similar to axenic research on *S. pectinata* (Ailstock et al. 1991), Zinecker et al. (2007) found that stem cuttings of *P. perfoliatus* grown in peat/oyster shell resulted in higher biomass than oyster shell alone, soil/sand or sand only substrates. The goal of that research was to find an appropriate horticultural mix to optimally propagate plants to produce seeds, cuttings and turions for restoration projects, and was ongoing work from Kujawski and Thompson (2000). However, basic data characterizing onset of flowering, substrate texture, redox, pH, or nutrient levels, were not collected, nor were the substrates compared with SAV bed sediments.

The objective of this study was to investigate how *P. perfoliatus* turion growth in four horticultural substrates compared with growth in intact SAV bed sediment cores taken from *P. perfoliatus* beds. The propagation substrates were standard horticultural materials used in previous studies, and included washed sand, soil/sand, oyster, and oyster/peat (e.g. Kujawski and Thompson 2000, Zinecker et al. 2007). Washed sand was designated a low fertility control (Chapin 1980, Barko and Smart 1986), soil/sand was assumed to be a higher nutrient substrate treatment (particularly in labile N, P, C, and K) (Korsaeth 2012). Oyster, and oyster/peat, given their larger particle sizes, were assumed to be refractory, i.e. the oyster/peat substrate contained slow release nutrients, with the peat also containing large fractions of refractory lignin and cellulose (Ferdelman and Luther 1991, Korsaeth 2012, Burdige and Zheng 1998, Etcheber et al. 2007, Black et al. 2017). SAV bed sediment cores were taken from healthy SAV beds with a high level of growth. Therefore it was assumed that nutrients percent Total N (%TN), Mehlich3 P (mg/kg), percent organic matter (%OM), percent organic C (%OC), percent total C (%TC) would be at intermediate levels, (based on results reported from Barko and Smart 1986, Smart and Barko 1985, and Zhu et al. 2014).

Three hypotheses were considered in this research. First, it was hypothesized that the SAV bed sediment cores would exhibit features such as lower redox values (Eh) and pH, than all other substrate treatments; they would contain moderate/intermediate levels of percent total nitrogen, carbon (organic matter, organic C, % total C), phosphorous and potassium, and that peat/oyster shell and oyster shell would more closely share important nutrients in common with the sediments than either sand or soil/sand. Sand would be lowest in quantity of nutrients, and soil/sand would be highest in labile N, P, C and K.

Secondly, it was hypothesized that turions collected from the same two sites as the sediment cores would grow best on their own bed sediments, Kent Narrows or Sherwood Forest SAV bed sediment. Thirdly, it was hypothesized that the SAV bed sediment cores collected from two different *P. perfoliatus* beds would likely support higher turion growth than would horticultural substrates of sand, soil/sand, and oyster shell alone. Oyster shell and peat, based on previous, abovementioned experiments, would produce growth closer to the level of the intact sediment core treatments. Data collection for substrates and biomass followed a systematic approach: For substrate characterization, redox, pH, temperature, textural analysis, micro- and macro-nutrient analysis, organic matter analysis and total carbon and nitrogen content were collected; For biomass: plant tissue total carbon and nitrogen, inflorescences for each treatment, stem densities, stem lengths, above- and below-ground biomass data were collected and evaluated on the basis of substrate and planting effects.

MATERIALS AND METHODS

P. perfoliatus population identification and field collection of turions

Field surveys were conducted during the summers of 2007 and 2008 to identify large *P. perfoliatus* L. source beds for turion harvest and sediment cores for microcosm experiments. Two sites, one at Sherwood Forest on the Severn River (39°01'49.99" N 76°32'43.76" W), and the other at Kent Narrows (SAV beds near Muddy Creek and Marshy Creek (38°58'14.65" N 76°14'22.07" W), were of sufficient size and had access for observations, and turion and sediment core collection.

The Kent Narrows SAV beds were located in protected tidal embayments adjacent to *Spartina* marshes, boating activity, and marinas (soil classification Honga

peat (H_o), USDA-NRCS, Soil Survey, Queen Anne's Co.). The Sherwood Forest SAV bed is located on the Severn River adjacent to hilly, steep-sloped residential and wooded lands. The predominant soil classification for this area is the Monmouth-Collington Soil association (Davison and Rucker 1988). This association has well-drained sandy and loamy soils that developed in sediments containing glauconite or "greensands" well known for their slow-release nature of high mineral value in horticulture (Traunfeld and Nibali 2013) (Figure 2.1).

Ambient natural bed conditions for light (umol) were recorded biweekly using a Licor 250A light meter reading from a Licor underwater quantum sensor (LI-192). Dissolved oxygen, conductivity, salinity, and temperature were also recorded (YSI 85 Handheld, Xylem Corp.) or retrieved from MD-DNR (eyesonthebay.net).

Turions were collected between late December 2008 and early January 2009. Overwintering buds (turions) from each of the two sites were harvested using a shovel to dig up the sediment in the beds to a maximum depth of 30 cm. The sediment was placed on a wooden framed screen (0.75 cm diameter), and sieved. Approximately 100-150 turions were collected from each site, placed in containers with water from the site, and refrigerated (in darkness) within 1-2 hours of collection. All turions from both populations were a creamy white in color, tapered at each end, and approximately 2-3 mm in diameter.

Field sediment core collection and substrate preparation

Sediment cores from *P. perfoliatus* beds were collected and placed intact into a bowl the same size as the corer (18.5 cm diameter by 7 cm deep, total surface area was approximately 269 cm^2). The core size compares favorably with other and microcosm

experiments, most being between 6-15 cm in depth (Kautsky 1988, Caffrey and Kemp 1992, Goldsborough and Kemp 1988).

Microcosm bowls with sediment cores were covered with plastic to remain saturated and eliminate undue exposure to oxygen. Bowls were filled with water, covered with plastic, and refrigerated until further processing within 24-48 hours of field collection. Twenty-four sediment cores in total were collected in end November/early December 2008; twelve from Kent Narrows SAV beds near Marshy Creek, twelve from the Sherwood Forest SAV beds on the Severn River.

Microcosm experiment - experimental system

Research was conducted at the Greenhouse Research Complex located at the University of Maryland, College Park. The 750m^2 greenhouse was maintained at a temperature between 20-30˚C during the day and at a minimum of 15˚C at night. Natural sunlight was supplemented by metal halide lamps that supplied 400 umol par for a 14 hour photoperiod. Light measurements were taken to ensure that a uniform lighting regime. This was also confirmed by Sharpe (2009) in a similar greenhouse study at the same site.

The experimental system consisted of individual microcosms (volume 19L) randomly placed three rows deep on three separate greenhouse tables (Figure 2.2). Each microcosm was filled with water and contained one submersed bowl (Figure 2.3). Greenhouse tap water (Washington Suburban Sanitary Commission 2016, Appendix A, Table A.1) was used for this research as *P. perfoliatus* grows in fresh water in many places in the world (Ogden 1943). It also allows for maintaining consistent water column

chemistry and for removal of algae, and provides the likely environment in which most propagation scenarios occur for this species.

Each microcosm bowl was filled with either a substrate treatment alone ("unplanted"), or a substrate planted with turions from one of two selected *P. perfoliatus* populations ("planted"). A 90-watt Sweetwater® Linear II Diaphragm Air Pump generated airflow through a manifold consisting of three, 5.25 m lengths of standard ¾ inch (1.9 cm) PVC suspended above the height of the microcosms via support angles along the three tables. Vincon® flexible (5/16" outer diameter, 0.79cm) clear tubing was attached to valves screwed into the PVC pipe manifold that conveyed the air from the manifold to each microcosm tank via Sweetwater ® silica airstone diffusers (0.05 cfm or $2.5E^{-}5cu\ ms^{-1}$ or 1.42 liters per minute). One tube/airstone combination was submersed and attached to the side of each microcosm tank to encourage even mixing of water for optimum plant growth during the experiment (Figures 2.2 and 2.3)(Crossley et al. 2002).

Experimental design

Experimental treatments consisted of four (4) replicate microcosms (experimental units). Each microcosm was assigned one of three planting treatments (planted with Sherwood Forest turions, Kent Narrows turions, or unplanted), and one of six substrate treatments, for a 3 by 6 factorial design resulting in a total of 18 different planting combinations (18 x 4 replicates each treatment = 72 total microcosms)(Figure 2.2). In addition to the substrate treatments of SAV bed sediment cores from Kent Narrows and Sherwood Forest, four substrate treatments using standard horticultural materials were prepared: sand only (Sakrete silica quartz), 50:50 sand and common potting soil, 100% oyster shell only, and a 50:50 mixture of oyster shell and peat. The microcosm/planted

bowl design conceived for this research provided adequate space for plant growth for up to 15 weeks; the natural, seasonal equivalent in Chesapeake Bay necessary for most *P. perfoliatus* plants to flower once, set seed, and for some to begin early stages of senescence (Ailstock and Shafer 2006). This period of time was of sufficient duration to allow observation of growth relative to treatments without the plants getting "pot bound" or experiencing negative alterations in growth over the time period of the experiment (Zinecker et al. 2007).

Substrate preparation and planting:

Sediment cores were processed within 72 hours of field collection. The core was turned upside down out of the bowl on plastic sheeting to determine whether any benthic fauna or turions at the base of the core could be easily observed, and then sliced in small sections to discern any remaining roots or plant material, which were then traced to their origin in the core with a dissection needle or scalpel and removed within a 15 minute time window to reduce exposure to air. The core was then placed in its bowl, and immersed in microcosm water for conditioning before planting.

Substrates were placed into the same sized 18.5 cm inner diameter (ID) bowls into which the field sediments were placed. S and substrate and was thoroughly rinsed was then filled to the rim of the bowl. The sand and soil treatment was moistened sand and standard potting soil mixed in a 1:1 ratio. The mixture was filled to the last 1/5 of each microcosm bowl, and capped with sand to reduce exposure of soil nutrients and organic materials to the water column thereby creating undue algal growth in the microcosm. Oyster shell: oyster shell was thoroughly rinsed and placed in bowls. Oyster shell and Peat: The same rinsed oyster shell was well saturated and then combined until

homogeneous with saturated peat moss in a 1:1 ratio and filled to the top 1/5 of the bowl. The remainder of the bowl was topped with rinsed oyster shell to reduce any algal growth due to the organic presence of the peat moss and associated particulate matter.

The microcosm tanks were pre-washed with greenhouse tap water (from the public utility) and a 10% bleach solution to remove any residual dust, algae or other contaminants. The bowls with substrates and sediment cores were then placed in the microcosms for conditioning. The conditioning period allowed time before planting for the environment in the microcosms to equilibrate and also to ensure the final removal of any remaining biomass from the sediment cores.

Turions of each population were measured for length and mass, and separated into small, medium, and large size classes by weight (g). Turions were kept refrigerated (4 deg. C) and in darkness at all times before planting to inhibit preemptive growth before the start of the experiment. Forty-eight to seventy-two (48-72) hours after conditioning the substrates, three turions were selected randomly, one from each size class, and measured for length (cm) and mass (g), with total length and mass recorded. The substrate surface of the bowl was divided into thirds. In each third, a slit in the sediment surface was created with a knife and a turion inserted to a depth of approximately 1.5 cm, then recovered with substrate. The planted bowl was then placed back into the microcosm.

After planting in early March 2009, the microcosms were monitored every week for algal growth, topped off or refilled with water, and growth of turions observed. Algae was removed from the tank if in evidence on a biweekly basis or as needed, when tanks were cleaned or water added.

End of experiment microcosm conditions:

In order to assess whether in situ substrate treatments were different with respect to extent of anoxic or reduced conditions, end of experiment oxidation-reduction potential (Eh) was measured using five platinum (Pt) electrodes, a calomel reference electrode and modified multimeter per the instrumentation and methods in Rabenhorst (2009). The use of the calomel reference in soil oxidation reduction potential requires the addition of 244mV to each raw Eh reading to account for the difference between the calomel and the standard hydrogen electrode (Eh) (Rabenhorst 2009). Measurements were made at two depths: shallow (2-5 mm below substrate surface) and deep (~4cm below substrate surface). An Oakton pH meter (model WD-35614) was used in conjunction with a Hanna instruments pH meter to ensure accuracy and redundant measurement of pH and temperature at both shallow and deep measurements.

The microcosms were harvested 15 weeks after planting the turions. This time period allowed for *P. perfoliatus* plants to flower, and for a few inflorescences and stems had begun to senesce. Aboveground biomass (AGB) shoots were cut at the sediment surface of each bowl. Stem density, individual stem lengths (cm) and number of flowers on each stem were recorded. Aboveground biomass was then placed in a paper bag and oven dried at 70 deg. C for 24 hours and subsequently weighed (grams dry weight of biomass). Belowground biomass (BGB) was taken with substrate from the microcosm bowls, separated, rinsed, air dried, placed in 60 deg. C oven for 24 hours, and weighed. In order to determine plant tissue carbon and nitrogen content, a subset of aboveground and belowground samples (N=37) were first ground by plant grinder (Thomas Wiley/Model: Mini Mill), and then ground more finely on the roller mill for 24 h before

analyses for C and N by dry combustion (LECO CNS 2000 analyzer, St. Joseph, MI) (Plant samples analyzed were 6 grown on sand substrate, 6 grown on oyster, five on soil/sand, 6 on oyster/peat, 8 on Sherwood Forest and 8 on Kent Narrows sediment.

SAV bed sediment cores were placed into trays, and allowed to fully dry. A subset of the cores were characterized for color and redoximorphic features based on the soil color chart (X-Rite 2009). Each sample was then sieved and separated with a 2mm sieve to separate "soil" from refractory particles greater than 2mm. The larger (>2mm) particles including shells, pieces of bark, peat, etc. were oven dried and placed in a separate bag and weighed for the > 2mm fraction. A subset (N=32 for OM, LOI; N=29 for Total N and Total C)) of the < 2mm particles were reserved for soil analyses. The total for 32 samples included 2 sand, 2 oyster, 4 soil sand, 4 oyster/peat, 8 Sherwood Forest sediment, and 10 Kent Narrows sediment, with the Total N and C analysis (N=29) missing two oyster/peat and one Sherwood Forest samples from the list.

After oven drying for 24 hours at 60 deg. C, a quick particle size analysis (Kettler et al. 2001) was conducted to determine soil texture. In order to determine the carbon and Nitrogen content, dried subsamples were ground by roller mill for 24 h before analyses for C and N by dry combustion. Total Carbon (Loss on Ignition) in samples followed methods outlined in Nelson and Sommers (1996). These analyses were conducted at USDA-ARS in Beltsville, MD. Mehlich 3 Extraction (M3) tests of the substrates, were performed at the University of Delaware Soil Testing Laboratory (Mehlich 1984, and, for updated specifics: Sims et al. 2002). In order to determine the micro- and macro-nutrient (M3) composition, a representative subset of 17 substrate samples was analyzed: two

samples sand substrate, two samples oyster/peat, two samples oyster shell, two samples soil/sand, five samples Sherwood Forest and four samples Kent Narrows sediment.

Statistical Analysis

Fixed and main effects were reviewed with analysis of variance using the mixed procedure (proc mixed SAS Institute 2013). Where significant, means were evaluated using the Tukey-Kramer adjustment Honest Significant Difference (HSD). Covariates were applied to biomass analysis where relevant with respect to initial starting turion length. Statistical analysis was conducted using the SAS System for Windows 9.3 (SAS Institute 2013). All statistical tests were conducted at the 5% significance level.

RESULTS

Initial starting mass and length - turions

Turion length (cm) was found to be significantly different between Kent Narrows and Sherwood Forest populations ($F_{1,36}$ =7.08, p=0.0116) (Figure 2.4). Microcosms planted with Kent Narrows turions averaged a length of 24.4 $\pm$ 0.4 cm (range: 21.0-28.0 cm); and the Sherwood Forest average summed length of turions per microcosm was 25.7 $\pm$ 0.3 cm (range: 22.5-28.5 cm). No significant differences were found between turion lengths for the effect of substrate treatments, making the total input of starting biomass per substrate treatment equitable ($F_{5,36}$ =0.87, p=0.5088). Beginning average summed turion mass (g) was not significant between substrate treatments ($F_{5,36}$ =0.78, p=0.5716) or populations ($F_{1,36}$ =0.73, p=0.3978). Turion length and mass (g) were then used as a covariate in analyses of end of experiment length of stems (cm), stem density, and biomass (g) to ensure that the initial conditions were taken into consideration for each mesocosm (experimental unit).

Environmental conditions in microcosms

Water temperature and light remained consistent throughout the experiment with no differences in treatments observed. Natural and supplemental light in the greenhouse ranged from 500 to 1100 umoles at any given time within the 14 hour photoperiod ($F_{10,54}$ = 0.58, p = 0.82). Microcosm water column temperature ranged from a low of 20 degrees Celsius (during early season cool nights) to 30 degrees Celsius on the hottest days. Ambient water column temperatures for the treatment groups were not significantly different from any other when compared for fixed effects of plant population ($F_{2,52}$ = 1.62, p = 0.2070) or substrate treatment ($F_{5,52}$ = 1.79, p = 0.1315).

Water column pH in individual microcosms ranged from 8.6 to 10.0. Significant differences were detected between unplanted substrate treatments compared with planted substrates ($F_{2,52}$ = 10.40, p = 0.0002). The mean pH for unplanted microcosms was less alkaline, (pH 9.17 $\pm$ 0.08) compared with both the Kent Narrows populations (pH 9.50 $\pm$ 0.06) and the Sherwood Forest plant populations (pH 9.52 $\pm$ 0.05). There were no statistically significant differences between substrate treatment groups for water column pH ($F_{5,52}$ =2.08, p = 0.08).

End of experiment analyses

Substrate temperatures

Substrate temperature was measured at two depths in each microcosm, shallow (just below the surface (2 mm), and deep (4 cm). Average soil temperatures were significantly different for the main effect of planted vs. unplanted treatments ($F_{2, 71.9}$ =15.54, p < 0.0001). Among all planting and substrate treatment combinations, unplanted microcosms averaged lower temperatures (24.96 $\pm$ 0.12 °C), than either of the

planted microcosm treatments (Sherwood Forest plants 26.15 ± 0.39 °C and Kent Narrows plants 26.47 ± 0.23 °C). Average temperature differences between substrate treatments were not significant ($F_{5, 36.6}$ =2.43, p = 0.0531), nor were there significant results with respect to the main effect of depth ($F_{1,36.7}$ =0.00, p = 0.9581).

Substrate pH

Average pH of substrate treatments was measured at shallow (2mm) and deep (4cm) portions of each microcosm and was significantly different for the fixed effects of depth*substrate ($F_{5, 54}$ =2.01, p < 0.0001). Average pH was generally lower for deep measurements (4cm) than shallow measurements. Kent Narrows sediment (deep=pH 7.46 ± 0.28), followed by Sherwood Forest (deep=pH 7.51 ± 0.15), soil/sand substrate (deep=pH 7.77 ± 0.14), oyster shell/peat substrate (deep=pH 7.89 ± 0.19), sand (deep=pH 8.48 ± 0.16), followed finally by Sherwood Forest (shallow: pH 8.51 ± 0.21), and oyster (deep=pH 8.65 ± 0.13). Average pH for the remaining shallow measurements: (2mm) was lowest with oyster (shallow: pH 8.91 ± 0.09), followed by Kent Narrows (shallow: pH 9.04 ± 0.17), sand (shallow: pH 9.05 ± 0.08), soil/sand (shallow: pH 9.27 ± 0.10), and lastly, oyster/peat substrate (shallow: pH 9.28 ± 0.14) (Figure 2.5, Table 2.5). In addition to these findings, significant differences were found between the three factors of substrate*depth *planting treatment ($F_{10,53}$ =2.57, p = 0.0128), i.e. results indicated that unplanted controls were lower in pH than those that were planted.

Substrate redox (Eh), shallow and deep

Soil redox was measured at two depths in each microcosm, shallow (within 2mm of the surface), and deep (4cm). Significant differences were found between the main effects of substrate*depth for redox (Eh), measured in average millivolts ($F_{5,53}$ =6.19, p <

0.0001). The lowest overall averaged Eh measured was for Sherwood Forest sediment at 4 cm depth (sf-d) (187 ± 32), followed next by Kent Narrows deep (kn-d) sediment (229 ± 35), oyster shell/peat deep (oypt-d) (229 ± 15), Sherwood Forest sediment near the surface (sf-s) (236 ± 31), soil/sand deep (sosa-d) (246 ± 16), oyster shell/peat shallow (oypt-s) (254 ± 17), Kent Narrows shallow (kn-s) (268 ± 37), soil/sand shallow (288 ± 10), oyster shell substrate, deep (oyster-d) (293 ± 35), oyster shell shallow (oyster-s) (321 ± 42), and last, sand substrate-shallow (sand-s) (359 ± 28), sand, deep (sand-d) (394 ± 24) (Figure 2.6, Table 2.5).

Due to the significant variation in recording Eh and the variation of oxygenation in rooted soil, no significant differences were found for the fixed effects of planting treatment ($F_{2,53}$ =1.48, p = 0.2359), planting treatment* depth ($F_{2,53}$ =0.60, p = 0.5523), for the fixed effects of substrate and planting treatment ($F_{10,53}$ =1.00, p= 0.4540), or for the fixed effects of substrate*planting*depth ($F_{10,53}$ =0.70, p = 0.7220). An important trend, however, was seen between planted and unplanted, with the unplanted treatments having lower redox than the planted ones, regardless of treatment with the exception of sand.

Qualitative sediment characterization for evidence of soil redox

Qualitative data was collected on a subset of microcosm substrate characteristics and included X-Rite (2009) color evaluation, and characterization of the percent >2mm component of the substrates (Appendix B, Table B.1). Most of the substrates and all of the sediment cores but one showed evidence of reduced conditions (matrix chroma of 2 or less) and redoximorphic features such as oxidized root channels and mottles (Army Corps of Engineers 1987, X-Rite 2009). The SAV bed sediment cores >2mm fraction

consisted primarily of shell and refractory organic plant matter in addition to sediment concretions. Almost all SAV bed sediment cores showed evidence of bioturbation.

Sediment analysis for particle size, texture

Analyses for particle size and texture for a subset of 27 substrate samples are summarized in Table 2.1 and 2.5. Significant differences for percent greater than 2mm particle size fraction (% > 2mm fr) were found between substrate treatments for percent grams averaged for each 1 L experimental unit volume over the 6 treatments ($F_{5,44}$ =1,515.7, p > 0.0001). Kent Narrows sediment had the lowest ave % >2mm fr with 0.47 $\pm$ 0.06 percent, followed by sand 1.30 $\pm$ 0.21 percent, soil/sand 2.61 $\pm$ 0.17 percent, Sherwood Forest 3.60 $\pm$ 0.85 percent, oyster peat with 3.89 $\pm$ 1.77 percent, and the highest percent > 2mm fr was the oyster shell treatment with 71.60 $\pm$ 1.53 percent.

Textural analysis on a subset of 27 samples indicated primarily sandy, or sandy loam textures for all substrate treatments. Significant differences were found for the main effect of substrate for average percent sand ($F_{4,22}$ = 3.12, p = 0.0357), average percent silt ($F_{4,22}$ = 3.73, p = 0.0183), and average percent clay ($F_{4,22}$ = 4.00, P = 0.0139). Oyster/peat substrate samples were not included due to inconsistency of particle size in the samples, and so are not included in the results other than for > 2mm fraction.

Kent Narrows samples contained the lowest percent sand with a treatment average of 86.75 $\pm$ 1.76 %, followed by oyster shell with 87.14 $\pm$ 3.41% sand-sized particles, Sherwood Forest sediment with 91.16 $\pm$ 0.37%, soil/sand 92.54 $\pm$ 1.48%, and lastly, sand contained the highest percent sand with a treatment average of 97.61 $\pm$ 0.98%.

For average percent silt, sand contained the lowest percent silt with no recorded silt detectable in the sample, followed by Sherwood Forest sediment with 2.74 $\pm$ 0.39%,

oyster: 3.63 ± 2.09%, soil/sand: 4.00 ± 0.70%, and Kent Narrows sediment cores contained the highest % silt averaging 8.75 ± 1.76%.

For average percent clay particles, sand contained the lowest percent clay averaging 2.39 ± 0.98%, followed by soil/sand 3.46 ± 0.78%, Kent Narrows 4.51 ± 0.53%, Sherwood Forest: 6.10 ± 0.33%, and lastly oyster shell contained the highest % clay-like particle sizes averaging 9.23 ± 3.46%.

Mehlich3 (M3) analysis for sediments

Mehlich 3 analysis means comparisons were significant for substrate treatment as the main effect. Results indicated that either Sherwood Forest or Kent Narrows sediment cores (or both) averaged intermediate values for most of the nutrients between sand/soil and sand substrates (Table 2.2).

Means comparisons of M3 soil P (mg/Kg) for phosphorous were significantly different among substrates ($F_{5,11}$ =620.08, p = 0.0001). Sand had the lowest P (mg/Kg) (1.82 ± 0.05), followed by oyster (2.73 ± 0.38), oyster/peat (7.57 ± 0.97), Sherwood Forest (14.39 ± 0.50), Kent Narrows (17.78 ± 1.78) and soil/sand (322.12 ± 17.06).

Means comparisons of M3 soil K (mg/Kg) were significantly different among substrates ($F_{5,11}$ =4.87, P<0.0135). Sand had the lowest K (mg/Kg) (10.70 ± 0.91), followed by oyster (30.30 ± 3.41), Kent Narrows (45.90 ± 5.04), oyster/peat (51.79 ± 4.09), soil/sand (107.54 ± 10.99) and Sherwood Forest (110.14 ± 27.33).

Means comparisons of M3 soil calcium (mg/Kg) were significantly different among substrates ($F_{5,11}$ =1635, P<0.0001). Sand had the lowest Ca (mg/Kg) (80.12 ± 17.33), followed by Kent Narrows (376.66 ± 34.87), Sherwood Forest (458.40 ± 79.29),

soil/sand (3237.019 ± 206.391), oyster/peat (10,608.8 ± 970.10), and oyster (32,218.89 ± 413.85).

Means comparisons of M3 soil magnesium (mg/Kg) were significantly different among substrates ($F_{5,11}$ =25.9, P<0.0001). Sand had the lowest Mg (mg/Kg) (16.59 ± 2.48), followed by Kent Narrows (99.12 ± 7.92), Sherwood Forest (220.25 ± 17.00), soil/sand (243.64 ± 7.7), oyster/peat (284.44 ± 14.60), and oyster (308.87 ± 1.20).

Means comparisons of M3 soil Mn (mg/Kg) were significantly different among substrates ($F_{5,11}$ =4.99, P<0.0125). Sand had the lowest Mn (mg/Kg) (0.38 ± 0.04), followed by Kent Narrows (9.78 ± 4.51), oyster (10.52 ± 0.07), oyster/peat (11.01 ± 1.30), soil/sand (31.41 ± 0.35) and Sherwood Forest (67.29 ± 27.76).

Means comparisons of M3 soil Zinc (mg/Kg) were significantly different among substrates ($F_{5,11}$ =5.94, P<0.0067). Sand had the lowest Zn (mg/Kg) (0.74 ± 0.01), followed by oyster (3.54 ± 0.48), oyster/peat (6.88 ± 0.43), Sherwood Forest (8.19 ± 3.35), soil/sand (10.41 ± 0.53) and, Kent Narrows (16.73 ± 2.15).

Means comparisons of M3 soil Cu (mg/Kg) were significantly different among substrates ($F_{5,11}$ =53.1, P<0.0001). Sand had the lowest average Cu (mg/Kg) (0.21 ± 0.03), followed by Sherwood Forest (1.27 ± 0.12), oyster (1.30 ± 0.06), oyster/peat (1.65 ± 0.30), soil/sand (3.66 ± 0.21), and Kent Narrows (7.57 ± 0.29).

Means comparisons of M3 soil Fe (mg/Kg) for iron were significantly different among substrates ($F_{5,11}$ =22.51, P<0.0001). Oyster had the lowest Fe (mg/Kg) (14.88 ± 0.38), followed by sand (24.57 ± 1.34), oyster/peat (134.92 ± 2.35), soil/sand (189.04 ± 12.70) Kent Narrows (234.66 ± 21.96), and Sherwood Forest (269.92 ± 19.46).

Means comparisons of M3 soil B (mg/Kg) were significantly different among substrates ($F_{5,11}$ =25.5, P<0.0001). Sand contained no or negligible amounts of Boron (mg/Kg), followed by Sherwood Forest (0.29 ± 0.12), Kent Narrows (0.40 ± 0.08), oyster (0.53 ± 0.04), oyster/peat (1.05 ± 0.01), and soil/sand (1.09 ± 0.01).

Means comparisons of M3 soil S (mg/Kg) were significantly different among substrates ($F_{5,11}$ =21.12, P<0.0001). Sand had the lowest S (mg/Kg) (6.14 ± 0.43), followed by oyster (82.16 ± 0.70), Kent Narrows (243.90 ± 1.57), oyster/peat (292.94 ± 25.23), soil/sand (389.64 ± 52.60) and Sherwood Forest (418.25 ± 2.76).

Means comparisons of M3 soil Al (mg/Kg) were significantly different among substrates ($F_{5,11}$ =91.18, p < 0.0001). Oyster shell had the lowest Al (mg/Kg) (2.08 ± 0.81), followed by oyster/peat (4.64 ± 0.06), sand (54.37 ± 7.12), soil/sand (79.26 ± 4.78), Kent Narrows (216.26 ± 0.28) and Sherwood Forest (252.85 ± 1.97).

Carbon and nitrogen content of substrates

Carbon and nitrogen content of substrates were evaluated and results are summarized in Table 2.3. Significant differences were found between a subset of the substrates (N=32) for percent (%) organic matter lost from 5g samples ($F_{5,26}$ =1182.36, p < 0.0001). Sand contained the lowest organic matter loss on ignition averaging 0.073 ± 0.01 percent, followed by Sherwood Forest sediment 0.47 ± 0.04 %, oyster shell with 0.80 ± 0.02 %, Kent Narrows sediment averaging 0.86 ± 0.10 percent, soil and sand substrate with 3.47 ± 0.02 percent, and lastly, oyster shell and peat substrate contained the highest organic matter LOI averaging 18.3 ± 0.03 percent.

Significant differences for averaged percent organic carbon (OM/2) were determined ($F_{5,26}$ =1182.81, p<0.0001). Sand contained the lowest organic carbon 0.036 ±

0.006%, followed by Sherwood Forest, 0.23 ± 0.02%, oyster, 0.40 ± 0.008%, Kent Narrows, 0.43 ± 0.048%, soil/sand, 1.73 ± 0.15%, and lastly oyster/peat, 9.15 ± 0.23%.

Significant differences for averaged percent bicarbonate (based on Total % C – %OC) were found ($F_{5,22} = 5.32$, $p = 0.0024$). The Kent Narrows sediment contained the lowest percent bicarbonate averaging 0.103 ± 0.02 percent, followed by Sherwood Forest sediment (0.11 ± 0.02 percent), sand (1.20 ± 0.06 percent), soil/sand (1.51 ± 0.82 percent), oyster/peat (8.07 ± 7.28 percent), and lastly oyster (9.18 ± 4.60 percent).

Significant differences for averaged percent Total Carbon analysis by gas combustion were found ($F_{5,23} = 12.76$, $p < 0.0001$). The Sherwood Forest sediment cores contained the lowest percent total carbon averaging 0.35 ± 0.013 percent, followed by Kent Narrows bed sediment (0.49 ± 0.031 percent), sand (1.23 ± 0.05 percent), soil/sand (2.60 ± 0.949 percent), oyster (9.58 ± 4.61 percent), and lastly oyster/peat (16.95 ± 6.86 percent).

Significant differences were found for averaged percent total nitrogen analysis ($F_{5,23} = 12.51$, $p < 0.0001$). The Sherwood Forest sediment contained the lowest percent total nitrogen averaging (0.02 ± 0.016 percent) followed by Kent Narrows sediment (0.03 ± 0.015 percent), sand (0.08 ± 0.032 percent), soil/sand (0.13 ± 0.02 percent), oyster (0.15 ± 0.03 percent), and lastly oyster/peat (0.25 ± 0.32 percent).

Significant differences for averaged organic C:N ratios were found ($F_{5,23} = 6.56$, $p < 0.0006$). Sand contained the lowest C:N ratio averaging (0.42 ± 7.30), followed by oyster (3.1 ± 5.96), sand/soil (8.11 ± 5.16), Sherwood Forest native sediment (10.62 ± 3.65), Kent Narrows (12.96 ± 3.27), and lastly oyster/peat (50.18 ± 7.30 percent).

Insufficient samples were tested to determine whether there were significant differences between populations of *P. perfoliatus* for C:N ratio.

Common constituents of sediments and substrates important to plant productivity

When all 22 substrate parameters were compared with variables of SAV bed sediment cores on the basis of statistical significance, sand shared 6 parameters in common, oyster/peat 11, oyster 13, and soil/sand had the largest number (14) of statistically significant parameters in common with either Kent Narrows or Sherwood Forest sediments. When taking into account differences that were less important due to the refractory, coarse nature of the substrates, sand retained 6 variables in common, soil/sand retained 14, oyster shell substrate increased to 17 variables, and oyster/peat increased from 11 to 21 variables in common with SAV substrate (Table 2.5).

Percent Total Nitrogen, M3 P, and M3 K ratios were most similar between SAV bed sediment cores, oyster/peat, oyster, sand (Kent Narrows, 0.03%:1:2.6; Sherwood Forest, 0.02%:1:7.6; oyster/peat, 0.25%:1:6.8; oyster 0.15%:1:11 and sand, 0.08%:1:5.9). Soil/sand ratio was 0.13%:3:1 (Table 2.6).

End of experiment biomass measurements

Carbon and nitrogen content of aboveground and belowground biomass

Carbon and nitrogen content of AGB and BGB were evaluated and are summarized in Table 2.4. No significant differences were found for averaged C for aboveground biomass between any of the substrate treatments ($F_{5,31} = 1.42$, $p = 0.2441$). Since C:N ratios were significant, non-significant results for %TC results are reported here: soil/sand (36.48 $\pm$ 0.47 percent C), oyster (36.51 $\pm$ 0.80 percent C), oyster/peat

(36.68 ± 0.55 percent C), sand (37.63 ± 0.15 percent C), Kent Narrows (37.60 ± 0.69 percent C), Sherwood Forest (37.94 ± 0.21 percent C).

Significant differences for averaged percent N for aboveground biomass (AGB) were found ($F_{5,31} = 7.29$, $p < 0.0001$). Sand contained the lowest percent TN average (0.92 ± 0.10), followed by oyster and peat (1.05 ± 0.07 percent N), oyster (1.19 ± 0.06 percent N), Kent Narrows native sediment (1.21 ± 0.06 percent N), Sherwood Forest (1.22 ± 0.05 percent N), and lastly soil/sand (1.77 ± 0.11 percent N).

Significant differences for averaged organic C:N ratios for AGB were found ($F_{5,31} = 5.58$, $p = 0.0009$). Soil/sand contained the lowest C:N ratio averaging (20.91 ± 1.30), followed by oyster (31.31 ± 1.92), Sherwood Forest native sediment (31.48 ± 1.39), Kent Narrows sediment (33.35 ± 2.48), oyster/peat (35.88 ± 2.92), and lastly sand (42.09 ± 4.35). Insufficient samples were tested to determine whether there were significant differences between populations of *P. perfoliatus* for C:N ratio.

Significant differences were found for averaged C for belowground biomass ($F_{5,31} = 10.23$, $p < 0.0001$). Sherwood Forest sediment contained the lowest belowground biomass percent carbon (30.12 ± 0.83 % C), followed by soil and sand (34.12 ± 0.75 % C), sand (34.13 ± 2.60 % C), Kent Narrows sediment (36.39 ± 0.39 % C), oyster (36.52 ± 0.68 % C), and lastly oyster and peat (38.70 ± 0.94 % C). Insufficient samples were tested to determine whether there were significant differences between populations of *P. perfoliatus* for percent TC.

Significant differences for averaged percent N for belowground biomass were found ($F_{5,31} = 5.05$, $p < 0.0017$). Sand contained the lowest %TN average (0.92 ± 0.12), followed by oyster (1.11 ± 0.06 percent N), oyster and peat (1.12 ± 0.03 percent N),

Sherwood Forest native sediment (1.27 ± 0.06 percent N), Kent Narrows native sediment (1.42 ± 0.07 percent N), and lastly soil and sand (1.48 ± 0.16 percent N). Insufficient samples were tested to determine whether there were significant differences between populations of *P. perfoliatus* for %TN of belowground biomass.

Significant differences for averaged percent C:N ratios were found for BGB ($F_{5,31}$ = 9.75, $p < 0.0001$). Kent Narrows contained the lowest C:N ratio averaging (23.99 ± 1.26), followed by soil/sand (24.16 ± 2.64), Sherwood Forest native sediment (26.17 ± 1.39), oyster (33.40 ± 1.83), oyster/peat (34.79 ± 1.64), and lastly sand (37.88 ± 2.54). Insufficient samples were tested to determine whether there were significant differences between populations of *P. perfoliatus* for percent C:N ratio for belowground biomass.

Number of inflorescences

The average number of inflorescences present per microcosm at the end of experiment harvest was evaluated by substrate treatment and was significant ($F_{5,35}$ =7.38, $p < 0.0001$). Plants in sand treatments bore no inflorescences, soil/sand 1.29± 0.84, oyster/peat 3.13 ± 1.65, oyster shell 5.25± 2.37, Kent Narrows sediment 7.75 ± 2.06, and Sherwood Forest sediment 14.0 ± 2.48 inflorescences (Figure 2.7). No significant differences were found for population ($F_{1,35}$ =0.12, P<0.7314) or population and substrate ($F_{5,35}$ =0.91, $p < 0.4862$) for average number of inflorescences per treatment combination.

Stem density comparison between substrates

Stem density (number of aboveground stems) was evaluated for substrate treatment using the average per treatment summed turion lengths as a covariate. Significant differences were found between substrate means ($F_{5,34}$ =6.30, p = 0.0003). Sand treatments had the fewest stems and averaged 28.6 ± 3.5 stems, oyster 30.6 ± 3.0,

soil/sand 34.1 ± 2.0, Sherwood Forest sediment 36.9 ± 6.8, oyster/peat 43.5 ± 4.0, Kent Narrows sediment 59.0 ± 6.6 (Figure 2.8).

Summed stem lengths per substrate treatment

End of experiment summed and averaged stem lengths were compared between substrate treatments using initial turion summed length as a covariate and were statistically significant ($F_{5,34}$ =31.79, $p < 0.0001$). Sand substrate had the lowest summed stem lengths with the treatment averaging 237.56 ± 26.46 cm, soil/sand stem lengths averaged 414.21 ± 12.46 cm, oyster stems averaged 537.88 ± 45.33 cm in length, oyster peat averaged 675.38 ± 52.98 cm, Sherwood Forest averaged 903.65 ± 70.61 cm, and Kent Narrows summed stems lengths were longest averaging 1064.81 ± 73.49 cm (Figure 2.9). No significant differences were found for the effects of population ($F_{1,34}$ =0.26, P<0.6518) or population x substrate ($F_{5,34}$ =0.42, p = 0.8301) for summed average stem lengths.

Relationship between stem lengths, inflorescences, and added effects of substrate treatment and population

Fixed effects of individual stem lengths*population*substrate as they related to the presence and absence of inflorescences were statistically significant ($F_{5,1667}$ =44.59, p < 0.0001). *P. perfoliatus* plants with shorter stems (absence ≤ 23.5cm) bore flowers with lower frequency (0) than those with longer stems (presence ≥ 23.5cm). (Figure 2.10). Appendix C, Table C.1., provides data for each turion population comparing, average lengths, total number, and percent of stems bearing inflorescences in each treatment, and the same data for those stems not bearing inflorescences. Both turion populations grown in Sherwood Forest sediment, turions grown on Kent Narrows sediment, and Sherwood

Forest turions grown on oyster substrate, produced the highest percentage of longer stems (13.77%, 12.78%, 9.09%, 7.52%, of total stems, respectively), resulting in a higher number of stems in each treatment bearing inflorescences. Kent Narrows turions grown on oyster substrate produced just one stem bearing inflorescences and had the lowest total number of stems at (99) of almost any treatment other than Kent Narrows turions grown on sand (87). Kent Narrows turions grown on soil/sand produced inflorescences on 3.96% of the longer stems (total=4), but Sherwood Forest turions grown on soil/sand substrate produced one flower-bearing stem.

End of Experiment biomass (g)

Average aboveground biomass (g) of substrate treatments were compared using beginning turion summed mass (g) as a covariate, and were statistically significant ($F_{5,34}$ =18.27, $p < 0.0001$). Sand substrate had the lowest aboveground biomass averaging 0.49 $\pm$ 0.07 g, soil/sand: 0.89 $\pm$ 0.14 g, oyster 1.28 $\pm$ 0.07g, oyster/peat: 1.56 $\pm$ 0.13 g, Sherwood Forest: 1.82 $\pm$ 0.22 g, Kent Narrows: 2.16 $\pm$ 0.19 g (Figure 2.11).

Average belowground biomass (g) grown in the six different substrate treatments was compared using beginning turion summed mass (g) as a covariate, and were statistically significant ($F_{5,35}$ =3.65, $p = 0.0092$). Soil/sand substrate had the lowest belowground biomass averaging 0.44 $\pm$ 0.07 g, followed by oyster: 0.48 $\pm$ 0.05 g, sand 0.56 $\pm$ 0.09 g, Kent Narrows: 0.82 $\pm$ 0.05 g, oyster/peat: 0.88 $\pm$ 0.15 g, Sherwood Forest: 0.93 $\pm$ 0.18 g (Figure 2.12).

Aboveground biomass summed with belowground biomass (g) grown in the six different substrate treatments were compared and found to be statistically significant ($F_{5,35}$ =11.83, $p < 0.0001$). Sand substrate had the lowest total biomass averaging 1.05 $\pm$

0.15 g, followed by soil/sand: 1.32 ± 0.21 g, oyster 1.76 ± 0.11 g, oyster/peat: 2.44 ± 0.25 g, Sherwood Forest: 2.75 ± 0.36 g, and the highest biomass was Kent Narrows substrate: 2.98 ± 0.21 g (Figure 2.13).

Average R:S ratios (grams BGB:AGB) were compared for substrate as a fixed effect, and were statistically significant using sum of grams starting turion material as a covariate ($F_{5,35}$ =9.91, $p < 0.0001$). Oyster substrate had the lowest R:S ratio averaging 0.38 ± 0.04, followed by Kent Narrows: 0.40 ± 0.03, soil/sand 0.48 ± 0.04, Sherwood Forest: 0.51 ± 0.08, oyster/peat: 0.56 ± 0.07, and lastly sand: 1.26 ± 0.20 g (Figure 2.14).

DISCUSSION

This study focused on three primary objectives. The first objective was to characterize and compare two SAV bed sediments with four SAV propagation substrates. The (combined) second and third objectives were to compare growth of two different populations of *P. perfoliatus* turions in the SAV bed sediments and substrates. The SAV bed sediment treatments were undisturbed, intact cores collected from the same two locations as the turions, Kent Narrows and Sherwood Forest. The four substrate treatments were horticultural in nature and consisted of low fertility sand, higher fertility sand/soil, and intermediate fertility (refractory) substrates of oyster shell and oyster shell/peat.

Turions did not demonstrate preferential growth in the sediment from their site of origin, as initially hypothesized. Pre-experimental analyses for turion size indicated that Sherwood Forest site bed sediments supported turions that were slightly longer (cm), but not necessarily greater in mass, compared with turions from Kent Narrows bed sediments (Figure 2.4). Therefore it was concluded that sediment cores collected at Kent Narrows and Sherwood Forest sites offered SAV slightly different textural and nutritional

compositions that supported different growth parameters of turions. This indicated turion plasticity and ability to respond to sediment conditions regardless of site of origin. Given the similar growth responses to treatment substrates by the two different populations, analyses were limited to substrate level only and not population. The exception to this was consideration of presence of inflorescences and stems lengths. (Figure 2.10, Table 2.5, Appendix C, Table C.1).

In agreement with the hypothesis regarding substrates, SAV bed sediment cores exhibited a trend of lower redox and pH values than other treatments, with soil/sand and oyster/peat having values closest to the lower Eh and pH values of SAV bed sediments. Sand and oyster had much higher Eh and pH values, likely due in part to the absence of labile organic material and more oxygenation via increased diffusion due to larger pore spaces (Figure 2.5 and 2.6, Richardson and Vepraskas 2001). The slightly lower Eh in SAV bed sediment cores may have been due to a more established redox mosaic that included microbial biota and macroinvertebrates that also may have generated oxygen demand. More importantly, lower redox for sediment and oyster/peat and sand/soil substrates may have enabled a greater level of plant access to N in the form of ammonium, which is likely the preferred form of N for these plants (Meyer et al. 2013, Caffrey and Kemp 1992).

Also in agreement with hypotheses relating to substrate nutrients, SAV bed sediment core nutrient levels were intermediate to sand and soil/sand for variables of P, Ca, B, % OM, % OC, and % TC (Table 2.5). Magnesium and Mn were also intermediate for Kent Narrows, however Sherwood Forest had values closer to soil/sand. Surprisingly, SAV bed sediment cores were three to four times lower in %TN than sand substrate

(Tables 2.3, 2.5, Figure 2.16, 2.17). However, the N in sand substrate may have been less available due to the absence of organic matter and silt in washed sand replicates as well as higher Eh. Thus a moderate and naturally derived quantity % organic C, and an intact microbial community, likely placed the bed sediments with the ability to provide intermediate quantities of N (Brady and Weil 2002, Fraser et al. 2016), for %TN tissue uptake (Tables 2.4, 2.5 and 2.6, Figures 2.16, 2.18, 2.19). SAV bed sediment cores were highest for those elements originating from adjacent landforms such as Fe and Al (Sims et al. 2002).

Kent Narrows sediment alone was highest for Zn and Cu, possibly due to detrital input from the adjacent salt-marsh, higher silt, and lower sand content (Burke et al. 2000, Reboreda and Caçadora 2007, Reboredo and Ribeiro 1984), or from nearby marina and boating activities (further addressed in plant primary productivity below). The higher levels of Mn in Sherwood Forest sediment were possibly due to groundwater inputs rich in Mn (and Fe) from the Magothy aquifer (Curtin et al. 1997). Geological erosional inputs of glauconite ("greensands") were a possible source contributing to high Mg in Sherwood Forest bed sediments, but also Fe, K, and lime (Traunfeld and Nibali 2013, Davison and Rucker 1988). This type of sand substrate is also known for its supplies of refractory nutrients that are slowly released (Traunfeld and Nibali 2013). This is why simply identifying sediments from a generalized textural standpoint as "sand" or "loamy sand" only may not elucidate the actual contribution of nutrients to the plant/sediment system. Specific nature of texture is also relevant when considering quality and quantity of organic matter additions and origin. Paschal et al. (1982) collected data from a wide range of vegetated sediments (sandy, sandy loam, pebbles, shells) from the Potomac

River for P (89-420 mg/kg), Mn (11-860 mg/kg), Zn (3.9-170 mg/kg), Cu (10mg/kg), and Fe (4,800 mg/kg) among other constituents, pointing to the highly variable numerical ranges of nutrient conditions of the sediments in which aquatic plants grow.

Dissolved nutrients in the water column important to SAV, including Ca, Mg, Na, K, SO_4, Cl^- (Barko et al. 1991, Barko and Smart 1983) were likely present in the groundwater of the source beds for the SAV sediments cores (USGS 2017). In addition, most nutrients were present in the tap water supplied to microcosms in trace amounts in this study (Appendix A, Table A.1). Bicarbonate has been consistently reported as being an alternative carbon acquisition strategy to CO_2 in photosynthesis for *P. perfoliatus*. While present in sediments and substrates, HCO_3^- (and CO_2) were also likely present in the tap water, and in diffused air from airstones supplied to microcosms, in sufficient quantities (Barko et al. 1986, Lucas and Dainty 1977).

A total of 22 variables were sampled for sediment/substrate characteristics (pH, Eh, texture, nutrients (M3), %OC, %OM, %TC, %TN), and significant differences were determined using means comparison procedures. The soil variables for each horticultural substrate were compared against the numerical range that spanned both SAV bed sediment cores. The assumption was that, since the range of parameters of the SAV bed sediments supported healthy plant populations, an artificial substrate would ideally have as many of the 22 variables in common, i.e. approximate the "healthy SAV bed sediment standard." Oyster shell/peat (21/22), and oyster shell (17/22), were found to have a greater number of variables in common and within the range of the SAV bed sediments than any other substrates, but only when taking into account the refractory, slow release nature of the larger particle sizes of oyster alone and oyster and peat (Brady and Weil

2002). Oyster/peat consisted of 58% coarse fraction materials while oyster substrate consisted of 71.6% >2mm fraction, making this portion less immediately available to the plants.

Surprisingly, soil/sand shared 14 of 22 variables (including all fractions of texture, Eh, and pH), with the SAV bed sediments. While these values made it the most similar to the bed sediments from a statistical perspective, the values that were higher than the SAV bed sediments, labile %OC, %TC, %OM, %TN, and Mehlich 3 Phosphorus, were all an order of magnitude greater than the range of SAV bed sediments. Sand shared only 6 of 22 variables in common with the SAV bed sediments. It also lacked textural quantities of silt, as well as %OC and B, with the other elements likely available in very low quantities (Brady and Weil 2002), and this was likely caused by leaching due to washing. The number of variables in common with the range of SAV bed sediments for each substrate also followed overall plant productivity (Table 2.5, Figure 2.13).

Percent Total Nitrogen, Mehlich 3 P, and Mehlich 3 K ratios (%TN / M3P / M3K) were most similar between SAV bed sediment cores, oyster/peat, oyster, and sand (Kent Narrows, 0.03%:1:2.6; Sherwood Forest, 0.02%:1:7.6; oyster/peat, 0.25%:1:6.8; oyster 0.15%:1:11 and sand, 0.08%:1:5.9). Soil/sand, was markedly different in %TN:M3P:M3K ratios, having a ratio of 0.13%:3:1 (Table 2.6), i.e. an order of magnitude higher %TN, a 3-fold higher P, and a seven fold lower K. Primary productivity also followed the relative order of the soil variables that were most similar in %TN:M3P:M3K proportions and quantity to the highly productive SAV bed sediments (Table 2.6, Figure 2.13). Soil/sand was less able to generate yield of above- and

belowground biomass (to the point of being depressive – Figures 2.16, 2.17, 2.18). This may indicate that higher labile N availability, higher levels of P, or lower levels of K, were potentially a limiting factor to plants grown in soil/sand substrate. The %TN:M3P:M3K ratios in sand were likely not delivered in sufficient quantity (i.e. in deficiency) (Larcher 2003, Brady and Weil 2002) (Figure 2.15). For coffee crops, Zhang et al. (2017) found that there were highly significant differences between NPK ratios (1:0.5:0.8 vs. 1:0.8:0.5) and the relative quantity of fertilizer applied. An intermediate amount (lower quantity) of fertilizer, and increasing the P fraction from 0.5 to 0.8, and K from 0.8 down to 0.5, fundamentally affected yield and was more ecologically friendly. For this research, since there are not recommended levels, for mg/Kg of N, P, K and various other elements for SAV, the assumption was that the levels in the SAV bed sediment cores were reasonable for *P. perfoliatus* growth at the collection sites.

As hypothesized, Kent Narrows sediment, Sherwood Forest sediment, and oyster shell/peat substrate, supported the highest values of above-ground, belowground, and summed total biomass in grams dry weight (gDW), highest stem densities, and number of long stems, than any other treatments. In addition to %TN, in particular, the inputs of %OC (and %OM), were key factors that came into play to support plant growth (Table 2.4, 2.5, Figure 2.18, 2.19, 2.20, Wium-Andersen and Andersen 1972). Kent Narrows sediment exhibited the highest qualitative growth overall among these three best performing treatments with two exceptions. Kent Narrows higher values were likely due to qualitatively higher organic material, and a statistically significant higher silt percent that may have favored stem growth due to texture (Table 2.5, Figure 2.8, Ozimek et al. 1976).

Sherwood Forest sediment supported the highest number of inflorescences (by 50% or greater than all other treatments), and the highest belowground biomass (12% higher minimum), compared to all other treatments. Oyster/peat substrate was second highest in belowground biomass and stem densities. Oyster shell supported more inflorescences than oyster/peat (40% more inflorescences), but its texture and lack of easily available organic matter may have reduced above- and belowground biomass and stem densities. Sand/soil supported 45% lower biomass, 20% fewer stems, and 60% fewer inflorescences than oyster/peat substrate. Sand values were at least 60% lower in all growth parameters compared with oyster/peat substrate, with no evidence of inflorescences (Table 2.5, Appendix C, Table C.1).

Drivers of Plant Growth - Nutrient uptake, yield, and other response curves

The primary variables affecting *P. perfoliatus* growth in the six treatments appear to have been Eh, pH and texture, in the sense that these three affected presence and availability of the critical drivers of plant primary productivity. Since the SAV bed sediments supported healthy, self-sustaining, SAV plant populations, the assumption was that %TC, %OC, %OM, %TN, were sufficient to support appropriate plant nutrient tissue concentrations and growth by being optimal, rather than in deficient or in depressive quantities. The relationship between yield, elemental availability in the sediment, and uptake and concentration of nutrients in plant tissues, can be described using response curves, a relationship often cited for plant tissue nitrogen and yield (Figure 2.15)(Bates 1971, Jamieson et al. 2000, Wikström 1994).

Sherwood Forest and Kent Narrows SAV bed sediments supplied intermediate quantities of %OM and %OC (%OM from Loss on Ignition divided by two), and %TC

that appeared to be related to intermediate values of %TN in plant tissue (Figure 2.19). Kilminster et al. (2014), and Kamp-Nielsen (2002) also found a significant correlation between %organic matter and %TN in substrates (eg. 4.0 %OM/0.06%TN for seagrass beds vs. 5.4 %OM/0.09%TN for mangroves). For the refractory qualities of oyster, and oyster/peat, while the data indicated intermediate and high quantities of both %OM and %TN in the refractory substrate (less %OM for oyster), uptake resulted in intermediate values for %TN in plant tissue (Figure 2.16). Percent OM and %OC are able to create conditions that make nutrients more available, such as lower redox, and provide conditions that increase nutrition for microbes/mycorrhizal associations that facilitate plant uptake of N. Eutrophicants can also be decreased in the presence of organic C, as is seen in the reduction of nutrients by precipitation (of important nutrients such as P) due to formation of soluble, organically-based chelates; (Erftemeijer et al. 1994, Brady and Weil 2002, Larcher 2003, Cronk and Fennesey 2001).

Sand supported a slightly lower result of uptake of %TN in plant tissue which at 0.92%TN for both AGB and BGB, this value may represent a critical low end threshold %TN the minimum required for *P. perfoliatus* growth (Figure 2.16). Soil/sand %TN was highest and statistically different for AGB, however for BGB it was just 0.4% higher than Kent Narrows for %TN in root tissue. Because of this apparent close relationship to %TN content in AGB tissue, %OC quality and quantity in substrate also appears to affect yield, with Kent Narrows at optimal yield for AGB, but appearing as a slightly lower yield in comparison to Sherwood Forest for BGB (Tables 2.3 and 2.4, Figure 2.19, 2.20). Of particular note in all instances is that plant tissue uptake (%TN) levels off before percent soil organic carbon (as well as %OM), which appears to increase exponentially. This is

an important indicator that growth might approach depressive to toxic levels under conditions of high tissue %TN in well before what might be considered moderate labile organic matter conditions. This agrees with research by Misra (1938), and Barko and Smart (1986), where (a more labile) %OM became problematic for SAV between a 20% - 30% threshold depending on water depth, water movement, and quality of substrate.

Kilminster et al. (2006) found that additions of labile organic matter in the form of ground seagrass wrack reduced seagrass growth by 50%, while increasing leaf molar concentrations of N and P by 30%. The fraction of larger fines found in Kent Narrows were similar to those found in Sand-Jensen and Sondergaard (1979). They reported that low level, natural additions of organic materials, such as Terrados et al. (1998) reported higher species richness in sediments with a natural silt fraction of up to 12%, but a reduction in richness above 15% silt, which again may point to an optimal to luxury supply of nutrients, and then a transition to decreased growth after 15% (silt) (Fig. 2.15).

The plant yield curve plotting %TN tissue content and growth performance (Figure 2.18), indicates that sand provides deficient resources to support %TN uptake for *P. perfoliatus* growth, while soil/sand provides too much N to tissue, resulting in a depressed yield. The differences of how a substrate's refractory properties affect plant uptake are evident when the curve with all substrate treatments is plotted (both refractory (majority >2mm) and more labile (majority <2mm) are compared (Fig. 2.18 A and B). The relative decrease in yield for oyster compared with oyster peat may be due to the lack of available organic material. When considering the more labile treatments composed primarily of <2mm particles (C and D), the curve becomes a more predictable indicator of %TN tissue and yield. Most yield curves typically consider above- and belowground

dry matter produced together, however, as is seen with the relative differences between %TN in Figure 2.18, this can mask the individual factors that may support sustainability and growth dynamics. In the case of BGB, plants grown on Kent Narrows substrate may have produced lower belowground biomass due to sufficient plant requirements in comparison with lower tissue %TN and higher biomass in Sherwood Forest BGB. In other words, due to the fact that the whole plants were supplied with adequate nutrients, additional scavenging (root expansion) was not necessary in Kent Narrows plants (Larcher 2003, Chapin 1980). However, other factors may also have been affecting the belowground portions of plants grown in Kent Narrows sediment and this will be discussed. The reduced %TN and %OC of Sherwood Forest sediments, may produce rooting habits of greater belowground biomass, and in some cases larger turions, by being lower than Kent Narrows in %OM, which may subsequently (along with enriched but refractory glauconitic sands) provide a slightly lower supply of %TN in the substrate to the plants.

Oyster/peat substrate was lower in %TN AGB and BGB than oyster, yet still produced higher yield. Presumably peat, with its additional nutrients (Yoo et al. 2017), and texture (Kamp-Nielsen et al. 2002), may have facilitated higher nutrient availability and subsequent growth. It appears the high level of %TN recorded in the substrates for oyster and oyster/peat, were not available to the plants in a fashion similar to the more labile soil/sand substrate, and this refractory access resulted in higher yield. Yield can be affected by very small variations of C, N, P, or S. For example Kilminster et al. (2014), found that leaf and rhizome extension was reduced with very slight increases in C (0.30%TC), N (0.051%TN), P (80.8mgkg^{-1}) or S (0.015 AVS % dry wt) compared with

higher growth conditions (0.26 %TC; 0.047 %TN; 71.2 $mgkg^{-1}$ P; 0.0 AVS %Dwt). Meyer et al. (2013) also concurred that increased %N in leaves reduced % cover in *P. perfoliatus* as well as other community aquatic species.

Stem densities responded best to the finer textured, silty, Kent Narrows sediment, with lowest % sand of any treatment (Table 2.5, Figure 2.8, Appendix C, Table C.1). Oyster/peat was the only other substrate that responded with stem densities closest to Kent Narrows; it may have approximated the organic texture of Kent Narrows, and contained no sand. Other substrates were roughly equivalent to one another in moderate stem densities. Denny (1980) and Ozimek et al. (1976) found that rooting depth was greatly affected by sediment density due to texture. Jiang et al. (2008) also found that stem densities decreased, along with root allocation and overall biomass, when SAV was grown in a sand substrate. In this study, %TN in plant tissue appeared to be allocated differently in highest yields of above- and below- ground biomass. Sherwood Forest, Kent Narrows and oyster/peat had %TN allocations that were somewhat lower in AGB, and %TN was higher in BGB, whereas in oyster, and more markedly soil/sand, %TN was higher in AGB than BGB. Percent TC in AGB tissue was relatively consistent across all treatments, (~36.5-38.5%), and this range was also similar for BGB for %TC for oyster/peat, oyster, and Kent Narrows. However, the substrates containing the highest percentages of sand, and lower stem densities (in addition to oyster shell), also had the lowest %TC BGB: soil/sand (34.12% TC), sand (34.13%), and Sherwood Forest (30.12%TC).

Presence of inflorescences were highest with intermediate substrate %OC and intermediate substrate %TN in both above- and belowground biomass for the SAV bed

sediments (Table 2.5). This is likely due to the fact that intermediate %OC and %TN in biomass also supported the highest yield for SAV bed substrates (Table 2.5, Figures 2.11-2.13). More importantly, the higher aboveground yield also produced the longest stems (Figures 2.9, 2.10). Longer stems (in this study >23.5) were more likely to support inflorescences (Figures 2.9, 2.10, Appendix C, Table C.1). Oyster substrate was third highest for flowering, and this may have been attributable to the higher %TN in above- and belowground biomass, and the highest value of K in the NPK ratios (as well as high Ca in substrate), possibly enabling a small but important percentage of longer stems that bore more inflorescences (Taiz and Zeiger 2006). Although yield for oyster/peat was on par with the SAV bed sediments, plants grown in oyster/peat incorporated less %TN AGB than oyster substrate, but were similar to oyster for %TN BGB. Percent TN in soil/sand depressed yield, and as a result, decrease in biomass lead to fewer, longer stems, and fewer flowers. Johnson et al. (2017) found a positive correlation between increasing porewater ammonium and percentage of flowering shoots across locations with different sediment nutrient types with *Zostera marina*. However, because key aspects such as %TN in plant tissue, and redox were not measured, it is difficult to determine how the sandy and muddy sediments, number or length of plant spikes, or organic matter might have been affecting flowering. Jackson et al. (2017) also documented enhanced stem elongation and flowering with nutrient enrichment of seagrass beds of *Z. marina* using fertilizer stakes (N:P:K was 15:3:3), however it is unknown what the %TN values were for plant tissue for fertilized vs. unfertilized treatments.

While the reduced flowering (~50%) of Kent Narrows sediments compared with Sherwood Forest substrates indicated a trend, it was not statistically significant. The trend

might seem to suggest something depressive, or perhaps a tradeoff of vegetative vs. reproductive propagation, however, there may be other relevant factors. Potassium in the mg/Kg quantity and NPK ratios was among the lowest of all fractions across substrate treatments (Tables 2.5, 2.6). Güsewell and Koerselman (2002), and Lawniczak et al. (2009), emphasize the importance of determining whether nutrients other than just N:P, or N:K are limiting, and that nutrients are variable both seasonally and annually, thereby affecting yield.

There are typically two flowering events per growing season in *P. perfoliatus* in Chesapeake Bay (Ailstock and Shafer 2004, Olesen 1999). Plants grown on Sherwood Forest sediment may have flowered early due to the lower %TN and %OC resources. Higher belowground biomass production and early flowering are often associated with nutrient poor substrates (Larcher 2003), and is a common behavior of terrestrial monocots (Halstead and Lynch 1996). However, unlike the low values of aboveground biomass of washed sand and other infertile soils, there was still ample aboveground biomass for Sherwood Forest plants. In addition, pre-experiment measurement indicated Sherwood Forest sediment supported an average larger size class of longer turions (initially) than the turions collected at Kent Narrows (Figure 2.4). Large belowground biomass may be an important predictor for a greater number of inflorescences in *P. perfoliatus* where adequate nutrient thresholds have been met. In addition to the sand fraction, the coarse fraction of Sherwood Forest was larger by an order of magnitude compared with the finer sediment of Kent Narrows, and was slightly more reduced. This was somewhat unexpected given that sandier substrates are generally considered to be less reduced than siltier sediments with higher organic matter and silt. This may be due to

the type of sand found at the Sherwood Forest sediment. Sherwood Forest had higher K, Ca, and significantly higher Mg, Mn, and S. Potassium, Ca, and Mg are particularly important in *P. perfoliatus* growth during flowering, and Kent Narrows was in some instances an order of magnitude lower in these three elements than Sherwood Forest. In horticulture, robust root growth often results in larger perennial underground structures (e.g. bulbs, turions), which may result in more, and larger flowers (Corr and Widmer 1991). In low nutrient conditions, *Potamogeton crispus* turions also were found to be larger and have a larger carbohydrate reserve, and were smaller with higher nutrient reserves in high nutrient conditions (Xie et al. 2011). While the lower percentage of N and C in Sherwood Forest appeared to have been a critical reduction in vital resources, other micro- and macro- nutrients in ample supply may in general have been sufficient to encourage plasticity and attendant morphology that is easily adapted to conditions of patchy resource availability in the SAV habitat at Sherwood Forest.

Reduced flowering (and belowground biomass) may also have been due to Kent Narrows elevated elements of Cu and Zn compared with any other treatment in this research (six to nine times greater for Cu and two-fold greater for Zn than the values for Sherwood Forest). These elevated values were possibly a result of excretion of metal-containing salts and decomposition of detrital litter from the adjacent *Spartina* marsh. *Spartina* is known for concentrating Cu and Zn and other metals in the rhizosphere and/or leaves and release through the leaves (Burke et al. 2000), thereby becoming a possible source of metals in the adjacent SAV bed sediments in the sand, silt or clay fractions (Reboreda and Caçador 2007). Other workers have documented further scavenging of Zn, Cu, and other metals by *Spartina* detrital litter due to chelation exchange of metal ions

and microbial activity (Drifmeyer and Rublee 1981), and it has been documented that the more refractory portions of *Spartina* (lignin and cellulose) become less easily mineralized over time (Hodson et al. 1984). *Zostera marina*, growing in sediments adjacent to a salt marsh, was shown to be inhibited by 0.32 mg/Kg Cu (Lyngby and Brix, 1984).

Another explanation of outside influence on metals or organic sources or nutrient levels could be originating from the watermen that have historically cleaned their gear on a regular basis near the beds (CBEC staff, personal conversation), or inputs from a marina located nearby. Regardless, the elevated Cu may be a possible factor in the reduced belowground biomass, and subsequent reduced or delayed inflorescences (by 50%) in comparison to Sherwood Forest (Zhu et al. 2016, Doss and Christian 1979). Zhu et al. (2016) found that Cu reduced belowground biomass of *Vallisneria natans.* In addition, the presence of water column ammonium-N (in conjunction with elevated Cu) further restricted growth rate. Copper also can alter photoperiod and delay onset of flowering (Jin et al. 2015). Among the Potamogeton species, *P. perfoliatus* is considered the highest accumulator of Cu (Matache et al. 2013). Clearly, more research is necessary to determine the effects of small increments of Cu in natural sediments, as most studies experiment with Cu levels that are typically an order of magnitude higher than the values found in this research, or are added only in the water column (Zhu et al. 2016, Fritioff and Greger 2006, Monferran et al. 2009).

Nutrient depletion in the sediments of the microcosms, particularly the SAV bed sediments, did not occur as might have been expected. The few unplanted microcosms evaluated for %TN and %TC indicated values similar to end of study planted microcosms. Unplanted microcosms ranged from 0.28-0.33%TC, and from 0.012-0.029.

%TN for Sherwood Forest sediment. For Kent Narrows the range was from 0.31-0.39%TC and from 0.02- 0.04 %TN. Future studies would ideally perform before and after analyses of %TN, %TN, M3P, and possibly other levels of elements in sediments to further determine the extent of depletion of given nutrients. In addition, it would be useful to develop a sediment budget in cases where autochthonous organic matter can be separated from allochthonous in order to determine extent of resources depleted vs. resources that are added back in to the sediment/plant environment.

CONCLUSIONS

This study characterized and described growth responses of two different populations of *P. perfoliatus* turions to propagation substrates and SAV bed sediments. The data revealed that statistically different growth rates, flowering, and possibly time of flowering occurred in response to the nutritional composition of substrates and sediments. These responses appeared largely due to availability of, and ability of plants to uptake and use, %TN, and that %OM contributed to the presence of %TN, as well as the ability of plants to access it. The range of other soil elemental nutrients in substrate, coincided with increasing or decreasing yield based on similarity to SAV bed sediments, but analysis on plant tissue contents of these other nutrients was not examined. More research on the relationship between plant tissue uptake of nutrients and associated yield, as well as sediment nutrient content, would help gain further insight into growth dynamics for *P. perfoliatus*.

In spite of similar general textural classifications of sandy or sandy loamy, the origin and quality of the textural fractions determined the presence of elemental nutrients, and the redox and pH conditions that made it possible for plants to use those nutrients.

An understanding of the nature and percent accounting for the coarse fraction of a substrate or sediment appears to be key. The source of organic and other material, and degree of lability of organic C and availability of N, affect yield and reproductive capacity based on tissue uptake and concentration of N. Once an initial range of parameters for sediment C and N has been established for *P. perfoliatus*, other nutrients, their ideal levels for each species or species assemblages, and the role they play in plant nutrition and physiology, can be more easily evaluated, as suggested by Short (1987).

Nutrient response curves indicated that uptake of %TN in plant tissue in adequate ("intermediate") quantities was a key driver for optimal biomass yield and sexual reproduction with appropriate controlled conditions of light and water quality. Environmental conditions for *P. perfoliatus* beds are summarized in Figure 2.21, and are specific to data collected from the SAV beds in this and other studies. Basic controlling conditions are highly related to other feedbacks in the system, which comprise both natural and anthropogenically driven feedbacks. Ideally, these empirical values might serve as the point of departure upon which data is collected for other *P. perfoliatus* beds throughout the Chesapeake Bay and in other regions. In this way, it will be possible to better understand the range of controlling factors that play a role in *P. perfoliatus* persistence in natural habitats. With this data it will be possible to evaluate potentially new or similar system changes at different scales and settings, and to establish a fundamental guide to research, conservation, and restoration that is sensitive to landscape position and other factors.

This research determined that SAV bed sediments are the best materials for propagation and restoration of *P. perfoliatus.* Bed sediments are developed through

pedogenic processes not easily reproduced using horticultural substrates (Demas and Rabenhorst 2001). While restoration ecologists are increasingly using inoculation with microbes and mycorrhizae to improve degraded lands (Asmelash et al. 2016, Wubs et al. 2016), it would be advantageous to further evaluate the fractions of organisms, sediment textures, and other components most commonly found in healthy Chesapeake Bay mesohaline SAV bed sediment cores. This would help to determine the suite of factors most relevant to plant yield and the sustainability of *P. perfoliatus* and other SAV species beds.

Given that oyster/peat substrate was closest in yield to Sherwood Forest and Kent Narrows bed sediments, it appears to be the best alternative as a propagation substrate at present, and may increase yield if inoculated with SAV bed sediment. However, further experiments to optimize production of inflorescences while also maximizing biomass would be ideal, as flowering was not particularly robust for oyster/peat. The high %OM in oyster/peat substrate, although apparently refractory in nature for this experiment, might become problematic if it were to increase in lability over time (i.e. via oxygenation), creating an environment too rich in humic substances. Use of glauconitic sands or small percentage (<9% OM) natural wrack or detritus from SAV, *Spartina* marshes or other sources may also prove to be useful organic matter additions to propagation substrates given that they are naturally occurring. SAV wrack and marsh detritus were likely sources of OM in the high yield Sherwood Forest and Kent Narrows bed sediments. Highly successful germination was found in *Posidonia australis* by Statton et al. (2013) using seagrass wrack and marine dredge sediments. Algae and other SAV species have been used for particulate organic matter additions, however in both

experiments the vegetative materials were ground up and passed through a 1-2 mm sieve (Barko and Smart 1986, Kilminster et al. 2006), and therefore did not add to a similar level of coarse materials and organic matter found in natural SAV bed sediments or the oyster and oyster/peat substrates in this study.

A number of specific experimental approaches in this investigation were undertaken that may prove beneficial in future efforts of propagation, conservation, and restoration of *P. perfoliatus* and other SAV species:

1/ The duration of the experiment was 15 weeks, which in this case allowed plants to begin to flower and to begin foliar turnover/senescence. It was particularly useful to consider onset of flowering and how it might be related to %TN, %OC partitioning in the plant as well as how belowground and aboveground biomass respond to the presence of nutrients in the substrate. Multiple seasons and sampling times would provide more data to better understand how substrates affect seed fecundity, turion production and yield of above and belowground biomass over multiple seasons. This research established that sediments and substrates encouraged various components of yield (flowering, aboveground, belowground biomass, stem densities, lengths), with the possibility of consequently improving restoration success. Next steps to consider would be methodologies for transplantation into the field, where, if optimal conditions don't exist, there is the possibility to create an environment that is able to introduce optimal circumstances whereby plants can successfully establish.

2/ Use of intact field cores of actual SAV bed sediments in microcosm experiments allowed for more complete investigation into the edaphic conditions that favor healthy SAV beds. It appears this approach has never been fully considered for any

of the mesohaline SAV bed sediments in Chesapeake Bay. In addition, intact, undisturbed cores provided insight into why SAV bed sediments, with their low redox, microbial populations, intermediate elemental levels, and moderate %TN levels, are able to produce the highest yield when compared with lower and higher fertility substrates that still appear to support biomass. It also emphasized the fact that the mere presence of aboveground biomass may not guarantee plant sustainability if flowering and yield are reduced.

3/ Similarities of textural fractions may be limited in name only, as fractions may not be of the same geological origin. Even where the elemental constitution appears similar, seemingly small differences in NPK ratios and organic matter can substantially change how a substrate or sediment affects yield and reproduction.

4/ The coarse fraction (>2mm) of sediment and substrates may add important elements that assist rooting or support other biota in the sedimentary environment, that in turn may increase yield. In addition, the coarse fraction may be of a nature to provide refractory materials that are metabolized at a different rate more amenable to plant nutrition than more labile materials, thereby also potentially affecting yield (Benelli et al. 2017). In this study and others, the coarse fraction portions of sediments consisting of woody debris and shells was used by plants to interweave root biomass, thereby providing anchorage for plants, and additional refugia for microorganisms and macroinvertebrates, subsequent enrichment of nutrients, and diffusion of porewater. This quality is compromised or altogether lost in natural environments where highly sorted, near-shore fining is found near breakwaters and other areas that disrupt natural wave to shoreline interactions (Palinkas and Koch 2012, Statton et al. 2013).

5/ Elemental nutrient evaluation using Mehlich 3 analysis allowed for characterization of both natural and horticultural substrates in a way that parameters for the highest yields were compared with those that resulted in reduced yields. NPK substrate ratios were of particular interest in that they can be tracked most closely and compared across species in many different settings. Because the reduced sedimentary environment is different from terrestrial settings, %TN may be one of the best ways to compare between treatments and availability to plants, whereas the P:K fraction may remain best compared using Mehlich 3. Given that lower levels of P corresponded with higher yield, it continues to be confirmed that P is much less important than N and K for *P. perfoliatus*, (in agreement with Talevska 2004), however additional values created across SAV species would be valuable, particularly when looking at multiple species assemblages.

6/ Percent TN in substrate, particularly if comprised of a majority (>90%) labile, <2mm fraction with >1.7% OM, is more easily assimilated by plants, and as a consequence may depress yield and reproduction (Figures 2.16, 2.17, 2.18, 2.19, 2.20).

P. perfoliatus is an important mesohaline and freshwater species found in temperate bodies of water globally (Ogden 1943). In Chesapeake Bay, restoration is still not entirely successful. In Germany where portions of Rhine river hydrology have been restored, *P. perfoliatus* is one of two species (the other being *S. pectinata*, also a mesohaline species) that did not revegetate naturally (Meyer et al. 2013). Meyer et al. (2013) concluded, after seven years, these two species are unlikely to regenerate on their own. This indicates that there may be something fundamentally different about *P. perfoliatus* ecology that makes it more difficult to restore than other species. Given its

importance to the Chesapeake Bay estuarine ecology, and in light of the fact that there are still a number of healthy *P. perfoliatus* beds, additional management actions to restore this species are highly advised. This research will ideally follow up with the establishment of appropriate *P. perfoliatus* site conditions in Chesapeake Bay that can serve as a model for more successful and sustainable restorations (Figure 2.21).

CHAPTER 2:

Tables

Table 2.1. Analysis of substrate particle size and texture for <2mm fraction for a subset of samples for each treatment, and %>2mm fraction of sample (number in parenthesis represents total (n) samples. Greater interest was placed on native SAV bed sediments. Letters denote statistically significant differences for % sand, silt and clay. Oyster/peat heterogeneity contained too large a percent of refractory matter, and so it was excluded it from <2mm analysis.

	<2mm fraction			>2mm	
Substrate (No. of samples)	**% sand**	**% Silt**	**% clay**	**%**	**Texture**
Sand (2)	97.61 ± 0.98 [b]	0.00	2.39 ± 0.98 [a]	1.3 ± 0.2[ab]	sand (2)
Oyster (3)	87.14 ± 3.41 [a]	3.63 ± 2.09 [a]	9.23 ± 3.46 [b]	71.6 ± 1.5[d]	sandy loam (1), loamy sand (1), sand (1)
Soil/sand (2)	92.54 ± 1.48 [a]	4.00 ± 0.70 [a]	3.46 ± 0.78 [ab]	2.6 ± 0.2[ab]	sand (2)
Oyster/peat	NA	NA	NA	58.0 ± 1.8[c]	NA
Sherwood Forest (9)	91.16 ± 0.37 [a]	2.74± 0.39 [a]	6.10 ± 0.33 [ab]	3.6 ± 0.9[b]	sand (8), loamy sand (1)
Kent Narrows (11)	86.75 ± 1.76 [a]	8.75 ± 1.76 [b]	4.51 ± 0.53 [a]	0.47 ± 0.1[a]	loamy sand (7), sandy loam (1) sand (3),

Table 2.2. Mehlich 3 results for four greenhouse substrates and two native sediments taken from *P. perfoliatus* beds. All values are mg/Kg. **Bolded = $F_{(5,11)}$.P value.** *Subscript letters next to mean indicate significant difference (at the 5% level).

	Substrates (number of samples)					
Property	sand (2)	oyster (2)	soil/ Sand (2)	oyster/ peat (2)	Sherwood Forest (5)	Kent Narrows (4)
P						
Range	1.8 - 1.9	2.3 - 3.1	305 – 339	6.6 – 8.5	14 – 16	16 – 21
*Mean	1.8[a]	2.7[a]	322[b]	7.6[a]	14.4[a]	18[a]
S.E.	0.05	0.4	17	1.0	0.5	1.8
F=620.08, 0.0001						
K						
Range	9.8 – 11.6	26.8 – 33.7	96.6 – 118.5	48 – 56	54 – 166	36 – 59
Mean	10.7[a]	30.3[ab]	107.5[ab]	52[ab]	110[b]	46[ab]
S.E.	0.91	3.41	11.0	4.1	27	5
F=4.87, 0.0135						
Ca						
Range	64–97	31805–32633	3031–3443	9639 – 11579	352 – 629	271 – 516
Mean	80[a]	32218[d]	3237[b]	10609[c]	458[a]	377[a]
S.E.	17	414	206	970	80	35
F=1635. 0.0001						
Mg						
Range	14 – 19	307 – 310	236 – 251	269 – 299	168 – 285	72 – 113
Mean	17[a]	309[c]	244[c]	284[c]	220[bc]	100[a]
S.E	2.5	1.2	7.7	14.6	17	8
F=25.9, 0.0001						
Mn						
Range	0.3 - 0.4	10.4 – 10.6	31.1 – 31.8	9.7 – 12.3	35.0 – 122.8	6.1 – 18.9
Mean	0.38[a]	10.5[ab]	31.4[ab]	11.0[ab]	67.3[b]	9.78[a]
S.E.	0.04	0.07	0.35	1.30	27.76	4.51
F=4.99, 0.0125						
Zn						
Range	0.73 – 0.75	3.07 – 4.02	9.88 – 10.93	6.46 – 7.31	5.10 – 16.68	12.72 – 23.50
Mean	0.74[a]	3.54[a]	10.41[ab]	6.88[ab]	8.19[ab]	16.53[b]
S.E.	0.01	0.48	0.53	0.43	3.35	2.15
F=5.94, 0.0067						
Cu						
Range	0.19 – 0.24	1.24 – 1.36	3.45 – 3.87	1.35 – 1.94	1.10 – 1.45	6.25 – 9.01
Mean	0.21[a]	1.30[a]	3.66[b]	1.65[ab]	1.27[a]	7.57[c]
S.E.	0.03	0.06	0.21	0.30	0.12	0.29
F=53.1, 0.0001						
Fe						
Range	23.2 – 25.9	14.50 – 15.26	176.3 – 201.7	132.6 – 137.3	234 – 320	189 – 306
Mean	25.0[a]	14.88[a]	189.0[bc]	134.9[ab]	270[c]	235[bc]
S.E.	1.34	0.38	12.70	2.35	19	22
F=22.51, 0.0001						
B						
Range	0	0.49 – 0.57	1.08 – 1.10	1.04 – 1.07	0.11 – 0.56	0.31 – 0.60
Mean	0[a]	0.53[b]	1.09[c]	1.05[c]	0.29[ab]	0.40[b]
S.E.	NA	0.04	0.01	0.01	0.12	0.08
F=25.5, 0.0001						
S						
Range	5.7 – 6.6	81 – 83	337 – 442	267 – 318	315 – 469	173 – 304
Mean	6.1[a]	82[ab]	390[cd]	293[cd]	418[d]	244[bc]
S.E.	0.4	0.70	53	25	2.8	1.6
F=21.12. 0.0001						
Al						
Range	47.2 – 61.5	1.3 – 2.9	75 – 84	4.6 – 4.7	237 – 275	177 – 243
Mean	54.4[ab]	2.1[a]	79[b]	2.6[ab]	253[c]	216[c]
S.E.	7.12	0.8	4.8	0.06	2.0	0.3
F=91.18, 0.0001						

Table 2.3. Substrate Treatments % Total Carbon, % Organic Carbon, Bicarbonate, Nitrogen and organic Carbon:Nitrogen ratio based on Loss on Ignition (LOI) and %TC and %TN analysis. Subscript letters next to mean±S.D. indicate significant differences (5% level).

Substrate	%OM	% OC (LOI OM/2)	%Bicarbonate	%Tot C	%TN	%OC:%TN
sand	0.073 ± 0.01[a]	0.036 ± 0.006 [c]	1.20 ± 0.06[a,b]	1.23 ± 0.05[b,c]	0.08 ± 0.032[b,c]	0.42 ± 7.30[b]
Sherwood Forest	0.47 ± 0.04[a]	0.23 ± 0.02 [c]	0.11 ± 0.02[b]	0.35 ± 0.013[c]	0.02 ± 0.01[c]	10.62 ± 3.65[b]
oyster	0.80 ± 0.02[a]	0.40 ± 0.008[c]	9.18 ± 4.60[a]	9.58 ± 4.61[a,b]	0.15 ± 0.03[a,b]	3.13 ± 5.96[b]
Kent Narrows	0.86 ± 0.10[a]	0.43 ± 0.048[c]	0.103 ± 0.02[b]	0.49 ± 0.031[c]	0.03 ± 0.014[c]	12.96 ± 3.27[b]
soil/ sand	3.47 ± 0.02[b]	1.73 ± 0.15[b]	1.51 ± 0.82[a,b]	2.60 ± 0.95[b,c]	0.13 ± 0.02[a,b]	8.12 ± 5.16[b]
oyster/ peat	18.3 ± 0.03[c]	9.15 ± 0.23[a]	1.23 ± 0.050[b,c]	16.95 ± 6.86[a]	0.25 ± 0.03[a]	50.18 ± 7.30[a]

Table 2.4. % Total Carbon, % Total Nitrogen and C:N ratios for aboveground and belowground biomass. Subscript letters next to mean±S.D. indicate significant differences (5% level).

	% Total Carbon		% Total Nitrogen		C:N	
Substrate	AGB	BGB	AGB	BGB	AGB	BGB
sand	37.63 ± 0.15[a]	34.13 ± 2.60 [abc]	0.92 ± 0.10[a]	0.92 ± 0.12[a]	42.09 ± 4.35[a]	37.88 ± 2.54[b]
Sherwood Forest	37.94 ± 0.21[a]	30.12 ± 0.83[a]	1.22 ± 0.05[a]	1.27 ± 0.06[a]	31.48 ± 1.39[c]	26.17 ± 1.39[ab]
oyster	36.51 ± 0.80[a]	36.52 ± 0.68[c]	1.19 ± 0.06[a]	1.11 ± 0.06[a]	31.31 ± 1.92[a,b]	33.40 ± 1.83[ab]
Kent Narrows	37.60 ± 0.69[a]	36.39 ± 0.39[bc]	1.21 ± 0.06[a]	1.42 ± 0.07[ab]	33.35 ± 2.48[c]	23.99 ± 1.26[ab]
soil/ sand	36.48 ± 0.47[a]	34.12 ± 0.75[ab]	1.77 ± 0.11[b]	1.48 ± 0.16[b]	20.91 ± 1.30[a]	24.16 ± 2.64[b]
oyster/ peat	36.68 ± 0.55[a]	38.70 ± 0.94[c]	1.05 ± 0.07[a]	1.12 ± 0.03[a]	35.88 ± 2.92[a]	34.79 ± 1.64[b]

Table 2.5. Data from substrate pH, texture, Mehlich3, organic matter, carbon, and nitrogen analyses on six substrates. Sediment cores taken from healthy SAV beds were analyzed and used as a "ideal nutrient profile" to which other substrate values were compared, and bolded if in statistical agreement. (("o") Indicates not within order of magnitude). Superscript letters indicate statistical significance ("a") at the 0.05 level. Refractory materials ("®") indicate peat or oyster shell in coarse (>2mm) particles that are less reactive.

substrate ↓ substrate variable → treatment	Kent Narrows	Sherwood Forest	Oyster shell/Peat	Oyster	Soil/ Sand	Sand
pH (4 cm depth)	7.3-7.7 ^{a}	7.4-7.6 ^{a}	**7.7-8.0** ^{ab}	8.5-8.7 ^{c}	**7.6-7.8** ^{a}	8.3-8.6 ^{bc}
Eh (mV, 4cm depth)	195-255 ^{ab}	150-210 ^{a}	**215-245** ^{ab}	**260-325** ^{bc}	**230-300** ^{b}	370-415 ^{c}
% sand	84-87^{a}	90-92^{a}	no data$^{®}$	**(87)** $^{a\,®}$	**91-94**^{a}	96-99^{b}
% silt	7.5-9.5^{b}	2.50-3.0^{a}	no data$^{®}$	**(3.6)** $^{a\,®}$	**3.3-4.7** ^{b}	0
% clay	4.0-5.0^{a}	5.8-6.4^{ab}	no data$^{®}$	**(9.23)** $^{b\,®}$	**2.7-4.3** ^{b}	**1.5-3.3**^{a}
> 2%	0.40-0.50^{a}	2.4-4.4^{ab}	58.0$^{®}$	71$^{®}$	**2.4-2.8**^{ab}	**1.1-1.5**^{ab}
phosphorus (P)	16 – 21^{a}	14 – 16^{a}	**6.6 – 8.5**^{a}	**2.3 - 3.1**^{a}	305 – 339^{b}	1.8 - 1.9^{a,o}
potassium (K)	36 – 59^{ab}	54 – 166^{b}	**48 – 56**^{ab}	**26.8–33.7**^{ab}	**96.6–118.5**^{ab}	**9.8–11.6**^{a}
Calcium (Ca)	271 – 516^{a}	352–629^{a}	9639–11579$^{c®}$	31805–32633$^{b®}$	3031–3443 ^{b}	64–97 ^{a,o}
Magnesium (Mg)	72 – 113^{a}	168 – 285^{bc}	**269 – 299**^{c}	**307 – 310**^{c}	**236 – 251**^{c}	**14–19**^{a}
Manganese (Mn)	6.1–18.9^{a}	35.0–122.8^{b}	**9.7 – 12.3** ^{ab}	**10.4–10.6**^{ab}	**31.1–31.8**^{ab}	0.3-0.4^{a,o}
Zinc (Zn)	12.7–23.5 ^{b}	5.1–16.7^{ab}	**6.5–7.3**^{ab}	**3.1–4.0**^{a}	**9.9–10.9**^{ab}	**0.73–0.75**^{a}
Copper (Cu)	6.3–9.0^{c}	1.1–1.5^{a}	**1.35-1.94**^{ab}	**1.2–1.4**^{a}	**3.5–3.9**^{b}	0.19–0.24^{a,o}
Iron (Fe)	189–306^{bc}	234–320^{c}	**132.6–137.3**^{c}	14.5–15.3^{a}	**176.3–201.7**^{bc}	23.2–25.9^{a}
Boron (B)	0.31–0.60^{b}	0.11–0.56 ^{ab}	1.04–1.07^{c}	**0.49 – 0.57**^{b}	1.08–1.1^{c}	0
Sulfur (S)	173–304^{bc}	315–469^{d}	**267 – 318** ^{cd}	81–83^{d,o}	**337–442**^{cd}	5.7 – 6.6^{a}
Aluminum (Al)	177–243^{c}	237–275^{c}	4.6 – 4.7^{ab}	1.3–2.9^{a}	75 – 84^{b}	47.2-61.5^{ab}
% organic matter (OM)	0.86±0.10^{a}	0.47±0.04^{a}	18.3±0.03$^{c\,®}$	**0.80±0.02**^{a}	3.47±0.02^{b}	0.073±0.01^{a,o}
% organic carbon (OC)	0.43±0.048^{c}	0.23±0.02 ^{c}	9.15 ± 0.23$^{a\,®}$	**0.40±0.008**^{c}	1.73±0.15^{b}	0.036±0.006 ^{c,o}
% bicarbonate (%BIC)	0.103±0.02^{b}	0.11±0.02^{b}	**1.23±0.050**^{bc}	9.18±4.60^{a}	1.51±0.82^{ab}	1.20±0.06^{ab,o}
% Total Carbon	0.49±0.031^{c}	0.35±0.013^{c}	16.95±6.86$^{a,®}$	9.58±4.61$^{ab,®}$	2.60±0.95^{bc,o}	1.23±0.05^{bc,o}
% Total Nitrogen	0.03±0.014^{c}	0.02±0.01^{c}	0.25 ± 0.03$^{a®}$	0.15±0.03$^{ab,®}$	0.13±0.02^{ab}	**0.08±0.032**^{bc}
Statistically Significant w/in same order mag **(bold)**: S.S., in range, + including "refractory" material (gray) :			11/22 21/22	13/22 17/22	13/22 No change	6/22 No change
Biomass Response						
Aboveground biomass (g)	2.16±0.19^{d}	1.82±0.22^{cd}	1.56±0.13^{bc}	1.28±0.07^{bc}	0.89±0.14^{ab}	0.49±0.07^{a}
Belowground biomass (g)	0.82±0.05^{a}	0.93±0.18^{b}	0.88±0.15^{a}	0.48±0.05^{a}	0.44±0.07^{a}	0.56±0.09^{a}
Total biomass (AGB+BGB) (g)	2.98±0.21^{b}	2.75±0.36^{b}	2.44±0.25^{b}	1.76±0.11^{a}	1.32±0.21^{a}	1.05±0.15^{a}
Total no. stems	59.0±6.6^{b}	36.9±6.8^{a}	43.5±4.0^{ab}	30.6±3.0^{a}	34.1±2.0 ^{a}	28.6±3.5 ^{a}
Summed Stem lengths (cm)	1064.8±73.5^{b}	903.7±70.6 ^{ab}	675.4±53.1^{ab}	537.9±45.3^{a}	414.2±12.5^{a}	237.6±26.5^{a}
*Ave. stem length (w/flowers) (cm)	46.5±15.3	61.6±26	46.9±14	48.6 ± 18	39.6 ± 5	0
No. inflorescences	7.75±2.06 ^{ab}	14.0± 2.48 ^{b}	3.13±1.65^{a}	5.25±2.4^{a}	1.29±0.84^{a}	0
*Separate statistics were not performed on flowering stem lengths of combined turion populations, see Appendix C, Table C1 and Figure 2.10.						

Table 2.6. Total %N, Mehlich 3 plant available P and K (M3P, M3K) values, for six different substrates that supported variable growth of *P. perfoliatus*. All values are in mg/Kg other than percent Total Nitrogen (%TN). Soil sand is the only substrate with a P fraction higher than 1.

Substrate Trt → **Soil variable ↓**	**Kent Narrows**	**Sherwood Forest**	**Oyster/Peat**	**Oyster**	**Soil Sand**	**Sand**
%TN	0.03	0.02	0.25	0.15	0.13	0.08
M3P	18	14.4	7.6	2.7	322	1.8
M3K	46	110	52	30.3	107	10.7
%TN:M3P:M3K	0.03%:1:2.8	0.02%:1:7.6	0.25%:1:6.8	0.15%:1:11	0.13%:3:1	0.08%:1:5.9

CHAPTER 2:

Figures

Figure 2.1. Locations for source sites where plant population (turions) treatments and SAV bed sediment cores were obtained for microcosm experiments. Sherwood Forest/Brewer's Creek on the Severn River, and Kent Narrows/Chesapeake Bay Environmental Center (CBEC) near Marshy Creek were the sites of obtaining the sediment for two different submersed aquatic sediments: "Sherwood Forest" and "Kent Narrows" sediments. Muddy Creek and Severn River were the sources for the two plant treatment populations of "Kent Narrows" and "Sherwood Forest" turions used for the experiment.

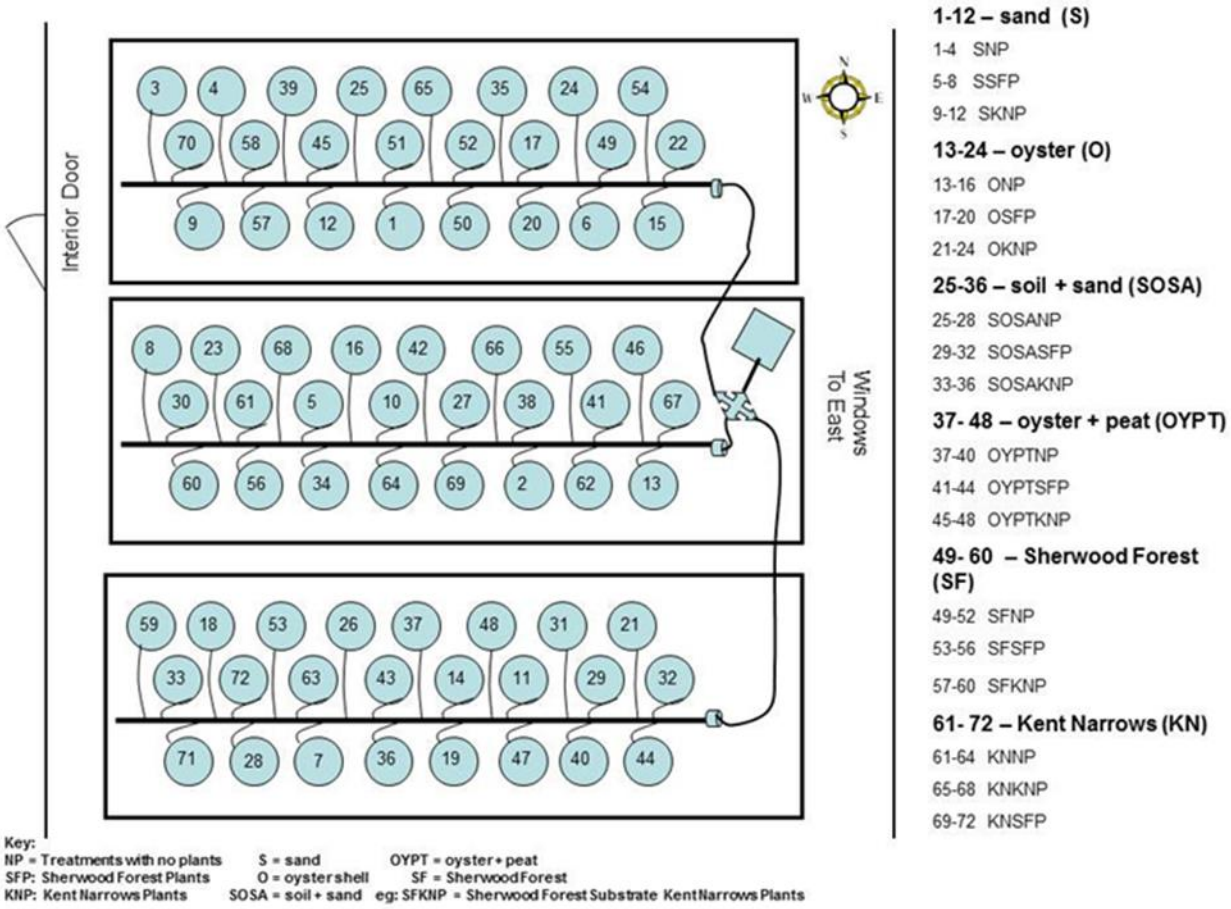


Figure 2.2. Microcosm array in the greenhouse. The circles with numbers indicate the random placement of the microcosm treatments described and numbered at right.

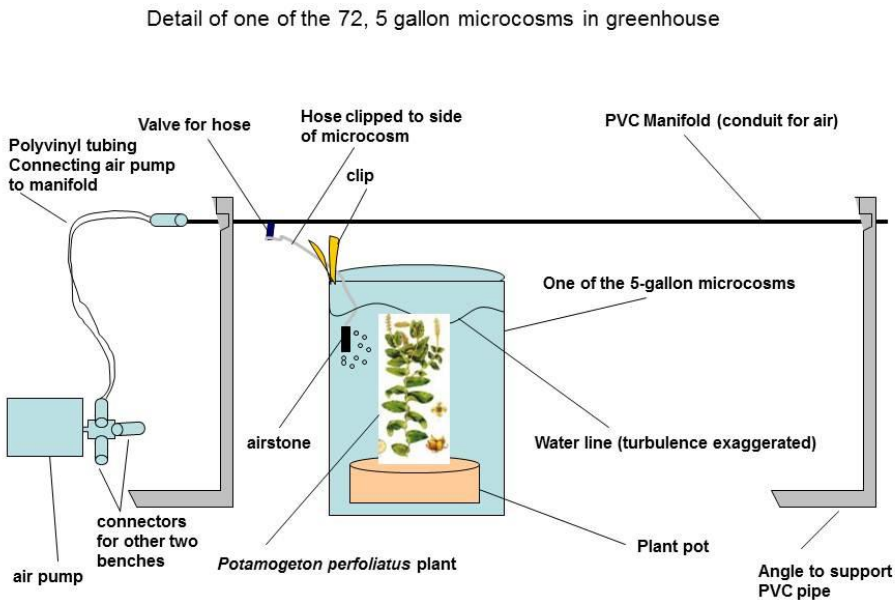


Figure 2.3. Design of manifold with one of the microcosms. In the experimental set-up the 72 microcosms were arranged side by side on three benches as in Figure 2.2.

Figure 2.4. Average turion length (summed - mm) per microcosm was slightly higher Sherwood Forest turions than for Kent Narrows turions, although the standard error was more variable for Kent Narrows. Turion length was equitable among substrate treatments.

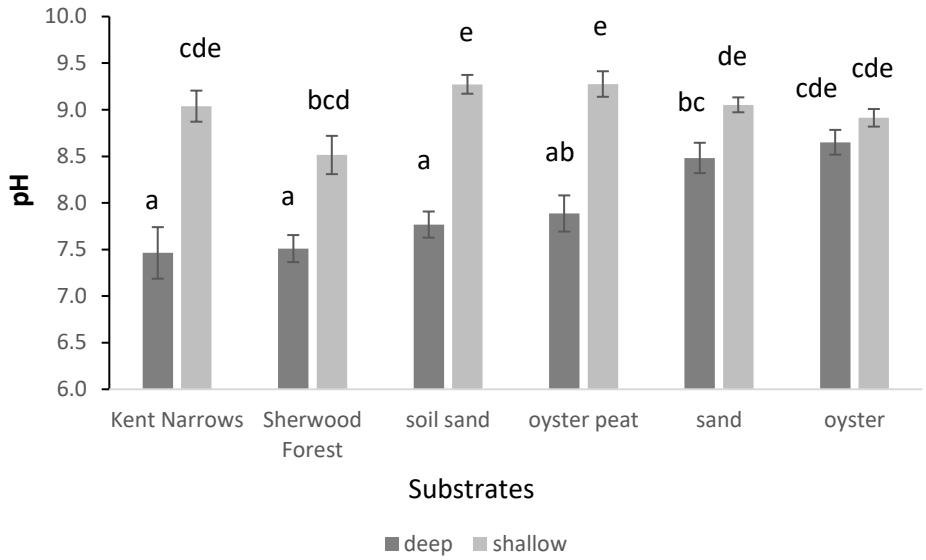


Figure 2.5. Average pH measured near the surface of the sediment (shallow) and at four cm (deep) for six substrate treatments in microcosms. Subaqueous sediments taken from SAV beds at Kent Narrows and Sherwood Forest, along with soil sand substrate, had the lowest pH, while the more alkaline caps of oyster and sand had slightly higher pH.

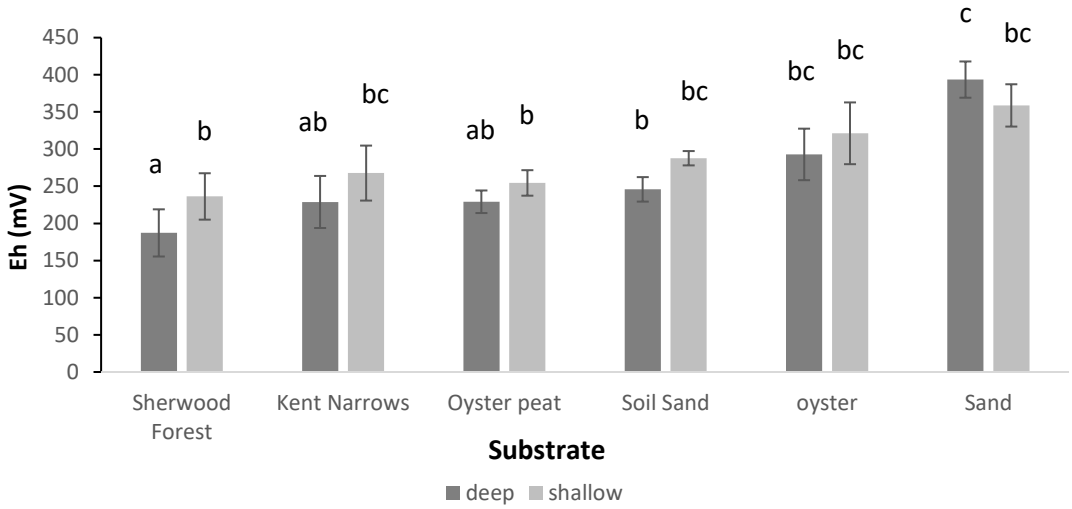


Figure 2.6. Sediment oxidation reduction (redox) measurements for shallow and deep substrates. Eh (in mV) was measured at just below the sediment surface (shallow), and at 4 cm depth (deep). Deep measurements for Kent Narrows and Sherwood Forest, and the oyster shell and peat substrate had the most reduced environments.

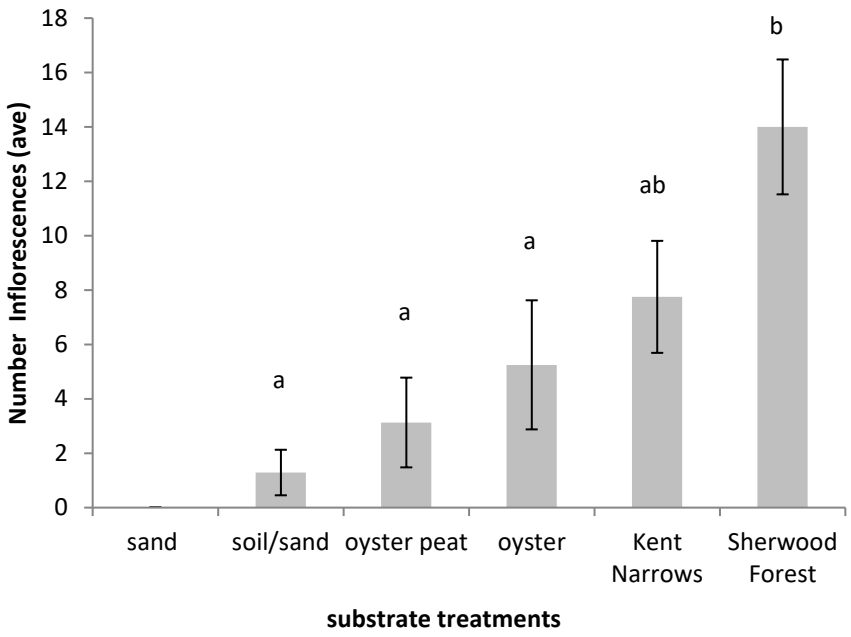


Figure 2.7. Average number of inflorescences at end of experiment harvest per substrate.

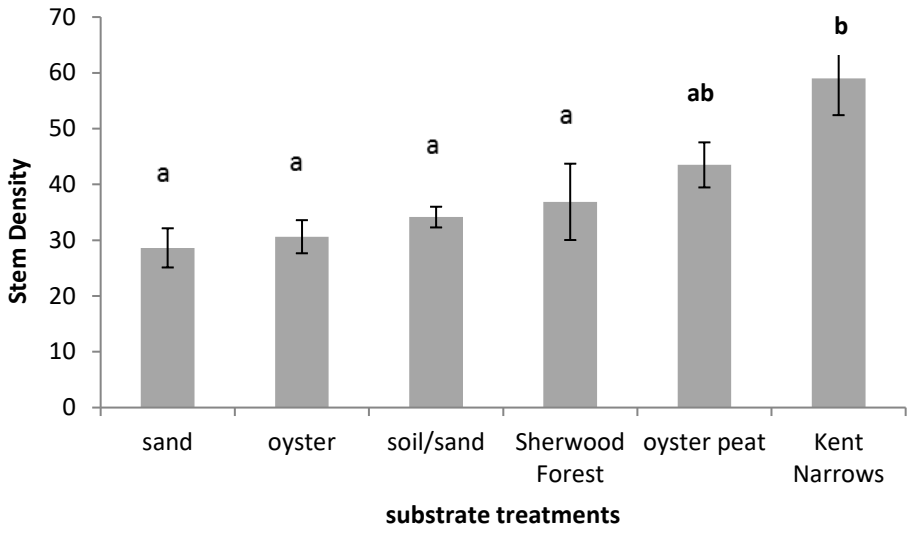


Figure 2.8. Stem densities for six different substrate treatments.

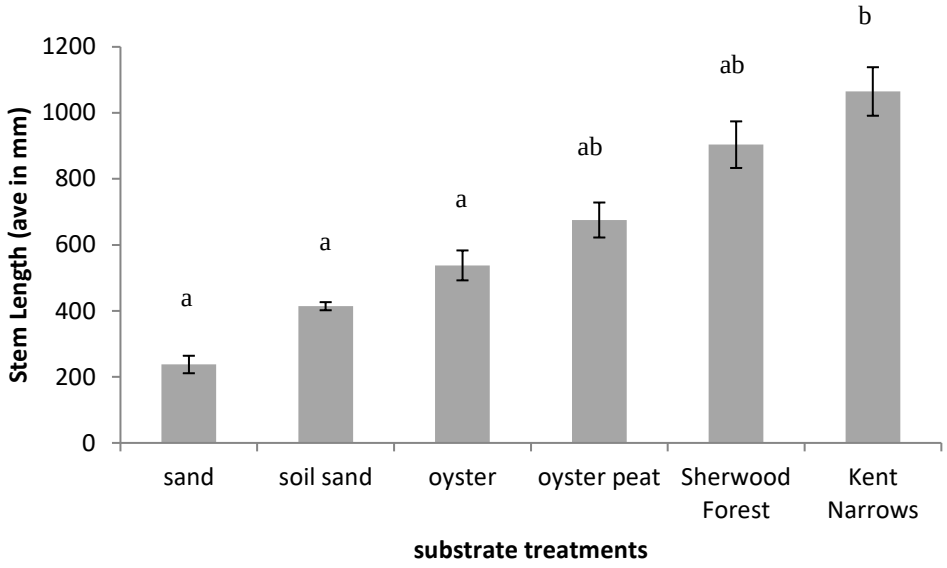


Figure 2.9. Average stem lengths (mm) per microcosm for six different substrate treatments.

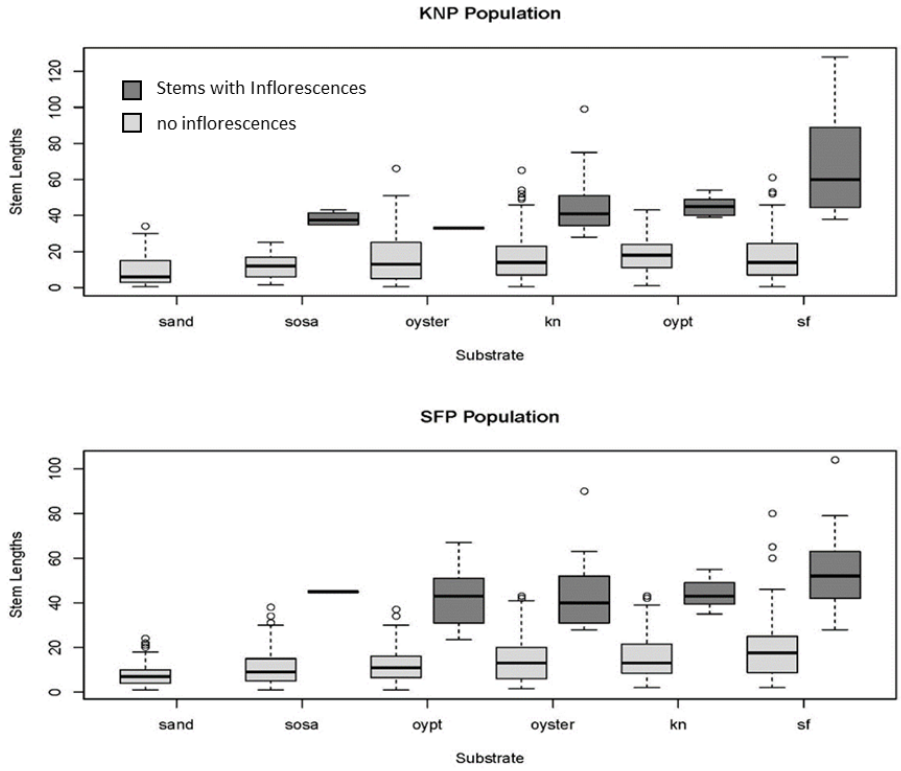


Figure 2.10. Stem length as it correlates to presence and absence of flowers for six substrate treatments and two *P. perfoliatus* populations. The data indicates that longer stems (23.5 cm and greater) have a greater likelihood to bear flowers, than stems measuring under 23.5 cm (zero flowers borne on those stems). Substrate influences both stem lengths and is also a factor in presence/absence of flowers. Data in Tabular Form in Appendix C, Table C.1.

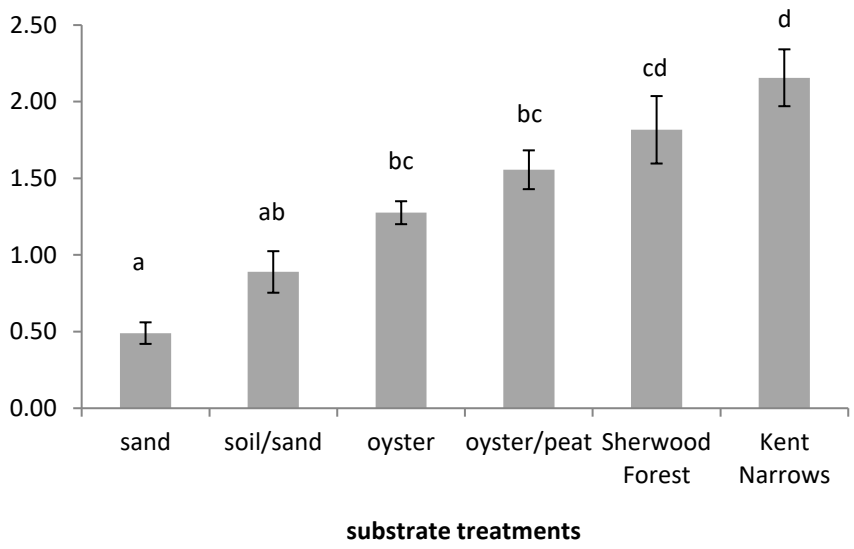


Figure 2.11. End of experiment aboveground biomass (g) growth for six substrates (15 weeks).

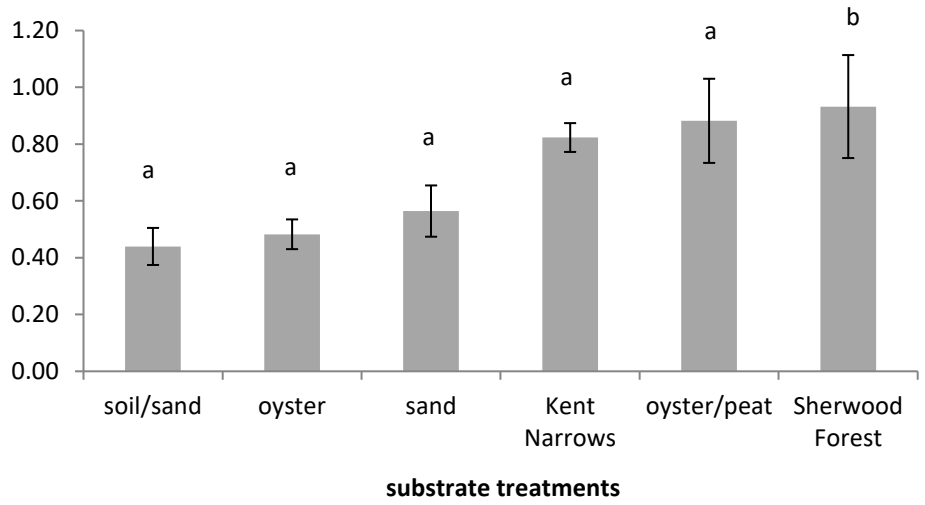


Figure 2.12. Belowground biomass (g) for *P. perfoliatus* grown on six different substrates, two subaqueous soils (Kent Narrows, Sherwood Forest) and the four greenhouse substrates.

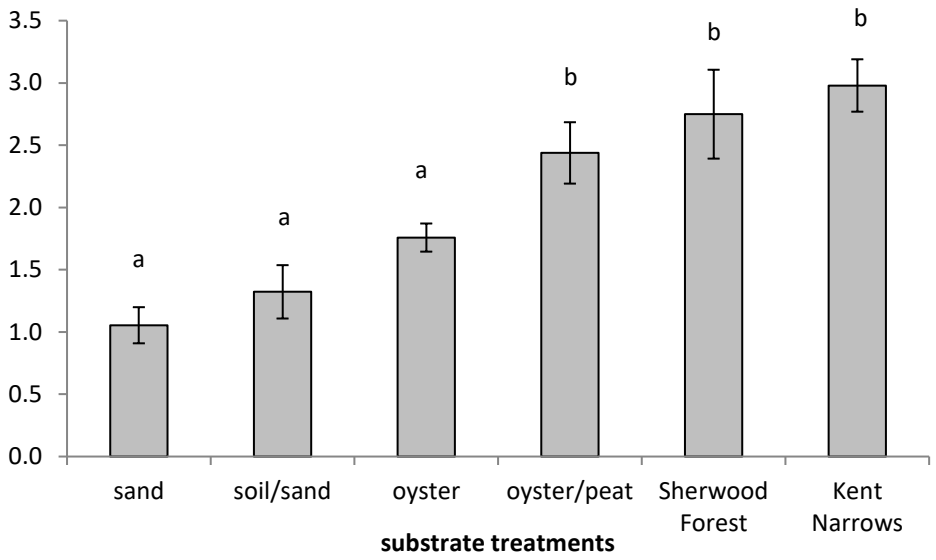


Figure 2.13. Aboveground and belowground biomass summed (g) for each substrate, both plant populations combined.

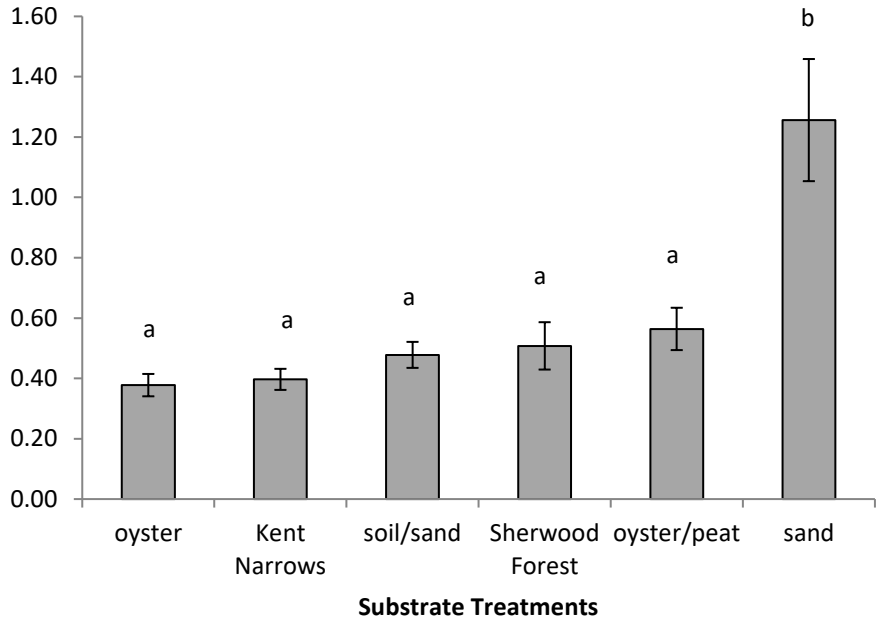


Figure 2.14. Root:shoot ratios for grams BGB:AGB for *P. perfoliatus* turions grown in six substrate treatments.

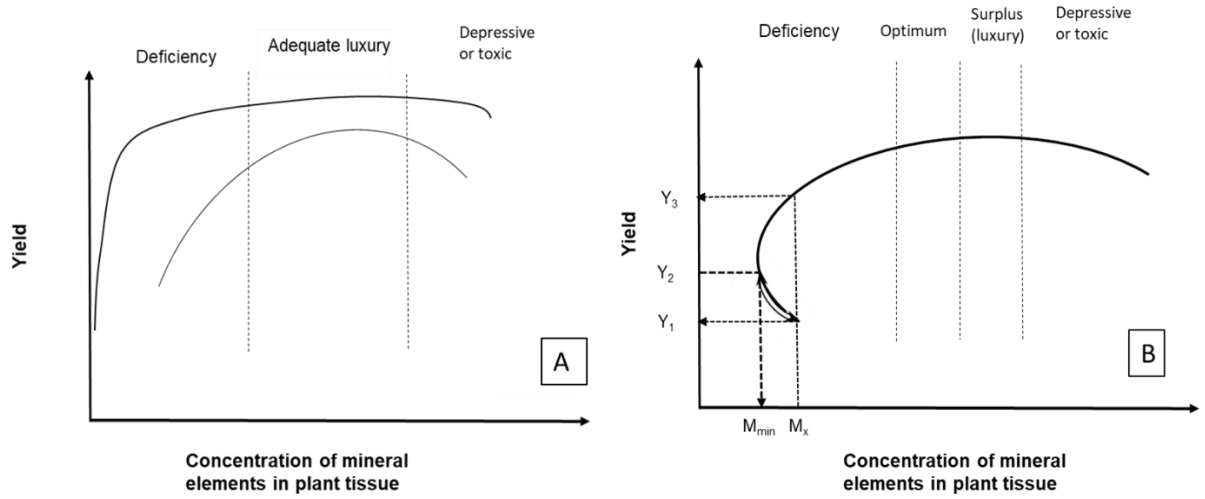


Figure 2.15. Schematic representations of nutrient uptake and yield curves. A: (redrawn from Reid 2002, Bates 1971) illustrates two growth scenarios with nutrient concentration into plant tissue with different availability in soil. B: (redrawn from Larcher 2003, ref. Wikstrom 1994, Bates 1971) During rapid growth the uptake of mineral substances is slower than the increase in biomass ($Y_1 \rightarrow Y_2$), the concentration of mineral substances in plant tissue may even drop temporarily from M_x to M_{min} ("dilution effect"). This occurs when mineral uptake is not proportional to another important element, such as C. Element concentrations are usually sufficient to support plant tissue for "optimal" yields ($Y_1 \rightarrow Y_3$). "Surplus" or luxury levels in plant tissue may be a benefit to the plant, or, as can be the case with depressive levels of N, prematurely increase shoot development while decreasing root biomass. In some species there can also be a delay in reproduction, or increase vulnerability to herbivory. Plants tolerate a larger range of macronutrients before they are at depressive or toxic levels, while it takes a much smaller range of trace nutrients to disrupt plant growth.

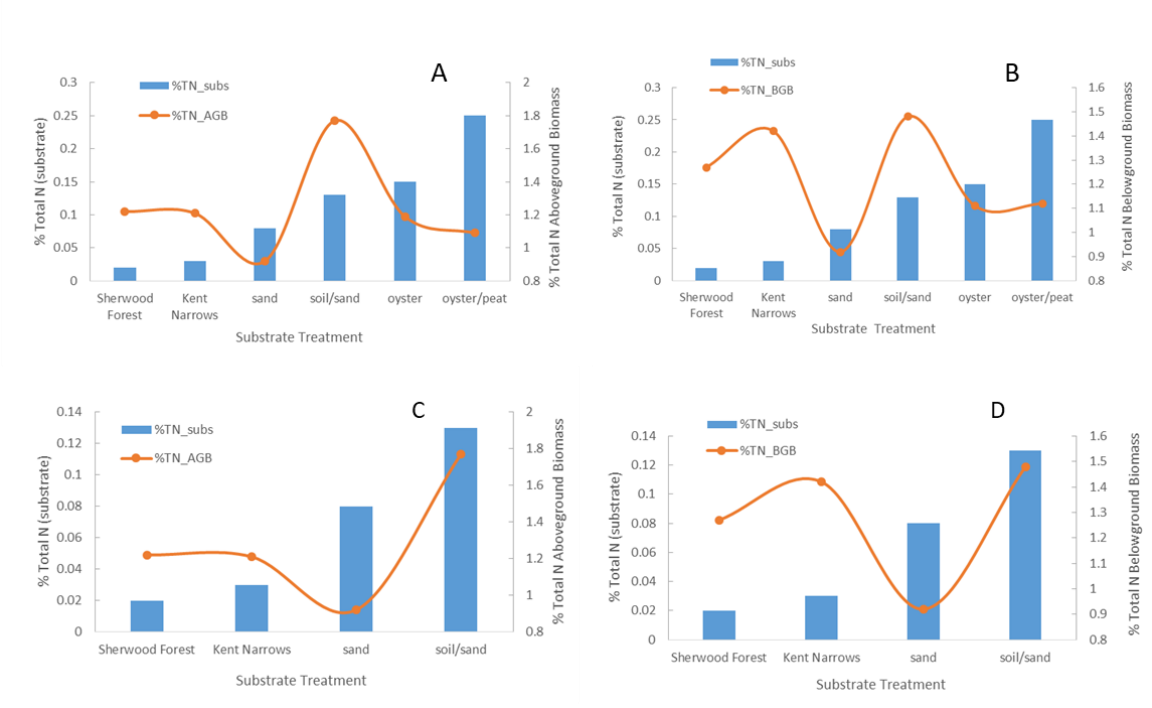


Figure 2.16. The relationship between % Total N in the substrate and corresponding %TN in plant tissue (AGB and BGB). As reported elsewhere, there is rarely a consistent relationship between increase in N in sediment and N taken up by the plant (Larcher 2003). The N uptake responses shown here are after 15 weeks growth, and may be variable depending early, middle (reproductive), and senescence portion growth cycles.

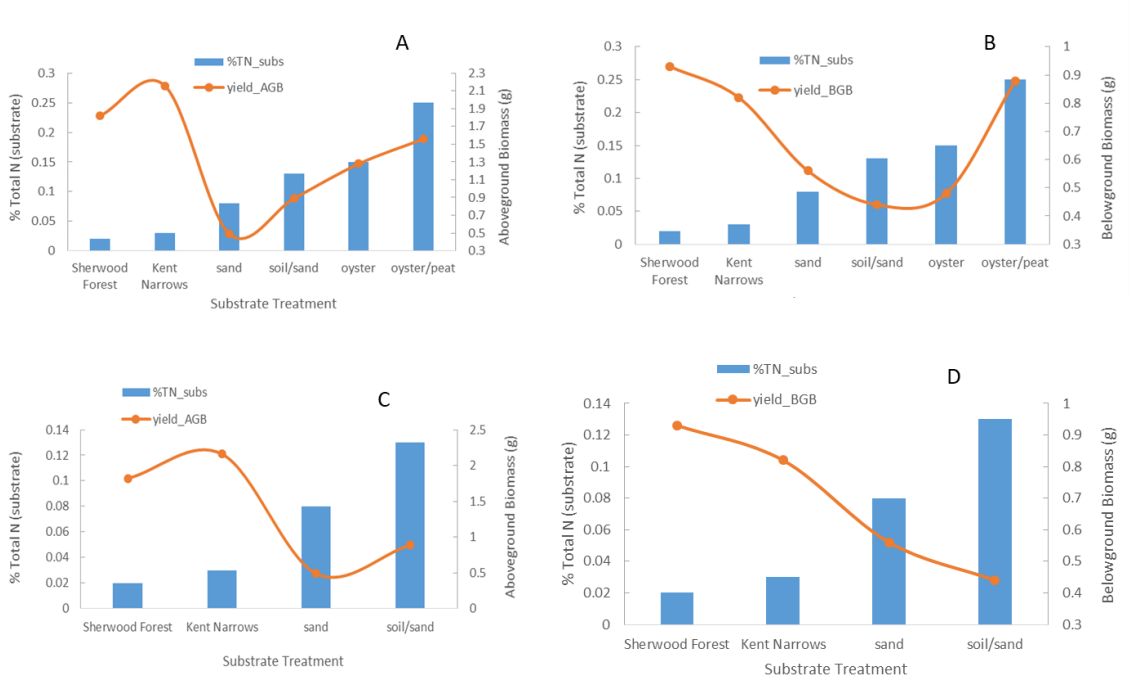


Figure 2.17. The relationship between % Total N in the substrate and corresponding yield in plant tissue (AGB and BGB). Figures A and B indicate the differences in substrate treatment lability (availability) of N and corresponding yield. When the most labile, (<2mm) substrate treatments are considered only (C and D), it appears that a substrate containing the %TN similar to either washed sand or soil/sand may negatively affect *P. perfoliatus* growth.

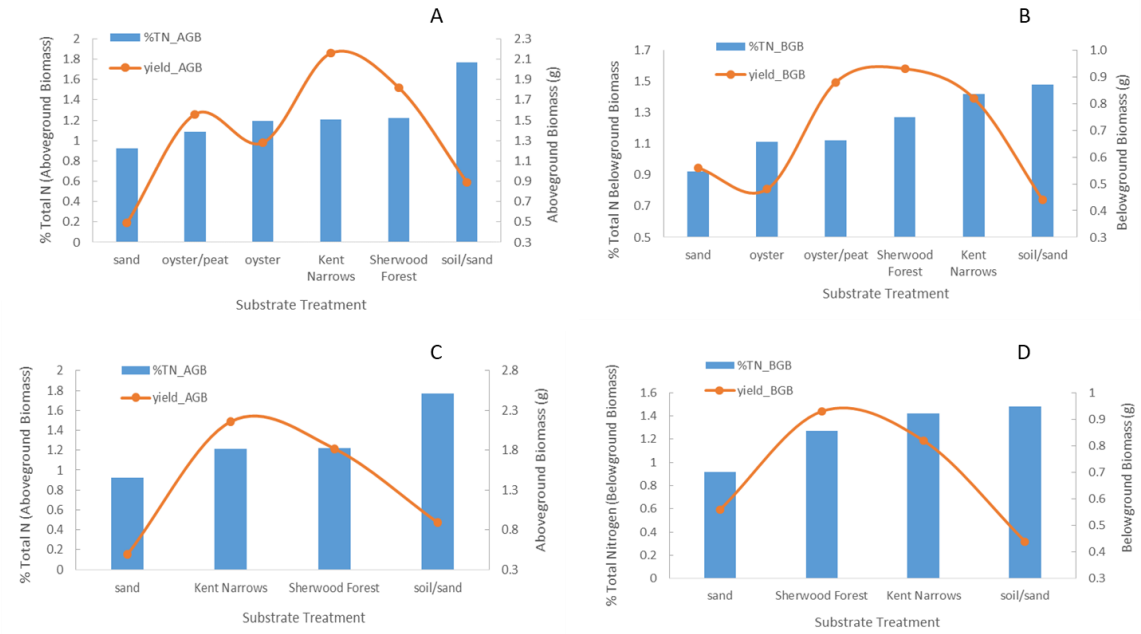


Figure 2.18. Relationship between % Total N in above- and belowground plant tissue and yield (aboveground and belowground biomass). The trend shows slight decreases in yield for refractory oyster, due to the fact that there is a lower quantity of labile, available N. When considering substrate treatments with the 87% or greater fine fraction (C and D), depressive growth is particularly apparent with increasing tissue concentration of %TN. This dynamic is in agreement with the theoretical curve in Figure 2.15 (Larcher 2003).

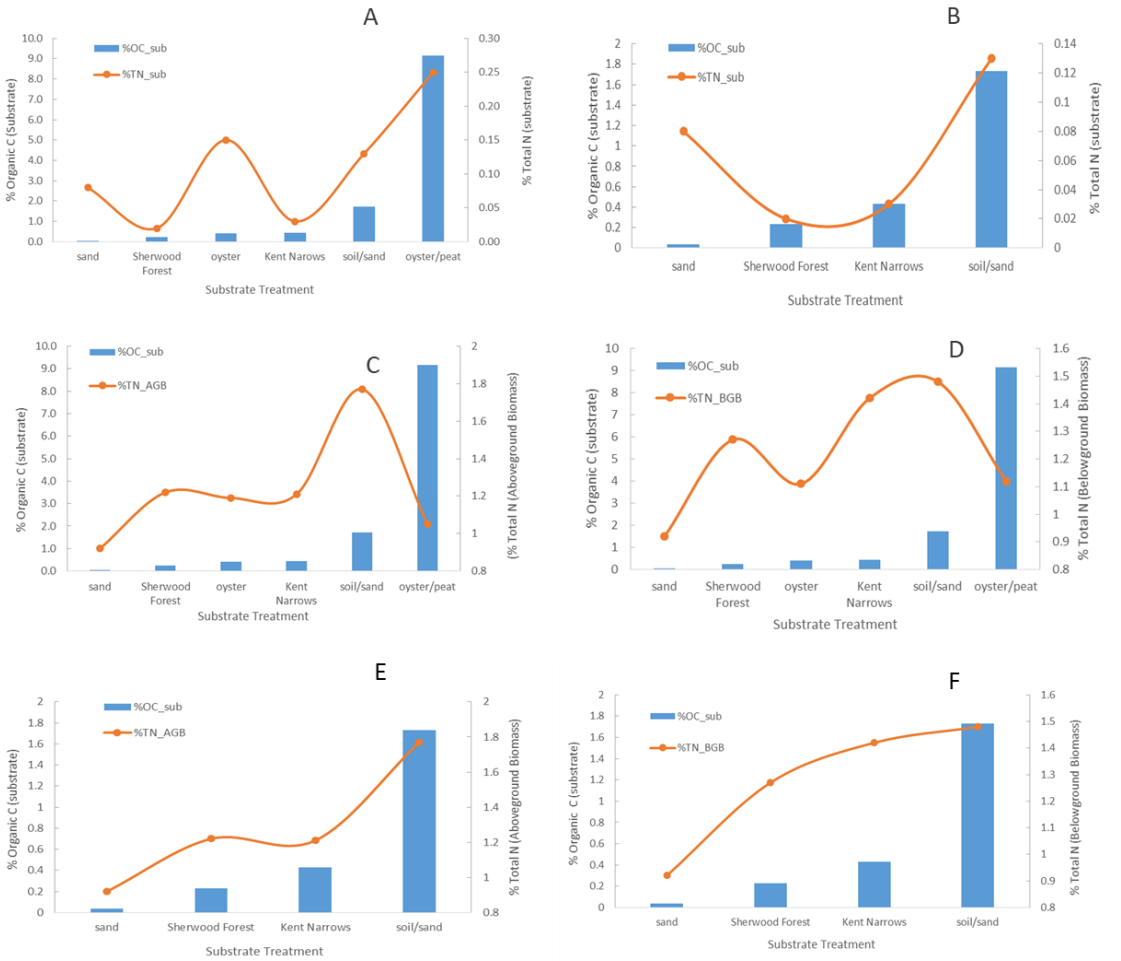


Figure 2.19. With the exception of washed sand, A and B indicate that increasing % Organic C may be a reasonable predictor of increasing in %TN in substrate, but only if particle size and nature are taken into account. Percent OC and %TN in plant tissue (C, D, E and F) are more strongly correlated than %OC with %TN substrate. Oyster and oyster/peat indicate lower %TN uptake with their highly refractory %OC contents compared with the more labile substrate treatments. The < 2mm (E and F) fraction indicates a general trend with increasing %TN uptake in plant tissue for labile fractions containing increasing %OC. Percent TN uptake is variable and dependent upon season and life stage of the plant (i.e. whether early season, flowering, peak growth, or senescing, phases). This indicates that the quality and quantity of organic C (organic matter, humic substances, etc.) may serve as an important vessel for plant nutrients including N, and may be a more reliable indicator than %TN in substrate for plant tissue uptake of %TN for the <2mm, labile treatments considered here (E and F).

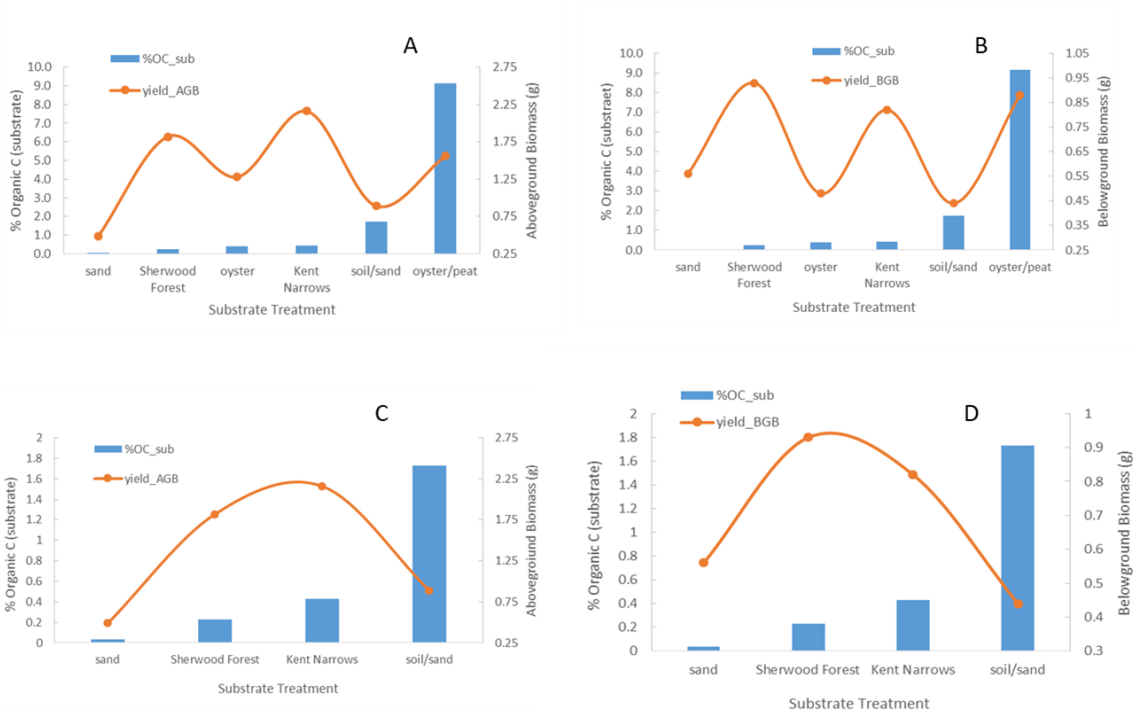


Figure 2.20. The relationship between %OC and yield for both > 2mm and < 2mm substrates. Larger and more refractory particle sizes of the substrate treatments (A and B) appear to reduce effect of any %TN contained in the organic component of the substrate and therefore have a less negative impact on yield. Finer particles and more labile %OC in substrate appear to be a better indicator of yield response than %TN in substrate, and are more similar to the yield response from actual %TN in plant tissue.

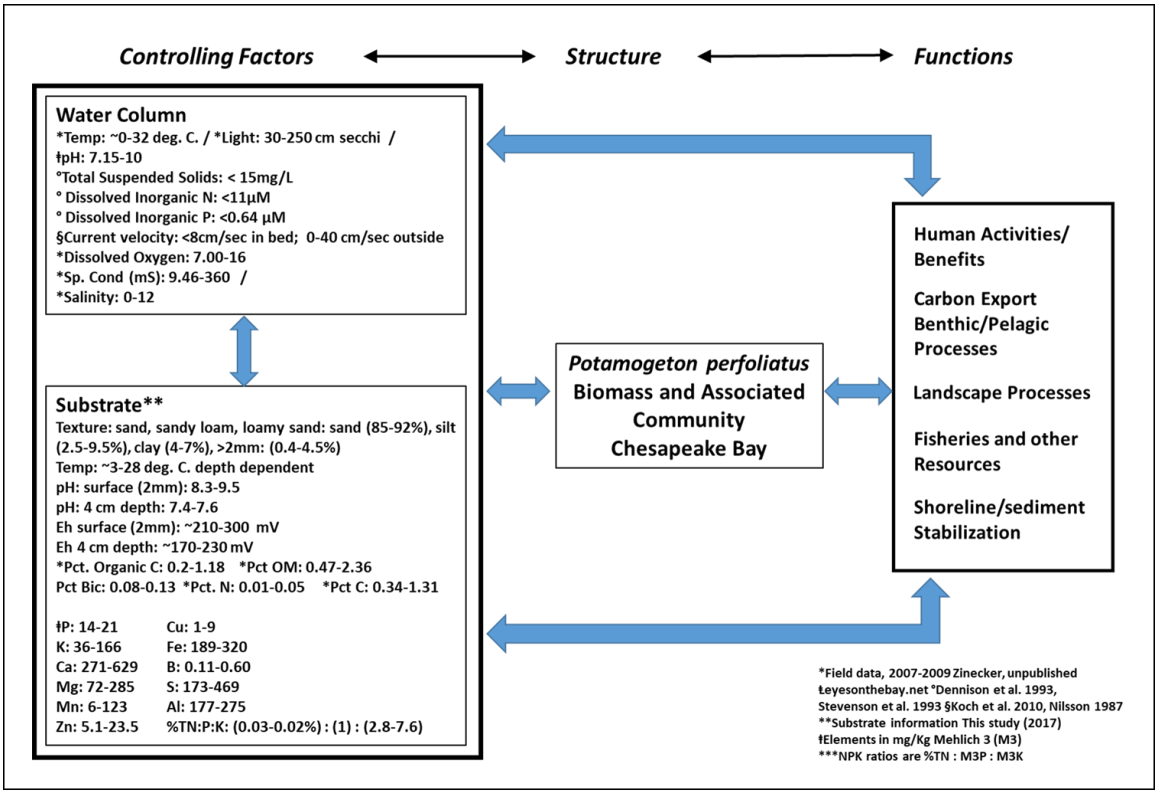


Figure 2.21. Some important environmental factors that sustain *P.* perfoliatus beds and the functions they support (based and expanded from Thom et al. 2005, for eelgrass). Here the basic elements of controlling factors, structure, and functions are shown to feedback on one another. Values for substrata have been poorly defined in the field. The substrate data are based on sediment samples and cores taken from *P. perfoliatus* beds in Chesapeake Bay, (some of which are in Zinecker CH2, this study, some unpublished). Sediment analyses are based on <2mm fraction of soil, however, the >2mm fraction, and the microbial component, may also play an important role in SAV bed sustainability, structure and function).

CHAPTER 3:

Use of a biodegradable pot, and seeds from different harvest years to improve *Potamogeton perfoliatus* L. restoration

INTRODUCTION

Diminished submersed aquatic vegetation (SAV) beds in littoral zones around the globe, and at some sites in Chesapeake Bay, are recovering where management efforts have reduced phosphorous, nitrogen, and suspended sediment loadings (Gurbisz and Kemp 2014, Waycott et al. 2009). These water quality factors may often improve in conjunction with a reduction of environmental disturbance, or where meteorological or climate-related patterns such as drought create more favorable conditions for SAV bed expansion (Gurbisz and Kemp 2014, Orth et al. 2015, Stevenson et al. 1993).

However, in the mesohaline (10-18 parts salinity) portion of Chesapeake Bay, SAV bed inventories have indicated more modest increases in coverage. The recovery has largely indicated that beds are more fragmented than in previous years, and contain fewer species than historic coverages (Orth et al. 2015, 2017). Redhead grass (*Potamogeton perfoliatus* (L.)) was historically a dominant species in Middle Chesapeake Bay, but at present covers only 30 percent of its original range prior to the 1960's (Brush and Hilgartner 2000, Orth et al. 2015). The SAV species widgeon grass (*Ruppia maritima* (L.)), now occupies much of its former habitat (Orth et al. 2015). This shift in species is of concern because studies of both terrestrial and aquatic habitats have demonstrated that reductions in biological diversity can affect overall productivity, community stability and ecosystem function (Shields and Moore 2016, Zak et al. 2003, Engelhardt and Ritchie 2002, Folke et al. 2004, Booth and Grime 2003, Tilman 2006).

SAV communities in Middle Chesapeake Bay are highly impacted by land-based runoff pollution and shoreline modifications (Zhang et al. 2015, Murphy et al. 2014,

Dennison et al. 1993, Landry and Golden 2017). Disturbance caused by riprap and bulkhead shoreline protection in the populous areas of the mesohaline have resulted in only 24% of the potential SAV habitat to be covered with vegetation in a given study area (Patrick et al. 2016). As of 2010, pollution from chemical contaminants had impaired up to 72% of the Bay and its tidal river segments (USEPA 2011), and a portion of this percentage is accounted for in the Middle Bay tributaries that correspond with higher population, housing, and industry density (Sexton et al. 2013, USEPA 2011).

In light of these anthropogenic impacts, there continues to be a need for improved SAV restoration approaches in Chesapeake Bay and many other impacted, aquatic coastal habitats. Existing SAV beds may benefit from supplemental plantings to become more diverse and sustainable (Lotze et al. 2011, Cuttriss et al. 2013). SAV restoration technologies have the potential to create stronger feedbacks for grass beds using recruitment and transplantation methods that are site specific, and demonstrate an understanding of the life histories of the plants (Strazisar et al. 2016). However, research providing a detailed description of their preferred edaphic conditions is still lacking (Shields and Moore 2016, Muenscher 1938, Orth et al. 1994, Marion and Orth 2010b).

Restoration success may be diminished by propagation and transplant methods that disturb propagules in transit and relocation, don't properly evaluate site or substrate conditions, or fail to establish sufficient contact/security in the sediment (Shields and Moore 2016, Golden et al. 2010, Orth et al. 2008, Marion and Orth 2010a). Addy's work in the 1940's outlined general requirements for *Zostera marina* L. (eelgrass) restoration using both transplants and seeds, including harvest and storage guidelines (1947a, 1947b, Addy and Aylward 1944, Fonseca 2011). Research specific to substrate and restoration

for seagrasses was also reported by van Breedveld (1975). These beginnings formed the foundation for more recent, large scale restoration planting techniques with eelgrass seeds. These restorations are in the polyhaline portions of Chesapeake Bay, and have been successful primarily due to availability of appropriate substrate, ample light, acceptable water quality, and eelgrass seed biology (Pickerell et al. 2005, 2006, Orth et al. 2003, Granger et al. 2002, Orth et al. 2010, Orth et al. 1988). Higher than usual water temperatures have been attributed to native and restored eelgrass bed die-offs, and this continues to be a concern (Orth et al. 2010).

At present, the emphasis on restoration of *P. perfoliatus* in Middle Chesapeake Bay, continues to be on using prior and developing knowledge of seed biology and subsequent protocol development to use seeds for large scale restorations (Ailstock et al. 2010a, Ailstock et al. 2010b, Shafer and Bergstrom 2010). Seeds represent a lower level of effort and output to store, prepare, and distribute in the natural environment rather than transplanting each adult shoot by hand, however, the prospect of loss or movement in the sediment after broadcast seeding can range from highly variable to complete lack of success (Stephen Ailstock, personal conversation, Orth et al. 1999, Orth et al. 2008).

Research on seed viability for seagrass species such as *Z. marina*, indicates a range of months in the longevity of seeds that may be due to different harvest and storage conditions. For example, under active aeration, optimal cold storage conditions and proper salinity, *Z. marina* seeds may retain high viability for a little over a year (Jarvis 2014; Dooley et al. 2013; Granger et al. 2002). Jarvis et al. (2014) found that seeds in *Z. marina* bed seedbanks germinated at a rate of less than 5% of the remaining seeds after 15 months. Dooley et al. (2013) found that while 32% of *Z. marina* seeds germinated

after four years in cold storage, approximately only 5% of the germinated seeds produced seedlings that developed leaves. For *Vallisneria americana* L., Campbell (2005) reported seed viability up to 2-3 years in unaerated, sealed storage containers at 3-4 deg. C. Because of concerns for contamination and precocious germination of *V. americana*, Kauth and Biber (2014) experimented with relative humidity and temperature experiments and determined that optimal viability of *Vallisneria* could be retained for up to six months when stored at 3 deg. C. at 25% moisture content. Statton et al. (2013) found that various tank culture conditions enabled seedlings of *Posidonia australis* Hook. F. to be available for purposes of restoration for at least seven months.

Seed collection, storage and germination research on *P. perfoliatus* by Ailstock et al. (2010a) indicated *P. perfoliatus* seeds require aeration to avoid germination while in cold storage (at 4 deg. C), and also for optimal germination at the time of post-storage induction. Ailstock et al. (2010a) also found that the best storage conditions for germination post-harvest included water conditioned within the range of 0-15 parts salinity, and that optimal germination induction ranged from 6-9 months of storage. Ailstock et al. (2011) found that seeds of *P. perfoliatus* germinated in freshwater cold storage (sometimes as high as 30%) in passive aeration (container open to air), if left longer than six months in storage before germination induction. Muenscher (1938) found this same dynamic with other seeds of Potamogeton species, and that seed germination was high upon induction after five to six months cold storage, but then reduced after 12 months of cold storage. Muenscher (1938) additionally determined that germinated seeds kept in cold storage continued to be viable when later exposed to favorable conditions of growth induction. These storage experiments confirmed what Muenscher (1938)

observed in natural habitat conditions. Seeds from this genus may germinate in early spring or even late autumn after seed release, and remain germinated in the sediments, available to establish before predation or heat become a liability for the young plants during the early growing season.

Storage methods of *P. perfoliatus* seeds that retain an extended viability beyond one or two years may be valuable, but no published studies appear to have been conducted thus far that report on the comparative net primary productivity of germinated seeds from various production years. Nor do studies typically follow growth for complete cycle of dispersed, mature, germinated seeds to adult, flowering plants (Zinecker CH2). The ability to pre-germinate seeds in storage before broadcasting at the restoration site could be of high utility, thereby guaranteeing germination in the field. However, guaranteed germination does not necessarily mean guaranteed establishment of seedlings to adult plants. In SAV beds where seeds are naturally dispersed, researchers have observed that up to 85% of *Ruppia maritima* seeds germinated (Strazisar et al. 2016), and up to 40% of *Vallisneria americana* seeds germinated (Jarvis and Moore 2008), but few to no seeds grew to maturity in either study. In the case of the species *Amphibolus arctica*, current velocities inhibited plant establishment by dislodging up to 100% of seedlings in a sandy substrate (Rivers et al. 2011). These values are not dissimilar to seedling mortality in terrestrial grasslands, where mortality can account for up to 85% of seedlings (Silvertown and Dickie 1980).

Research has conclusively shown that temperate and tropical SAV species have differential responses to disturbance and ability to recover (Kirkman 1997), and this also may be true for the relative success of restoration projects (Fonseca 2011, Meyer et al.

2013). While large scale planting efforts have been successful for eelgrass and other seagrasses, large scale planting for *P. perfoliatus* and other temperate, estuarine SAV species may be more problematic based on the unreliable and quickly changing site/weather conditions in the mesohaline and oligohaline-fresh Chesapeake Bay.

Given the high natural mortality of seedlings in natural SAV beds, researchers have searched for methods to exert more control over how seeds are deployed at a given restoration planting site in order to better enhance recruitment, i.e. increase planting success (Twilley et al. 1999). The idea of making a type of pot or vessel to propagate and plant SAV propagules to increase restoration success is not new. Many approaches using holdfasts, or in-situ planting containers, have been refined and developed over the last few decades. Peat pots (Bergstrom 2006, Orth 2006, Lewis et al. 2006), burlap matting with seeds attached (Orth 2006), burlap or polyethylene bags wrapped around shoots (Thorhaug and Austin 1976, van Breedveld 1975), have all been used with varying degrees of success. In a patent, Anderson (2005), described both shells and containers made of various organic materials that serve as vessels and holdfasts for planting seeds or shoots under water. While many of these methods use organic materials that may eventually biodegrade, some holdfast materials, such as metal staples, remain in place as waste (Zhang et al. 2015, Fonseca et al. 1994).

Lee and Park (2008) described a natural shell technique whereby *Zostera* sp. shoots can be more easily anchored to establish in sediment. However they found that establishment was better facilitated in muddy sediment than sandy sediment. This demonstrates the need for specialized planting systems that are adaptable to the range of environments where a given SAV species may grow, but is less likely to naturally

establish. In the case of *P. perfoliatus*, research indicates that seedling growth and establishment is also less effective on sandy sediment and does not tolerate burial much below the surface (Ailstock et al. 2010b, Ailstock et al. 1991, Ozimek et al. 1976). Growth responds much better to a low threshold of organic fines, or will tolerate larger percentages of organics provided they are refractory in nature and there is adequate light, otherwise yield may be depressed (Haslam 1978, Misra 1938, Zinecker CH2). Some of the most successful experimental yields for *P. perfoliatus* and other mesohaline species have used a mix of oyster shell and peat as a substrate (Ailstock et al 1991, Kujawski and Thompson 2000, Zinecker et al. 2007). In additional experiments, it was further determined that oyster/peat was most closely aligned with those yields found for *P. perfoliatus* growth on its own bed sediment (Zinecker CH2). While these findings have enabled better substrate preferences targeting, restoration methods such as using plugs or peat pots (Bergstrom 2006), sods or turf (Mark Lewandowski, MD-DNR, personal conversation 2017), or broadcasting seeds (S. Ailstock, 2016, personal conversation), have met with limited success. Given these challenges, there appears a need for continued efforts to develop restoration technologies that work with plant biology, seedling establishment, and edaphic requirements combined.

The goal of this research was to develop a biodegradable plant pot that would improve propagation, deployment and establishment of SAV propagules at restoration sites, with a focus on *P. perfoliatus*. The placement of seeds or propagules (i.e. turions), in a fully biodegradable, rigid vessel filled with growth enhancing substrate, is unlike the other, abovementioned methods. The pot is made of biodegradable, mold-injection grade PHA (polyhydroxyalkanoate) plastic. Certain types of bacteria produce

polyhydroxyalkanoates under stressful conditions, or where essential nutritional factors are in short supply, i.e. nitrogen, phosphorous, sulphur, oxygen and/or magnesium, and in the presence of excess carbon (Muhammadi 2015, Lemoigne 1926). PHAs consist of hydroxycarboxylic acids, carbon and other compounds produced for the purpose of the cell's emergency energy usage. In some cases, these reserve PHA's can account for 90-97% of the cell's dry weight (Braunegg et al. 1998, Khanna and Srivastava 2005). Plastic consisting exclusively of PHAs is the only 100% biodegradable polymer other than PCL, polycaprolactone plastic (Ishigaki et al. 2004). Under aerobic conditions, microbial degradation breaks down PHA into carbon dioxide and water, and to methane under anaerobic conditions (Khanna and Srivastava 2005; Mas-Castella 1995; Volova et al. 2010). A number of species of bacteria and fungi are able to metabolize PHA through extracellular secretion of the specific PHA depolymerase enzymes that break down the PHA polymers (Ashby et al. 2007).

The ASTM (American Society for Testing and Materials) provides quantitative, experimental benchmarks for technologies such as biodegradable plastics. As part of this standardized research, PHA material has been tested in septic sludge (Gutierrez-Wing 2010), compost (ASTM D6400, ASTM D868-11), and marine environments (ASTM D7081-05) (withdrawn), but published research relating to specific applications using the plastic and demonstrating its utility in natural environments, and extent of biodegradation in that capacity, are few. Khan et al. (2001), and Mas-Castella (1995), tested PHA materials in anaerobic sludge and bacterial mats in the field, respectively, but only to test biodegradability, not to evaluate a specific use. PHA plastic has been successfully used in the making of a biodegradable cull ring for blue crab traps. When pots become lost from

the line and settle on the Bay floor, the cull ring panel biodegrades after a year, thereby providing an escape for bycatch that accidentally find and enter the derelict crab pots (Bilkovic et al. 2012). PHA pots are used in applications for terrestrial horticultural purposes, and degradation has been compared alongside other biodegradable pots, and in different soil environments (Castronuovo et al. 2015, Kratsch et al. 2015, Lim et al. 2005). In addition, medical applications are becoming more widespread (e.g. Grage et al. 2009, Lim et al. 2017). However, no prior research has reported on the use and degradation of PHA plant pots developed for the purposes of aquatic plant restoration or propagation.

The extent and characterization of types of plastic debris in water bodies across the globe (Cole et al. 2011, Mani et al. 2015), and in Chesapeake Bay (Yonkos et al. 2014), are well documented. A number of studies have established the ingestion of, or harmful effects of, plastics on aquatic fauna including macroinvertebrates, coral, zooplankton, birds, sea mammals, and bivalves (Mendes et al. 2015, Green et al. 2017, Lamb et al. 2018, Wilcox et al. 2015, Hall et al. 2015). Polyethylene may absorb various pesticides and other contaminants (Nerin et al. 1996, Joyce et al. 2015), creating the possibility of additional environmental health hazards associated with fossil-fuel based plastic. Microbes and fungi are able to degrade both conventional and biodegradable plastics in various environments (Hadad et al. 2005, Bonhomme et al. 2003, Volova et al. 2010, Boyandin et al. 2013, Kanmani et al. 2016). However, no studies have documented the effects of plastics, fossil-fuel based or biodegradable plastics such as PHA, on the growth of aquatic plants, specifically seagrasses and other species of SAV (only

terrestrial plants, i.e. Kratsch et al. 2015). They have also not documented the effect aquatic plants might have on the degradation of these materials.

This research evaluated the biodegradation of PHA aquatic plant pots developed for restoration of *P. perfoliatus* using seeds, and to assess the potential for the pots to improve aquatic plant growth. The idea behind this planting system was to reduce disturbance to propagules during transplantation, and increase certainty as to seed fate and planting location as the plants establish and the pot degrades as it is no longer required to assist the plant. Research consisted of two microcosm experiments under controlled greenhouse conditions, and a field trial at two different salinities (one tidal freshwater site and one mesohaline site). For both microcosm experiments, microcosms were filled with the same mixture of peat and oyster shell with marsh sediment inoculant. All experimental pots were filled with an SAV bed sediment taken from SAV beds and a mix of fine oyster shell (2mm diameter) and peat. The PHA biodegradable container, filled with SAV bed sediment, hypothetically would allow the seeds adequate time to establish in the substrate contained in the pot, and for gradual contact with surrounding sediment as the pot degrades and the plants grow.

Microcosm experiment I (MEI) compared degradation of the PHA pots and the growth performance of *P. perfoliatus* seeds, with seed growth in polyethylene (PE) control pots. Unplanted pots made of both materials served as controls. It was hypothesized that seeds grown in PHA pots over a sixteen (16) week period would yield higher biomass than when grown in control PE pots; and that PHA pots would degrade more rapidly than the less degradable PE pots. It was anticipated that lower Eh or redox conditions (as a proxy for microbial activity) often found in deeper portions of the

sediment (Zinecker CH2) would correspond with greater PHA pot biodegradation than shallower areas (presumably with less negative Eh). Finally it was hypothesized that there would likely not be any differences in degradation between planted pots vs. unplanted pots given the lack of significant differences in redox in planted vs. unplanted substrates in Zinecker (CH2).

Microcosm experiment II compared degradation of PHA pots both planted and unplanted. In addition, *P. perfoliatus* seed growth in PHA pots was compared to the restoration method of broadcasting seeds directly onto the sediment (in this case at the smaller scale of the microcosm). Seeds from two different harvest years were used. Growth of older seeds that had been stored, pregerminated, for a little over 4.5 years (harvested in summer 2006), was compared with growth of seeds harvested and cold-stored from the summer 2010, eight months before the experiment. It was hypothesized that seeds from either harvest year would grow better in inoculated PHA pots than on bare microcosm sediment over a period of twelve weeks. It was also postulated that the more recently harvested seeds (2010) would grow better than those seeds which were harvested during the summer of 2006, inducted, and stored in cold storage over 4.5 years previous to the experiment. Finally it was hypothesized that there would likely not be any differences in degradation between planted pots vs. unplanted pots given the lack of significant differences in redox in planted vs. unplanted substrates in Zinecker (CH2). Lastly, it was hypothesized that differences in redox measurements would coincide with lower degradation in the top portion of the spindle and greater diameter loss in the deeper portion of the spindle.

The goal of the field investigation was to measure pot degradation over five months in SAV bed sediment at sites in two different salinity zones in Chesapeake Bay – mesohaline, and tidal fresh. It was hypothesized that pot degradation would be greater at the mesohaline site than in the tidal fresh site due to qualitatively loamier sediment content, differences in temperature, and higher salinity at the mesohaline site (thereby providing better conditions for microbial activity (Richardson and Vepraskas 2001, Lim et al. 2005, eyesonthebay.net).

MATERIALS AND METHODS

This research entailed the use of a biodegradable plastic pot made from a mold developed for the purpose of this project, and a granular form of mold injection-grade polyhydroxyalkanate (PHA) plastic (Mirel^{TM} bioplastic). Control pots using the same mold were made from petroleum-based polyethylene (PE) plastic. The pot is a 10 ml volume, V-shaped trough that holds a given species propagule and an appropriate quantity and type of substrate or inoculant that supports plant establishment, providing a microenvironment that may reduce transplant shock. A spindle runs through the bottom of the trough through the pot to the top center of the trough to make a "T" shape. The spindle is tapered to enable better placement when transplanted in sediment at the restoration site. The bottom spindle diameters ranged from 4.01-4.15 mm in diameter and the top ranged from 5.72-6.16 mm in diameter. Pot mass ranged from 3.52 $\pm$ 0.01 - 4.26 $\pm$ 0.27g. Control and PHA pots were produced in two different lots during mold injection, resulting in small variations in mass and spindle diameter between pots. Pre-experimental mass was determined for each pot. A high precision caliper (500 series, Mitutoyo Corporation, USA), was used to determine minimum and maximum spindle diameter for the "top" of each pot (just below the keel) and "bottom" (the portion that was buried

more deeply in the sediment when planted (Table 3.1, Figure 1). Both mass and top and bottom spindle diameter were used to measure degradation in all experiments.

For microcosm experiments I and II, seed stock from harvest years 2009 and 2010 (respectively) was donated from researchers at Anne Arundel Community College (AACC) per the protocols for optimizing seed collection and storage in Ailstock et al. (2011). Seeds were harvested from Marshy Creek, a small stream outlet located approximately 1km Southeast of Kent Narrows (Zinecker CH2). The seeds were stored at 4 deg. C, and aerated until microcosm planting.

Additionally, for Microcosm Experiment II, seeds were collected from SAV tanks at the USDA Norm Berg National Plant Materials Center (PMC) in the summer of 2006. The seeds were collected from the sediment of propagation trays after seeds had matured and dispersed from *P. perfoliatus* plants onto the sediment of the trays. Seeds were spun down in a blender, rinsed and separated from the liquid remainder of the plant detritus that accompanies seeds. The seeds were then put in a loosely sealed, unaerated glass jar filled with tap water, and left in 4 degree C cold storage. Within six months, almost all seeds had germinated due to unaerated conditions, with the ivory-colored radicle emerged from the seed. The seeds then remained in this condition for four years in darkened cold storage until microcosm planting.

SAV bed sediment for the pots was collected from Marshy creek SAV beds, as well as sediment from saltmarshes adjacent to the SAV bed where the pot inoculant was collected. The sediment was placed in rubber containers, covered with water, and refrigerated until microcosms were filled with substrate. The goal was to provide inoculant for the pots, and a larger quantity of microbial rich marsh substrate to add to the

oyster shell/peat substrate used to fill the microcosms in order to ensure pot degradation. Since microcosms were prepared all at once for both experiments, but were months apart, and it was anticipated that some additional mineralization might occur to the microcosms in experiment II from the marsh sediment.

Experimental System

For microcosm experiments I and II, research was conducted at the Greenhouse Research Complex located at the University of Maryland, College Park. The 750m^2 greenhouse was maintained at a temperature between 20-30˚C during the day and at a minimum of 15˚C at night. Natural sunlight was supplemented by metal halide lamps that supplied 400 umol par for a 14 hour photoperiod. Light measurements were taken to ensure a uniform lighting regime, and automated shades protected plants from the most intense sunlight. Uniformity of lighting on all treatments in the greenhouse was confirmed by Sharpe (2009) and Zinecker (CH2) in similar greenhouse studies at the same site.

The manifold and microcosm design were similar to Zinecker (CH2). Microcosms (19L) were filled to about 4.5L (12 cm depth, 22 cm diam.), with a substrate of 2/3 oyster shell/peat moss thoroughly mixed with 1/3 marsh sediments. The mixture was then covered with an oyster shell cap to reduce diffusion of substrates into the water column that might cause algal growth. The peat/oyster shell substrate produced high *P. perfoliatus* growth in a previous experiment (Zinecker CH2). The oyster/peat, along with the marsh sediment, served to provide reduced, anaerobic conditions. Tap water was added to the microcosms for conditioning, and aerated via airstones in preparation for planting for both microcosm experiments.

Experimental Design

For Microcosm Experiment I (July-November 2010 – 16 weeks), to determine significant differences between PHA and PE pot treatments for pot degradation and planting treatment, a completely randomized 2x2 factorial design was set up with sixteen (n=16) replicate microcosms for each treatment (n=64) (Table 3.2). In order to test whether soil redox, temperature, or pH conditions were affected by any of the treatments, an additional treatment control of no pots and no plants was included with seven (n=7) replicates (for a 2x3 experimental design configuration for the purposes of the substrate tests) for a total of seventy-one microcosms (experimental total n=71). At the time of this experiment, methodological approaches for ensuring seeds would stay securely on the sediment were not sufficient to support a "plants only, no pots" treatment for a fully balanced 3x3 design. Efforts to develop a "hand broadcasting" simulated restoration at the microcosm scale was established for microcosm experiment II.

For microcosm experiment II (March-June 2011 - 12 weeks), growth of redhead seeds from two different harvest years (2006 and 2010) was compared between PHA pots and "broadcast seeding" on the microcosm sediment surface. A 2x2 factorial design tested average biomass response (n=20). The investigation of average pot degradation response per planting combination was evaluated using a simple three treatment combination (n=15) of unplanted pots, pots with 2006 seeds, and pots planted with 2010 seeds. In order to test whether soil redox, temperature, or pH conditions were affected by any of the treatments, a full 2x3 factorial treatment design was used (n=30), employing all microcosm replicates (Table 3.3).

Microcosm preparation and planting

In the microcosm experiments the interest was not in germination rates, therefore (in addition to the already germinated seeds from 2006), seeds from 2009 and 2010 were pre-germinated by induction. To induce germination, seeds were soaked in a 10% bleach solution for 2 minutes, rinsed, and rubbed lightly against a fine mesh screen for one minute, 25 or 50 seeds at a time. They were then placed on petri dishes in groups of 6-10 seeds per dish. For microcosm experiment I, 300 seeds were used for germination tests in order to ensure viability of at least 192 required viable seeds: 6 seedlings per microcosm x 16 replicates x 2 treatments). The petri dishes were placed in a growth chamber with a cycle of 12 hours of fluorescent lighting (70 $umolm^{-2}s^{-1}$) at 22 deg. C. Within 6-12 days approximately 75 percent of the seeds germinated. The seedlings were kept in the petri dishes submersed in DI water in the growth chamber for several more days until planting.

For microcosm experiment I, three PHA or PE control pots were weighed and summed for each microcosm. Each pot was filled with only the SAV bed sediment inoculant, and planted with two germinated seeds using a forceps, and "capped" with fine-grained oyster shell (approximately 1-2 mm depth), to reduce escape of nutrients and buoyant seeds from the top of the pot. Each group of three pots was planted in a microcosm. Buoyant seedlings that became dislodged were replanted or replaced within 48-72 hours of planting.

For microcosm experiment II, 2006 and 2010 seed planting in PHA pots followed the same protocol as microcosm experiment I, however, weighing and planting included just two pots in each microcosm with two seeds each. To handle the four seeds for hand transplanting directly onto each half of the microcosm sediment, seeds were covered by a

1-2 mm shell layer to anchor the buoyant, germinated seeds in place without inhibiting plant growth, as with the PHA pots. However, this often required multiple attempts, and in some cases seed replacement, if seeds appeared damaged (i.e. radicle had fallen off).

Microcosm maintenance and monitoring

After the microcosms were planted, they were monitored every week to remove algal growth, topped off with fresh water, and the growth of seedlings observed. Temperature of the tanks was monitored weekly. Shade cloth was installed on the windows to maintain temperature in the microcosms at 30 deg. C. or below, typical for summer ambient water temperatures similar to seed site origin (Zinecker CH2).

End of Experiment Microcosm Conditions

Before microcosm harvest, redox, pH and temperature measurements were taken to see if there were any differences between the treatments at the surface and at 4 cm depth per the methods in Zinecker (CH2). Aboveground biomass (AGB) was clipped with a scissors as close to the substrate surface as possible including rhizomes reaching across the surface of the sediment, and the number of inflorescences noted. Belowground biomass, with no pots, was separated from sediments, rinsed, and air dried. Belowground biomass treatments with PHA bioplastic and PE control pots were taken out of the microcosms, rinsed with water while removing any residual substrate or pot pieces, and roots untangled from pots. Once harvested and cleaned, inflorescences on plants in each microcosm were counted. Both AGB and BGB samples were dried at 70 deg. C for 24 hours, weighed, and analyzed for percent total carbon (%TC) and percent total N (%TN) by combustion analysis (LECO CNS 2000 analyzer, St. Joseph, MI). Sediment from microcosms was dried for 24 hours. Combustion analysis was also used to measure %TC

and %TN of the substrate, and evaluated in a subset of three replicates for each of the treatments. Loss on Ignition of organic matter (%OM) was calculated from a 5 gram sample. Percent organic carbon (%OC=%OM/2) and percent bicarbonate %Bic (based on %TC – %OC) were calculated based on data from CHN analysis and LOI. All biomass and substrate analyses followed the same methods as described in Zinecker (CH2).

Pots were oven dried at 30 deg. C., weighed and compared with pre-experimental weights. Caliper measurements of the spindle diameter were taken in two places, just below the pot keel (shallow), as well as at the deep portion of the spindle (4 cm). Measurements at shallow and deep Eh activity (redox also served as a potential proxy for microbial degradation).

Field Experiment

Site Descriptions

The field experiment investigated PHA pot degradation in estuarine sediments located near two different SAV beds, one tidal fresh in Upper Chesapeake Bay, the other in the mesohaline Middle Bay. Pots were deployed in approximately 0.5 m depth water at low tide. The mesohaline site was located on Ragged Point Road at Ragged Point, near Cambridge, MD in Middle Chesapeake Bay, (38˚33’37.72”N 76˚16’49.48” W), and at a tidal fresh site located at Carpenter’s Pt. Rd., Carpenter’s Pt., Perryville, Md. in the Upper Chesapeake Bay (39˚32’20.28” N 76˚00’30.20”W). While Ragged Point has historically supported both *P. perfoliatus* beds and *Ruppia maritima*, *R. maritima* was the dominant species (Orth et al. 2015, Zinecker, personal observations). Carpenter’s Point supports *Vallisneria americana* (L.) monoculture beds with *Myriophyllum spicatum* and

much less frequently *P. perfoliatus* and other SAV species dispersed in other locations (Orth et al. 2010, Zinecker, personal observations). The tidal fresh site consisted of low organic, sandy gravelly substrates, whereas the mesohaline site consisted of finer grained sandy loamy sediments.

Pot preparation and field planting

Twenty pots for each site were selected, numbered, and weighed. Fishing line was cut to several different lengths corresponding to random placement of pots within a 5 m diameter plot, and were arrayed around a center holdfast, at each of the sites. Once in the field, the fishing line was tethered to a tie-out stake and each pot was tied to the fishing line with an aluminum number plate, then planted in the sediment. Initial degradation at both sites was imperceptible after 30 days, and thus it was decided to wait another 30 days. However, storms affected visibility and did not allow for sampling on or around the same dates. Thus it was decided to do a pre- post experimental harvest similar to the microcosms.

After finding the central planting stake, pots were dug out of the sediment. The sediment was removed, brought back to the lab, and dried at 30 deg. C. for 24 hours. They were weighed (g. dry wt.) after removal from the oven similar to the microcosm experiments, and caliper measurements made on shallow (portion of spindle just below keel) and deep (bottom of spindle) spindle diameter depths.

Statistical Analysis

Fixed and main effects were reviewed with analysis of variance using the mixed procedure where appropriate (proc mixed SAS Institute 2013). Where significant, means were evaluated using the Tukey-Kramer adjustment Honest Significant Difference

(HSD). Repeated measures were used to evaluate pot spindle measurements, sediment redox, temperature, and pH at two different depths in the substrate: shallow (surface of substrate), and deep (4 cm). Statistical analysis was conducted using the SAS System for Windows 9.3 (SAS Institute 2013). All statistical tests were conducted at the 5% significance level.

RESULTS

Environmental conditions for microcosm experiments I (MEI) and II (MEII)

Microcosm ambient conditions of light were consistent across all microcosms, similar to conditions reported by Zinecker (CH2), and Sharpe (2009). Water column temperature across all treatments was also consistent, and was within one to two degrees Celsius regardless of date taken (Spring/Fall Ranges (Lower end): 23-26°C, Summer Ranges (higher end): 30-32°C.

End of experiment measurements

Substrate temperature (MEI)

Substrate Temperatures °C, Shallow and Deep: Before harvest, substrate temperature was measured at two depths in each microcosm, at shallow (just below the surface - 2mm), and deep (4 cm). Average soil temperatures were not significant for the fixed effects of pot treatment ($F_{2, 38.4}$ =2.01, p = 0.1476), planting treatment ($F_{2,39.8}$ =0.50, p = 0.6123), and nearly significant for depth ($F_{1,16.8}$ =3.75, p = 0.0699). Temperature averages ranged from 22.8 $\pm$ 0.3 to 23.6 $\pm$ 0.7°C (shallow), and 22.6 $\pm$ 0.2°C to 23.5 $\pm$ 0.4°C (deep).

Substrate pH (MEI)

Microcosm substrate pH was measured at two depths in a subset of microcosms for each treatment just before harvest, concurrent with temperature, and Eh (redox)

measurements. Average substrate pH values were significantly different for the main effect of depth (shallow vs. deep measurements) ($F_{1, 13.3}$ =122.96, $p < 0.0001$)(Table 3.7). Average substrate pH values were not significant for the fixed effect of pot treatment ($F_{2, 28.8}$ =0.09, p = 0.9137), or planting treatment ($F_{2, 42.1}$=1.21, p = 0.3093).

Substrate Redox (Eh) – Shallow and Deep (MEI)

Sediment redox in millivolts (mV), was measured at two depths similar to temperature and pH. Significant differences were found for the fixed effect of depth ($F_{1, 26}$ =286.07, $p < 0.0001$) (Table 3.7). No significant differences were found for the fixed effects of pot treatment ($F_{2, 26}$ = 1.66, p = 0.2096), or for planting treatment ($F_{1,26}$ = 0.82, p = 0.3742).

Carbon and nitrogen content of microcosm substrate (MEI)

The same substrate was used in all microcosm treatments, and is likely the reason no significant differences were found for percent organic matter (%OM), percent organic carbon (%OC= %OM/2), percent total carbon (%TC), percent total nitrogen (%TN), % Bic (%TC-%OC), and % organic carbon to % total nitrogen ratio (C:N). Since the relationship and importance of organic matter, %TN and yield was established in Zinecker (CH2), statistical results for pot*plants as well as average values $\pm$ S.D. are summarized in Table 3.4 to demonstrate the consistency of values across microcosm treatments, and for comparison with microcosm experiment II. Variables reported in table are briefly described below with F and p-values.

Loss on ignition (LOI) analyses were conducted to determine percent organic matter (%OM) loss from a 5g sample of (post-experiment) microcosm substrate. %OM,

for the fixed effects of pot treatment ($F_{2,10}$ =0.42, p = 0.6667) planting treatment, ($F_{1,10}$ =3.27, p = 0.1008) or pot*planting treatment ($F_{1,10}$ =2.53, p = 0.1431).

No significant differences were found for averaged %OC for the fixed effect of pot treatment ($F_{2,10}$ =0.09, p = 0.9141), planting treatment, ($F_{1,10}$ = 2.62, p = 0.1365), or pot*planting treatments ($F_{1,10}$ =1.84, p = 0.2053).

No significant differences were found for averaged %TC analysis by combustion for the fixed effect of pot treatment ($F_{2,9}$ =1.13, p = 0.3653), plant treatment ($F_{1,9}$ =0.25, p = 0.6325) or pot*plnt treatment ($F_{1,9}$ =0.50, p = 0.4966).

No significant differences for averaged %Bic (%TC –%OC) were found for pot treatment ($F_{2,9}$ = 0.59, p = 0.5739), planting treatment ($F_{1,9}$ = 1.08, p = 0.3254), or pot*plant ($F_{1,9}$ = 0.14, p = 0.7137).

No significant differences between treatments were found for averaged %TN analysis for fixed effects of pot treatment ($F_{2,9}$ =2.23, p = 0.1637), plant treatment ($F_{1,9}$ =0.94, p = 0.3579), or pot*plant treatment ($F_{1,9}$ =0.77, p = 0.4027).

No significant differences were found for averaged %OC:%TN (C:N) ratios for pot treatments ($F_{2,9}$ = 0.93, p = 0.4310), plant treatments ($F_{1,9}$=2.71, p = 0.1340), or pot*plant treatments ($F_{1,9}$ = 0.88, p = 0.3718).

Carbon and nitrogen content of aboveground and belowground biomass (MEI)

Results for carbon and nitrogen content of biomass are summarized in Table 3.5. No significant differences were found for averaged %TC for aboveground biomass between control PE and PHA pot treatments ($F_{1,11}$ = 2.05, p = 0.1796). Significant differences were found for %TN for aboveground biomass (AGB) ($F_{1,11}$ = 6.39, p =

0.0281). Nearly significant differences were found, for averaged C:N ratios for AGB ($F_{1,11} = 4.42$, $p = 0.0594$).

Significant differences were found for averaged %TC for belowground biomass ($F_{1,12} = 12.82$, $p = 0.0038$). Significant differences for averaged %TN for belowground biomass were found ($F_{1,12} = 5.47$, $p = 0.0375$). Significant differences were found for averaged C:N ratios ($F_{1,12} = 7.96$, $p = 0.0154$).

Number of inflorescences (MEI)

Significant differences for number of inflorescences at end of experiment harvest were found for the fixed effect of pots ($F_{1,28} = 5.15$, $p = 0.0311$). Plants grown in PHA pots bore no inflorescences, whereas plants grown in control PE pots produced a total of 39 flower heads distributed in seven of 15 microcosms, for an average of 2.5 $\pm$ 1.1 inflorescences per microcosm (Table 3.6).

Above- and belowground biomass (g)

Results for biomass results are summarized in Table 3.6. No significant differences were found for aboveground biomass of plants for the fixed effect of pot treatment ($F_{1,28} = 2.39$, $p = 0.1331$). No significant differences were found for belowground biomass (g) ($F_{1,28} = 1.05$, $p = 0.3153$), or for summed AGB + BGB for the fixed effect of pot treatment ($F_{1,28} = 1.92$, $p = 0.1763$). PHA pots had the lowest total AGB+BGB biomass averaging 2.42 $\pm$ 0.32 g, while control PE pots averaged 3.00 $\pm$ 0.27 g. No significant differences were found for R:S ratios (grams BGB:AGB) for the fixed effects of pots ($F_{1,28} = 2.04$, $p = 0.1644$). R:S ratios were lowest in control pots (0.62 $\pm$ 0.05), while R:S ratios for PHA pots averaged 0.74 $\pm$ 0.07.

PHA and PE pot mass (g) % loss (MEI)

Highly significant differences for the response variable of pot mass lost (% g from total initial grams) were found for the fixed effects of pots (*% g lost*: $F_{1,60}$ =2,368.41, p < 0.0001), and planting treatment (*% g lost*: $F_{1,60}$ =10.12, p = 0.0023). Nearly significant differences were found for % pot mass lost for the fixed effects of pot*plant (*% g lost*: $F_{1,60}$ =3.01, p = 0.0877). Since nearly significant differences were found for the pot*plant treatment, these results are reported with significant differences of biomass and spindle diameter % lost in Table 3.6.

For the significant differences found for the fixed effects of pot treatment, % mass lost for control, PE plastic pots, (whether planted or unplanted) decreased only slightly, (0.43 $\pm$ 0.18%), whereas PHA pots, regardless of planting treatment, decreased significantly more on average (61.32 $\pm$ 1.35 %) after four months. For significant differences of fixed effects of plant treatment, % mass lost for unplanted pots, regardless of whether it was a PHA biodegradable pot or a nonbiodegradable plastic control, resulted in an overall decrease in % mass of 28.89 $\pm$ 5.36 %. Planted pots averaged a higher overall decrease in % pot mass lost regardless of pot type, losing on average 32.86 $\pm$ 5.72 %.

PHA and PE pot spindle diameter (mm) % loss (MEI)

PHA pots % spindle diameter was significantly degraded and decreased substantially in diameter, in comparison to control pots. The most relevant significant results are summarized in Tables 3.6 and 3.7.

Significant differences for the response variable of minimum/maximum range estimates of percent (%) spindle diameter lost were significant for fixed effects of pots

(min: $F_{1,60}$ =1874.10, P<0.0001 / max: $F_{1,60}$ =1782.49, p < 0.0001), planting treatment (min: $F_{1,60}$ =27.33, p < 0.0001 / max: $F_{1,60}$ =29.98, P < 0.0001), depth (min: $F_{1,60}$ =195.71, P<0.0001 / max: $F_{1,60}$ =167.22, p < 0.0001), pot*plant (min: $F_{1,60}$ =10.12, p = 0.0023 / max: $F_{1,60}$ =8.57, p = 0.0048), and pot*depth (min: $F_{1,60}$ =169.00, p < 0.0001 / max: $F_{1,60}$ = 164.68, p < 0.0001). No significant differences were found for spindle diameter % lost for the fixed effects of planting treatment*depth (min: $F_{1,60}$ =0.03, p = 0.8543 / max: $F_{1,60}$ = 0.30, p = 0.5874), or for the fixed effects of pot*plant*depth (min: $F_{1,60}$ =0.72, p = 0.3994 / max: $F_{1,60}$ = 0.02, p = 0.8791).

Substrate temperature (MEII)

Substrate temperature in °C was measured at two depths in each microcosm, at shallow (just below the surface - 2mm), and deep (4 cm), just before harvest. Average soil temperatures were not significant for the fixed effects of pot treatment ($F_{1, 23.5}$ =0.03, p = 0.8594), planting treatment ($F_{2, 23.5}$ =1.43, p = 0.2594), or depth ($F_{1, 22}$ =2.11, p = 0.1604). Temperature averages for treatments measured at the surface of the microcosm sediment ranged from 28.9 $\pm$ 0.2 to 29.9 $\pm$ 0.3°C, and at 4 cm depth the range was 29.2 $\pm$ 0.5°C to 30.3 $\pm$ 0.3°C.

Substrate pH (MEII)

Microcosm sediment average soil pH values were significant for the main effect of pot ($F_{1, 24}$ =6.01, p = 0.0218), plants, ($F_{2, 24}$ =5.33, p = 0.0121), depth ($F_{1, 24}$ =858.04, p < 0.0001), and planting trt*depth ($F_{2, 24}$ =4.95, p = 0.0159). Interestingly, average sediment pH for the main effect of PHA pot was lower in average pH compared with sediment containing no pots (8.51$\pm$ 0.20 pH and 8.76 $\pm$ 0.19 pH, respectively). Depth was the most highly significant for pH among treatment effects (Table 3.11). For the fixed effects of plant*depth, significant pH indicated plants raised pH at shallow and

deep measurements in comparison to non-planted sediment (Table 3.12). Average sediment pH values were not significant for the fixed effect of pot*plant treatment ($F_{2, 24}$ =5.33, p = 0.5662), or pot*plant*depth treatment ($F_{2,24}$=0.08, p = 0.9215).

Substrate redox (Eh), shallow and deep (MEII)

Significant differences were found for average sediment redox (Eh) for the main effect of depth ($F_{1, 24}$ =477.26, p < 0.0001) (Table 3.11). No significant differences were found for the fixed effects of pot treatment ($F_{1,24}$ = 2.42, p = 0.1330), or planting treatment ($F_{2,24}$ = 0.93, p = 0.4098).

Carbon and nitrogen content of microcosm substrate (MEII)

Carbon and nitrogen content of substrate were anticipated to be homogeneous, but since organic carbon and nitrogen appear to be closely associated with yield (Zinecker CH2), results are reported in Table 3.8. No significant differences were found for %OM for the fixed effects of pot treatment ($F_{1,24}$ =0.44, p = 0.5156), planting treatment ($F_{2,24}$ =0.23, p = 0.7932) or pot*planting treatment ($F_{2,24}$ =1.93, p = 0.1664). No significant differences were found for averaged (%OC) for the fixed effect of pot treatment ($F_{1,24}$ =0.43, p = 0.5161), planting treatment, ($F_{2, 24}$ = 0.23, p = 0.7933), or pot*planting treatments ($F_{2, 24}$ =1.93, p = 0.1664). Percent organic carbon ranged from 3.25 $\pm$ 0.17 %OC to 4.05 $\pm$ 0.51 %OC.

No significant differences were found for average %TC analysis by gas combustion for the fixed effect of pot treatment ($F_{1,24}$ =0.61, p = 0.4426), plant treatment ($F_{2,24}$ =1.79, p = 0.1882) or pot*plnt treatment ($F_{2,24}$ =2.13, p = 0.1412).

No significant differences were found for averaged percent bicarbonate (based on %TC –%OC) for the main effect of pot ($F_{1,24} = 0.26$, $p = 0.6172$), or planting treatment ($F_{2,24} = 2.34$, $p = 0.1175$).

No significant differences between treatments were found for averaged percent total nitrogen analysis combustion for pot treatment ($F_{1,24} = 0.07$, $p = 0.7906$), plant treatment ($F_{2,24} = 0.22$, $p = 0.8036$), or pot*plant treatment ($F_{2,24} = 0.17$, $p = 0.8475$).

No significant differences were found for averaged organic C:N ratios for main effects of pot treatments ($F_{1,24} = 0.27$, $p = 0.6103$), plant treatments ($F_{2,24} = 0.67$, $p = 0.5202$), or pot*plant treatments ($F_{2,24} = 2.51$, $p = 0.1023$).

Carbon and nitrogen content of above- and belowground biomass (MEII)

No significant differences were found for %TC or %TN, however C:N for belowground biomass was significant, and therefore all values for carbon and nitrogen are summarized in Table 3.9. Percent TC for aboveground biomass for the main effect of pots was not significant ($F_{1,8} = 2.44$, $p = 0.1567$), nor for the main effect of 2006 vs. 2010 plants: ($F_{1,8} = 1.04$, $p = 0.3376$), or for pots*plants ($F_{1,8} = 1.11$, $p = 0.3229$). Percent TN for aboveground biomass (AGB) was not significant for pot vs. no pots, ($F_{1,8} = 3.38$, $p = 0.1033$), or plant treatment ($F_{1,8} = 0.44$, $p = 0.5249$). Percent total nitrogen for aboveground biomass ranged from 1.650 ± 0.095 to 1.909 ± 0.007. No significant differences were found for averaged C:N ratios for AGB for the main effect of pot ($F_{1,8} = 0.33$, $p = 0.5806$), or for plant ($F_{1,8} = 0.71$, $p = 0.4239$).

No significant differences were found for averaged %TC for belowground biomass for pots ($F_{1,8} = 0.38$, $p = 0.5563$), or for plants ($F_{1,8} = 0.98$, $p = 0.3502$).

No significant differences for averaged %TN for belowground biomass were found for the main effect of pot ($F_{1,8} = 0.13$, $p = 0.7259$) or planting treatment ($F_{1,8} = 2.90$, $p = 0.1270$). Significant differences were found for averaged BGB C:N ratios for the fixed effects of planting treatment ($F_{1,8} = 7.82$, $p = 0.0233$). No significant differences were found for averaged C:N ratios for the fixed effects of pots, ($F_{1,8} = 0.53$, $p = 0.4888$).

Number of inflorescences (MEII)

Differences for number of inflorescences at end of experiment harvest were significant for the fixed effects of pots vs. nopots, ($F_{1,13} = 5.10$, $p = 0.0417$), and for plants, ($F_{1,13} = 7.91$, $p = 0.0147$). Part of the source of these differences may be due to one outlier microcosm that produced 21 inflorescences (2006 seeds, PHA pot), there were an uneven number of missing values for inflorescences between treatments (3/10 for 2010, 1/10 for 2006), and variation in flowering times. Irrespective of seed year, seeds planted in PHA pots bore somewhat more inflorescences on average (3.22 ± 2.13), than seeds hand planted directly into the microcosm sediment (1.43 ± 0.68). 2006 plants bore, on average, more inflorescences (3.44 ± 2.12), than 2010 plants (1.14 ± 0.59) (Table 3.10).

Above- and belowground biomass (g) (MEII)

While not significant, a slight trend indicated a difference between aboveground biomass of plants for the fixed effect of PHA pots vs. handplanting treatment ($F_{1,16} = 3.28$, $P=0.0888$), and are reported here due to the fact that AGB is a predictor for relative presence of inflorescences (Zinecker CH2). AGB appeared to be lowest in hand planted treatments regardless of seed harvest year (1.08 ± 0.14g), with higher AGB found in plants in PHA pot treatments: (1.47 ± 0.18g). No significant differences were found for the fixed effect of harvest year ($F_{1,16}=0.55$, $p = 0.4686$) or for pots*plants ($F_{1,16}=2.26$, $p =$

0.1522). Highest average aboveground biomass was measured in microcosms planted with 2006 seeds in PHA pots. No significant differences were found for belowground biomass (g) for pot treatments, ($F_{1,16}$ =1.07, p = 0.3156), or for plants ($F_{1,16}$ =0.03, p = 0.8639). Similar to AGB, belowground biomass growth averaged lowest in those treatments with just seeds and no pots.

No significant differences were found for summed overall biomass (AGB + BGB) for the fixed effect of pot treatment ($F_{1,16}$ =2.62, p =0.1248), or plants ($F_{1,16}$ =0.23, p = 0.6345). Lowest summed biomass averages were found in treatments hand planted with 2006 seeds (no pots): (1.43 $\pm$ 0.24 g), followed by 2010 plants (no pots) (1.64 $\pm$ 0.33 g), 2010 seeds planted in PHA pots: (1.77 $\pm$ 0.36 g), and the highest summed biomass was found for 2006 seeds planted in PHA pots (2.27 $\pm$ 0.26 g). No significant differences were found for R:S ratios for the fixed effects of pots ($F_{1,16}$ =1.08, p = 0.3133) and plants ($F_{1,16}$ =0.99, p = 0.3356). R:S ratios were lowest in microcosm treatments with 2006 seeds planted in PHA pots (0.33 $\pm$ 0.01), followed by 2010 seeds with no PHA pots (0.42 $\pm$ 0.05), 2010 seeds planted in PHA pots (0.45 $\pm$ 0.04), and the highest root:shoot ratio was produced by 2006 seeds not planted in PHA pots (0.46 $\pm$ 0.05).

Stem density (MEII)

No significant differences were found for stem densities for the fixed effects of pots ($F_{1,13}$ =0.06, p = 0.8086), or plants ($F_{1,13}$ =0.02, p = 0.8785). Stem densities were lowest in microcosm treatments with 2006 seeds not planted in PHA pots, (52.60 $\pm$ 5.10), followed by 2010 seeds planted in PHA pots (52.67 $\pm$ 18.70), 2010 seeds not planted in PHA pots (61.0 $\pm$ 11.53), with the highest stem density similar to the highest AGB, 2006 seeds planted in PHA pots (69.20 $\pm$ 10.14).

PHA pot mass (g) % loss (MEII)

No significant differences for the response variable of percent pot mass lost were found for the fixed effects of planting treatment (*% g lost*: $F_{2,12}$ =0.49, p = 0.6240) (Table 3.10).

PHA pot spindle diameter (mm) % loss (MEII)

End of experiment results on biodegradation of pots indicated that all pots in all planting treatments lost spindle diameter at both the top of the spindle, in more shallow sediment, and in deeper sediment near the bottom of the PHA pot spindle. However, no significant differences were found for the response variable of minimum/maximum range estimates of percent (%) spindle diameter lost for fixed effects of plant treatment (min/max: $F_{2,12}$ =1.47, p = 0.2688), depth (min: $F_{1,12}$ =1.23, p = 0.2883 / max: 0.68, p = 0.4264), or depth*plnts (min: $F_{2,12}$ =0.62, p = 0.5557 / max: $F_{2,12}$ = 0.63, p = 0.5516). Spindle diameter % loss (min/max) was highly variable both within and among treatments. Although variances in % spindle diameter loss was too great to be significant, shallow and deep portions of PHA pot spindles for all treatments appeared to respond to depth.

Field Experiment

Field Conditions for temperature and salinity

Salinity at field sites were significant for fixed effects of month ($F_{4,70}$ = 14.32, p < 0.0001), site: ($F_{1,70}$ = 1604.89, p < 0.0001), and month*site ($F_{4,70}$ = 14.32, p < 0.0001). Salinity was consistently fresh, or 0, at Carpenter's Point throughout the study. At the mesohaline site near Cambridge, June salinity averaged 8.38 $\pm$ 0.68 parts, July averaged

10.41 ± 0.74, August averaged 13.15 ± 0.53 parts, September averaged 9.48 ± 0.24, and October averaged 7.45 ± 0.11 (see Table 3.17 for ranges).

Temperatures (◦C) at field sites were significant for the fixed effect of month ($F_{4,68}$ = 192.47, p < 0.0001), but not month*site ($F_{1,68}$ = 2.39, p = 0.1268), and this was largely due to lack of data points, and the fact that temperature trends do not follow calendar dates. Therefore, trends (in text, here) and ranges (Table 3.17) are reported to reflect relative seasonal temperature changes that may have affected microbial activity and pot degradation at the time of the field trial. Means comparison procedures detected significant differences in temperatures between all months except July and August, which appeared somewhat similar in temperature ranging from 27.21 ± 0.70 ◦C to 29.50 ± 0.06 ◦C. June temperatures for the fresh site averaged 23.93 ± 0.50◦C compared to the mesohaline site at 22.48 ± 0.64◦C. September and October showed the greatest variation in temperature differences between months and sites. In September, the fresh site averaged 18.66 ± 0.04 ◦C, and the mesohaline site averaged 21.79 ± 0.15◦C. In October, the fresh site temperature was 15.63 ± 0.09 ◦C and the mesohaline site at 18.04 ± 0.06◦C.

PHA pot mass (g) % loss (field experiment)

Biodegradation of PHA pots was measured at sites of fresh and mesohaline salinities after 5 months. Degradation was extensive enough so that pots were substantially degraded and many small pieces had broken up into the sediments. While retrieval of all pieces was unlikely, extent of fragmentation, and lack of ability to find portions of the pot, were used as a determinant for extent of degradation for percent mass lost in addition to percent spindle diameter loss. Significant differences were found for the fixed effect of site for PHA pot % mass (g) lost: $F_{1,6}$ =7.67, p = 0.0324. PHA pots

buried in the upper Bay, oligohaline sediments lost on average less mass compared with those buried in mesohaline sediments (Table 3.13).

PHA pot spindle diameter (mm) (%) loss (field experiment)

No significant differences were found for the response variable of average percent (%) spindle diameter lost for the fixed effects of site: $F_{1,6}$ =0.97, p = 0.3524) or depth (% diam. lost: $F_{1,6}$ =1.91, p = 0.2164). Evidence of biodegradation was heterogeneous at each salinity site and on each pot spindle, although pots at the mesohaline site overall appeared more degraded. Diameter loss was lowest at the upper Bay site vs. the mesohaline site. While overall a trend seemed to follow that the tidal fresh site had lower degradation than the mesohaline site, the variance was too great to be of any statistical significance regarding consistent differences between average spindle degradation any one treatment.

DISCUSSION

This research focused on two primary objectives. The first objective was to determine whether biodegradable pots made for SAV restoration would improve *P. perfoliatus* seedling establishment and growth. To address this objective, microcosm experiment I (MEI) compared growth of seeds from the same harvest year when placed in PHA vs. polyethylene (PE) control pots. Microcosm experiment II (MEII) evaluated whether seedlings from two different harvest years might establish and grow better in the PHA pots than on bare sediment.

The second objective investigated whether presence/absence of plants, or sediment conditions, affected biodegradation of the PHA pot. Microcosm experiment I compared degradation of planted and unplanted PHA pots with degradation of PE control pots. Microcosm experiment II compared degradation of the PHA pots planted with seeds

from two different harvest years, with unplanted pots. The field experiment provided an opportunity to observe degradation of pots planted in SAV beds at mesohaline or fresh water sites on two different sediment types.

Sediment nutrients and associated microbial and fungal populations, redox, and pH, all played a key role in both experimental objectives because they supplied the conditions to sustain aquatic plant growth (Crump and Koch 2008), and to degrade PHA and PE pots (Dharmalingam et al. 2015, Shahnawaz et al. 2016). A substrate mix of marsh sediment, oyster shell, and peat was added to all microcosms for MEI and MEII. As a result, it was not surprising that for each experiment, there were no within-experiment significant differences for any sediment parameters related to % organic matter (%OM), % organic carbon (%OC), % total carbon (%TC), % total nitrogen (%TN), or % bicarbonate (%BIC) (Table 3.4 and 3.8).

However, MEII took place several months after MEI, and as a consequence, the sediment used for the microcosm environment become further reduced, or the microcosm may have been disturbed over time. Organic matter in the microcosms in MEII may have diffused upward over time, as the organic matter from the peat was visible in the oyster shell cap. This may have altered the environment in the upper microcosm of MEII, changing mineralization specific to the upper portions of sediment near the surface, as well as general nutrient enrichment from changes in microbial metabolic processes over several months (Brady and Weil 2002, Barko and Smart 1986, 1983, Smart and Barko 1985).

Redox in the shallow portions of sediment for both microcosm experiments were likely anaerobic, as each microcosm generally averaged well below +330 mv

(Ponnamperuma 1984). On average, Eh for the shallow sediment surface of MEI was often above or just at the point where nitrate is reduced to NH_4, (and was around 248mv or higher), whereas for MEII, Eh was often well below this average by 30 mv (averaging around 200mv). NH_4 is the preferred form of nitrogen for many aquatic plants, its presence thereby affecting N availability and biomass yield (Meyer et al. 2013, Caffrey and Kemp 1992). Deep Eh measurements at 4 cm were roughly equivalent between the experiments, both in the negative range of mv or just above zero mv. Although significant for both experiments, the differences between deep and shallow Eh were greater for MEI than for MEII. Microcosm experiment I shallow/deep range was approximately 251mv on average, but a smaller range of 220 mv between shallow and deep Eh averages for MEII (Table 3.7, Table 3.11, Figure 3.2). This stratification between deep and shallow Eh measurements was in agreement with the hypothesis stating that there would be lower Eh at 4cm depth than near the surface sediment. Wigand et al. (1997) also found shallow/deep Eh values for Vallisneria beds in Upper Chesapeake Bay, where in the shallow root zone Eh was +125 mv, and at 4 cm. -5 mv. Esteban et al. (2015) also found stratification and changes in pond sediment bacterial populations in shallow and deep portions of Winogradsky columns over several months. Over time, populations became less similar to the initial pond microbial community. The authors suspected bacteriophage predation, but did not measure Eh, pH, or nutrient information along with microbial population stratification data.

Average pH was similar to Eh, in that deeper portions of the microcosms were significantly more acidic than shallow measurements of pH, likely in conjunction with the mineralization occurring. The general pH environment of the microcosm substrate in

MEI and MEII was consistent with respect to deep/shallow stratification also seen in Zinecker (CH2). Additionally, for MEII, pH followed a (significant) hierarchy of noplant (lowest pH), 2006 plants (intermediate pH), and 2010 plants (highest pH), respectively. A trend of lower pH was observed in shallow pH for unplanted vs planted, but was not significant. Lower pH for unplanted vs. planted treatments was also observed in Zinecker (CH2) and was significant.

Spartina alterniflora marsh sediment was used as a microbial inoculant added to the oyster shell/peat mix in the microcosms. As a result, the microcosms were relatively high in labile organic C and N, particularly compared with natural SAV bed sediments and horticultural substrate mixes (Table 3.14, 3.15, 3.16, Zinecker CH2). While it would have been ideal to use 100% SAV bed sediment in the substrate fill for the microcosms, the volume needed was too great given the low acreages of *P. perfoliatus* beds in Chesapeake Bay. Spartina marsh sediment is known for microbial richness (Cordova-Kreylos et al. 2006). Thus while the marsh sediment presented an opportunity for lower redox, high microbial activity, and greater potential biodegradation of pot material than a sandy or a more refractory substrate, it also added a large quantity of labile carbon that was more available to diffuse and mineralize over time between the two experiments. As a consequence, it increased organic carbon and nitrogen, which can be problematic for plants (Brady and Weil 2002, Richardson and Vepraskas 2001, Misra 1938, Barko and Smart 1986).

Spartina sediment %OC in one study averaged 28.1 $\pm$ 6.4 %, with a C/N ratio of 26.5 (Boschker et al. 1999). In Zinecker (CH2), the %OC of the oyster shell /peat substrate (with no inoculant) was 9.15 $\pm$ 0.23 %OC with a C/N ratio of 50.18, and was

considered highly refractory due to the larger particle sizes of shells and peat moss. SAV bed sediment %OC averaged an order of magnitude lower at 0.43 $\pm$ 0.05 with a C/N ratio of 12.96 $\pm$ 3.27. Percent organic C in this study for MEI and MEII ranged from treatment averages of 2.86-4.05 %OC. As a result, the microcosm values for %OM, %OC, %Bic, %TC, and %TN for this study were on average higher than what might be considered normal for *P. perfoliatus* beds (0.23% to 1.18%) (Tables 3.14, 3.15, 3.16, Zinecker CH2, Zinecker unpublished data from Severn River locations). And, in conjunction with changes in Eh and pH, sediment parameters in microcosms appeared to qualitatively increase during the time that elapsed between MEI and MEII (Figure 3.2, Table 3.4, Table 3.8).

For plant growth, the primary objective for experiments MEI and MEII was to determine if plants established and grew better in the PHA pots compared with PE pot controls or on bare sediment. In MEI, results were somewhat inconclusive with respect to the fact that *P. perfoliatus* plant growth would be significantly greater in PHA pots vs. PE pots. In contrast to this hypothesis, PE pots produced slightly greater above and belowground biomass than in PHA pots (Table 3.6, Table 3.15, Figure 3.3). While not significant, this qualitative difference likely influenced the significant differences in inflorescences, as they occurred in PE pots only, with no inflorescences in PHA pots. Based on this information, it might be presumed, that plants would grow better in PE pots. But it was likely %OC in the substrate and the uptake of %TN in plant tissue were the greatest indicators, as PE pots had qualitatively lower %OC, and statistically significant lower plant tissue %TN compared with the PHA pot treatments (Table 3.4, Table 3.15, Figure 3.2). This trend agrees with observations in Zinecker (CH2), where

greater aboveground biomass was associated with the possibility to produce longer stems that reached at or above the water surface. As a result the plants were able to bear inflorescences. This yield correlated with an intermediate %OC, and a (subsequent) intermediate uptake of %TN in above- and below- ground biomass that increased plant growth. It was found that higher levels of %OC in the substrate and %TN in plant tissue depressed growth.

Plants in PHA pots accumulated almost 24% more %TN in plant tissues than did plants grown in PE pots. It is not known if the polyethylene had any effect on the surrounding biogeochemistry of the soil that would create differences in available %OC, or the availability of N, and requires more study with *P. perfoliatus* and other SAV. Noureddin et al. (2004) found that the edible water plant, *Ipomoea aquatica,* absorbed bisphenol A, a plastic additive, and metabolized it, however, it is unknown if the metabolites are a health threat. Other studies have documented the ability of PE and other types of fossil-fuel based plastic to sorb and interact with pollutants, the soil and plants. Because plastics (both biobased and non-biobased) are made of an amorphous and crystal structure, this matrix makes a "bidirectional migration" of molecules in and out of the plastic possible (Ramos et al. 2015). Also to consider, in MEI, there is the possibility that increased %TN was due to the fact that the actual PHA pot itself increases mineralization and under reduced conditions, as bacteria metabolizing PHA form methane. Bacteria present may also, as part of the metabolic process, couple denitrification to methane oxidation to form carbon dioxide, thereby augmenting nitrogen fixation (Nauhaus et al. 2005; Raghoebarsing et al. 2006, Ettwig et al. 2010). This sediment based mechanism has not been investigated in *P. perfoliatus*.

For the comparison of biomass for MEII, it appeared that, qualitatively, plants grown in PHA pots established slightly better than those grown on bare sediment (Table 3.16). For each seed year, the seeds planted in PHA pots established qualitatively greater yields both for aboveground and belowground biomass. This likely occurred for two reasons: the substrate in the pot was SAV bed sediment with oyster shell and peat, an ideal substrate for optimal *P. perfoliatus* growth (Zinecker CH2), and also the seed was planted securely in the pot rather than being buried under a very thin layer of oyster shell fine grains. The small differences did not carry through with any consistency with inflorescences, save the 2006 PHA pot treatment, which appeared to have 30% more biomass in aboveground biomass, and almost an order of magnitude more inflorescences per msq than any other treatment (Table 3.16). In light of the fact that the effect of pots for each seed year, as well as substrate, were more important for growth and establishment than seed age, there is no agreement with the hypothesis stating that 2010 seeds would produce greater yield than those harvested, germinated, and stored from 2006. This was particularly true given that the 2006 seeds ultimately produced the highest biomass qualitatively, and the highest inflorescences statistically.

The lack of correlation of %TN in AGB, yield, and presence of inflorescences may be due to the shorter duration of this experiment (84d). If the experiment would have been run for a longer duration, the differences in inflorescences may have changed over time or "caught up" to more clearly represent AGB biomass, as this experiment was shorter than either MEI (112d) or Zinecker (CH2) (105d). Of import to consider is that the highest number of inflorescences in Zinecker (CH2) was with intermediate %OC and %TN tissue. The substrate values in this study could not effectively be considered

intermediate. Turions were used in Zinecker (CH2), vs. seeds in MEI and MEII. There have been no studies comparing seeds and turions side by side with various nutrient regimes to determine any subsequent effects on yield or inflorescence production. Measurement of relative stem lengths would also have been of greater use than stem density, to potentially better understand how abiotic and microbial factors were affecting overall plant performance. From a mechanical perspective, there was clearly more effort required to securely plant seeds on bare sediment than the PHA pots when placed in the sediment of the microcosms.

Similar to MEI, it is likely that MEII biomass yield was likely not significantly different because sediment parameters were homogeneous. Qualitative differences between treatments, %OC and %TN in biomass, again affected qualitative differences in biomass. This was particularly true with respect to %TN in aboveground biomass, and the response of yield in belowground biomass, i.e. higher %TN in aboveground biomass, corresponded to lower yield in belowground biomass. This resulted in a 20 percent reduction in NPP in comparison to MEI. (Table 3.14, Table 3.15, Table 3.16, Figure 3.3). This response was also noted in Zinecker (CH2). This may be the key to the similar thinking that while Spartina marshes appear to be healthy, in actuality, their belowground biomass is reduced/depressed and the community may begin to fragment (Deegan et al. 2012).

For the purposes of better understanding these dynamics in the experimental environment, it might be further investigated how, over time, NPP would manifest itself with respect to sediment conditions, tissue uptake of N, with observations over a much longer time period, i.e. several growing seasons. Manipulation of both PE and PHA pots,

planting densities (with different species), and soil nutrient regimes would likely further elucidate the contributions of these variables to plant yield. Field sampling at sites where nutrient conditions have consistently been at different levels would also be very useful to determine whether the dynamics now confirmed in salt marshes (Deegan et al. 2012) are true for *P. perfoliatus* and other species.

Most critical is the fact that use of the marsh sediment instead of a more refractory, or low nutrient substrate, resulted in biomass yields in both MEI and MEII that were approximately half of what was seen in NPP for SAV bed sediments. As was emphasized in Zinecker (CH2), the relative reduction in stem lengths and belowground biomass affect the plants ability to sustain populations with seeds as well as have a network of roots for nutritional scavenging, and to protect against erosion or other disturbance. These findings are very preliminary, and it will be useful to further investigate the significance and contribution of microbial communities to yield and reproduction potential under various substrate and other environmental conditions (Deegan et al. 2012, Kirwan and Megonigal 2013).

For pot degradation, MEI results were in agreement with the hypothesis that PHA pots would degrade more rapidly than PE pots over the course of 112 d. This was true for both mass lost (% g) and % spindle diameter lost (shallow and deep) (Tables 3.6, 3.7, 3.15). PHA-based pots degraded (lost mass) on average approximately 98% more than PE pots, which either appeared to gain mass or lose a small quantity (<2%). Percent spindle diameter loss near the sediment surface for PE pots ranged from fractional diameter gain, to a loss of less than < 1%, whereas for PHA loss was on average 95% higher than PE pots. Bottom % spindle loss for PE was greater than shallow though not

significant. PHA spindle diameter loss at 4cm was 99% more than PE pots. PHA% spindle diameter loss at 4 cm was almost 43% greater than shallow PHA %spindle loss. Of particular note, and apparently not documented in any of the peer reviewed literature, was that the presence of plants, (from 6 seeds growth over three pots) produced significantly greater pot loss than unplanted controls for both PE and PHA (Table 3.6), as well as greater loss in deep compared with shallow redox conditions (Table 3.7). This same phenomenon of enhanced degradation in planted treatments was likely not seen in MEII due to the reduced duration of experiment (84 d vs. 112 d), reduced overall biomass (4 seeds vs. 6 seeds), and fewer pots (2 pots vs. 3 pots in MEI). Jia et al. (2016) attributed faster dissipation of sediment contaminants due to *Avicennia marina* (Forsk.) Vierh exudates that served to increase microbial populations near roots, thereby facilitating contaminant metabolism (i.e. rhizodegradation), and a similar phenomenon may have occurred in MEI. Lim et al. (2005) documented that in forest soils, a medium chain length PHA made from palm kernel oil biodegraded more quickly than either PE (no degradation) or PHB (polyhydroxybuturate) films, and they attributed this to acidity of the soil and possible micro rganisms present, but did not mention plant presence or absence. Harrison et al. (2014) documented that bacteria in many situations will colonize PE plastic, and that microbial communities on plastics will vary with different sediment type. However, they found very little degradation of the PHA in highly organic mangrove sediments, no degradation for PE, and high degradation for PHB. Shahnawaz et al. (2016) used bacteria from the rhizosphere in a laboratory environment, pH was manipulated, and not part of an in situ test system. Degradation and loss in tensile strength was variable depending on different treatments and pH, however pH and

bacterial isolate appeared to be the only metrics of interest and so other environmental parameters were not shared.

These studies show how the variations in degradation of bio-based and fossil-fuel based polymers can be radically different, making comparison between studies probematic. Products labelled “PHA” can be quite different depending on polymer chain length, and be degradable only by specific organisms able to secrete the particular depolymerase to degrade a given polymer (Boyandin et al. 2013, Manna and Paul 2000).

Microcosm Experiment II reported no significant differences in degradation between unplanted pots vs. planted PHA pots, with qualitatively greater % pot mass loss in unplanted controls, followed by medium % degradation with highest biomass (2006 plants) and lowest % pot degradation with lowest biomass (2010 plants). Differences between deep and shallow % spindle diameter degradation were also qualitative only. The differences were likely due to the changed, less stratified redox in the microcosm environment. In MEII, redox values were spread more evenly throughout the degradation range rather than being clustered in deep/greater % spindle loss or shallow/lower % spindle loss. The degradation shifts did not necessarily mean the microbes were doing less work, just that there were less differences in the extent of biodegradation occurring to spindles between deep and shallow throughout the microcosm. This meant that degradation measured 25% of the spindle regardless of whether Eh was -50 or +200mv, while the greatest degradation still occurred between -50 mv to 0 mv, though for fewer data points (Figure 3.4C). Thus daily % spindle diameter loss was almost the same for deep and shallow Eh measurements, except for deep, unplanted, pots, which still appeared on average to retain a qualitative larger range between deep and shallow

portions of the spindle degraded (Table 3.16). Because microbes were not directly measured, it can only be suggested that the differences between experiments in Eh behavior represents a shift in microbial populations due to environmental parameters. This may have included locations of the microbes and proximity to the pots, the species assemblages, or their availability to metabolize PHA plastic. These types of changes may have been similar to the changes over time and sediment changes that fundamentally altered the community in the Winogradsky Columns in Esteban et al. (2013).

The Field Experiment hypothesized that PHA biodegradable pots would degrade more quickly in the mesohaline, sandy loamy sediments than in the sandy gravelly substrate of tidal fresh Upper Bay. During the course of the study, two major storms occurred, Hurricane Irene (Last week of August 2011), and Hurricane Lee (first week of September 2011). The mobility of the finer grained sands near the armored shoreline at Ragged Point caused the pots to become more deeply buried, while in the upper Bay, a thick mat of Vallisneria wrack and gravelly substrate protected the pots and kept them in place where initially planted, just at the sediment surface rather than those more deeply buried at Ragged Point.

As a result of these conditions, the significantly higher mass loss (20% greater) at Ragged Point site was likely due to the ability of pots to remain completely buried even deeper than initially planted, than the shallower, more exposed pots in the upper Bay. In addition, while the storm disturbances affected temperature and salinity, the mesohaline site was slightly warmer, for a longer seasonal period, than the tidal fresh site. More research on these differences would provide a clearer idea of the microbial environment, and the relationship of temperature, nutrient regime, and salinity.

Percent diameter spindle degradation appeared to be qualitatively more extensive at the mesohaline site compared with freshwater Carpenter's Point site. At the Ragged point mesohaline site the spindles overall lost ~33% more diameter for both shallow and deep measurements compared with Carpenter's Point. Differences between shallow and deep within each treatment were also approximately 33-35% in loss between shallow and deep. The spindle diameters at both sites were heterogeneously degraded, i.e. the bottoms were not always more degraded than the tops (and so no significant differences were found). In addition, in many cases a portion of the center of a spindle was more highly degraded than either the top or the bottom, etc. This presents an interesting question about the heterogeneous distribution of microorganisms able to metabolize PHA once this material is in a natural environment, and how a disturbance such as storms, affect this metabolism or microbial distribution. Clearly additional field and microcosm experiments would provide opportunities to evaluate the distribution of microbial communities that are able to metabolize PHA and similar materials.

CONCLUSIONS

The greenhouse experiments confirmed that, under controlled conditions of the microcosms, *P. perfoliatus* plants grew reasonably well whether in PHA pots, nonbiodegradable PE control pots, or by hand transplants. Reasonably well in this case was defined with the knowledge that overall yield was approximately half to 60% of what yield could be under appropriate substrate conditions (Zinecker CH2). While non-significant, a trend indicated that in most cases, for both aboveground and belowground biomass, biomass was greatest in the non-degradable PE pots, followed by PHA pots, and

the lowest biomass growth overall was with seeding by hand on bare microcosm sediment. Given the level of effort involved in the bare sediment planting vs. planting in pots, there is persuasive evidence that the ability to place seeds easily and quickly in an environment that transitions immediately and securely to the field restoration is an advantage.

Differences in plant reproductive fitness in the form of flowers produced was inconsistent across both microcosm experiments and most treatments, with approximately one microcosm in each experiment having disproportionately high numbers of inflorescences. Again, the production of inflorescences was approximately half of what it might have been given appropriate substrate conditions (Zinecker CH2, Table 3.15). Ideally more research would be undertaken to understand the balance between aboveground, belowground biomass yields and the production of inflorescences.

Overall seed fitness, for the 2010 and 2006 seeds, while seeming to respond favorably to pots compared with hand transplanting, requires additional investigation, as this was one of the first microcosm experiments using SAV seeds with the PHA pot. Also, given that this is the first time seeds of this age (4.5 years) were used to successfully produce plants with flowers of any of the species found in Chesapeake Bay, this may provide the motivation for further research to develop longer term seed storage and preservation techniques. This has the potential to enable greater flexibility in restoration planning or propagation scenarios, particularly when a large number of seeds is required.

Chapter 3

Tables

Table 3.1. Experiment pot mass (g) and minimum and maximum ranges of top and bottom spindle diameters (mm) for biodegradable PHA pots and polyethylene plastic (PE) control pots. Mold injection and plastic formulation were variable, consequently mass varied and spindles were not perfectly symmetric. Starting mass, and diameter ranges are given below. Top and bottom ranges of spindle diameter were used to calculate minimum and maximum % diameter lost.

		Diameter Range (mm)	
Pot Type	**Mass (g)**	**Top of Spindle (mm)**	**Bottom of Spindle (mm)**
Microcosm Experiment I			
PHA	3.78 ± 0.02	5.89-5.95	4.08-4.09
PE Planted	3.68 ± 0.01	5.77-5.84	4.03-4.09
PE Unplanted	3.52 ± 0.01	6.06-6.16	4.13-4.15
Microcosm Experiment II and Field Experiment			
PHA	4.26 ± 0.27	5.72-5.97	4.01-4.15

Table 3.2. Microcosm Experiment No. One: Four different treatment combinations replicated 16 times each, and one treatment was replicated 7 time (as a control for substrate testing), to test plant biomass response to pot treatment, and extent of degradation of pot treatments when planted with plants or with no plants. Redox, Loss on Ignition Total C, and N measurements were made on substrates in all five treatments. The same substrate was used in all microcosms. A completely orthogonal design including "No Pots, Plants" was initially planned, however seedling quality and methodologies were not yet sufficiently developed to support this design.

Treatments	**Plants**	**No Plants**
Biodegradable pots	BPP x 16	BPNP x 16
Control polyethylene pots	CPP x 16	CPNP x 16
No Pots	---	NPNP x 7 (even replicates unnecessary)

Table 3.3. Microcosm Experiment II: Six different treatment combinations replicated 5 times each, for a balanced 2x3 factorial design for testing growth of seed year in PHA biodegradable pots and "hand-broadcasting".

Treatments	2010 Plants	2006 Plants	No Plants
Biodegradable pots	5 reps	5 reps	5 reps
Hand-plant, no pot	5 reps	5 reps	5 reps

Table 3.4. % organic matter (%OM), % organic carbon (%OC = (LOI OM/2)), % bicarbonate (%TC - %OC), %Total Carbon, %Total Nitrogen and organic Carbon:Nitrogen ratio based on %OC and %TN. Substrates were not significantly different as pots were not highly concentrated in the substrate.

Treatment	%OM $F_{1,10}$=2.53 p = 0.1431	% OC $F_{1,10}$=1.84 p = 0.2053	%Bicarbonate $F_{1,9}$=0.14 p = 0.7137	%TC $F_{1,9}$=0.50 p = 0.4966	%TN $F_{1,9}$=0.77 p = 0.4027	C:N $F_{1,9}$=0.88 p = 0.3718
PHA pots No plants	6.33 ± 0.80	3.16 ± 0.40	2.44 ± 0.19	5.21 ± 0.12	0.10 ± 0.12	28.47 ± 4.17
PHA pots Plants	6.19 ± 0.28	3.10 ± 0.14	2.73 ± 0.38	5.83 ± 0.48	0.12 ± 0.02	26.85 ± 3.36
PE pots No plants	7.77 ± 0.91	3.61 ± 0.25	2.55 ± 0.37	6.16 ± 0.62	0.13 ± 0.01	28.21 ± 0.70
PE pots Plants	5.73 ± 0.11	2.86 ± 0.06	3.19 ± 0.66	6.05 ± 0.07	0.13 ± 0.01	22.28 ± 1.39
No pots No plants	6.74 ± 0.91	3.37 ± 0.26	3.00 ± 0.66	6.37 ± 0.18	0.13 ± 0.00	25.71 ± 1.24

Table 3.5. % Total Carbon, % Total Nitrogen and C:N ratios for aboveground and belowground biomass. Subscript letters next to mean±S.D. indicate significant differences (5% level). **Bolded = $F_{(5,11)}$.P value.** *Subscript letters next to mean indicate significant difference (at the 5% level).

Substrate	% Total Carbon		% Total Nitrogen		C:N	
	AGB $F_{1,11}$=2.05 p = 0.1796	BGB $F_{1,12}$=12.82 p = 0.0038	AGB $F_{1,11}$=6.39 p = 0.0281	BGB $F_{1,12}$=5.47 p = 0.0375	AGB $F_{1,11}$=4.42 p = 0.0594	BGB $F_{1,12}$=7.96 p = 0.0154
PHA pots Plants	37.14 ± 0.35[a]	34.84 ± 0.72[a]	1.63 ± 0.11[a]	1.45 ± 0.11[a]	23.12 ± 1.38[a]	25.04 ± 2.21[a]
PE pots Plants	36.53 ± 0.26[a]	37.60 ± 0.36[b]	1.24 ± 0.10[b]	1.08 ± 0.11[b]	31.33 ± 3.22[b]	36.84 ± 3.12[b]

Table 3.6. Percent (%) mass, top and bottom spindle diameter, with biomass results. Percent mass lost for fixed effects of pots was highly significant ($F_{1,60}$ = 10.12, p=0.0023), however, the trend for pots*plants is shown in conjunction with other significant values for similar fixed effects.

Treatment	% Mass Lost $F_{1,60}$=3.01 **p = 0.0877***	%Spindle Lost Min / Max $F_{1,60}$=10.12 / $F_{1,60}$=8.57 **p = 0.0023 / p = 0.0048**	Biomass AGB $F_{1,28}$=2.39, p = 0.1331	 BGB $F_{1,28}$=1.05, p = 0.3153	 Inflorescences **$F_{1,28}$=1.05,** **p = 0.0311**
PE pots No plants	-0.47 ± 0.13[a]	-0.89 ± 0.15[a] / 0.17 ± 0.12[a]	NA	NA	NA
PE pots Plants	1.33 ± 0.13[a]	0.21 ± 0.13[a] / 1.54 ± 0.13[a]	1.85 ± 0.15[a]	1.15 ± 0.14[a]	2.5 ± 1.1[a]
PHA pots No plants	58.24 ± 1.94[a]	20.75 ± 1.45[b] / 21.27 ± 1.40[b]	NA	NA	NA
PHA pots Plants	63.93 ± 1.53[a]	25.29 ± 1.38[c] / 25.78 ± 1.32[c]	1.46 ± 0.20[a]	0.96 ± 0.13[a]	0[b]

Table 3.7. Pot treatments for %spindle loss at deep and shallow pH/redox measurements. Subscript letters next to mean±S.D. indicate significant differences (5% level). % spindle lost (min/max)(pot*depth): ($F_{1,60}$=169, p < 0.0001 / $F_{1,60}$=164.68, p<0.0001). For redox: ($F_{1,26}$=286.07, p < 0.0001); For pH: $F_{1,13.3}$=164.68, p<0.0001

Treatment Pot	%Spindle Loss (Top - surface) Min / Max **Eh/pH conditions: 248.0 ± 0.02[a] mV / 9.34 ± 0.01[a]**	%Spindle Loss (Bottom – 4 cm) Min / Max **Eh/pH conditions: -3.30 ± 8[b] mV / 7.90 ± 0.1[b]**
PE	-0.59±0.19[a]/0.83 ± 0.16[a]	-0.09 ± 0.13[a] / 0.88 ± 0.19[a]
PHA	16.22±0.64[b]/17.07 ± 0.64[b]	29.81 ± 0.97[c]/ 29.98 ± 0.97[c]

Table 3.8. % organic matter (%OM), % organic carbon (%OC = (LOI OM/2)), % bicarbonate (%TC - %OC), %Total Carbon, %Total Nitrogen and organic Carbon:Nitrogen ratio based on %OC and %TN. Treatments were not significantly different as substrate treatment remained consistent and fewer pots were in the soil

Treatment	%OM $F_{2,24}$ =1.93 p = 0.1664	% OC $F_{2,24}$ =1.93 p = 0.1664	%Bicarbonate $F_{2,24}$= 0.26 p = 0.6172	%TC $F_{2,24}$ = 2.13 p = 0.1412	%TN $F_{2,24}$ =0.17 p = 0.8475	%OC:%N $F_{2,24}$ =2.51 p = 0.1023
No pots 2010 plants	7.27 ± 0.19	3.64 ± 0.10	3.21 ± 0.44	6.85 ± 0.40	0.14 ± 0.01	26.75 ± 1.76
PHA pots 2010 Plants	7.04 ± 0.58	3.52 ± 0.29	2.71 ± 0.33	6.23 ± 0.36	0.13 ± 0.01	26.58 ± 2.50
No pots 2006 plants	7.13 ± 0.45	3.57 ± 0.22	1.95 ± 0.24	5.51 ± 0.45	0.14 ± 0.01	26.02 ± 0.81
PHA pots 2006 Plants	6.69 ± 0.50	3.34 ± 0.25	3.32 ± 0.07	6.66 ± 0.25	0.15 ± 0.02	23.50 ± 2.14
PHA pots No Plants	8.10 ± 1.02	4.05 ± 0.51	3.00 ± 0.18	7.05 ± 0.61	0.15 ± 0.02	27.85 ± 1.09
No pots No plants	6.50 ± 0.35	3.25 ± 0.17	3.50 ± 0.29	6.75 ± 0.42	0.15 ± 0.01	23.06 ± 0.92

Table 3.9. % Total Carbon, % Total Nitrogen and C:N ratios for aboveground and belowground biomass. All analyses conducted at the 5% level.

Treatment	% Total Carbon		% Total Nitrogen		C:N	
	AGB	BGB	AGB	BGB	AGB	BGB
	$F_{1,8}$ =1.04 p = 0.3376	$F_{1,8}$ =0.98 p = 0.3502	$F_{1,8}$ =0.44 p = 0.5249	$F_{1,8}$ =2.90 p = 0.1270	$F_{1,8}$ =0.71 p = 0.4239	**$F_{1,8}$ =7.82 p = 0.0233**
2010 plants	32.99 ± 2.76[a]	36.49 ± 0.44 [a]	1.72 ± 0.06[a]	1.35 ± 0.08[a]	19.06 ± 1.35[a]	**27.46 ± 1.75 [a]**
2006 plants	35.73 ± 0.86[a]	33.52 ± 2.79[a]	1.78 ± 0.07[a]	1.59 ± 0.12[a]	20.21 ± 0.83[a]	**21.19 ± 1.72 [b]**

Table 3.10. % mass, top and bottom spindle diameter, with soil chemistry information. Percent mass lost for fixed effects of pots was highly significant however, the trend for pots*plants is shown in conjunction with other significant values for similar fixed effects.

Treatment	% Pot Mass Loss	Biomass			
		AGB	BGB	Stems	Inflorescences
	$F_{2,12}$ =0.49 p = 0.6240	$F_{1,16}$ =3.28, p = 0.0888	$F_{1,16}$ =0.10, p = 0.7612	$F_{1,13}$ =1.41, p = 0.2561	$F_{1,13}$ =5.10, **p = 0.0417**
No pots 2010 plants	NA	1.16 ± 0.22[a]	0.48 ± 0.12[a]	69.67 ± 15.6[a]	1.67 ± 1.3[ab]
PHA pots 2010 plants	37.65 ± 3.63[a]	1.23 ± 0.25[a]	0.55 ± 0.11[a]	58.00 ± 14.3[a]	0.75 ± 0.43[a]
No pots 2006 plants	NA	0.99 ± 0.25[a]	0.44 ± 0.07[a]	53.00 ± 4.9[a]	1.25 ± 0.85[a]
PHA pots 2006 plants	38.12 ± 9.05[a]	1.72 ± 0.21[a]	0.56 ± 0.05[a]	70.8 ± 0.21[a]	5.20 ± 3.97[b]
PHA pots No plants	46.41 ± 7.31[a]	NA	NA	NA	NA

Table 3.11. Pot treatments for % spindle loss at deep and shallow pH/redox measurements shows heterogeneity of spindle loss, even when redox and pH for shallow and deep are significantly different. Subscript letters next to mean±S.D indicate significance (5% level). % spindle lost (min/max) (planting treatment*dep): ($F_{2,12}$ =0.62, p=0.5557 / $F_{2,12}$ =0.63, p=0.5516). For redox (depth): $F_{1,24}$=477.26, p < 0.0001; For pH (plant*depth): $F_{2,24}$ =4.95, p=0.0159.

Treatment PHA Pot	%Spindle Loss (shallow) Min / Max	Eh/ (shallow) pH	%Spindle Loss (deep) Min / Max	Eh/ (deep) pH
2006 Plants	13.20 ± 3.35[a]/16.83±3.21[a]	203.87 ± 13[a] / 9.75 ± 0.14[a]	15.09 ± 5.65[a] / 17.95 ± 5.46[a]	17.81 ± 32[b] / 7.63 ± 0.12[c]
2010 Plants	9.11 ± 3.30[a]/12.91 ± 3.17[a]	184.44 ± 20[a] / 9.90 ± 0.10[a]	9.05 ± 0.95[a] / 12.12 ± 0.92[a]	-37.69 ± 13[b] / 7.70 ± 0.12[c]
No plants	14.77 ± 2.28[a]/18.34 ± 2.18[a]	208.0 ± 10[a] / 9.27 ± 0.09[b]	21.50 ± 7.32[a] / 24.14 ± 7.07[a]	-24.86 ± 10[b] / 7.56 ± 0.07[d]

Table 3.12. Averaged pot treatments for %spindle loss at deep and shallow pH/redox measurements. Maximum % spindle loss in conditions of shallow redox/pH is higher than the minimum end of Eh/pH for deep. Subscript letters next to mean±S.D. indicate significance (5% level). % spindle lost (min/max)(depth): ($F_{1,12}$ = 1.23, p = 0.2883 / $F_{1,12}$ =0.68, p=0.4264).For redox (depth): $F_{1,24}$=477.26, p < 0.0001; For pH (depth): $F_{1,24}$ =858.04, p<0.0001.

Treatment	%Spindle Loss (shallow) Min / Max	Eh/ (shallow) pH	%Spindle Loss (deep) Min / Max	Eh/ (deep) pH
PHA	12.36 ± 1.74[a] /16.03 ± 1.66[a]	212.4 ± 6.9[a] / 9.64 ± 0.08[a]	15.21 ± 0.82[a] / 18.07 ± 3.17[a]	-10.4 ± 9[b] / 7.63 ± 0.06[b]

Table 3.13. Pot treatments for % mass loss, and spindle loss at “deep” and “shallow” pH/redox measurements for tidal fresh and mesohaline field sites. Subscript letters next to mean±S.D. indicate significance (5% level). **% mass lost: $F_{1,6}$ =7.67, p=0.0324**; % spindle lost (min/max) (depth): $F_{1,6}$ =3.45, p = 0.1126 / $F_{1,6}$ =2.61, p = 0.1576.

Site/ Salinity	Conditions Substrate/Temp.	% Mass (g) Lost	%Spindle Loss (shallow) (min/max)	%Spindle Loss (deep) (min/max)
Fresh (0 parts)	gravelly/sandy 15-30 deg C	**54.67 ± 1.71[a]**	4.11 ± 1.12[a] / 8.12 ± 1.07[a]	9.35 ± 2.01[a] / 12.41 ± 1.94[a]
Mesohaline (5.9 -15.7 parts)	sandy 18-28 deg C	**68.80 ± 2.85[b]**	8.39 ± 2.25[a] / 12.23 ± 2.15[a]	15.34 ± 7.88[a] / 18.19 ± 7.61[a]

Table 3.14. Net primary productivity, % organic C, and percent plant tissue N for aboveground and belowground biomass (data from microcosm experiments (Zinecker CH2). The data serve as reference values for the relationship between productivity, %OC, %TN in plant tissues, and the related redox conditions.

Substrate Type 105 days	AGB gDW m^{-2} day^{-1}	BGB gDW m^{-2} day^{-1}	AGB+ BGB gDW m^{-2} day^{-1}	No. Stems m^{-2} 105 days	Infl. m^{-2} 105 days	%OC Sub	%N AGB	%N BGB	Eh (mV) top	Eh (mV) deep
sand	0.17 ± 0.02	0.20 ± 0.03	0.37 ± 0.05	1064.13 ± 130.48	0	0.036 ± 0.006	0.92 ± 0.10	0.92 ± 0.12	359 ± 28	394 ± 24
Soil/ Sand	0.31 ± 0.05	0.16 ± 0.02	0.47 ± 0.08	1269.25 ± 73.65	47.80 ± 31.12	1.73 ± 0.15	1.77 ± 0.11	1.48 ± 0.16	288 ± 10	246 ± 16
Oyster	0.45 ± 0.03	0.17 ± 0.02	0.62 ± 0.04	1157.06 ± 117.44	199.81 ± 92.39	0.40 ± 0.008	1.19 ± 0.06	1.11 ± 0.06	321 ± 42	293 ± 35
Oyster/ peat	0.55 ± 0.04	0.31 ± 0.05	0.86 ± 0.09	1617.10 ± 150.19	122.15 ± 70.59	9.15 ± 0.23	1.05 ± 0.07	1.12 ± 0.03	254 ± 17	229 ± 15
Sherwood Forest	0.64 ± 0.08	0.33 ± 0.06	0.97 ± 0.13	1380.11 ± 250.31	462.03 ± 82.27	0.23 ± 0.02	1.22 ± 0.05	1.27 ± 0.06	236 ± 31	187 ± 32
Kent Narrows	0.76 ± 0.07	0.29 ± 0.02	1.05 ± 0.07	2193.31 ± 244.68	288.10 ± 76.56	0.43 ± 0.048[c]	1.21 ± 0.06	1.42 ± 0.07	268 ± 37	229 ± 35

Table 3.15. PE and PHA pot mass loss on a daily basis throughout a microcosm experiment for 112 days. While PE pots degraded a small fraction, PHA pots degraded one to two orders of magnitude faster on a daily basis.

PHA vs PE 112 Days	AGB gDW m^{-2} day^{-1}	BGB gDW m^{-2} day^{-1}	AGB+ BGB gDW m^{-2} day^{-1}	Infl. m^{-2} 112 days	%OC Sub	%N AGB	%N BGB	Eh (mV) top	Eh (mV) deep	Pot Mass % Loss day^{-1} approx.	Spindle %loss top day^{-1} approx.	Spindle % loss deep day^{-1} approx.
PE pot No plants					3.61 ± 0.25			288 ± 11	6.12 ± 11	0	0.002	0.001
PE pot Plants	0.41 ± 0.03	0.26 ± 0.03	0.67 ± 0.06	65.00 ± 28.64	2.86 ± 0.06	1.24 ± 0.10	1.08 ± 0.11	265 ± 12	1.5 ± 28	0.01	0.01	0.01
PHA pot No plants					3.16 ± 0.40			248 ± 0	4 ± 12	0.52	0.13	0.25
PHA pot Plants	0.33 ± 0.04	0.21 ± 0.03	0.54 ± 0.07	0	3.10 ± 0.14	1.63 ± 0.11	1.45 ± 0.11	226 ± 30	-15 ± 11	0.57	0.17	0.29

Table 3.16. Growth of plants in PHA pots vs. bare sediment. Bare sediment qualitatively afforded less ideal circumstances in which to establish and grow.

Pot vs nopot 84 days	AGB gDW m^{-2} day^{-1}	BGB gDW m^{-2} day^{-1}	AGB+ BGB m^{-2} day^{-1}	Stems m^{-2} 84 days	Infl. m^{-2} 84 days	%OC Sub	%N AGB	%N BGB	Eh (mV) top	Eh (mV) deep	Pot Mass % Loss day^{-1} approx.	Spindle top % loss day^{-1} approx.	Spindle deep% loss day^{-1} approx.
Nopot 2010 Seeds	0.34 ± 0.07	0.14 ± 0.04	0.49 ± 0.10	1741.67 ± 504.63	41.7 ± 42	3.64 ± 0.10	1.75 ± 0.08	1.32 ± 0.13	233 ± 18	31 ± 24			
Phapot 2010 Seeds	0.36 ± 0.08	0.16 ± 0.03	0.53 ± 0.11	1450.00 ± 399.87	18.6 ± 12.0	3.52 ± 0.29	1.70 ± 0.12	1.29 ± 0.10	184 ± 20	-38 ± 13	0.45	0.15	0.14
Nopot 2006 Seeds	0.30 ± 0.05	0.13 ± 0.02	0.43 ± 0.07	1325.00 ± 122.73	31.3 ± 23.7	3.57 ± 0.22	1.91 ± 0.01	1.48 ± 0.05	227 ± 11	-9 ± 14			
Phapot/ 2006 Seeds	0.51 ± 0.06	0.17 ± 0.02	0.68 ± 0.08	1770.00 ± 258.41	130.0 ± 99.2	3.34 ± 0.25	1.65 ± 0.10	1.71 ± 0.23	204 ± 13	18 ± 32	0.45	0.20	0.21
PHApot No plants						4.05 ± 0.51			208 ± 10	-25 ± 10	0.58	0.21	0.29

Table 3.17. Field experiment comparing mesohaline (Middle Bay) and fresh (Upper Bay) degradation of PHA pots in sediment near SAV beds. For the mesohaline, temperatures stayed warmer for the last two months of the experiment for the mesohaline, and sediment type was more amenable to burial of the pots.

PHA pot field trial ~5 months /156 days	Salinity Range (parts)	Temperature Range	Pot Mass % Loss day^{-1} approx.	Spindle top % loss day^{-1} approx.	Spindle deep% loss day^{-1} approx.
Fresh	0	15.63 ± 0.09 – 29.50 ± 0.06	0.35 [a]	0.05	0.08
Mesohaline	7.45 ± 0.11 - 13.15 ± 0.53	18.04 ± 0.06 – 27.62 ± 0.37	0.44 [b]	0.08	0.12

Chapter 3
Figures

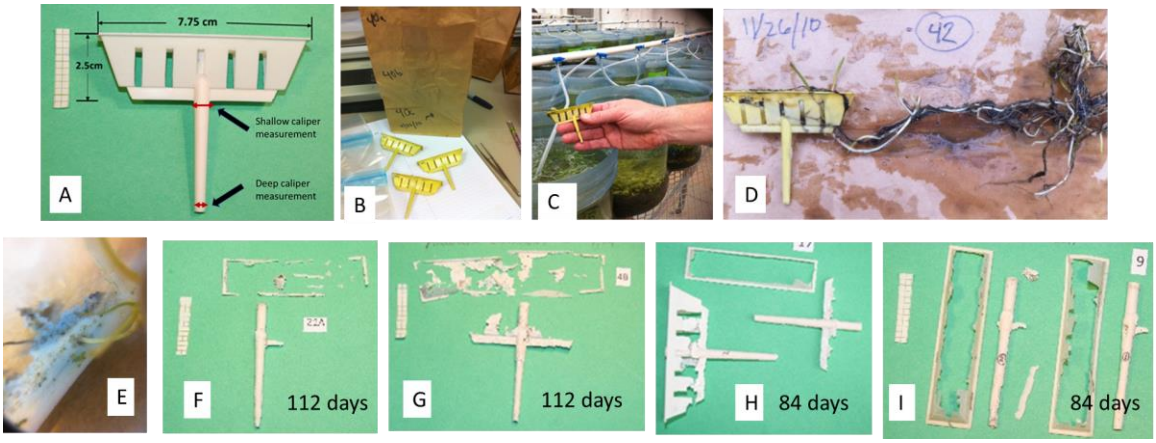


Figure 3.1. A. PHA pot dimensions and locations where deep and shallow caliper measurements were made. B, C. The polyethylene control pots (yellow) were made from petrol-based plastic that did not degrade appreciably. D. Plants in PE pots were still able to grow as well as plants in PHA pots. E. The plants readily grew through any available degraded openings in the PHA pots. F, G, H, I. Degradation after 112 days (Microcosm Experiment I) yielded results that appeared more degraded than degradation after 84 days, with the results appearing more heterogeneous (Microcosm Experiment II).

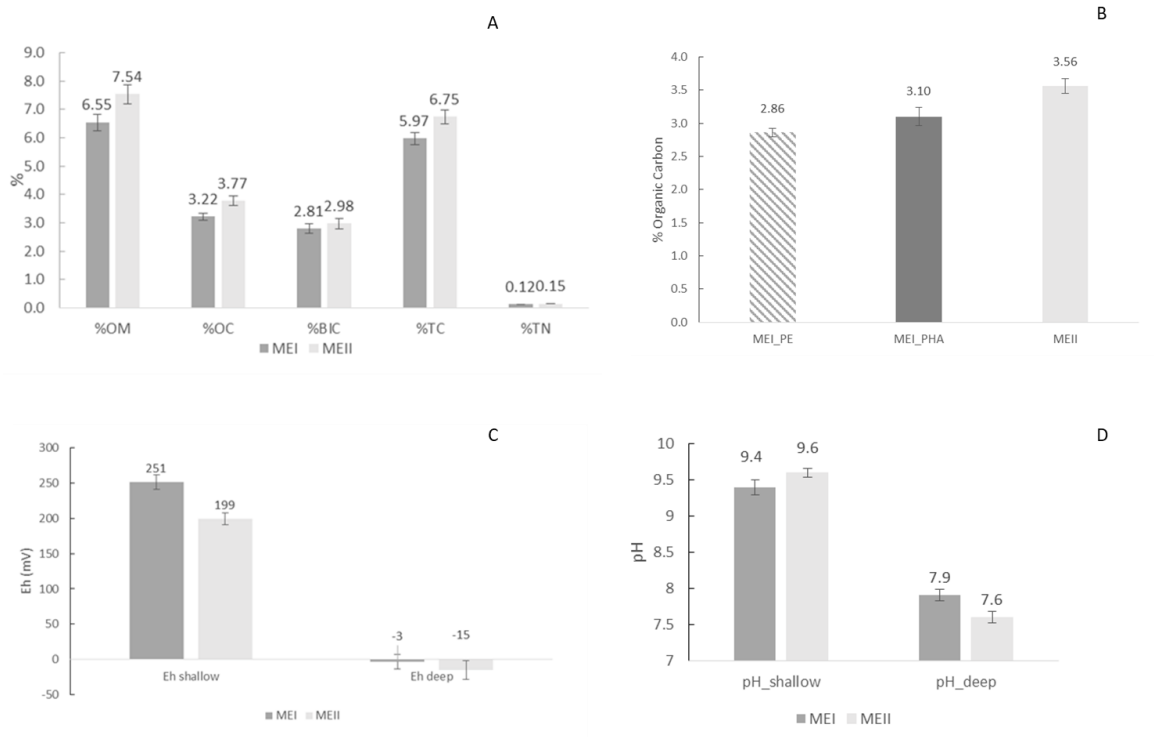


Figure 3.2. A. Microcosm conditions for MEI and MEII for sediment conditions. It appears all values increased over the longer period of time the microcosms were prepared for MEII. B. Specific breakdown between %OC for PE, PHA in MEI and PHA in MEII. The lower %OC in PE resulted in the lowest and siginificant values of AGB and BGB for %TN (Figure 3.3).

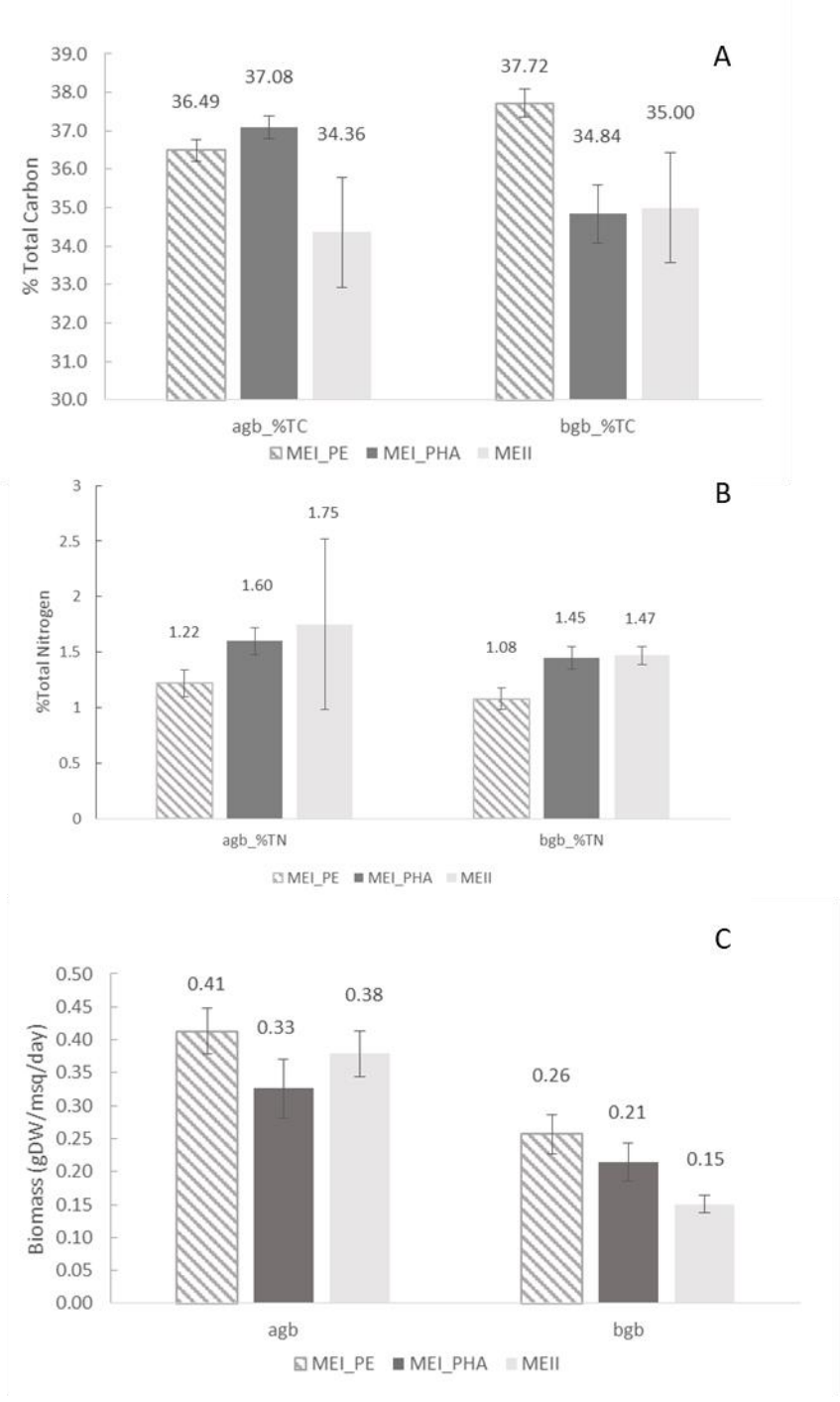


Figure 3.3. A. Microcosm conditions for MEI and MEII for %TC. B. The significant increase in %TN in biomass. C. Increasing plant tissue %TN appeared to affect yield, particularly with respect to belowground biomass.

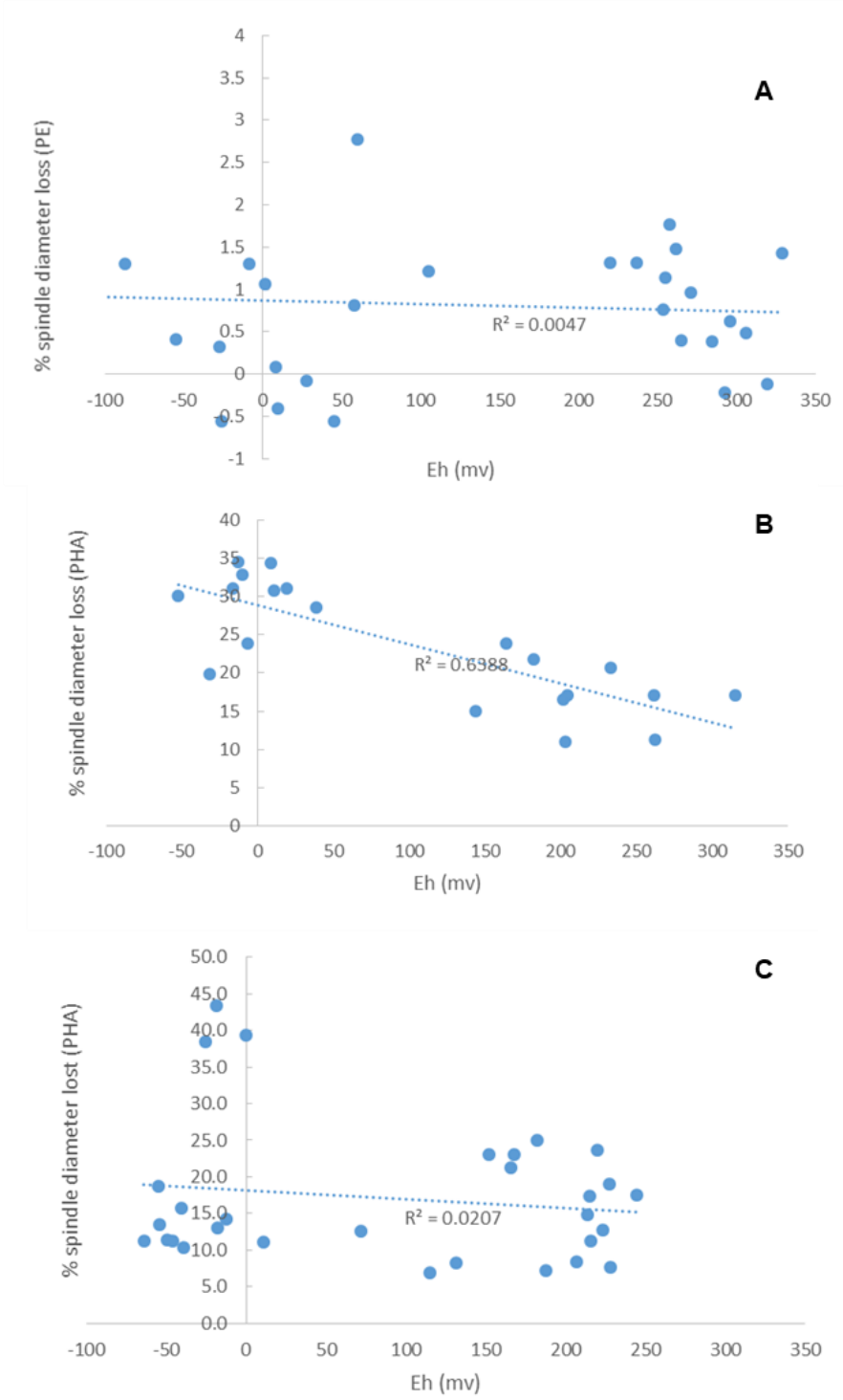


Figure 3.4. MEI and MEII correlation of Eh and % degradation of combined deep and shallow portions of the spindle. A. In MEI there was very little correlation between Eh and extent of degradation for PE plastics. B. There was good agreement for PHA with a relatively strong difference between greater spindle loss and more negative redox in MEI. C. In MEII the largest qualitative % spindle diameter loss still occurred at or below 0 mv, however, it appeared there were more consistent moderate percent losses across the -75mv-250 mv range unlike B, and losses between treatments or depth were not statistically significant. This may have been due to microbial migration,i.e. a shift in microbial populations in the microcosm over time, or some combination of factors.

CHAPTER 4:

Emergy analysis of two *P. perfoliatus* L. restoration methods: biodegradable pots with seeds vs. hand transplanting of sods

INTRODUCTION

Potamogeton perfoliatus L., or redhead grass, is a submersed aquatic angiosperm species in mesohaline Chesapeake Bay that has declined dramatically since the 1960s and has not shown evidence of recovery (Hengst et al. 2010, Ailstock et al. 2010a, Gruber et al. 2011, Orth et al. 2015, 2016). Submersed aquatic vegetation (SAV) communities are a high-value component of coastline ecosystems and other shallow water bodies (Thayer et al. 1975, Millennium Ecosystem Assessment 2005). They preserve shorelines (Fonseca and Cahalan 1992), conserve sediment through rooting and microbially-mediated biogeochemistry (Risgaard-Petersen and Jensen 1997, Fierer et al. 2009, McGlathery et al. 2007), provide critical habitat (Hughes et al. 2009, Heck et al. 2003, Lubbers et al. 1996), and sequester carbon (Macreadie et al. 2015). Economic-ecologic valuations on SAV and other closely related coastal resources range anywhere from \$3.00 USD/m^2 (Costanza et al. 1997, 2014), to \$89.00 USD/m^2 (de Groot et al. 2012), and \$204.00 USD/m^2 (Vassallo et al. 2013). The marked difference in these numbers coincides with a progression over the years of incorporating humans' perceived needs with the requirements for the system's sustainability (Vassallo et al. 2013). Vassallo's (2013) emergy valuation of a seagrass meadow took into account both "receiver (user)" and "donor" perspectives of the value of SAV, as have others in forests (Campbell and Brown 2012) and theoretical systems (Pulselli et al. 2011).

SAV restoration, while deemed critically important, is a management tool that continues to be refined and developed (Suding et al. 2011, Abelson et al. 2016, Orth et al. 2017). Where water quality (Kemp et al. 2004, Borum and Sand-Jensen 1996), and other

environmental conditions for submersed aquatic habitat are inappropriate (Bourque et al. 2015, Short and Wyllie-Echevveria 1996, Arnold et al. 2000), SAV restorations are less successful (Shafer and Bergstrom 2010).

Restoration efforts in the Chesapeake Bay using *P. perfoliatus* shoots, seeds, and sods (also known as turfs), have not yet met with consistent, sustainable successes (STAC 2011, Shafer and Bergstrom 2010). *P. perfoliatus* seed broadcasting can at times be challenged with unfavorable meteorological events that may disturb the restoration site sediment and in-situ seeds (Steve Ailstock, 2016, personal conversation). Whole sods of *P. perfoliatus* may persist successfully for one or two seasons but then may slowly decline due to herbivory, poor water quality or sediment erosion (Mark Lewandowski, MD-DNR, personal conversation 2017). Because smaller numbers of *P. perfoliatus* restorations have been successful, and budgets do not always allow for flexibility to test the efficacy of various planting approaches and to explore the suitability of multiple sites (Bergstrom 2006), managers have been less able to share the potential benefits restored SAV beds provide through follow-up and monitoring (Neckles et al. 2012, Bell et al. 2014, Fonseca et al. 1998).

This may also be why restoration managers are less likely to publish the costs and relative successes of their projects with sufficient regularity and detail (Lewis 2006). Lewis (2006), and others (Abelson et al. 2016, Barbier 2012, Millenium Ecosystem Assessment 2005), urge the SAV and coastal restoration communities to share information about their projects to raise awareness of not only the advantageous economic and ecological values that SAV restoration potentially presents, but to garner

more funding for conservation and support for further development of restoration techniques to sustain these imperilled resources.

Thus far the valuation of SAV restoration projects has focused less on ecosystem services, and more on the manager-defined costs of the projects (Shafer and Bergstrom 2008, Lewis 2006, Fonseca et al. 1982). Among the many SAV restorations that have been implemented, just a handful of the projects have provided a general idea of the costs on a per hectare (area) or a per propagule basis (Busch et al. 2010, Fonseca et al. 1982, Lewis 2006, Thorhaug and Austin 1976). U.S. Dollar values for restoration efforts for seagrasses range from more detailed budgets with relatively high inputs: USD$ 338.7/m^2 (Lewis et al. 2006), to less resource intensive approaches that entail more modest budgets, (and in some cases exclude one-time large purchases): \$4.29 m^2 to \$11.70 m^2 (Busch et al. 2010). Recent data that document *P. perfoliatus* restoration have ranged from \$8.34 m^2 to \$24.46 m^2 (Bergstrom 2006, Shafer and Bergstrom 2008) (Table 4.11).

In this hypothetical case study, both emergy analysis, a form of environmental cost accounting (Odum 1996), and standard USD$, were used to evaluate the environmental inputs and costs of two restoration techniques for *P. perfoliatus*. One method, a hand transplanting of whole sods, has been successfully implemented with some seagrass species, (Ranwell et al. 1974); the second method involves seed transplantation at the site using small pots made from fully biodegradable polyhydroxyalkanoate (PHA) plastic (Zinecker CH3, Zinecker and Kangas 2011a).

In a microcosm experiment, *P. perfoliatus* seeds grown in the PHA biodegradable pots exhibited higher net primary productivity (0.61 gdw/m^2/day) than the growth of seeds broadcast onto the microcosm sediment alone (0.46 gdw/m^2/day)

(Zinecker CH3). The growth rate in the PHA pots was also higher compared with the growth of *P. perfoliatus* turions on a sand/soil (0.47 gdw/m^2/day). This substrate is typically used for *P. perfoliatus* propagation for hand transplanting of sods for restoration projects (Table 4.1) (Zinecker CH2, Zinecker and Kangas 2011b). The idea behind the pots was to improve the potential for SAV restoration success through better control of placement of seeds in the sediment via the planting vessel, and through the inoculation of the seeds with SAV bed sediment to encourage growth and establishment (Zinecker CH3).

Emergy analysis, or embodied energy analysis, enables the investigator a transparent framework through which to characterize and describe a given process or system, enumerate the types of inputs and flows, such as labor, fuel, transportation, building materials, and propagules (Odum 1996). These inputs from both "user" and "donor" sides then combine within the defined system to generate a given output, product or service (Campbell and Brown 2012). The sum of the inputs are converted to solar equivalencies so that comparisons can be made across inputs in the emergy unit, solar emjoules (Odum 1996, Ciotola et al. 2011). The values of the system yield, ratios and indices then serve as the basis upon which to determine such factors as the sustainability of the two restoration systems being evaluated, their costs to the environment, and the USD$ costs.

When systems are evaluated using emergy, the percent inputs purchased from the economy or those primarily derived from human labor, are compared with renewable, environmentally-based inputs. This then elucidates the aspects of a given system that are less efficient, and more costly from an energetic standpoint. Ciotola et al. (2011) used

emergy analysis to determine differences between two renewable eco-conscious anaerobic digesters, the one that produced biogas, and not biogas and electricity, was more sustainable and placed less of a load on the environment. Williamson et al. (2015) compared two different aquaculture techniques for oysters, and determined the one that used boats for transportation and placed the aquaculture site a distance from the shore was simply more energy consumptive, and therefore less sustainable. These two examples illustrate the utility of emergy to discern between methods that might not be that different from a monetary or even logistical perspective, but place different demands on the natural environment contributing to the system.

To date, there have not been any published, integrative studies that go beyond $USD valuation for SAV restoration. However, the existing studies (e.g. Busch et al. 2010, Shafer and Bergstrom 2008, Lewis et al. 2006, Bergstrom 2006), identify inputs such as labor, propagules for transplant, materials, and services, which figure prominently in almost any form of environmental cost accounting or sustainability evaluation. In addition, this study is among the first to economically evaluate fully biodegradable materials for any purpose, in this instance a use case analysis for SAV restoration. Bilkovic et al. (2012) explored the use of a biodegradable "cull" panel in crab pots. They found that if the panels were made from polyhydroxyalkanoate (PHA) plastic, when gear was lost (storms, weak lines, etc.), the cull panels would biodegrade within a few months and avoid bycatch of marine life. However, it is not yet clear whether the panel will be widely used by watermen, as it adds to the $USD cost of the crab pots (Chirp Shannahan, personal conversation). No quantitative economic analysis has yet been conducted to show benefits of the PHA cull panel to the environment compared

with the more conventional materials/methods. Akiyama et al. (2003) determined through a lifecycle inventory that PHA plastic was more environmentally friendly than petrochemical polymers. Kim and Dale (2005) determined that the PHA footprint on the environment with respect to such aspects as general environmental impact and waste stream was also more favorable than polystyrene, however, with corn as the feedstock starch for its production, environmental eutrophication was a concern. Efforts are being made to employ alternative feedstocks to produce PHA and other similar polymers at lower cost (Dobroth et al. 2011); this would allow for an even more sustainable and closed cycle process (Dias et al 2006, Braunegg et al. 1998), particularly with reutilization of waste streams as part of the process (Koller et al. 2017, Koller et al. 2011). However, in this research, a pure form of PHA was used due to its' previously demonstrated rapid biodegradation as an aquatic plant pot in anaerobic SAV bed sediments (Zinecker CH3).

Also important, the budgetary SAV restoration studies cited above identify two important, but very different phases of SAV restoration: the first part of a restoration involves identifying the restoration site, harvest or procurement of propagules, treatment, storage, and preparation/propagation for restoration (Busch et al. 2010, Ailstock et al. 2011, Marion and Orth 2010a). The second part is the work involved in transporting propagules to the site, the planting methodology chosen, and actual deployment of the transplants at the restoration site (Marion and Orth 2010b, Bergstrom 2006). In his way, discrete parts of larger processes or systems can be evaluated separately, allowing the possibility to gain efficiencies, or to see a particularly resource intensive component that might be improved upon, while still considering the system as a whole. This is

particularly useful when there is a scale or energetic change between two systems (Allen and Starr 1982).

The primary objective of this hypothetical case study was to use emergy analysis to model and compare the hand transplant of sods, and inoculant/seed/PHA pot restoration methods (as described in Zinecker CH3). Given the cost and lack of consistent reliability of SAV restorations (STAC 2011, Lewis et al. 2006), it is important to model and assess costs, effectiveness, and environmental sustainability gained from new prospective planting methods compared with more traditional methods. Resources and dollars can be saved, and improvements made, with an initial case study comparing restoration methods. Consequently, this research asked whether the net emergy yield, and the sustainability indices based on emergy analysis of the PHA pot and seed restoration technique, would be an improvement over the more traditional hand transplanting of sods technique. In addition, the study posited that the PHA pot method would produce more square meters of restored SAV bed, resulting in a greater cost savings on a USD$/m^2 basis.

METHODS

Study site - propagation phase

This case study was based on propagation methods and field transplanting methods which have been used previously for restorations or other research. For the sod transplant method, *P. perfoliatus* turions were planted in propagation trays, submersed in propagation tanks, and allowed 8-weeks to grow and form sods in the trays which were then sent to the planting site. The propagation tank used as the model in this study was located at the Norm Berg National Plant Materials Center, in Beltsville, MD

(39.017208°N, -76.852155°W), (dimensions: 2.59m x 2.59m (6.71 m^2), depth = 0.60m). Sixty-four (64) propagation trays (0.26m x 0.35m x 0.08m), fit into the propagation tank.

While the PHA/pot method did not require submersion in a propagation tank, PHA pots were placed in the same type plastic propagation trays as the sod/turions, irrigated, and allowed to condition. As a consequence, the size of the propagation tanks, and the number of trays that comfortably fit in the tanks (64), served as the bounding factor for the case study.

Study site - restoration phase

The case study restoration site for this research was located on a tributary in the mesohaline Chesapeake Bay previously documented to support populations of *Potamogeton perfoliatus.* At the time of the study, the hypothetical restoration site would have been evaluated for presence of *P. perfoliatus* or any other species of SAV recently documented at or near the site using the VIMS SAV inventories (e.g. Orth et al. 2015).

Propagation phase – preparation of propagules

For the sod transplant method each propagation tray was filled ¾ full with a 50:50 sand/soil substrate topped with 100% sand (to reduce algal growth), planted with eight turions and then submersed in the water of the propagation tank. The turions were then grown in each of the 64 trays for a period of eight weeks, removed from the tank, covered with wet newspaper to prevent unnecessary stress from drying and heat, and transported to the restoration site. The turion growth rate of 0.47 g DWm^{-2}/day in this study was based on growth from turions over fifteen weeks in soil/sand substrate in a microcosm experiment (Zinecker, CH2). While this soil/sand combination demonstrated poor overall growth performance in comparison to SAV bed cores and other horticultural substrates, it

was important to keep the method the same (as has been used previously for hand-planted sods), to illustrate differences in potential net primary productivity based on the two methodologies (Table 4.1, Appendices 4 & 5).

For the seeds/PHA pot method, PHA pots were filled with a substrate of 1/3 oyster shell, 1/3 peat mix, and 1/3 SAV bed sediment as an inoculant to encourage growth. In prior microcosm research this substrate mix was found by Zinecker (CH3) to be among the best substrates for enhanced primary productivity of *P. perfoliatus* seeds (0.61 $gDWm^{-2}day^{-1}$) (Table 4.1, Appendix 7). Approximately 35 seeds were planted in the substrate mix in each pot, and then topped with a layer of oyster shell that kept the seeds immobile during transport and planting. Forty (40) pots were placed in each of the 64, 0.26 m x 0.35 m plastic propagation trays, for a total of 2,560 pots in the 64 trays. The trays were filled with a water to condition the seeds in the substrate. After approximately two weeks of conditioning, the seeds were transported to the transplant site.

Restoration phase – transplanting of propagules

For this case study, an exclosure of construction fencing served to delineate the restoration areas and reduce any disturbance from animals, debris and wake from natural river processes or boats, and reduced the possibility of herbivory from larger fish species, crustaceans, or waterfowl. Per the methods used by MD-DNR (Mark Lewandowski, personal conversation), each square meter of area of SAV bed restored would require four sods. Since there were sixty-four sods produced during propagation in total, the restoration transplanting would result in 16 m^2 of restored SAV bed (64 sods / 4 per m^2 =16 m^2). Sods were hand-transplanted by digging out a space in the bare sediment in the

m^2 plot the same size as the propagation tray. The sod was inverted onto an empty tray, and then placed, root side down, into the prepared depression. When transplanted, the four sods (grown from 32 turions) filled approximately 36 % of the restoration plot. Initial starting turions at the beginning of the season for a healthy, existing SAV bed ranges from 42-179 turions/m^2, and so the % biomass planted in this research approximated a proportional percentage of a natural bed (Zinecker CH2, Wolfer and Straile 2004). Vari and Toth (2017) found the vegetative spread of *P. perfoliatus* employs a strategy to preferentially spread to unvegetated areas during peak growing season (post seed-germination/turion emergence), and does so consistently, and for a longer period of the growing season (through September).

For the seed/PHA pot restoration method, approximately 35 seeds per pot, and five pots were planted in each m^2 plot for a total of 175 seeds planted per m^2. The original number of 2,560 PHA pots (40 pots in each of the 64 plastic trays) was then divided by five pots for each m^2 yielded a total restoration area of 512 m^2. The total of 175 seeds/m^2 approximates only 12 percent of the potential (but quite variable) 1,440 m^{-2} seed yield estimate produced for a healthy P. perfoliatus bed (Ailstock and Schafer 2004). The in situ growth rate from seed to adult plants of 0.61 g DWm^{-2}/day in this study (Appendix 7, Table 4.1) was a conservative average estimate based on growth from seed in PHA pots over 12 and 16 weeks in two different microcosm experiments (NPP ranged from 0.53-0.68 DWm^{-2}/day, Zinecker CH3). It was also 30% lower than turion growth on undisturbed SAV bed sediment cores in microcosms (Zinecker CH2, Table 4.1). While the actual mature seed yield from a natural SAV bed seed yield may range from 34-60%, this does not take into account the significant factors of burial (Ailstock et al. 2010b),

herbivory, and unsuccessful fruiting, which has not been documented in *P. perfoliatus*. These values have been estimated in other species, and can be as high as 87% (Balestri and Cinelli 2003). Zinecker (CH3) found that 6-8 *P. perfoliatus* seeds grew biomass in microcosms (area 0.04 m^2 or approximately 20% of a sq. m.) with healthy growth in less than two months. Therefore, between the disturbance posed by transplanting, weather, wind, and wave action in a field environment, and the potential for seeds to somehow become dislodged from the pot, a much larger number than necessary quantity of seeds (35) was identified to be placed in each of the five pots transplanted in each of the 512 m^2 of plots at the restoration site. Pots were inserted at the end of a PVC pole that attached to a spindle located on the pot, and then pushed into the sediment.

Standard procedure for emergy evaluation

The emergy evaluation was proposed by H.T. Odum (1996) to take into account the inherent thermodynamic and environmental value of nature's products while also taking into account fundamental economic valuation and accounting. This accounting method is a framework that delineates, considers, and describes a process, product, service, or output (yield). The raw materials, or inputs that are used in the characterization of the process are defined, themselves, in energy units that consider past energies (usually in units of Joules) that resulted in their own composition through the use of a value called a "transformity". The term transformity in this case is defined as the product of basic mathematical calculations (environmental cost-accounting), that take into account the aggregate factors used to create a raw material or input, product or process. That transformity is then used as a multiplier of the amount of that raw material or input used in the accounting of the costs of the current process defined in the present

framework or process being considered. The sum of products are then used to arrive at a final transformity unique to that specific process as defined within that framework. To achieve this accounting, the emergy analysis comprises the following basic steps: 1/ Completing a systems diagram (an illustrated description) of the system or process of interest with symbols that specifically define the components and interaction of components in the system, from lower to higher energies (Figure 1); 2/ Constructing a table inventorying the inputs (see Appendices 4-7), 3/ Calculating the emergy flows, ratios and indices (see Tables 4.2-4.7); and 4/ Making policy recommendations based on a comparison of the ratios and indices.

Energy systems diagrams

The systems diagram design is first conceptualized by identifying the boundaries of the system, product, or process of interest. The diagram is then drawn using energy systems symbols (Odum 1971, Odum and Odum 1976, Odum 1996) (symbols defined under Figures 4.1-4.5). These symbols are the major components that contain, contribute, produce/store/consume emergy in a system. They are used to depict the inputs such as labor, propagules, sediment, economic users, fuels, materials, sun, biomass, water, wind, tides, etc. Arrows and lines are drawn between these various inputs and components to indicate the flow of emergy in the system and the direction in which energies are either concentrated or dissipated. In most cases, the emergy concentration in the system increases from left to right, with renewable inputs on the left side of the diagram (e.g. sun, rain), and nonrenewable, purchased inputs and services (goods, labor, markets), at the top and right sides of the diagram. SAV restoration is comprised of different phases that are often specific to the species being restored: the propagule collection and

propagation stage, and the actual site restoration phase. Consequently, in this study there are two energy systems diagrams for propagation: one for the hand-transplanting of sods, and one for the seed/PHA pot propagation method (Figures 4.1 and 4.2). There are also two systems diagrams for the actual restoration deployment for each method being considered (Figures 4.3 and 4.4). A more generalized diagram describes the ultimate goal of SAV restoration (4.5).

Emergy analysis tables

Table 4.1 provides examples from the literature to illustrate primary productivity and reproductive potential of *P. perfoliatus* in natural and restoration settings. Tables 4.2-4.5 are standard emergy analysis summary tables that provide the $USD cost in addition to energy system data, transformities, the product of the two, and references of renewable environmental, non-renewable, and purchased inputs (Ulgiati and Brown 1998). All line items in the table are first calculated in a separate Appendix (4-7) in greater detail, with footnotes elaborating the units and dimensions that make up the raw data or "Required Amount" in the accompanying Table. Thus each value is assigned a unit, such as grams, joules, dollars. The units are then calculated on an annual basis per m^2, and the most appropriate transformity is multiplied by the amount for a given input. The transformity enables a given input's units/year to be expressed on a standardized solar emjoules (sej) per unit basis, and is defined as "the quotient of a product's emergy divided by its emergy" (Odum 1996). These inputs were first quantified in raw units (Odum 1996), and assigned a published transformities found in one of the many folios and other references available in the literature. The PHA plastic production manufacturing process has been deemed more sustainable than conventional, fossil fuel-

based PE by several LCA's (Akiyama et al. 2003, Kim and Dale 2008, Koller et al. 2017). However an emergy analysis has not yet been conducted. However, given that it is a more sustainable, microbially-based fermentation-based industrial process similar to other fermentation industries, the transformity of ethanol derived from switchgrass was used as a proxy (Felix and Tilley 2009). In addition, some transformities for other inputs of the SAV propagation and restoration methods don't yet exist, therefore some assumptions needed to be made (and are noted) when transformities from existing data sets or derived from papers dealing specifically with energetics of the inputs are made. As an example, oyster shell, used in *P. perfoliatus* propagation in this research, does not have an annual input value, and therefore the transformity for limestone was used in its place.

Emergy ratios and indices

System inputs were aggregated to form various ratios and indices for each systems diagram (Odum 1996). The aggregated inputs in this research included renewable environmental (R), non-renewable (N), and purchased inputs (F) as outlined in Ulgiati and Brown (1998). Throughout all systems diagrams and associated tabular inventorying and accounting, the primary concern is the final emergy yield (Y). It is the total emergy in the output of the system measured in solar emjoules Y= (R+N+F) (Ciotala et al. 2011). Emergy yield is the sum of all of the emergy inputs, which, together are used to calculate various ratios and indices. The emergy yield ratio is illustrated in the more generalized energy circuit diagram in Figure 4.5. The transformity (τ) (τ = Y/ε) of a process cannot be generated without yield, in addition to ε, which signifies the energy of the total output generated by the process being considered. For this research, the indices used for the two

restoration processes included: the fraction renewable: ϕR= R/(R+N+F), the emergy yield ratio: EYR= Y/F, the environmental loading ratio: ELR= (F +N)/R and the environmental sustainability index ESI=EYR/ELR (Ciotala et al. 2011, Ulgiati and Brown 1998).

US dollar costs

U.S. dollar amounts were calculated for all inputs (expenditures) for propagation and restoration phases, in order to compare the two methods from the perspective of standard $USD accounting and budgetary requirements, and to be able to compare with other studies (Table 4.11).

RESULTS

All results for both propagation and restoration phases are presented as per m^2 SAV bed restored/year (Tables 4.2-4.5). The sods transplant method resulted in a restored area of 16 m^2, and the PHA pot method resulted in 512 m^2 restored. Additional values and summaries for area values calculated specific to the propagation system, and the total area restored, are found in the Appendices (4-7). U.S. Dollar amount results are presented as a function of m^2 SAV bed restored as well as costs per annum and total $USD investment plus first year restoration (Tables 4.8, 4.9, 4.10).

Renewable resources - propagation

The total annual emergy input from the sun for the sod propagation was 2.11E+09 sej/m^2/yr while the seed/pot method was 5.71E+07 sej/m^2/yr. Precipitation accounted for 4.53E+10 sej/m^2/yr for sod propagation, and evaporation accounted for 8.24E+10 sej/m^2/yr, necessitating irrigation for the tanks. For the seed/pot method, precipitation

accounted for 1.23E+09 sej/m^2/yr, and evaporation accounted for 2.24E+09 sej/m^2/yr, also requiring irrigation (Tables 4.2 and 4.4, Appendices 4 and 6).

Renewable resources - restoration

For the restoration phase of the evaluation, total annual emergy input from the sun for restoration using sods was 5.88E+09 sej/m^2/yr, while for the seed/pot method it was 5.91E+09 sej/m^2/yr. Tidal input for both the sods and pot/seeds was 3.60E+10 sej/m^2/yr . River geopotential for handplanting was 3.08E+13 sej/m^2/yr, and for seeds/pots it was 3.91E+12 sej/m^2/yr (Tables 4.3 and 4.5, Appendices 5 and 7).

Purchased resources - propagation

The inputs in this category were purchased in order to propagate and prepare stocks for either method of restoration. Other than irrigation, no other inputs such as electricity or heating were necessary. It was estimated to be warm enough outside to start propagation and preparation for both methods. Additions of water for irrigation due to tray or tank evaporation were required for both methods and is considered a semi-nonrenewable, purchased resource. For the sod method, irrigation for the tanks was 9.58E+11 sej/m^2/yr, and for seeds/PHA pots, irrigation was 1.16E+08 sej/m^2/yr.

Substrate combinations for the propagation phase required quite different volumes of inputs for each method. The sod method required purchased sand, (3.86E+13 sej/m^2/yr) and soil, (2.61E+13 sej/m^2/yr). The substrate mix for the seed/pha pot method comprised SAV bed substrate (with its accompanying bacteria), oyster shell and peat. SAV bed sediment emergy was 1.39E+06 sej/m^2/yr, while the accompanying inoculant bacteria contained in the sediment was 1.56E+04 sej/m^2/yr. Oyster shell emergy, using the limestone proxy for a transformity, was 1.86E+10 sej/m^2/yr, and the peat was

6.08E+04 sej/m^2/yr. Propagules for each method were purchased, with turions for propagation sods having an emergy of 7.02E+08 sej/m^2/yr, and the larger number of seeds having an emergy of 1.16E+10 sej/m^2/yr. The emergy of the 64 plastic trays to hold each configuration of propagules was different due to the resulting restoration area, and was therefore a higher input of 9.86E+10 sej/m^2/yr for the sods method compared with 3.08E+09 sej/m^2/yr for the seed/pot method. Inputs for seeds/pot method included an additional input in the design and manufacture of the PHA planting pots, which was fiscally but not energetically as expensive, with an emergy of 1.71E+06 sej/m^2/yr. The sods method required tanks for growout and site preparation. The materials used included pressure treated lumber (1.07E+13 sej/m^2/yr), cedar planking (5.41E+10 sej/m^2/yr), hardware (4.27E+10 sej/m^2/yr), pond liner (4.04E+11 sej/m^2/yr), and PVC for irrigation 2.26E+11 sej/m^2/yr). For the sods method, shipping costs for propagules, and labor to set up the growing trays, and to build and maintain the large tanks added up to 1.67E+14 sej/m^2/yr. Shipping costs and labor for the seeds/PHA pot method added up to 8.30E+11 sej/m^2/yr (Tables 4.2 and 4.4, Appendices 4 and 6).

Purchased resources - restoration

The inputs in this category were purchased to support transport and transplantation of plant stocks at the site for the sod or seed/pot methods of restoration. For both methods, propagules, the primary output from the propagation phase, carried their transformity through from the propagation phase. The grown-out plants emergy for the sods was 2.45E+14 sej/m^2/yr, whereas the seed/pha pot propagules emergy was 8.58E+11 sej/m^2/yr. Emergy for exclosure fencing, and uprights of steel for hand transplanted sods was 3.01E+11 sej/m^2/yr). The plastic fencing, and a combination of

PVC and steel uprights were used for the seed/pha pot method exclosure due to its greater area restored and was 5.65E+10 sej/m^2/yr. Fuel and machinery included the purchase of a truck for both methods. For sods, the emergy summed input of machinery and fuel was 1.15E+14 sej/m^2/yr , and for seed/PHA pot it was 3.33E+12 sej/m^2/yr. Labor for driving, loading, unloading and transplanting for the sod restoration method was 3.53E+13 sej/m^2/yr, and for the seed/PHA method was 1.10E+12 sej/m^2/yr.

Emergy signature diagrams - propagation

The largest input for emergy in the propagation phase was labor for both sod hand-transplanting (1.67E+14 sej/m^2/yr at 68.4%) and seed/PHA method (8.30E+11 sej/m^2/yr, at 97%). The second largest input for the sods propagation was the propagation substrates (sand and soil together: 6.47E+13 sej/m^2/yr at 26.4%). Combined substrate for the seed/PHA pot method was 1.86E+10 sej/m^2/yr, accounting for only 2.18% of inputs. The transformity used for the PHA manufacturing process was 1.71E+06 sej/m^2/yr, a value presuming that the PHA industrial process is closer to cellulosic fermentation for ethanol (Felix and Tilley 2009) rather than the process for polyethylene conventional plastic (Kim and Dale 2005), and accounts for less than 0.001% of the emergy for this system.

Emergy signature diagrams - restoration

The two methods each had different highest percentage inputs in their restoration phase. The highest percent input for sod restoration was the grown-out propagules with 2.45E+14 sej/m^2/yr, at 57.51%, followed by the purchase of a truck: 1.14E+14 sej/m^2/yr at 26.73%, third, labor: 3.53E+13 sej/m^2/yr at 8.27%, and last, a renewable input, river geopotential was 3.08E+13 sej/m^2/yr, accounting for 7.23% of the inputs. The highest

emergy input for seed/pots was river geopotential 3.91E+12 sej/m^2/yr at 40.95% of the emergy, followed by the truck purchase: 3.56E+12 sej/m^2/yr, (37.25%), labor: 1.10E+12 sej/m^2/yr at 11.50%, and last, the input emergy of the seed/PHA pots system was 8.58E+11 (8.98%) of the total emergy of the restoration system.

Emergy yields, transformities, ratios and indices - propagation

The emergy yield (Y) for the hand transplant propagation phase was 2.45E+14 sej/m^2/yr with a system transformity (τ) of 2.58E+13 sej/m^2/yr. The seeds/PHA pot method emergy yield (Y) was 8.56E+11 sej/m^2/yr with a transformity (τ) of 1.75E+12 sej/m^2/yr. The Fraction Renewable (ϕR), Emergy Yield Ratio (EYR), Environmental Loading Ratio (ELR), and Environmental Sustainability Index values for the propagation phase (Table 4.6) were calculated from values generated in Tables 4.2 and 4.4. ϕR for the hand-transplant of sods was 5.3E-04 sej/m^2/yr, and for seeds/PHApots it ranged from 4.25E-03 sej/m^2/yr. EYR for sods was 1.00 sej/m^2/yr, and was also 1.00 sej/m^2/yr for seeds/PHA pots. ELR was 1.88E+03 sej/m^2/yr for sods, and 2.34E+02 sej/m^2/yr for seeds/PHA pots. For the ESI, the value for sods was 5.33E-04, and for seeds/PHA pots it was 4.29E-03 sej/m^2/yr.

Emergy yields, transformities, ratios and indices - restoration

The emergy yield (Y) for the hand transplant restoration phase was 4.26E+14 sej/m^2/yr with a system transformity (τ) of 1.43E+13 sej/m^2/yr. The seeds/PHA pot method emergy yield (Y) was 9.55E+12 sej/m^2/yr with a transformity (τ) of 1.04E+11 sej/m^2/yr. The Fraction Renewable (ϕR), Emergy Yield Ratio (EYR), Environmental Loading Ratio (ELR), and Environmental Sustainability Index values for the restoration phase (Table 4.7) were calculated from values generated in Tables 4.3 and 4.5. ϕR for

the hand-transplant of sods was 7.24E-02 sej/m^2/yr, and for seeds/PHApots it was higher at 4.14E-01 sej/m^2/yr. EYR for sods was 1.08 sej/m^2/yr, and was 1.71 sej/m^2/yr for seeds/PHA pots. ELR was 1.28E+01 sej/m^2/yr for sods, and 1.42 sej/m^2/yr for seeds/PHA pots. For the ESI, the value for sods was 8.42E-02, and for seeds/PHA pots it was 1.21 (Table 4.7).

US dollar costs - propagation

Propagating sods resulted in a total of $USD 73.56/$m^2$ SAV bed restored, vs. $USD 8.75/$m^2$ SAV bed restored for PHA pots. Annual costs for propagation for sods was lower at $USD 2,496.82/yr compared with PHA pots costing $USD 4,479.36/yr. Total project start-up plus first season restoration cost amounted to $USD 4,179.76 for hand-transplanting sods, and the propagation involved with PHA pot/seed method was more than twice expensive at $USD 8,442.85 (Table 4.8).

US dollar costs - restoration

Actual field deployment for hand transplanting sods on a per m^2 basis was $USD 279.30, and lower for PHA pots at $USD 15.23. Annually sods cost $USD 4,468.71, compared with $USD 7,795.99 for PHA pots. Finally, the total project startup costs and for the first year and the first restoration project was $USD 32,484.21 and $USD 39,920.25 for sods and PHA pot/seed methods, respectively (Table 4.9).

US dollar costs – propagation and restoration combined

$USD costs for propagation and restoration were combined to show overall project costs both with and without depreciation as noted below. The combined cost on a per m^2 basis for sods hand transplanted was $USD 352.86, and again, much lower for PHA pots at $USD 23.98. Annually combined propagation and restoration cost for the

sods technique was $USD 6,965.53, compared with an almost doubled value of $USD 12,275.35 for seed/PHA pots. Finally, the combined cost for total project startup for the first year plus the first year restoration project (with no depreciation considered, etc.), for sods was $USD 36,663.97 and $USD 48,363.10 for PHA pot/seeds. The $USD cost per each m^2 SAV bed restored for initial start-up plus first year project was $USD 2,291.50 for sods and $USD 94.46 for seeds/PHA pots. (Table 4.10).

DISCUSSION

The goal of this study was to compare the relative emergy and dollar cost of two different SAV restoration methods: hand transplanting sods of almost mature plants, and planting of PHA pots that contained an inoculated substrate and seeds. The results of this research conclude that the seed/pot method restored a much larger area than the hand transplanting of sods and the transplants. The PHA pot method also had a higher likelihood of performing better once transplanted in the larger area due to higher potential yield of plants. The overall dollar cost was approximately 20-25 percent greater for the PHA pots (Table 4.10&4.11). However, the larger total initial US dollar investment created 97% more restored SAV bed, with a potential to produce 67% more biomass per square meter. It did this with 93% more sustainability, at an overall lower cost to the environment (89% lower).

System inputs

Aside from renewable inputs such as energy from the sun, precipitation, evaporation, and water movement such as tides and river geopotential (see items 1-4 in Tables), contributions to the two restoration systems on the whole were based on conventional materials purchased at market value, and implemented through the equity of

labor to achieve the ultimate goal of restoration. In this case labor is considered a purchased, non-renewable input (Ingwersen 2010, Williamson et al. 2015). In other emergy analyses, typically conducted in less developed countries, a portion of labor can account for up to 68% of total labor, and the nonrenewable portion for 32% of total labor (Ciotola et al. 2011, Martin et al. 2006, Rydberg and Jansen 2002, others). Using these less conservative values may result in reporting increased sustainability of a process or system, however in more developed countries, this is deemed inappropriate due to the preponderance of purchased inputs that make up human labor (Elliot Campbell, personal conversation). Evaluating the restorations with respect to the two phases provided an opportunity to assess efficiency and resource consumption for each, including the differences in level and costs of human efforts to implement ecological restoration of SAV. Comparisons to other values in the literature will not be made for the propagation phase data as most emergy analyses (and their systems diagrams) do not generally separate staging and implementation phases, and thus the numbers for propagation are less comparable than end of system total.

System inputs – propagation

The propagation phase elucidated the very different inputs for both methods and demonstrated the large emergy expenditures involved in "setting up and preparation" for the actual restoration. For sod hand transplanting, expenditures on labor and materials are reflected in the large amounts of substrate required by a grow-out tank system, and the eight weeks of tank maintenance during preparation for restoration (99%+ emergy was accounted for in the tank construction and substrate/propagation system). The advantage of the tanks is they can be used for propagation and as a source of other propagules

(stems, seeds, turions) for years, and could provide seeds for the PHA pot technique. This would also enable the calculation of an initial propagation phase transformity for seeds and turions rather than using the fair market purchase price in $USD.

For propagation for the PHA pot method, after the large investment in labor emergy inputs (96.85%), the next largest emergy expenditures were the output of the PHA pots. Because the transformity for PHA plastic was not generated as part of this research, the PHA plastic transformity was one of the few instances where the proxy value for the industrial process of ethanol production was provided (Felix and Tilley 2009). If the pots were made of conventional plastic (Buranakarn 1998), their emergy would have likely accounted for a larger percent of the inputs rather than being a negligible input. Oyster shell, seeds, and the plastic propagation trays still accounted for the third largest emergy inputs (approximately 2.59%) – even though they were far less than the sods planting system. The materials used for the seed/PHA pot method are not extremely resource intensive and are small in volume compared with sods – 1 percent of what the sods method required on a per m^2 basis. Areas to gain efficiencies in sustainability might include reducing the value input of design and production of the PHA pot, but keep its utility the same, as well as reducing the time to fill the pots. The seeds input might also represent a good opportunity to regain efficiencies through on-site propagation or through wild-harvesting and outdoor storage.

System outputs, ratios and indices - propagation

The overall system output (ε) for the propagation phase for sods was almost 95% greater than PHA pots for each g/m^2 of SAV bed restored (Table 4.6, calculations for sods, Appendix 4, Item 17a: calculations for PHA pots: Appendix 6, Item 14a). This

difference is not surprising given that the comparison is grown-out plants (over an 8-week period) to recently shipped seeds. Because of this high level of effort for less SAV bed area restored, the emergy yield was almost 100% higher for sods. The higher sod emergy yield subsequently results in a higher transformity, and this places the sod propagation throughout the calculations for the various ratios and indices at a disadvantage. The renewable fraction (ϕR) for both methods is quite small, as stated above, due to the quantity of purchased resources, however the PHA pots have a slightly higher fraction of renewable resources than the sod method. The Emergy Yield Ratio for both propagation methods is 1, which indicates, as stated earlier, that a majority of inputs are purchased for both propagation methods. Similarly, the Environmental Loading Ratio, which ideally is low when a process is sustainable and maximizing use of local, renewable resources, in this case is fairly high, with the PHA pot and seed method retaining its place as requiring an order of magnitude less purchased resources than the sods method on a per msq basis. The Environmental Sustainability Index (ESI, or EYR/ELR), which increases when fossil fuel and other purchased resources are low, was not a standout for either of these methods, as suspected. However, the PHA propagation method is still more sustainable than the sod hand transplant propagation method by an order of magnitude.

System inputs – restoration

Similar to the propagation phase, the restoration phases for the sod hand transplant and PHA pot planting required two very different levels of effort, again resulting in a difference in m^2 SAV bed restored by an order of magnitude. The total number of trays transported to the site are the same number, 64. However the onsite

transplanting operation is easier and faster with the PHA pot planting, even though there are more square meters to plant, and thus more fencing to install. The sods require a greater level of effort to excavate the 64 spaces for 64 sods over $16m^2$, whereas the PHA pots are placed into position into the sediment using a PVC pole – 5 per sq. m over 512 sq m, making placement much faster. The restoration phase for sod hand transplanting had propagules as its highest single input, due to the fact that the transformity was value-added and carried through from the propagation phase and included eight weeks of growout, which is expensive energetically (Table 4.3). The second largest input into the sod system was machinery (primarily the purchase of a truck for delivery of propagules to the site), followed by labor, and subsequently a renewable: river geopotential. Seed/PHA pot restoration had as its largest input river geopotential due to the size of the area restored most likely, followed by machinery (the truck), labor and propagules. The lower position of the propagules in the PHA pot method compared with the sod restoration system reflects the lower level of effort devoted to the propagation/preparation phase, and the transformity for propagules for the seed/PHA pot method, is an order of magnitude lower than the sod restoration transformity. Labor was the last input of particular significance for the seed/PHA pot system, accounting for 11.50% of the total yield for its system. While this is a greater proportion labor (8.28%) than for the sod restoration method, however, numerically, it accounted for over 100% of the system value. This is similar to labor output for Williamson et al (2015). Oyster culture for bottom cages required more labor than floating cages, and the difference between systems was an order of magnitude difference for labor requirements.

As a renewable input, river geopotential was significant for both restoration methods, although it was larger for the seed/PHA system than for the sod transplant system due to the restoration sites being so different in area (m^2), a phenomenon also found in the different aquaculture site sizes in Williamson et al. (2015). This higher renewable value figured prominently into the end of first season restoration output (ε) as well as system ratio and indices. Since restoration site parameters such as the influence of tides, fetch, and river geopotential play large roles in propagule retention, plant stability and photosynthetic output (Best et al. 2008, Koch 1994), these should figure into the inputs of any SAV restoration emergy (or other) analysis in the future.

System outputs, ratios and indices - restoration

For restoration of sods grown in soil/sand, the final system output (ε) was just over one third (33.5%) the gDW/m^2 less than the biomass grown in the plots with the PHA pot/seed method. This indicates that the net primary productivity (NPP) of the PHA pot method was higher due to the use of the pot and type of substrate. The growth rate of the sand/soil mix that is typically used for propagation in the sod method was empirically determined to be lower than the inoculated oyster/peat in the PHA pot (Zinecker CH2 and CH3 and Table 4.1 - Calculations are found in Appendix 5, Item 10a – $0.47gDW/m^2/day$, and Appendix 7, Item 11a $0.61gDW/m^2/day$). The reduced resources required to produce more biomass at the restoration site using the PHA pots seed method were manifested in the growth rate, output (ε), and subsequently the transformity (τ).

Lu et al. (2006) describe the importance of the changing soil chemistry that occurs in various restored forest systems and leads to soil improvement, and reports that emergy analyses rarely take the substrate dynamic fully into account. In their analysis, Lu et al.

(2006) compares the transformity for the soils in their restored agro-forestry systems to other natural systems to illustrate the overall efficiency of the restoration. Lu et al. (2011) similarly demonstrate that it is the transformity of the actual outputs of restored forested systems that enable a true comparison of the net primary productivity of the system and its ability to build biomass and soil organic matter as foundational ecosystem services within the systems. Vassallo et al. (2013) document the ecosystem services role and importance of sediment trapping that the seagrass *Posidonia oceanica* provides, but they do not necessarily document the productivity of the bed relative to the sediment retained.

Therefore, while the sod transplant method starts off with more biomass in the field, it would hypothetically experience a lower overall growth rate due to the initial use of a less ideal substrate. The seeds/PHA pot started off with much lower biomass in the form of seeds, but with a higher density of inoculated, high energy propagules than necessary, and this would in theory favor a higher growth rate than the sod method (Table 4.1). In this way it is helpful, when doing an emergy, ecosystem services, or other evaluation of a restoration, to take into account the potential resulting NPP.

The transformities between the PHA pot and sod method by the restoration phase were different by two orders of magnitude different, i.e. sods transformity was approximately 137 times the transformity of the PHA pots/seed method due to the lower yield that was based in increased restored area and lower overall costs. Lagerburg and Brown (1999) found that tomatoes cultivated in an oil-heated greenhouse had a much higher transformity (13 times higher) than either Florida field-raised tomatoes or wood-pulp-heated greenhouse tomatoes simply due to the form of energy used. Depending on the market price of petroleum products and the market demand for fresh tomatoes, these

aspects, while seemingly obvious, are often not figured into restoration/production schemes (Cleveland 1995, Odum 1996). More subtle effects were found in the sustainable agroforestry approaches in Lu et al. (2011), who found that transformities for the final output of biomass accumulation and soil organic matter improvement ranged from being almost five times greater for soil organic matter and 2.5 times greater biomass than the more sustainable acacia plantation.

In this case study, fossil fuels and transportation also affected percent emergy of both methods with the purchased input of a gas-fueled pickup truck to transport workers and propagules to the restoration site. For sods the purchase accounted for 27% of total emergy and for PHA pots 37% of total emergy. The emergy yield (Y) is higher for the sods than the PHA pots, and this results, again, in overall higher transformities for all summed input categories, but particularly for purchased inputs. And this also results in a disadvantage with respect to sustainability. The renewable fraction (ϕR) for both methods is small, but higher than the propagation phase. The PHA pots had a much higher fraction of renewable resources than the sod method. The Emergy Yield Ratio for both propagation methods was above 1, for hand planting sods it was 1.08 and for PHA pot/seed planting it was 1.71, which indicates that the reduction of purchased inputs to the ratio of the renewable river geopotential improved the value. Emergy Yield Ratios similarly showing increased efficiencies are also demonstrated in Williamson et al. (2015) in reduced resources used in oyster farming, and in passive vs. more resource intensive sewage treatment (Winfrey and Tilley 2016), (Table 4.12).

The Environmental Loading Ratio also improved, lowering by two orders of magnitude both the sod restoration method and the seed/PHApot. Because SAV

restoration is high input and creates demand on environmental resources (ideally in just the short term), ELR was much closer in magnitude to such processes as oyster farming and wastewater treatment, than agro-forestry and production of sustainable energy in less developed countries (Table 4.12). The Environmental Sustainability Index (ESI, or EYR/ELR) also was reduced one order of magnitude for the sods restoration method, and substantially more, two orders of magnitude for seed/PHA pots, again, the overall favored method for restoration from a sustainability perspective. This is due to the fact that the fraction of nonrenewables and market purchases placing a burden on the environment were spread out over a larger restored area, i.e. m^2 SAV bed restored.

Dollar costs

The inclusion of dollar costs for each relevant input in the emergy analysis allowed for a more complete budget, and this may be why the costs for the project overall may be higher than other SAV restorations (Table 4.11). In this study, monitoring and time for pre-site evaluation were not necessary and were not included, but these additional inputs would have increased the costs appreciably (Busch et al. 2010). Fonseca et al. (1998) estimated that follow-up monitoring may be up to 60% of the cost for a SAV restoration/mitigation project. In the absence of monitoring, Thorhaug and Austin (1976) estimated that planting was the most costly portion at 60% of the project, with the goal of restorations being ultimately to increase planting efficiencies while cutting costs.

Dollar cost in general in meters squared SAV bed restored was lower for the PHA pot/seed method than for hand transplanting sods due to the fact that the pots/seed method covered 97% more area than the sods method. However, because the manufacturing costs were higher for the PHA pot, and required more materials for area

restored, overall total project costs were almost 20-25% higher. In addition, while fundamentally the PHA pots seemed to be a simpler operation in the propagation phase, they added very little longstanding value other than the mold, whereas the sod method resulted in a flexible, durable good – a propagation tank - that could be available for use in multiple years and multiple projects. The tanks at the propagation location have been used to grow plants to generate seeds, turions, cuttings, etc., and thereby may reduce the costs for future restoration projects. The values on a dollar costs per m^2 basis for other projects in the literature compare favorably only with those planting approaches that provided a more comprehensive budget, such as Lewis et al. (2006), at USD$ 338.7/m^2, a mitigation project of three different seagrass species in Florida. Other dollar costs/ m^2 from projects include Bergstrom (2006), which provided a range for *P*. perfoliatus from \$8.34-24.46/m^2. Busch et al. (2010), provides a summary of seagrass projects (primarily *Zostera marina*) averaging from \$4.29 m^2 to \$11.09/ m^2, and communicates that large costs are excluded, and that the emphasis was on materials alone, as opposed to labor, which tends to be costly. These values were similar to Fonseca et al. (1982), who reported a range of costs estimates from \$7.70-11.70/m^2.

If managers wish to try various approaches but lack financial resources, pilot projects are a valid way to test new techniques at smaller scales to determine whether methods result in established plants at a feasible cost (Bergstrom 2006).

CONCLUSIONS

This study demonstrated that emergy analysis can be used to identify fundamental, energetic differences between two different SAV restoration methods. In addition, inputs from the human market economy can be assigned dollar values that will

also determine which method provides better financial value, particularly in light of the emergy analysis. The solar transformities based on the characteristics of input and output and potential productivity of the plants enabled a comparison between the techniques, an approach similar to that used by Lu et al. (2011). Most conventional approaches to methodological evaluations of restoration projects do not have this type of data nor is there a means to compare it in this manner. The differences in area of SAV bed restored also was dramatically higher using the PHA pot/seed method due to the increased plant productivity, but also due to efficiencies gained in using a pot as a biodegradable planting vessel and holdfast.

The emergy analysis can be made flexible enough to take into account design variations that modify emergy while optimizing success, an idea similar to the approach Bergstrom (2006) used in designing variable approaches over multiple years. When restorations are successful, energy circuit diagrams and analyses can be modified with the developing system over time. Emergy and ecosystem services accounting can then be used during the course of ecosystem recovery to track the inputs and outputs of the system on an annual basis, with more details of valuable ecosystem services output revealed as the restoration incorporates with the ambient environment where it was intended to establish and contribute (Kangas 2004).

SAV seeds and propagules are no different from any seed crop, they require adequate sediment quality, water quality that allows sufficient light and below threshold levels of pollutants, suspended solids and nutrients, and physical disturbances. In Chesapeake Bay, this may at present be a tall order in the mesohaline (Dennison et al. 1993, Moore et al. 1996, Orth and Moore 1983, Orth et al. 2006). In spite of this,

researchers have determined how SAV seeds respond to substrate type (Alagna et al. 2015, Ailstock et al. 2010b), and that SAV net primary productivity (NPP) is also affected by substrate (Sculthorpe 1967, Ozimek et al. 1976, Barko and Smart 1986, Zinecker and Kangas 2011b, Zinecker CH2). Terrestrial restoration projects over the last twenty years have consistently demonstrated benefit and increased success of vegetation plantings from additions of appropriate microbes or arbuscular mycorrhizal fungi (Asmelash et al. 2016, Chanway 1997, Meena et al. 2017). Research is now beginning to characterize the diversity of sediment, rhizomatous, and other SAV plant-associated microbes and fungi (Crump and Koch 2008, Donnelly and Herbert 1999), even identifying variability of microbial populations as they change with disturbance, and restoration scenarios (Jiang et al. 2015, Bourque et al. 2015, Christiaen et al. 2013).

Substrate and growth rates have been acknowledged as being important inputs and factors to consider as part of rehabilitating degraded systems (Asmelash et al. 2016, Bai et al. 2008). Rutgers et al. (2012) present a schematic process for working with various stakeholders to identify and prioritize ecosystem services as part of four different agricultural land management practices. They observe that ecosystem services and land management may be active at very different spatial and temporal scales, and this may also be the case for SAV restoration. As SAV restoration science and management of Chesapeake Bay water quality continues to be developed and refined, aspects of project design, transplanting techniques, site/sediment suitability parameters and growth potential of propagules, can be strengthened by environmental accounting. The outcomes and rationale can then be used further to inform stakeholders and practitioners about techniques that will more effectively change the state of the degraded ecosystems they

are intended to restore (Kangas 2004, Odum and Odum 2003, Odum 1984, Fonseca et al. 1998, 1988).

Chanprateep (2010) articulates the idea of cost vs. net benefit of goods and the role that adequate marketing and communication, national policies, and even legal measures can potentially play to support use of PHA biodegradable plastics technology. However, in the case of this study, the outcome of gained value is both fiscal and ecosystem service-related (i.e. the pot is cheaper and delivers more restored SAV bed area per emergy unit and per dollar). This is the ideal scenario of the introduction of new products into a more environmentally sustainable, market-based economy (sensu Barbier 2012). Fonseca (2006, 2011) tempers the discussion of the excitement of new SAV restoration technologies with how little control managers and scientists actually have over open systems such as estuarine submersed aquatic plant communities. In light of these conditions, results of positive pilot field studies would provide further evidence that this SAV restoration method of seeds/PHA pots merits consideration as a restoration technique.

CHAPTER FOUR

Tables

Table 4.1 Values for primary productivity in *P. perfoliatus* in field and in microcosms, and one other mesohaline species, *Ruppia maritima*. Values for NPP gdw $m^{-2}day^{-1}$ on a daily basis were taken from 6/ and 9/ (bolded) below for the sods and PHA pot transplant methods, respectively.

Species /salinity/ Location	Peak AGB g dw m^{-2}	Peak BGB g dw m^{-2}	NPP gdw m^{-2} for (AGB+ BGB)	NPP gdw $m^{-2}day^{-1}$ (AGB+ BGB)	RSR	Refs.
1/ *R. maritima* / brackish/ Chesapeake Bay, VA	80-150			0.92		1
2/ *P. perfoliatus* / Mikolajskie Lake, Poland					0.39	2
3/ *P. perfoliatus* / brackish / Choptank estuary, Md – TURIONS					0.12	3
4/ *P. perfoliatus* grown in Sand substrate/ fresh / Greenhouse Microcosms (15weeks - TURIONS)	18.21 ± 2.62	20.98 ± 3.36	39.19 ± 5.39	0.37 ± 0.05	1.26 ± 0.20	4
5/ *P. perfoliatus* grown in Soil/Sand substrate/ fresh/ Greenhouse microcosms (15 weeks - TURIONS)	33.05 ± 5.39	16.34 ± 2.43	49.16 ± 7.96	**0.47 ± 0.08**	0.50 ± 0.04	4
6/ *P. perfoliatus* grown in Oyster/peat substrate/ fresh/ Greenhouse microcosms (15 weeks - TURIONS)	57.84 ± 4.71	32.79 ± 5.51	90.63 ± 9.16	0.86 ± 0.09	0.56 ± 0.07	4
7/ *P. perfoliatus* grown in SAV bed sediment cores/ fresh/ Greenhouse microcosms (15 weeks - TURIONS)	80.15 ± 6.89	30.60 ± 1.89	110.76 ± 7.82	1.05 ± 0.07	0.51 ± 0.08	4
8/ *P. perfoliatus* seeds grown in oyster+peat+Inoculant+PHA pots Greenhouse microcosms (16 weeks - SEEDS)	36.8 ± 4.4	13.8 ± 1.5	50.6 ± 5.6	0.61 ± 0.07	0.39 ± 0.03	5
9/ *P. perfoliatus* seeds grown in oyster+peat+Inoculant+ BARE SEDIMENT Greenhouse microcosms (16 weeks – SEEDS)	26.9 ± 3.4	11.5 ± 1.6	38.4 ± 4.9	0.46 ± 0.06	0.44 ± 0.03	5

1/ Wetzel and Penhale 1983
2/ Ozimek et al. 1976
3/ Goldsborough and Kemp 1988
4/ Zinecker CH2
5/ Zinecker CH3

Table 4.2. Emergy analysis table for **propagation and preparation** of plants for deployment by the **hand transplanting** method of SAV restoration. †**"Data" and "USD$" are based on each m² of SAV bed restored as a contribution from that input (with exception of some final outputs in Item no. 17).** See Table 4.6 for Emergy ratios and Indices. Data for this table was generated through calculations in Appendix 4.

Item No.	Item, Unit	†$USD /m²/yr	†Data /m²/yr	*Transformity (sej/unit)	Emergy (sej/m²/yr)	% of total EMERGY	Ref. for Trnsfmity
***Renewable Resources* (R) and Non-renewable Resources (N)**							
1.	Sunlight, J	$0	2.11E+09	1.00E+00	2.11E+09	0.00	7
2.	Rain, chemical, J	$0	2.49E+06	1.82E+04	4.53E+10	0.02	7
3.	Evaporation, J	$0	2.69E+06	3.06E+04	8.24E+10	0.03	7
4.	Irrigation **(N)**, J	$0.30	1.76E+06	5.45E+05	9.58E+11	0.39	3
		———			———	———	
	Total Emergy of Environmental Inputs to system (R, N):	***$0.30***			***1.09E+12 (R+N)***	***0.44***	
***Purchased Products* (F = F_M (purchased material items (5-13)) + F_S (paid human services (14-16))**							
5.	Pressure treated landscape timbers, g	$3.45	3.06E+03	3.50E+09	1.07E+13	4.4	2
6.	Cedar top-frame, g	$0.29	6.15E+01	8.80E+08	5.41E+10	0.02	4
7.	Hardware, g	$0.21	9.92	4.30E+09	4.27E+10	0.02	7
8.	Pond liner, g	$0.47	1.49E+02	2.71E+09	4.04E+11	0.16	5
9.	PVC, g	$0.09	2.28E+01	9.90E+09	2.26E+11	0.09	4
10.	Soil, J	$1.42	3.54E+08	7.38E+04	2.61E+13	10.7	8
11.	Sand, g	$3.28	1.81E+04	2.13E+09	3.86E+13	15.8	1
12.	[1]Turions, J	$30.4	1.21E+04	5.80E+04	7.02E+08	0.00	7
13.	Plastic, g	$2.40	1.68E+01	5.87E+09	9.86E+10	0.04	4
		———			———	———	
	Total Emergy F_M:	***$42.01***			***7.62E+13 (F_M)***	***31.14***	
	F = Economic Feedback resources that support the system						
Purchased / services (F_S)							
14.	Labor-Tanks*, J	$2.5	6.54E+06	6.74E+06	4.41E+13	18.1	6
15.	Labor – Propagation*, J	$27.50	1.83E+07	6.74E+06	1.23E+14	50.4	6
16.	Shipping UPS, USD$	$1.25	-	1.47E+10	1.84E+10	0.00	9
		———			———	———	
	Total Emergy F_S:	***$31.25***			***1.67E+14 (F_S)***	***68.42***	
		----------			----------	----------	
	System Yield $USD/m²:	***$73.56=***					
	System Yield/m² (Y) = R + N + (F_M+F_S)=				***2.45E+14* (Y)**	***100.00***	
17.	System Biomass output (***ε***):						
	Transformity (τ)= Y/*ε* = 2.45E+14 / 17a, b, c, or d:						
a.	P. perfoliatus, DW, g/m²		9.5	2.58E+13	2.45E+14		
b.	P. perfoliatus, DW, J/m²		1.39E+05	1.76E+09	2.45E+14		
c.	P. perfoliatus, DW Total, g		152	1.61E+12	2.45E+14		
d.	P. perfoliatus, DW Total, J		2.23E+06	1.10E+08	2.45E+14		

See Table 4.6 for Emergy ratios and Indices

Transformities are based on the old baseline Odum et al. 2000.

[1]Values for seedlings were used as a substitute for the transformity for turions given their similarities

References

1/ Arias and Brown (2009)
2/ Brown and Buranakarn (2003)
3/ Buenfil (2001)
4/ Buranakarn (1998)
5/ Campbell and Ohrt (2009)
6/ Ingwersen (2010)
7/ Odum (1996)
8/ Odum, Brown, Brandt-Williams, Folio#1, (2001)
9/ Odum (2007)

Table 4.3. Emergy analysis table for **deployment in the field** by the hand transplanting sods method for SAV restoration. (Occurs after 8 weeks of growout from purchased turions in propagation tank). Values are based on a per meter basis restored, in this case 16 m^2. †**("USD" and "Data" are based on each m^2 of SAV bed restored as a contribution from that input).** See Table 4.7 for Emergy ratios and Indices. Calculations for this table can be found in Appendix 5.

Item No.	Item, Unit	†$USD /$m^2$ /yr	†Data /m^2 /yr	*Transformity (sej/unit)	Emergy (sej/m^2/yr)	% of total EMERGY	Ref. for Trnsfmity
***Renewable Resources* (R)**							
1.	Sunlight, J	$0.00	5.88E+09	1.00E+00	5.88E+09	0.00	6
2.	Tides, J	$0.00	7.29E+05	4.94E+04	3.60E+10	0.01	1
3.	River, geopotential, J (estuarine circulation)	$0.00	Range: 0 to 9.69E+08	3.18E+04	3.08E+13	7.23	5
Total Emergy of Environmental Inputs to system (R):		***$0.00***			***3.09E+13 (R)***	***7.24***	
***Purchased Products* (*F = F_M (purchased material items (4-8)) + F_S (paid human services (9))**							
4.	Plastic, g Construction barrier	$0.20	2.53E+01	5.85E+09	1.48E+11	0.03	3
5.	Steel fence T-post, g	$0.05	3.68E+01	4.15E+09	1.53E+11	0.04	8
6.	Machinery and, g equipment	$125.00	7.75E+03	1.47E+10	1.14E+14	26.73	7
7.	Fuel, J	$0.36	2.03E+07	3.86E+04	7.84E+11	0.18	2
8.	Propagules *P. Perfoliatus,* DW, g/m^2	$73.69	9.5	2.58E+13	2.45E+14	57.48	9
Total Emergy (F_M):		***$199.30***			***3.60E+14 (F_M)***	***84.49***	
**F = Economic Feedback resources that support the system*							
Purchased / services (F_S)							
9.	Labor-Loading*, J Unloading, driving, Planting	$80.00	5.23E+06	6.74E+06	3.53E+13	8.27	4
Total Emergy (F_S):		***$80.00***			***3.53E+13 (F_S)***	***8.27***	
System yield $USD		***$279.30***					
System Yield (Y) = R + N + (F_M+F_S)=					***4.26E+14* (Y)**	**100.00**	

10. Restoration site growth – end of season output:
Transformity (τ) = Y/ε = 4.26E+14 / 11 a, b, c, or d

a. *P. perfoliatus*, DW, g/m^2	2.98E+01	1.43E+13	4.26E+14
b. *P. perfoliatus*, DW, J/m^2	4.37E+05	9.76E+08	4.26E+14
c. *P. perfoliatus*, DW, g	4.77E+02	8.94E+11	4.26E+14
d. *P. perfoliatus*, DW, J	6.99E+06	6.10E+07	4.26E+14

See Table 4.7 for Emergy ratios and Indices
Transformities are based on the old baseline Odum et al. 2000.

References
1/ Campbell (2004)
2/ Bastianoni et al. (2009)
3/ Brown and Buranakarn (2003)
4/ Ingwersen (2010)
5/ Martin (2002)
6/ Odum (1996)
7/ Odum (2007)
8/ Ortega (2000)
9/ Zinecker (CH2) see Table 4.2, Item 17

Table 4.4. Emergy analysis table for **propagation and preparation** of submersed aquatic seeds for deployment by the seed and PHA biodegradable pot transplant method for SAV restoration. †**"Data" and "USD$" are based on each m² of SAV bed restored as a contribution from that input (with exception of some final outputs in Item no. 14).** See Table 4.6 for Emergy ratios and Indices. Calculations for this table can be found in Appendix 6.

Item No.	Item, Unit	†$USD /m²/yr	†Data /m²/yr	*Transformity (sej/unit)	Emergy (sej/m²/yr)	% total Emergy	Ref. for Transformity
***Renewable Resources* (R)**							
1.	Sunlight, J	$0.00	5.71E+07	1.00E+00	5.71E+07	0.01	7
2.	Rain, chemical Potential, J	$0.00	6.74E+04	1.82E+04	1.23E+09	0.14	7
3.	Evaporation, J	$0.00	7.31E+04	3.06E+04	2.24E+09	0.26	7
4.	Water (irrigation), J	$1.79E-04	2.13E+02	5.45E+05	1.16E+08	0.01	1
5.	SAV Bed Sediment, g	$0.00	2.20E+01	6.30E+04 topsoil	1.39E+06	0.00	7
6.	Sediment bacteria, g	$0.00	2.20E-01	7.10E+04	1.56E+04	0.00	3
Total Emergy of Environmental Inputs to system (R, N):		***$0.00***			***3.64E+09 (R+N)***	***0.43***	
***Purchased Products* (*F = F_M (purchased material items (5-11)) + F_S (paid human services (12-13))**							
7.	Oyster Shell, g	$0.06	1.90E+01	9.81E+08 limestone	1.86E+10	2.18	7, 4
8.	Peat moss, g	$0.00	3.2	1.9E+04	6.08E+04	0.00	7
9.	*Seeds, J	$0.23	7.33E+03	5.80E+04	4.25E+08	0.05	7
10.	Plastic, g	$0.08	5.25E-01	5.87E+09	3.08E+09	0.36	2
11a.	PHA pots, Lo, g	$6.43	1.88E+01	9.07E+04 (ethanol)	1.71E+06	0.00	2
Total Emergy F_M:		***$6.79***			***2.21E+10 (F_M)***	***2.59***	
***Purchased / services* (S)**							
12/	Labor – purchased, J PHA deployment	$1.88	1.23E+05	6.74E+06	8.29E+11	96.85	6
13/	Shipping UPS, USD$	$0.08	-	1.47E+10	1.15E+09	0.13	8
Total Emergy F_S:		***$1.96***			***8.30E+11 (F_S)***	***96.99***	
System Yield $USD:		***$8.75***					
System Yield (Y) = R + N + (F_M+F_S)=					***8.56E+11 (Y)***	***100.00***	
14/ System Biomass output: **Transformity (τ)= Y/ε = 8.56E+11 / 14 a, b, c, or d**							
a.	P.p.seeds, DW, g/m²		0.49	1.75E+12	**8.56E+11**		
b.	P.p.seeds, DW, J/m²		7.33E+03	1.17E+08	**8.56E+11**		
c.	P.p.seeds, DW, g		2.51E+02	3.41E+09	**8.56E+11**		
d.	P.p.seeds, DW, J		3.75E+06	2.28E+05	**8.56E+11**		

See Table 4.6 for Emergy ratios and Indices

Transformities are based on the old baseline Odum et al. 2000.

*Value in Joules for seeds was factored in Appendix 6. Values for grams basis are also given.

References
1/ Buenfil (2001)
2/ Buranakarn (1998)
3/ Campbell (2012)
4/ Campbell (2000)
5/ Felix and Tilley (2009)
6/ Ingwersen (2010)
7/ Odum (1996)
8/ Odum (2007)

Table 4.5. Emergy analysis table for deployment and restoration of seeds planted in biodegradable PHA pots.. †The "Required amount" (below) is based on each m^2 of SAV bed restored as a contribution from that input. See Table 4.7 for Emergy ratios and Indices. Calculations for this table can be found in Appendix 7.

Item no.	Item, Unit	†$USD /$m^2$/yr	†Data /m^2/yr	*Transformity (sej/unit)	Emergy (sej/m^2/yr)	% total Emergy	Ref. for Transformity
***Renewable Resources* (R) and *Non-Renewable Resources (N)**							
1.	Sunlight,J	$0	5.91E+09	1.00E+00	5.91E+09	0.06	6
2.	Tides, J	$0	7.29E+05	4.94E+04	3.60E+10	0.39	1
3.	River, geopotential, J (estuarine circul'n)	$0	1.23E+08	3.18E+04	3.91E+12	40.95	5
Total Emergy of Environmental Inputs to system (R):		***$0.00***			***3.95E+12 (R)***	**41.39**	
***Purchased Products* (*F = F_M (purchased material items (4-8)) + F_S (paid human services (9))**							
4a.	Propagules (& pots), g	$8.75	4.90E-01	1.75E+12	8.58E+11	8.98	9
4b.	Propagules (& pots), g	$8.75	4.90E-01	1.97E+12	9.65E+11	-	9
5.	PVC fencing posts, g	$0.00	5.08E-01	5.85E+09	2.97E+09	0.03	3
6.	Plastic exclosure, g	$0.06	7.11E+00	5.85E+09	4.16E+10	0.44	3
7.	Steel fence T-post, g	$0.00	2.87E+00	4.15E+09	1.19E+10	0.12	8
8.	Machinery and Equip., g	$3.91	2.42E+02	1.47E+10	3.56E+12	37.25	7
9.	Fuel, J	$0.01	7.04E+05	3.86E+04	2.72E+10	0.28	2
Total Emergy F_M:		***$12.73***			***4.50E+12 (F_M)***	***47.10***	
*F = Economic Feedback resources that support the system							
***Purchased / services* (S)**							
10.	Labor-Loading*, J Logistics	$20.00	1.63E+05	6.74E+06	1.10E+12	11.50	4
Total Emergy F_S:		***$20.00***			***1.10E+12 (F_S)***	***11.50***	
System Yield $USD/$m^2$:		***$32.73***					
System Yield (Y) = R + N + (F_M+F_S)=					***9.55E+12 (Y)***	***100.00***	
11. System Biomass output (***ε***):							
Transformity (τ)=Y/Ɛ=9.55E+12 / 11a, b, c, or d							
a.	P. perfoliatus, DW, g/m^2		9.15E+01	1.04E+11	9.55E+14		
b.	P. perfoliatus, DW, J/m^2		1.34E+06	7.12E+06	9.55E+14		
c.	P. perfoliatus, DW Total, g		4.68E+04	2.04E+08	9.55E+14		
d.	P. perfoliatus, DW Total, J		6.86E+08	1.39E+04	9.55E+14		

See Table 4.7 for Emergy ratios and Indices
Transformities are based on the old baseline Odum et al. 2000.
*No Non-renewable Resources were input into this process

References
1/ Campbell (2004)
2/ Bastianoni et al. (2009)
3/ Brown and Buranakarn (2003)
4/ Ingwersen (2010)
5/ Martin (2002)
6/ Odum (1996)
7/ Odum (2007)
8/ Ortega (2000)
9/ Zinecker (CH3) see Appendix 6

Table 4.6. Emergy ratios and indices for the production of two different propagule and container delivery systems for SAV restoration. The relative transformities for propagules as an output of the propagation system, i.e. the relative Emergy Yield contained in propagule products just before restoration site delivery, is also given.*

Index/Ratio	**Calculation**	**Hand-transplant sods***	**Seeds and PHA pots***
System Output (ε)	Y/τ	9.5 gDW/m^2	0.49 gDW/m^2
Emergy Yield (Y)	R+N+F	2.45E+14	8.56E+11
Transformity for System (τ)	Y/Ɛ	2.58E+13	1.75E+12
Fraction Renewable (ϕR)	R/(R+N+F)	5.30E-04	4.25E-03
Emergy Yield Ratio (EYR)	Y/F	1.00	1.00
Environmental Loading Ratio (ELR)	(F+N)/R	1.88E+03	2.34E+02
Environment Sustainability Index (ESI)	EYR/ELR	5.33E-04	4.29E-03

* All units are in Sej/m^2/yr except ESI

Table 4.7. Emergy ratios and indices for the deployment and planting of two different delivery systems for SAV restoration. The relative transformities for propagules as an output of the restoration, i.e. the relative Emergy Yield contained in final products as a result of one season of growth after restoration planting, is also given.*

Index/Ratio	**Calculation**	**Hand-transplant sods***	**Seeds and PHA pots***
System Output after first season (ε)	Y/τ	29.80 gDW/m^2	91.5 gDW/m^2
Emergy Yield (Y)	R+N+F	4.26E+14	9.55E+12
Transformity for System (τ)	Y/Ɛ	1.43E+13	1.04E+11
Fraction renewable (ϕR)	R/(R+N+F)	7.24E-02	4.14E-01
Emergy Yield Ratio (EYR)	Y/F	1.08	1.71
Environmental Loading Ratio (ELR)	(F+N)/R	1.28E+01	1.42
Environment Sustainability Index (ESI)	EYR/ELR	8.42E-02	1.21

* All units are in Sej/m^2/yr other than noted

Table 4.8. Dollar valuation for the production of two different propagule and container delivery systems for SAV restoration: hand transplanting and PHA biodegradable pots loaded with seeds and inoculant. The costs compare the relative per m^2 annually USD$ value, the whole system annual USD$ costs, and third, the total USD$ costs for operational startup plus first year project.*

Propagation	Source	Hand-transplant sods	Seeds and PHA pots
USD$ per m^2	Tables 4.3&4.5	73.56	8.75
USD$ per annum	Appendices 4&6	2,496.82	4,479.36
USD$ initial investment + 1st project	Appendices 4&6	4,179.76	8,442.85

Table 4.9. Dollar valuation for the deployment and planting of two different for SAV restoration methods. The costs compare the relative per m^2 USD$ annual value, the whole restoration annual USD$ costs, and third, the initial USD$ costs for operational startup plus first year project.*

Restoration	Calculation	Hand-transplant sods	Seeds and PHA pots
USD$ per m^2	Tables 4.4&4.6	279.30	15.23
USD$ per annum	Appendices 5&7	4468.71	7795.99
USD$ initial investment + 1st project	Appendices 5&7	32,484.21	39,920.25

Table 4.10. Combined propagation and restoration dollar valuations for sod and PHA pot/seed SAV restoration methods. The costs compare the combined per m^2 USD$ annual value, annual USD$ costs, initial USD$ costs for operational startup plus first year project costs, and the $/$m^2$ SAV bed restored for initial investment plus first year's restoration.

Propagation and Restoration Combined	Calculation	Hand-transplant sods	Seeds and PHA pots
USD$ per m^2	Tables 4.8&4.9	352.86	23.98
USD$ per annum	Tables 4.8&4.9	6,965.53	12,275.35
USD$ initial investment + 1st project	Tables 4.8&4.9	36,663.97	48,363.10
USD$ initial investment + 1st year project (without depreciation)	**$/$m^2$ SAV bed restored**	$36,663.97 / 16$m^2$ = **2,291.50**	$48,363.10 / 512$m^2$ = **94.46**

Table 4.11. Restoration methods, natural bed densities, area planted, and cost. In many cases transplant densities are the result of trial and error, and differ greatly from natural beds. In some cases transplant density is dependent on the hydrologic energy signature of the site, or substrate considerations. This infers that any emergy analysis would benefit from the ability to be flexible and easy to modify for any given system that it diagrams, in order for a given restoration plan to change in response to the dynamic conditions of a given site.

Species/ location	**Method**	**Natural SAV bed density of propagule/ m^{-2}**	**Area planted ha or m^{-2}**	**Planting density ha^{-1}/ m^{-2}**	**Restoration Cost/planting unit**	**Cost m^{-2}**	**Restoration Study**
P. perfoliatus L. /mesohaline Cheapeake Bay	Shoot transplants from turions	[1, 8] Stems: 20-350 m^{-2} (variable)	176 m^2 Over three seasons	64 m^{-2}	$6.60/shoot unit	$8.34-24.46	1
P. perfoliatus L. / *Ruppia* maritima L. / Poplar Island, MD 2004	Mesh floats w/seeds	Seeds: 1,440 m^{-2} (data for P. perfoliatus)	4.86 ha	107,639/ 10.76 m^{-2} (each species)	NA	NA	2
P. perfoliatus L. /mesohaline Chesapeake Bay	Hand transplant of sods	[1, 8] Stems: 20-350 m^{-2} Turions: 30-100 m^{-2}	16 m^2	32 turions m^{-2} / 115 stems m^{-2}	$8.72/turion	$352.86	This study
P. perfoliatus L. / mesohaline Chesapeake Bay	Seeds in PHA pots	Seeds: 1,440 m^{-2}	512 m^2	175 seeds m^{-2}	$0.08/seed	$23.98	This study
Zostera marina L. Polyhaline Chesapeake Bay	Seeds via hand broadcasting[4] or seed buoy[5]	Seeds: 9,000 m^{-2}	25.5 ha over five years	51 m^{-2}	$0.01-0.34 (ave: $0.17)	$4.29-11.09	4, 5
Zostera marina L. polyhaline Chesapeake Bay	15 Clustered Shoots w/anchor =planting unit (pu)	[7]257-2193 shoots m^{-2}	NA	1.59-2.69 planting units m^{-2} (23-40 shoots m^{-2})	$4.34-4.84/pu or $0.29-0.33/shoot	$7.70-11.70**	6

References:

1/ Bergstrom (2006)
2/ Shafer and Bergstrom (2008)
4/ Busch et al. (2010)
5/ Pickerell et al. (2005)
6/ Fonseca (1982)

*Shoots consist of a 15 shoots per planting unit

**1982 USD converted to 2017 USD, originally: $30,000-45,000 ha^{-1} for low and high energy sites

Table 4.12. Comparisons of ecologically engineered or restored systems using emergy indices similar to this study.

Study	Yield	Fraction renewable	Emergy Yield Ratio	Environmental Loading Ratio	Environmental Sustainability Index	REF
Biogas	1.59E+16 sej/yr	0.66	2.93	0.52	5.67	1
Electricity	2.02E+16 sej/yr	0.52	2.07	0.93	2.22	1
Raft oysters	23.7E+12 sej/m^2/yr	0.29	1.40	2.5	NA	2
Cage oysters	29.6E+07 sej/m^2/yr	0.24	1.31	3.2	NA	2
Wastewater Treatment (passive)	4.1E+12 sej/m^2/yr	0.04	1.04	24	0.04	3
Wastewater Treatment (active)	2.3E+13 sej/m^2/yr	0.007	1.01	140	0.07	3
Forest Restoration	NA	NA	2.16	0.01	190.85	4
Orchard Restoration	NA	NA	3.07	1.13	2.72	4
Grassland Restoration	NA	NA	1.44	0.62	2.30	4
Fish pond Restoration	NA	NA	2.84	0.42	6.84	4
SAV Handplant Sods Propagation	2.45E+14 sej/m^2/yr	0.001	1.0	1880	0.001	5
SAV PHA pots/seeds Propagation	8.56-9.66E+11 sej/m^2/yr	0.004	1.0	234-265	0.004	5
SAV Handplant Sods Restoration	4.26E+14 sej/m^2/yr	0.07	1.08	12.8	0.08	5
SAV PHA pots/seeds Restoration	9.55-9.66E+12 sej/m^2/yr	0.5	1.71	1.42-1.44	1.2	5

1/ Ciotola et al. (2011)
2/ Williamson et al. (2015)
3/ Winfrey and Tilley (2016)
4/ Lu et al. (2006)
5/ This study

CHAPTER FOUR:

Figures

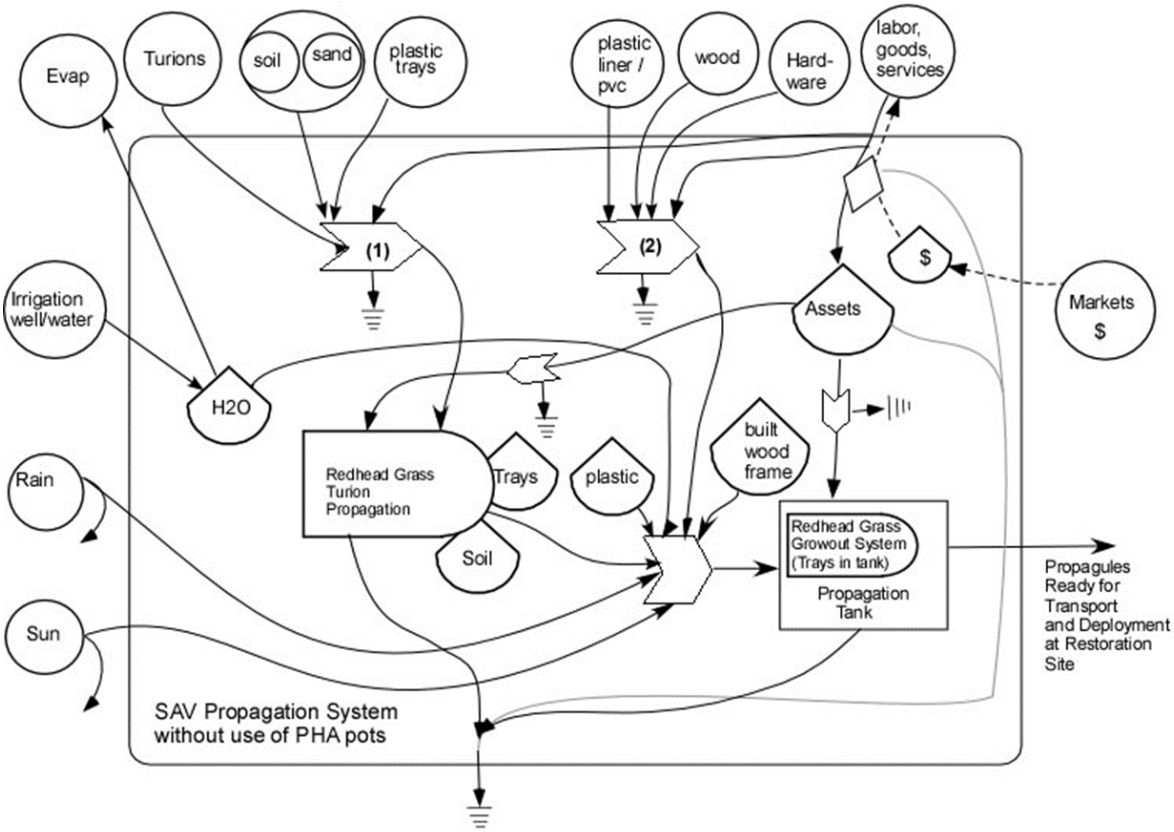
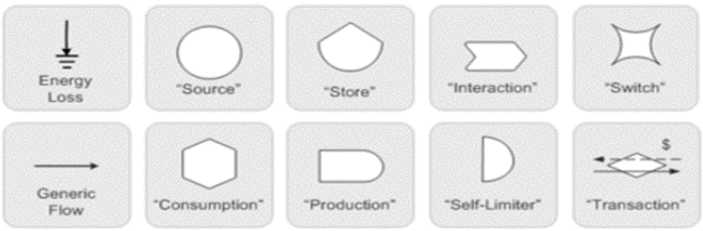


Figure 4.1. Traditional SAV propagation process for *P. perfoliatus*, using turions to propagate sods. (1) entails assemblage of propagation trays and (2) is the interaction of all the components that then fit into the *P. perfoliatus* growout tank system.
See guide below for meaning of symbols. From: http://prosperouswaydown.com/diagramsimages/



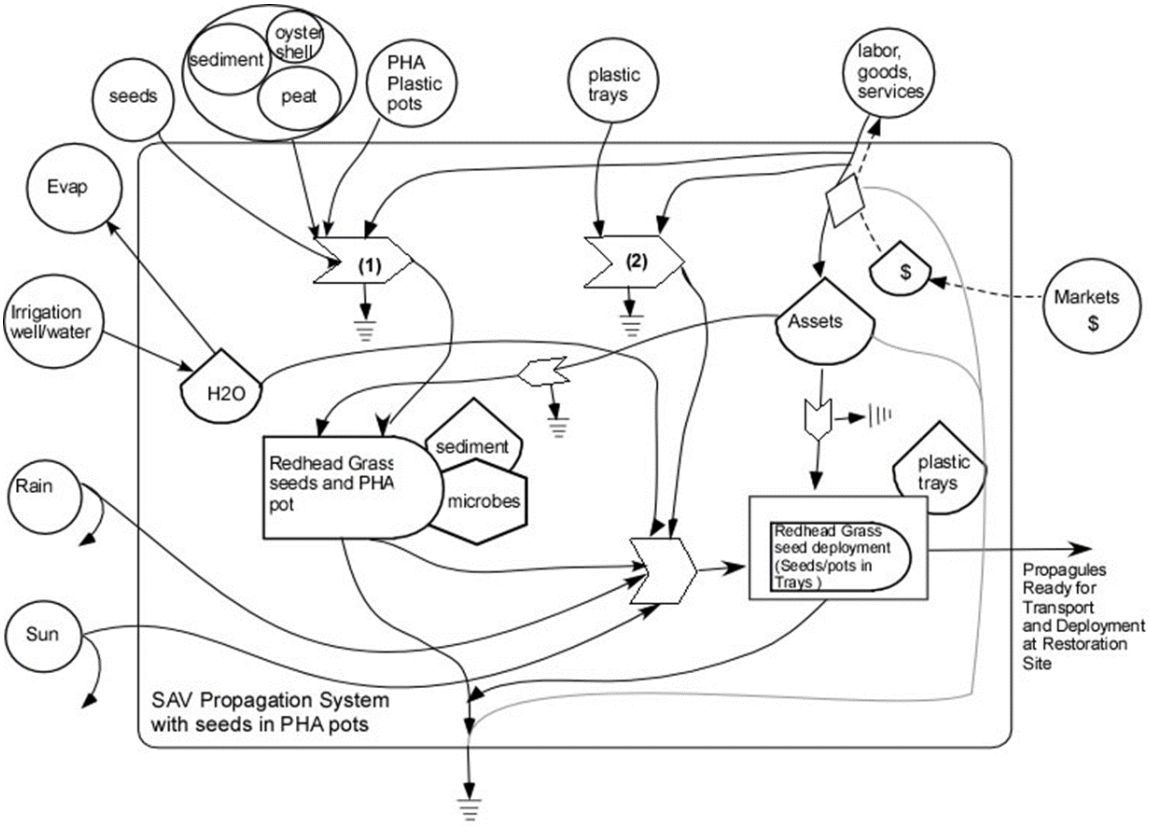
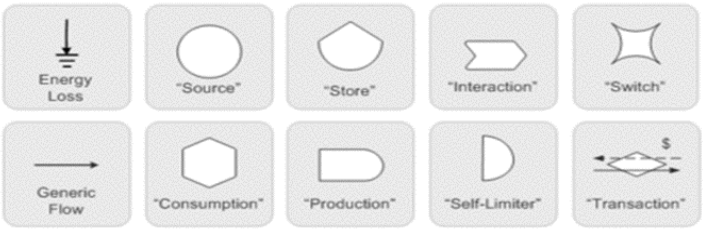


Figure 4.2. *P. perfoliatus* propagation tanks to prepare plants for restoration using biodegradable pots. (1) entails portion system devoted to obtaining seeds and pots; (2) is actual tray system that includes the output of (1). See guide below for meaning of symbols.
From: http://prosperouswaydown.com/diagramsimages/



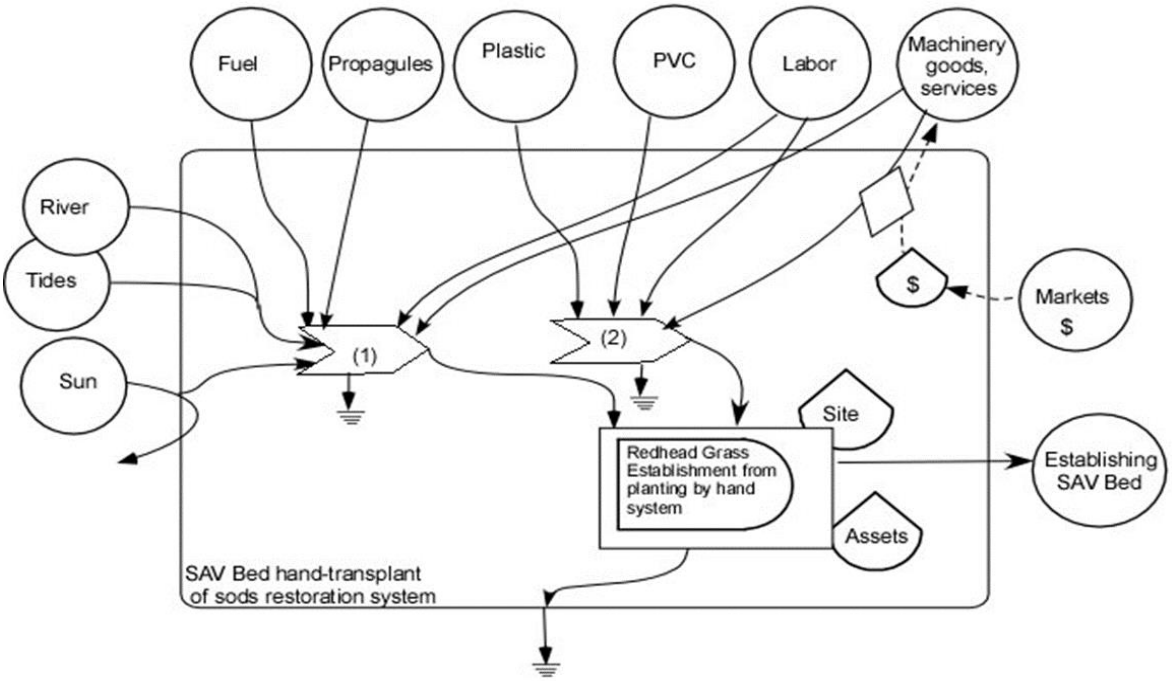
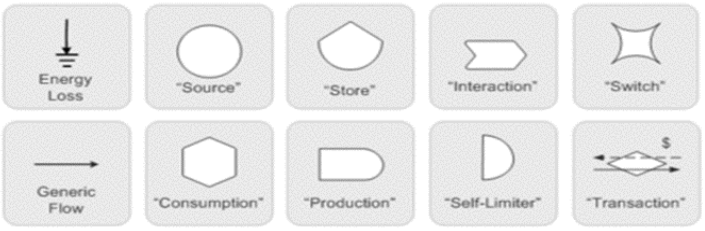


Figure 4.3. *P. perfoliatus* restoration site for restoration using hand transplant method. (1) entails transport and setup of fencing and (2) is actual planting. See guide below for meaning of symbols. From: http://prosperouswaydown.com/diagramsimages/



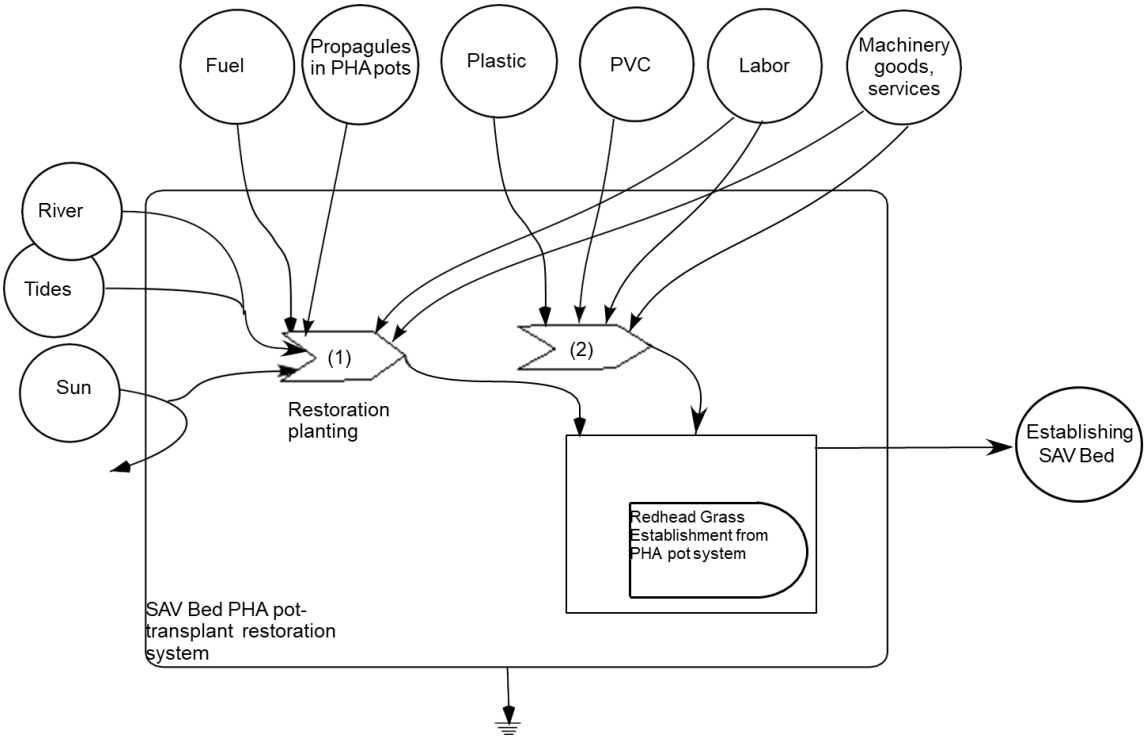
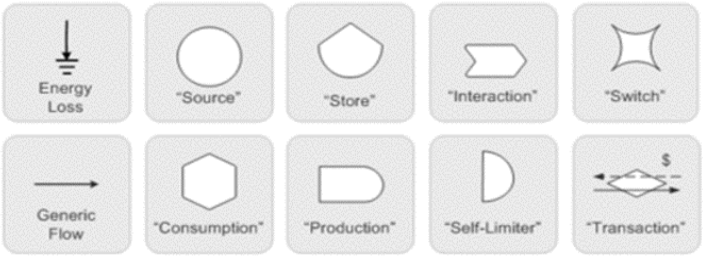


Figure 4.4. *P. perfoliatus* restoration site for restoration using PHA pot transplant method. (1) entails transport and setup of fencing, and (2) is actual planting.
See guide below for meaning of symbols. From: http://prosperouswaydown.com/diagramsimages/



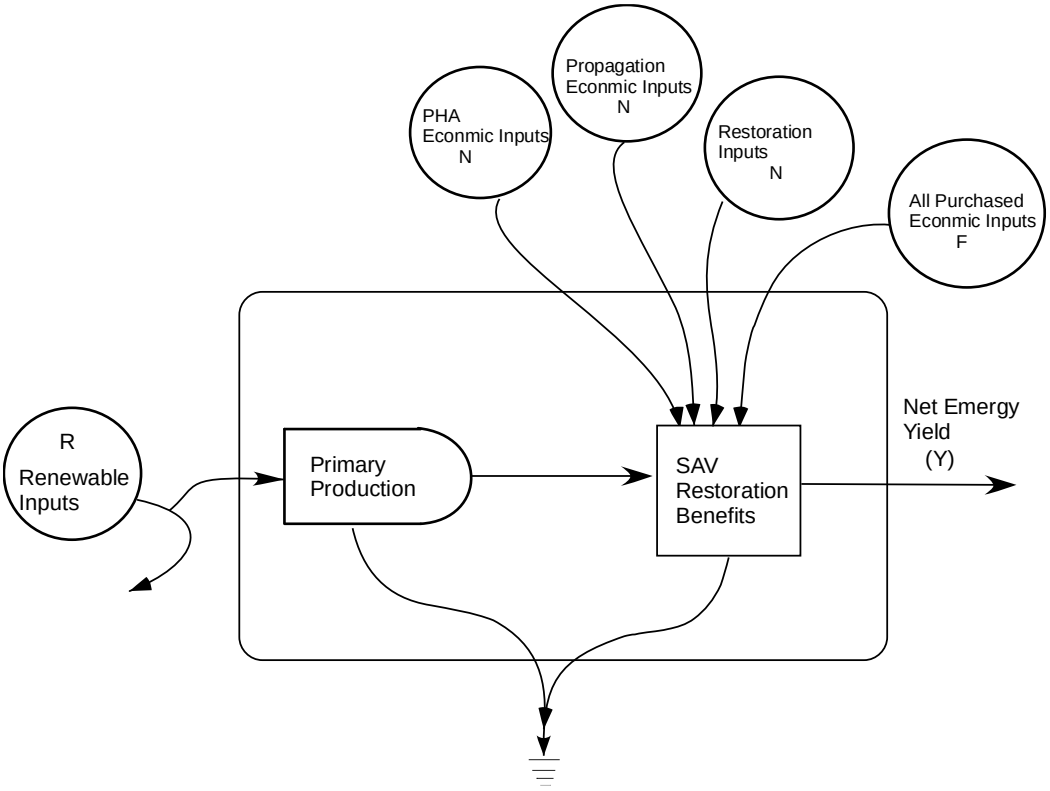
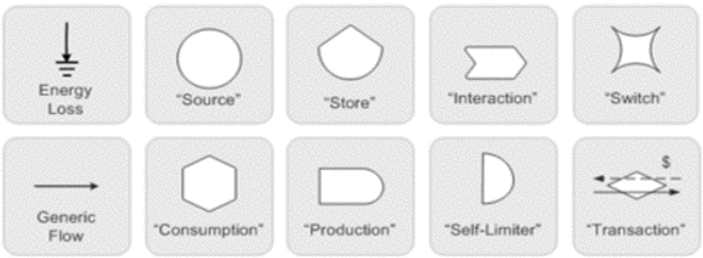


Figure 4.5. This simplified, generic emergy yield diagram evaluates the benefit of a successfully restored submersed aquatic vegetation ecosystem. This ratio is the emergy restoration export, or yield from the system (Y). The emergy yield ratio: (EYR) = Y/F
See guide below for meaning of symbols. From: http://prosperouswaydown.com/diagramsimages/



CHAPTER 5:

Conclusions and recommendations for restoration of SAV: *Potamogeton perfoliatus* L.

Background and chapter findings

Submersed aquatic vegetation in Chesapeake Bay is recovering in many areas, however *P. perfoliatus* habitat is being lost at an alarming rate of tens to hundreds of hectares every few years (Orth et al. 2001-2015). Given this loss in Chesapeake Bay, and the lack of documented recovery in impacted *P. perfoliatus* habitat elsewhere (Meyer et al. 2013), it appears a higher level of effort will be required to rehabilitate and restore habitat previously occupied by this important aquatic species. The overarching goal of this dissertation was to improve restoration of *P. perfoliatus* in Chesapeake Bay. The microcosm experiments and field trial provided data to support this goal through evaluation of *P. perfoliatus* growth responses to SAV bed sediment and substrates, development of a new PHA pot/seed restoration technique, and economic and environmental cost accounting to evaluate the costs and sustainability of the biodegradable pot and sod transplant restoration methods.

The microcosm experiment in Chapter Two concluded that *P. perfoliatus* turions did not show exclusive preference to the sediment from which they were originally taken; each population demonstrated similar growth characteristics for all treatments but preferred either bed sediment over horticultural substrates with the exception of oyster shell peat, which was closest to the bed sediments in nutrient characteristics. SAV bed sediments (and refractory oyster shell/peat), were unique in most aspects compared with horticultural substrates in that they were able to provide the plants with optimal yield with low-intermediate %OC and %TN in substrate, and an intermediate uptake of %TN plant tissue concentration.

In Chapter 3, microcosm experiments and a field trial were used to evaluate a newly developed biodegradable PHA pot/inoculant/germinated seed approach to restoration. This study incorporated the results from the Chapter 2 substrate experiments to better understand seed establishment and yield benefitting from an "ecological assist" through the use of PHA pots filled with a microbial inoculant for restoration transplanting. The MEI experiment lead to the conclusion that plant growth was comparable between PHA and PE pots. While it was confirmed that PHA degrades two orders of magnitude faster than PE, both PHA and PE degrade faster in the presence of the plants. MEII results lead to the conclusion that seeds from either harvest year would grow equally well; the PHA pot provided a favorable growth environment (qualitatively) for seed establishment when compared alongside seed broadcasting onto bare sediment. Observing the establishment and growth of seeds from two different harvest years (<1 year and 4.5 years) in both treatments demonstrated the possibility that current seed storage protocols may not have yet reached their maximum potential for conserving and storing harvested seeds for use in restoration or propagation (i.e. > 1yr old). Pot degradation was greater in the deep portions compared with shallow portions of the spindle for MEI but was more homogeneous for MEII (although a qualitative trend of shallow and deep similar to MEI was seen). This was due to a gradient of stratification that indicated less negative Eh in shallow portion of sediment for MEI and subsequent lower degradation. Pot degradation in the field experiment was distributed along the whole spindle of the pot in an inconsistent fashion, somewhat more similar to MEII than MEI. In addition, degradation overall (for % mass) was greater in the mesohaline than in the tidal fresh portions of the Bay.

Chapter 4 evaluated inputs from propagation and restoration phases of the PHA pot/seed technique and the sod transplant technique. Economic and emergy analysis were used to assess the sustainability and cost effectiveness of two restoration approaches: Hand transplanting of sods, and the PHA pot/seed restoration method. The primary findings of the research is that the PHA/pot seed restoration method was more cost effective and sustainable than the hand-transplant/sod method, providing more total sq. meters SAV bed restored for a lower cost /sq. m.

Conclusions

Three conclusions can be drawn from the main chapters of this research: 1/ It was possible to approximate some characteristics of natural bed sediment of *P. perfoliatus* to achieve comparable yield. Additional modifications in substrates and sediment will make propagation and restoration more sustainable and promote reproductive potential of *P. perfoliatus*; 2/ The PHA pot technique handled two problematic life stages of *P. perfoliatus* (germination and establishment). In addition, proper restoration site selection, and onsite substrate and water quality modifications will likely further facilitate restoration success; and 3/ Restoration of *P. perfoliatus* is expensive both financially and from a resource use perspective. Restoration efforts for this species should continue to increase efficiencies and sustainability, and reduce dollar costs and level of effort. The following summary includes conclusions and recommendations that frame the important findings of this dissertation research, and lays the foundation for future projects.

▪ Using intact, undisturbed sediment cores from field sites enabled one of the first holistic descriptions of SAV bed sediments in Chesapeake Bay, and an opportunity to compare

how they differ or are similar to horticultural substrates used to propagate *P. perfoliatus* and other SAV species.

▪ Textural descriptions (<2mm and the % coarse >2mm fractions) are key indicators of the availability of important elements such as C and N, and the general condition of the substrate, sediment, even the habitat.

▪ SAV bed sediments and oyster shell/peat substrate demonstrated highest yield overall, and contained intermediate levels of nitrogen, phosphorous and organic C, and most other essential elements for plants, in contrast to the higher levels (particularly N) found in soil/sand that depressed growth.

▪ SAV bed sediments contained the highest levels of land-based elements such as copper, iron, and aluminum, making them accurate indicators of landscape processes.

▪ %TN:M3P:M3K ratios of substrates most closely matching the NPK of (high yield) SAV bed sediments were also closest in yield. A lack of organic material, but similarity in NPK ratio, may indicate an overall lack of concentration of nutrients in a given substrate (as with sand).

▪ Redox stratification between shallow (more positive Eh) and deep (lower Eh) portions of the microcosms, and its alteration over time (CH3), served as an indicator of anaerobic microbial activity – which is also important for PHA degradation. Reduction of sediments increased availability of nitrogen in the form of NH_4, the form of Nitrogen most used by many aquatic plants.

▪ Reduced conditions facilitate access to NH_4, and in the presence of %OC in substrate, create greater facilitation for plant uptake of %TN, thereby affecting yield. In this

research, higher levels of %TN in plant tissue reduced yield particularly in belowground biomass.

▪ Refractory oyster shell/peat substrate appeared to be one of the best options for a propagation substrate if used in the short term. But if disturbed, over time, it could have the potential to mineralize or increase in lability and reduce yield. In the case of CH3, oyster shell peat mixed with marsh sediment inoculant supported lower yield than oyster/peat substrate or SAV bed sediments (CH2) due to its higher lability.

▪ Reduction in aboveground biomass due to excess %TN (soil/sand in CH2, and to a lesser extent the substrates in CH3 MEI and MEII), or inadequate nutrients (as in sand substrate), limited the number of longer stems that then are able to develop inflorescences.

▪ The common garden approach to evaluating two populations of turions showed that turions had similar ability to adapt to the various substrates and similar biomass. Turions from Kent Narrows grew a percentage of longer stems with a greater number of flowers on Sherwood Forest sediment (the "top performing" sediment overall) demonstrated the utility of evaluating both vegetative and sexual reproductive characteristics of a population, and that sediment can be a highly influential factor in both.

▪ Seeds taken from different sites of origin, and years (<1 yr vs. 4.5 yrs), using different storage methods, were not highly different in their responses to treatments when placed in similar substrates and treatments.

▪ Seeds of either harvest year planted in PHA pots produced qualitatively more biomass, than their counterparts planted on bare sediment.

▪ By managing germination, establishment, and placement in the sediment, the PHA pot method made handling and planting SAV far more efficient, and avoided the extra time and money to propagate shoots or sods of *P. perfoliatus* plants.

▪ *P. perfoliatus* plants grew qualitatively best in PE pots, followed by PHA pots, and then by growth on bare sediment. However yield was approximately half to 60% of what it could be under appropriate substrate conditions (Zinecker CH2).

▪ The propagation and preparation phase of the two restoration methods required quite different resources – the sod method required 95% more emergy, and was 88% more expensive on a per m sq. SAV bed restored basis than the PHA pot method.

▪ The restoration and deployment phase of the restoration were also quite different – the sod method required 73% more emergy than the PHA pot method, and was 95% more expensive on a per m sq. SAV bed restored basis.

▪ The propagules and substrates that each restoration method used were fundamentally different with their potential for getting plants in the ground and established. The sod method used soil/sand, which depresses plant yield, while the PHA pot method yield potential used an inoculant that stimulated plant growth, and produced (very conservatively) 25% higher NPP $gdwm^{-2}day^{-1}$.

▪ In addition to the PHA pot method being able to produce more biomass once in the field by the end of the first season, it restored 97% more SAV bed (total m^2), and cost 94% less per m^2.

▪ PHA pot mass loss rates (% g loss/day) were approximately 98% faster than PE pots. For MEI, PHA pots and PE pots degraded more quickly when planted than unplanted. This same trend did not occur in MEII. Instead unplanted pots had greater qualitative

degradation. In both cases, greater microbial degradation, appears to have coincided with qualitatively lower Eh.

▪ Rate of daily % spindle diameter degradation for PHA pots appeared to be comparable for both microcosm experiments. But spindle degradation in the field pots was 60% slower.

▪ Due to the differences in Eh between MEI and MEII in the shallow layer, there was greater microbial degradation of PHA in the upper layer of MEII than in MEI.

▪ In MEII, because of the migration of organic material to the surface of the microcosm, degradation was visibly more heterogeneous on the spindle. In addition, some pots appeared more degraded than others in the upper layer.

▪ For the field experiments, particularly in the mesohaline where the pots were more deeply and completely buried, degradation by mass was more significant, but in both cases, spindle degradation appeared to have no highly consistent relation to deeper vs. shallow portions of the spindle. This may indicate a more evenly distributed microbial community in actual estuarine sediments, as in MEII.

Recommendations

▪ More data on *P. perfoliatus* bed sediments and other SAV species is needed to further define plant/sediment relationships.

▪ Routine description of the <2mm and coarse fraction of sediment will better inform habitat quality and sediment structure.

▪ Further study would provide more information about the relationship between landscape geomorphological processes and sediment (habitat) suitability.

▪ Ideally NPK ratios, as well and organic matter content, micro- and macronutrients, would be identified for various SAV species bed sediments to learn more about the impacts on these habitats and sediment requirements for restoration, as well as to compare conditions in existing beds (i.e. reference sites).

▪ Collection of data on plant tissue nutrient concentrations and even toxics (in addition to %TC and %TN) during different times of the growing season would provide valuable information about plant resource use

▪ Research on microbial distribution in estuarine sediments and extent of degradation would further inform these dynamics.

▪ Sediments in aquatic habitats are rich in microbial species, many of which either form PHA inclusions or are able to metabolize PHA. Additional studies on these relationships in SAV beds could potentially advance the field of rhizodegradation and PHA production.

▪ Additional experiments are needed to test reproducibility of longer term storage of seeds in a germinated state, as well as to better evaluate production of inflorescences and the viability of the seeds subsequently produced by plants from seeds in long term storage.

▪ Additional experiments might refine propagation substrates to be better managed in the long term to avoid any issues presented by enrichment over time.

▪ Restoration substrates (onsite as well as during propagation) would ideally approximate as nearly as possible the SAV bed sediment parameters including texture and nutrient levels.

▪ More experiments are needed to understand, establish and optimize plant yield parameters and how they vary with different values of redox, pH, substrate, %TN, substrate quality (type and particle size) and quantity %OM thresholds and other nutritional parameters for both existing bed sediments, at restoration sites, and for propagation.

▪ Given the importance of flowering and productivity, more research may shed additional light on flowering responses to %TN plant tissue and %OC and other nutrients.

▪ Study of the relationship between sediment conditions, turion size, growth rates, and production of inflorescences will provide more information about vegetative regeneration and its contribution to the plant community regeneration.

▪ In the event that a permit is issued to destroy a *P. perfoliatus* bed, great care should be taken to conserve the sediment. Saving the plants/turions would also be beneficial.

▪ Light, temperature, salinity, plant species, and water column nutrient variables all were held constant for the microcosm experiments. It would be useful to manipulate these parameters while further exploring nutrient uptake and plant yield responses to the surrounding environmental conditions.

▪ Field experimentation and a pilot restoration are highly recommended in order to better understand the benefits of managing substrate and plant establishment.

Based on the data generated in this dissertation, and studies elsewhere, it seems clear that sustainable restoration and conservation of *Potamogeton perfoliatus* habitat in Chesapeake Bay may be possible where site parameters such as light, water, and sediment quality are appropriate. The best place to start may be expansion of existing *P. perfoliatus* beds, and the importance in doing this as soon as possible cannot be

emphasized enough. If restoration ecology projects are viewed as valuable lessons from which to learn, and are more carefully documented, restoration of SAV and other habitats will continue to improve and become a key conservation tool. In the book Restoration Ecology: A synthetic approach to ecological research, Jordan et al. (1987) discuss the idea of using ecological restoration as both a form of environmental technology and as a technique for basic research. They relate "The idea here is simply that one of the most valuable and powerful ways of studying something is to attempt to reassemble it, to repair it, and to adjust it so that it works properly." Ewel (1987) similarly shares: "The success of ecosystem restoration can be judged by five criteria…" [sustainability, invasibility, productivity, nutrient retention, and biotic interactions] "…The ecologist capable of creating an ecosystem that passes this rigorous test earns high marks; the one who fails is sure to gain new insights into ecosystem structure and function." This work was supported by two grants that were both committed to the idea of thinking creatively about restoration and technologies to get the best result. The hope of this research is that it may in some small way inspire others to conserve, repair, put back together, and perhaps even create new, valuable habitats.

APPENDICES

Appendix 1. Water quality profile in microcosms

Published values Washington Suburban Sanitary Commission report on chemical analysis of tap water used in this experiment from the Potomac River and Patuxent River watersheds. WSSC 2016.

WSSC TAP WATER ANALYSIS - 2015

POTOMAC WATER FILTRATION PLANT

PARAMETER	UNIT OF MEASURE	YEARLY AVERAGE	MAXIMUM	MINIMUM	EPA LIMIT [1]
GENERAL WATER QUALITY					
Alkalinity	mg/L	79	108	42	
Color	Units	1	11	0	15 (SMCL)
Hardness	mg/L	138	194	90	
pH	S.U.	7.4	7.8	7.1	6.5-8.5 (SMCL)
Specific Conductance	MicroSiemens/cm	444	1421	263	
Temperature	° C	15.6	29.5	0.6	
Threshold Odor	Units	1.0	1.0	1.0	3 (SMCL)
Turbidity [1]	NTU	0.04	0.22	0.01	TT=1 NTU; <0.3 NTU 95% of time
METALS					
Aluminum	µg/L	33	100	14	200 (SMCL)
Antimony	µg/L	n/d	n/d	n/d	6
Arsenic	µg/L	n/d	5	n/d	10
Barium	mg/L	0.036	0.053	0.027	2
Beryllium	µg/L	n/d	n/d	n/d	4
Cadmium	µg/L	n/d	n/d	n/d	5
Calcium	mg/L	38.6	54.9	25.5	
Total Chromium	µg/L	<2	2	n/d	100
Copper	mg/L	0.002	0.006	n/d	
Iron	mg/L	0.2	0.3	n/d	0.3 (SMCL)
Lead	µg/L	n/d	n/d	n/d	
Magnesium	mg/L	9.1	13.9	5.7	
Manganese	µg/L	12	49	<2	50 (SMCL)
Mercury	µg/L	n/d	n/d	n/d	2
Nickel	µg/L	2	2	<2	
Potassium	mg/L	3.2	4.8	2.1	
Selenium	µg/L	<2	13	n/d	50
Silicon	mg/L	2.3	4.6	0.5	
Silver	µg/L	n/d	<2	n/d	100 (SMCL)
Sodium	mg/L	32.8	220	17.0	
Thallium	µg/L	n/d	n/d	n/d	2
Zinc	µg/L	<2	3	n/d	5000 (SMCL)
INORGANICS					
Boron	mg/L	0.024	0.044	0.011	
Chloride	mg/L	66.7	394	38.2	250 (SMCL)
Residual Chlorine	mg/L	1.9	3.3	1.0	TT=>0.2
Fluoride	mg/L	0.68	0.87	0.54	4 (SMCL=2)
Nitrate	mg/L	1.4	2.3	0.5	10
Nitrite	mg/L	n/d	<0.05	n/d	1
Phosphorus	mg/L	0.27	0.36	n/d	
Sulfate	mg/L	40.2	97.6	11.8	250 (SMCL)
DISINFECTION BYPRODUCT PRECURSOR					
Total Organic Carbon	mg/L	1.8	2.8	0.9	TT
ORGANICS					
Haloacetic Acids (HAA5)	µg/L	15.3	24.4	8.4	
Total Trihalomethanes (TTHMs)	µg/L	14.4	29.1	5.4	
PESTICIDES & SYNTHETIC ORGANIC CHEMICALS (SOCs)					
2,3,7,8-TCDD (Dioxin)	pg/L	n/d	n/d	n/d	30
2,4,5 TP (Silvex)	µg/L	n/d	n/d	n/d	50
2,4-D	µg/L	n/d	n/d	n/d	70
3-Hydroxycarbofuran	µg/L	n/d	n/d	n/d	
Alachlor	µg/L	n/d	n/d	n/d	2
Aldicarb	µg/L	n/d	n/d	n/d	3
Aldicarb sulfone	µg/L	n/d	n/d	n/d	2

WSSC TAP WATER ANALYSIS - 2015

POTOMAC WATER FILTRATION PLANT

PARAMETER	UNIT OF MEASURE	YEARLY AVERAGE	MAXIMUM	MINIMUM	EPA LIMIT [1]
PESTICIDES & SYNTHETIC ORGANIC CHEMICALS (SOCs)					
Aldicarb sulfoxide	µg/L	n/d	n/d	n/d	4
Aldrin	µg/L	n/d	n/d	n/d	
Atrazine	µg/L	n/d	n/d	n/d	3
Benzo(a)pyrene	µg/L	n/d	n/d	n/d	0.2
Butachlor	µg/L	n/d	n/d	n/d	
Carbaryl	µg/L	n/d	n/d	n/d	
Carbofuran	µg/L	n/d	n/d	n/d	40
Chlorinated biphenyls (PCBs)	µg/L	n/d	n/d	n/d	0.5
Chlordane	µg/L	n/d	n/d	n/d	2
Dalapon	µg/L	n/d	<1	n/d	200
Dibromochloropropane (DBCP)	µg/L	n/d	n/d	n/d	0.2
Dicamba	µg/L	n/d	n/d	n/d	
Dieldrin	µg/L	n/d	n/d	n/d	
Di(2-ethylhexyl)adipate	µg/L	n/d	n/d	n/d	400
Di(2-ethylhexyl)phthalate	µg/L	n/d	<2	n/d	6
Dinoseb	µg/L	n/d	n/d	n/d	7
Diquat	µg/L	n/d	n/d	n/d	20
1,2-Dibromoethane (EDB)	µg/L	n/d	n/d	n/d	0.05
Endothall	µg/L	n/d	n/d	n/d	100
Endrin	µg/L	n/d	n/d	n/d	2
Glyphosate	µg/L	n/d	n/d	n/d	700
Heptachlor	µg/L	n/d	n/d	n/d	0.4
Heptachlor epoxide	µg/L	n/d	n/d	n/d	0.2
Hexachlorobenzene	µg/L	n/d	n/d	n/d	1
Hexachlorocyclopentadiene	µg/L	n/d	n/d	n/d	50
Lindane	µg/L	n/d	n/d	n/d	0.2
Metolachlor	µg/L	n/d	n/d	n/d	
Methomyl	µg/L	n/d	n/d	n/d	
Methoxychlor	µg/L	n/d	n/d	n/d	40
Metribuzin	µg/L	n/d	n/d	n/d	
Oxamyl (vydate)	µg/L	n/d	n/d	n/d	200
Pentachlorophenol (PCP)	µg/L	n/d	n/d	n/d	1
Picloram	µg/L	n/d	n/d	n/d	500
Propachlor	µg/L	n/d	n/d	n/d	
Simazine	µg/L	n/d	n/d	n/d	4
Toxaphene	µg/L	n/d	n/d	n/d	3
VOLATILE ORGANIC CHEMICALS (VOCs)					
1,1,1-Trichloroethane	µg/L	n/d	n/d	n/d	200
1,1,2-Trichloroethane	µg/L	n/d	n/d	n/d	5
1,1-Dichloroethene	µg/L	n/d	n/d	n/d	7
1,2,4-Trichlorobenzene	µg/L	n/d	n/d	n/d	70
1,2-Dichlorobenzene	µg/L	n/d	n/d	n/d	600
1,2-Dichloroethane	µg/L	n/d	n/d	n/d	5
1,2-Dichloropropane	µg/L	n/d	n/d	n/d	5
1,4-Dichlorobenzene	µg/L	n/d	n/d	n/d	75
Benzene	µg/L	n/d	n/d	n/d	5
Carbon Tetrachloride	µg/L	n/d	n/d	n/d	5
Chlorobenzene	µg/L	n/d	n/d	n/d	100
cis -1,2-Dichloroethene	µg/L	n/d	n/d	n/d	70
Dichloromethane	µg/L	n/d	n/d	n/d	5
Ethylbenzene	µg/L	n/d	n/d	n/d	700
Total Xylenes	µg/L	n/d	n/d	n/d	10000
Styrene	µg/L	n/d	n/d	n/d	100
Tetrachloroethene	µg/L	n/d	n/d	n/d	5
Toluene	µg/L	n/d	n/d	n/d	1000

WSSC TAP WATER ANALYSIS - 2015

POTOMAC WATER FILTRATION PLANT

PARAMETER	UNIT OF MEASURE	YEARLY AVERAGE	MAXIMUM	MINIMUM	EPA LIMIT [1]
VOLATILE ORGANIC CHEMICALS (VOCs)					
trans -1,2-Dichloroethene	µg/L	n/d	n/d	n/d	100
Trichloroethene	µg/L	n/d	n/d	n/d	5
Vinyl Chloride	µg/L	n/d	n/d	n/d	2
1,1,1,2-Tetrachloroethane	µg/L	n/d	n/d	n/d	
1,1,2,2-Tetrachloroethane	µg/L	n/d	n/d	n/d	
1,1-Dichloroethane	µg/L	n/d	n/d	n/d	
1,1-Dichloropropene	µg/L	n/d	n/d	n/d	
1,2,3-Trichlorobenzene	µg/L	n/d	n/d	n/d	
1,2,3-Trichloropropane	µg/L	n/d	n/d	n/d	
1,2,4-Trimethylbenzene	µg/L	n/d	n/d	n/d	
1,3,5-Trimethylbenzene	µg/L	n/d	n/d	n/d	
1,3-Dichlorobenzene	µg/L	n/d	n/d	n/d	
1,3-Dichloropropane	µg/L	n/d	n/d	n/d	
2,2-Dichloropropane	µg/L	n/d	n/d	n/d	
2-Chlorotoluene	µg/L	n/d	n/d	n/d	
4-Chlorotoluene	µg/L	n/d	n/d	n/d	
Bromobenzene	µg/L	n/d	n/d	n/d	
Bromochloromethane	µg/L	n/d	n/d	n/d	
Bromomethane	µg/L	n/d	n/d	n/d	
Chloroethane	µg/L	n/d	n/d	n/d	
Chloromethane	µg/L	n/d	n/d	n/d	
cis -1,3-Dichloropropene	µg/L	n/d	n/d	n/d	
Dibromomethane	µg/L	n/d	n/d	n/d	
Dichlorodifluoromethane	µg/L	n/d	n/d	n/d	
Hexachlorobutadiene	µg/L	n/d	n/d	n/d	
Isopropylbenzene	µg/L	n/d	n/d	n/d	
n-Butylbenzene	µg/L	n/d	n/d	n/d	
n-Propylbenzene	µg/L	n/d	n/d	n/d	
Naphthalene	µg/L	n/d	n/d	n/d	
p-Isopropyltoluene	µg/L	n/d	n/d	n/d	
s-Butylbenzene	µg/L	n/d	n/d	n/d	
t-Butylbenzene	µg/L	n/d	n/d	n/d	
trans -1,3-Dichloropropene	µg/L	n/d	n/d	n/d	
Trichlorofluoromethane	µg/L	n/d	n/d	n/d	
Nitrobenzene	µg/L	n/d	n/d	n/d	
Methyl-tert-butyl-ether	µg/L	n/d	n/d	n/d	
RADIONUCLIDES					
Gross Alpha	pCi/L	<2.0	<2.0	<2.0	15
Gross Beta	pCi/L	<4.0	<4.0	<4.0	50 [2]
Radium 228	pCi/L	<1.0	<1.0	<0.8	5 [3]
Tritium	pCi/L	<100	<100	<100	

CUSTOMER TAP [4]

PARAMETER	UNIT OF MEASURE	90th PERCENTILE [5]	# of SITES ABOVE AL	EPA ACTION LEVEL (AL)
Copper	µg/L	87.4	0 samples	1300
Lead	µg/L	1.17	0 samples	15

DISTRIBUTION SYSTEM

PARAMETER	UNIT OF MEASURE	YEARLY AVERAGE	MAXIMUM	MINIMUM	EPA LIMIT [1]
BACTERIOLOGICAL					
Samples Total Coliform Positive	%/month	0.33	1.05	0	5
Samples *E. coli* Positive	%/month	0	0	0	

WSSC TAP WATER ANALYSIS - 2015

DISTRIBUTION SYSTEM					
PARAMETER	**UNIT OF MEASURE**	**YEARLY AVERAGE**	**MAXIMUM**	**MINIMUM**	**EPA LIMIT [11]**
BACTERIOLOGICAL					
No.of E. coli Positive Routine Samples	Count	0	0	0	
No.of E. coli Positive Repeat Samples	Count	0	0	0	0
DISINFECTANT & DISINFECTION BYPRODUCTS					
Residual Chlorine	mg/L	1.26 [6]	4.50	n/d [7]	4 [8]
Haloacetic Acids (HAA5)	µg/L	43.4 [9]	87.7	2.9	60 [10]
Total Trihalomethanes (TTHMs)	µg/L	62.1 [9]	94.5	16.5	80 [10]

LEGENDS

n/d - not detected

mg/L - milligrams per liter, equal to parts per million (ppm). The equivalent of one minute in 2 years or one penny in $10,000.

µg/L - micrograms per liter, equal to parts per billion (ppb). The equivalent of one minute in 2,000 years or one penny in $10 million.

ng/L - nanograms per liter, equal to parts per trillion (ppt). The equivalent of one minute in 2,000,000 years or one penny in $10 billion.

pg/L - picograms per liter, equal to parts per quadrillion (ppq). The equivalent of one minute in 2,000,000,000 years or one penny in $10 trillion.

pCi/L - picocuries per liter (a measure of radiation)

S.U. - Standard Unit

NTU - Nephelometric Turbidity Unit

TT - Treatment Technique. A required process intended to reduce the level of a contaminant in drinking water.

AL - Action level. The concentration of a contaminant which, if exceeded, triggers treatment or other requirements which a water system must follow.

= equals

< less than

[1] - Filtered water, maximum of measurements taken every 15 minutes.

[2] - EPA considers 50 pCi/L to be the level of concern for beta particles.

[3] - The EPA limit of 5 pCi/L applies to combined Radium 226 and 228.

[4] - Most recent required sampling, between June and September 2014.

[5] - If more than 10% of sites exceed action level, system is required to take additional steps to control corrosiveness of their water.

[6] - Highest running annual average (RAA)

[7] - All samples deemed to have detectable disinfectant residual.

[8] - Maximum residual disinfectant level (MRDL), the highest level of a disinfectant allowed in drinking water; based on RAA.

[9] - Highest locational running annual average (LRAA)

[10] - Maximum contaminant level based on LRAA.

[11] - Shown as maximum contaminant levels (MCL) unless otherwise noted as secondary MCLs (SMCL). MCLs are enforceable health-based standards, whereas SMCLs are non-enforceable guidelines for contaminants that may cause aesthetic effects in drinking water.

WSSC TAP WATER ANALYSIS - 2015

PATUXENT WATER FILTRATION PLANT

PARAMETER	UNIT OF MEASURE	YEARLY AVERAGE	MAXIMUM	MINIMUM	EPA LIMIT [11]
GENERAL WATER QUALITY					
Alkalinity	mg/L	33	90	26	
Color	Units	0	5	0	15 (SMCL)
Hardness	mg/L	69	102	46	
pH	S.U.	7.4	7.9	7	6.5-8.5 (SMCL)
Specific Conductance	MicroSiemens/cm	221	285	192	
Temperature	°C	15.6	27.7	3.6	
Threshold Odor	Units	1.0	1.2	1.0	3 (SMCL)
Turbidity [1]	NTU	0.03	0.10	0.02	TT=1 NTU; <0.3 NTU 95% of time
Geosmin	ng/L	3.9	8.1	<2	
2-Methylisoborneol	ng/L	3.9	13.7	n/d	
METALS					
Aluminum	µg/L	19	308	11	200 (SMCL)
Antimony	µg/L	n/d	n/d	n/d	6
Arsenic	µg/L	n/d	<2	n/d	10
Barium	mg/L	0.027	0.033	0.023	2
Beryllium	µg/L	n/d	n/d	n/d	4
Cadmium	µg/L	n/d	n/d	n/d	5
Calcium	mg/L	16.1	19.2	13.6	
Total Chromium	µg/L	n/d	<2	n/d	100
Copper	mg/L	0.016	0.028	0.007	
Iron	mg/L	<0.2	<0.2	n/d	0.3 (SMCL)
Lead	µg/L	n/d	n/d	n/d	
Magnesium	mg/L	4.7	5.3	4.1	
Manganese	µg/L	<2	4	n/d	50 (SMCL)
Mercury	µg/L	n/d	n/d	n/d	2
Nickel	µg/L	<2	<2	n/d	
Potassium	mg/L	2.6	2.9	2.1	
Selenium	µg/L	n/d	2	n/d	50
Silicon	mg/L	2.3	3.2	0.6	
Silver	µg/L	n/d	n/d	n/d	100 (SMCL)
Sodium	mg/L	16.4	28.0	13.0	
Thallium	µg/L	n/d	<1	n/d	2
Zinc	µg/L	<2	3	n/d	5000 (SMCL)
INORGANICS					
Boron	mg/L	0.007	0.009	0.002	
Chloride	mg/L	40.4	62.8	34	250 (SMCL)
Residual Chlorine	mg/L	1.4	1.7	1.0	TT=>0.2
Fluoride	mg/L	0.67	0.83	0.44	4 (SMCL=2)
Nitrate	mg/L	1.0	1.7	0.3	10
Nitrite	mg/L	<0.05	<0.05	n/d	1
Phosphorus	mg/L	0.23	0.34	n/d	
Sulfate	mg/L	5.6	7.6	<4	250 (SMCL)
DISINFECTION BYPRODUCT PRECURSOR					
Total Organic Carbon	mg/L	1.4	2.9	0.9	TT
ORGANICS					
Haloacetic Acids (HAA5)	µg/L	21.2	31.7	12.6	
Total Trihalomethanes (TTHMs)	µg/L	21.5	34.8	9.9	
PESTICIDES & SYNTHETIC ORGANIC CHEMICALS (SOCs)					
2,3,7,8-TCDD (Dioxin)	pg/L	n/d	n/d	n/d	30
2,4,5 TP (Silvex)	µg/L	n/d	n/d	n/d	50
2,4-D	µg/L	n/d	n/d	n/d	70
3-Hydroxycarbofuran	µg/L	n/d	n/d	n/d	
Alachlor	µg/L	n/d	n/d	n/d	2

WSSC TAP WATER ANALYSIS - 2015

PATUXENT WATER FILTRATION PLANT

PARAMETER	UNIT OF MEASURE	YEARLY AVERAGE	MAXIMUM	MINIMUM	EPA LIMIT [11]
PESTICIDES & SYNTHETIC ORGANIC CHEMICALS (SOCs)					
Aldicarb	µg/L	n/d	n/d	n/d	3
Aldicarb sulfone	µg/L	n/d	n/d	n/d	2
Aldicarb sulfoxide	µg/L	n/d	n/d	n/d	4
Aldrin	µg/L	n/d	n/d	n/d	
Atrazine	µg/L	n/d	<1	n/d	3
Benzo(a)pyrene	µg/L	n/d	n/d	n/d	0.2
Butachlor	µg/L	n/d	n/d	n/d	
Carbaryl	µg/L	n/d	n/d	n/d	
Carbofuran	µg/L	n/d	n/d	n/d	40
Chlorinated biphenyls (PCBs)	µg/L	n/d	n/d	n/d	0.5
Chlordane	µg/L	n/d	n/d	n/d	2
Dalapon	µg/L	n/d	<1	n/d	200
Dibromochloropropane (DBCP)	µg/L	n/d	n/d	n/d	0.2
Dicamba	µg/L	n/d	n/d	n/d	
Dieldrin	µg/L	n/d	n/d	n/d	
Di(2-ethylhexyl)adipate	µg/L	n/d	n/d	n/d	400
Di(2-ethylhexyl)phthalate	µg/L	n/d	n/d	n/d	6
Dinoseb	µg/L	n/d	n/d	n/d	7
Diquat	µg/L	n/d	n/d	n/d	20
1,2-Dibromoethane (EDB)	µg/L	n/d	n/d	n/d	0.05
Endothall	µg/L	n/d	n/d	n/d	100
Endrin	µg/L	n/d	n/d	n/d	2
Glyphosate	µg/L	n/d	n/d	n/d	700
Heptachlor	µg/L	n/d	n/d	n/d	0.4
Heptachlor epoxide	µg/L	n/d	n/d	n/d	0.2
Hexachlorobenzene	µg/L	n/d	n/d	n/d	1
Hexachlorocyclopentadiene	µg/L	n/d	n/d	n/d	50
Lindane	µg/L	n/d	n/d	n/d	0.2
Metolachlor	µg/L	n/d	n/d	n/d	
Methomyl	µg/L	n/d	n/d	n/d	
Methoxychlor	µg/L	n/d	n/d	n/d	40
Metribuzin	µg/L	n/d	n/d	n/d	
Oxamyl (vydate)	µg/L	n/d	n/d	n/d	200
Pentachlorophenol (PCP)	µg/L	n/d	n/d	n/d	1
Picloram	µg/L	n/d	n/d	n/d	500
Propachlor	µg/L	n/d	n/d	n/d	
Simazine	µg/L	n/d	<1	n/d	4
Toxaphene	µg/L	n/d	n/d	n/d	3
VOLATILE ORGANIC CHEMICALS (VOCs)					
1,1,1-Trichloroethane	µg/L	n/d	n/d	n/d	200
1,1,2-Trichloroethane	µg/L	n/d	n/d	n/d	5
1,1-Dichloroethene	µg/L	n/d	n/d	n/d	7
1,2,4-Trichlorobenzene	µg/L	n/d	n/d	n/d	70
1,2-Dichlorobenzene	µg/L	n/d	<0.5	n/d	600
1,2-Dichloroethane	µg/L	n/d	n/d	n/d	5
1,2-Dichloropropane	µg/L	n/d	n/d	n/d	5
1,4-Dichlorobenzene	µg/L	n/d	<0.5	n/d	75
Benzene	µg/L	n/d	n/d	n/d	5
Carbon Tetrachloride	µg/L	n/d	n/d	n/d	5
Chlorobenzene	µg/L	n/d	n/d	n/d	100
cis -1,2-Dichloroethene	µg/L	n/d	n/d	n/d	70
Dichloromethane	µg/L	n/d	n/d	n/d	5
Ethylbenzene	µg/L	n/d	n/d	n/d	700
Total Xylenes	µg/L	n/d	<0.5	n/d	10000
Styrene	µg/L	n/d	n/d	n/d	100

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PATUXENT WATER FILTRATION PLANT

PARAMETER	UNIT OF MEASURE	YEARLY AVERAGE	MAXIMUM	MINIMUM	EPA LIMIT [1]
VOLATILE ORGANIC CHEMICALS (VOCs)					
Tetrachloroethene	µg/L	n/d	n/d	n/d	5
Toluene	µg/L	n/d	n/d	n/d	1000
trans -1,2-Dichloroethene	µg/L	n/d	n/d	n/d	100
Trichloroethene	µg/L	n/d	n/d	n/d	5
Vinyl Chloride	µg/L	n/d	n/d	n/d	2
1,1,1,2-Tetrachloroethane	µg/L	n/d	n/d	n/d	
1,1,2,2-Tetrachloroethane	µg/L	n/d	n/d	n/d	
1,1-Dichloroethane	µg/L	n/d	n/d	n/d	
1,1-Dichloropropene	µg/L	n/d	n/d	n/d	
1,2,3-Trichlorobenzene	µg/L	n/d	n/d	n/d	
1,2,3-Trichloropropane	µg/L	n/d	n/d	n/d	
1,2,4-Trimethylbenzene	µg/L	n/d	n/d	n/d	
1,3,5-Trimethylbenzene	µg/L	n/d	n/d	n/d	
1,3-Dichlorobenzene	µg/L	n/d	n/d	n/d	
1,3-Dichloropropane	µg/L	n/d	n/d	n/d	
2,2-Dichloropropane	µg/L	n/d	n/d	n/d	
2-Chlorotoluene	µg/L	n/d	n/d	n/d	
4-Chlorotoluene	µg/L	n/d	n/d	n/d	
Bromobenzene	µg/L	n/d	n/d	n/d	
Bromochloromethane	µg/L	n/d	n/d	n/d	
Bromomethane	µg/L	n/d	n/d	n/d	
Chloroethane	µg/L	n/d	n/d	n/d	
Chloromethane	µg/L	n/d	n/d	n/d	
cis -1,3-Dichloropropene	µg/L	n/d	n/d	n/d	
Dibromomethane	µg/L	n/d	n/d	n/d	
Dichlorodifluoromethane	µg/L	n/d	n/d	n/d	
Hexachlorobutadiene	µg/L	n/d	n/d	n/d	
Isopropylbenzene	µg/L	n/d	n/d	n/d	
n-Butylbenzene	µg/L	n/d	n/d	n/d	
n-Propylbenzene	µg/L	n/d	n/d	n/d	
Naphthalene	µg/L	n/d	n/d	n/d	
p-Isopropyltoluene	µg/L	n/d	n/d	n/d	
s-Butylbenzene	µg/L	n/d	n/d	n/d	
t-Butylbenzene	µg/L	n/d	n/d	n/d	
trans -1,3-Dichloropropene	µg/L	n/d	n/d	n/d	
Trichlorofluoromethane	µg/L	n/d	n/d	n/d	
Nitrobenzene	µg/L	n/d	n/d	n/d	
Methyl-tert-butyl-ether	µg/L	n/d	n/d	n/d	
RADIONUCLIDES					
Gross Alpha	pCi/L	<2.0	<2.0	<2.0	15
Gross Beta	pCi/L	<4.0	<4.0	<4.0	50 [2]
Radium 228	pCi/L	<1.0	<1.0	<0.9	5 [3]
Tritium	pCi/L	<100	<100	<100	

CUSTOMER TAP [4]

PARAMETER	UNIT OF MEASURE	90th PERCENTILE [5]	# of SITES ABOVE AL	EPA ACTION LEVEL (AL)
Copper	µg/L	87.4	0 samples	1300
Lead	µg/L	1.17	0 samples	15

DISTRIBUTION SYSTEM

PARAMETER	UNIT OF MEASURE	YEARLY AVERAGE	MAXIMUM	MINIMUM	EPA LIMIT [1]
BACTERIOLOGICAL					
Samples Total Coliform Positive	%/month	0.33	1.05	0	5
Samples *E. coli* Positive	%/month	0	0	0	

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DISTRIBUTION SYSTEM

PARAMETER	UNIT OF MEASURE	YEARLY AVERAGE	MAXIMUM	MINIMUM	EPA LIMIT [11]
BACTERIOLOGICAL					
No.of *E. coli* Positive Routine Samples	Count	0	0	0	
No.of *E. coli* Positive Repeat Samples	Count	0	0	0	0
DISINFECTANT & DISINFECTION BYPRODUCTS					
Residual Chlorine	mg/L	1.26 [6]	4.50	n/d [7]	4 [8]
Haloacetic Acids (HAA5)	µg/L	43.4 [9]	87.7	2.9	60 [10]
Total Trihalomethanes (TTHMs)	µg/L	62.1 [9]	94.5	16.5	80 [10]

LEGENDS

n/d - not detected

mg/L - milligrams per liter, equal to parts per million (ppm). The equivalent of one minute in 2 years or one penny in $10,000.

µg/L - micrograms per liter, equal to parts per billion (ppb). The equivalent of one minute in 2,000 years or one penny in $10 million.

ng/L - nanograms per liter, equal to parts per trillion (ppt). The equivalent of one minute in 2,000,000 years or one penny in $10 billion.

pg/L - picograms per liter, equal to parts per quadrillion (ppq). The equivalent of one minute in 2,000,000,000 years or one penny in $10 trillion.

pCi/L - picocuries per liter (a measure of radiation)

S.U. - Standard Unit

NTU - Nephelometric Turbidity Unit

TT - Treatment Technique. A required process intended to reduce the level of a contaminant in drinking water.

AL - Action level. The concentration of a contaminant which, if exceeded, triggers treatment or other requirements which a water system must follow.

= equals

< less than

[1] - Filtered water, maximum of measurements taken every 15 minutes.

[2] - EPA considers 50 pCi/L to be the level of concern for beta particles.

[3] - The EPA limit of 5 pCi/L applies to combined Radium 226 and 228.

[4] - Most recent required sampling, between June and September 2014.

[5] - If more than 10% of sites exceed action level, system is required to take additional steps to control corrosiveness of their water.

[6] - Highest running annual average (RAA)

[7] - All samples deemed to have detectable disinfectant residual.

[8] - Maximum residual disinfectant level (MRDL), the highest level of a disinfectant allowed in drinking water; based on RAA.

[9] - Highest locational running annual average (LRAA)

[10] - Maximum contaminant level based on LRAA.

[11] - Shown as maximum contaminant levels (MCL) unless otherwise noted as secondary MCLs (SMCL). MCLs are enforceable health-based standards, whereas SMCLs are non-enforceable guidelines for contaminants that may cause aesthetic effects in drinking water.

Appendix 2. Soil color, redox properties, and texture

Morphological descriptions of <2mm and >2mm fractions of randomly selected microcosm substrates based on treatment. Values are for substrates placed in a 1.098 L container (6.5cm depth) submersed in a 5 gallon microcosm of fresh water. Color determinations are for moist samples only.

Trtmnt	csm	Matrix	Redox	Redox (mv)		>2mm fraction – summarized per treatment		
		Color	Color	Shallow	Deep	**gL^{-1} or g/cosm	**$\%L^{-1}$	Desc.
sand	1	2.5Y 7/4	na	479 ± 6	563 ± 8	27.3 ± 4.4^{ab}	1.3 ± 0.2^{ab}	g, na
	2	2.5Y 7/4		284 ± 40	352 ± 13			
	4	2.5Y 8/2		331 ± 14	364 ± 6			
	9	2.5Y 7/4		458 ± 81	449 ± 26			
	10	2.5Y 7/4		252 ± 51	250 ± 12			
	11	2.5Y 7/4		332 ± 52	356 ± 68			
	12	2.5Y 6/4		337 ± 13	359 ± 9			
oyster	13	5Y 8/1		205 ± 33	150 ± 61	1280.0 ± 101.4^{d}	71.6 ± 1.5^{d}	sh, l, na
	15	2.5Y 8/1		274 ± 31	260 ± 47			
	19	2.5Y 7/1		357 ± 7	372 ± 22			
	23	2.5Y 6/1		667 ± 34	584 ± 30			
soil-	26	10YR 2/1	10YR 8/4	302 ± 24	274 ± 40	42.0 ± 30^{ab}	2.6 ± 0.2^{ab}	p, sc, or
sand	27	10YR 3/2	10YR 8/8	307 ± 22	278 ± 35			
(s)=sand	30	2.5Y 8/1(s)	2.5Y 7/8	308 ± 70	179 ± 66			
(so)=soil	32	2.5Y 8/1(s)	2.5Y 6/8	270 ± 19	256 ± 21			
	32	2.5Y 3/1(so)						
	33	2.5Y 8/1(s)	2.5Y 7/8	265 ± 11	289 ± 26			
	33	2.5Y 3/1(so)						
	36	2.5Y 8/1(s)	2.5Y 7/8	293 ± 30	305 ± 24			
	36	2.5Y 3/1(so)						
oyster-	40	10YR 2/1(p)	10YR 6/8	309 ± 21	298 ± 27	585.0 ± 18.7^{c}	58.0 ± 1.8^{c}	p, sh, or
peat	40	5Y 8/1(s)						
(p)=peat	44	10YR 2/1(p)						
(s)=shell	44	10YR 7/1(s)						
	45	10YR 2/1(p)		272 ± 29	244 ± 48			
	45	10YR 8/1						
Shrwood	49	5Y 4/2		234 ± 34	249 ± 26	68.1 ± 14.4^{b}	3.6 ± 0.9^{b}	sc, g, sh, or, b
Forest	50	5Y 4/2		100 ± 61	71 ± 73			
	52	2.5Y 4/1		128 ± 66	70 ± 80			
	53	2.5Y 3/1	7.5YR 4/6	181 ± 50	142 ± 60			
	58	2.5Y 4/1	10YR 4/4	311 ± 23	249 ± 29			
	59	2.5Y 3/1		215 ± 27	177 ± 40			
	60	2.5Y 3/6	10YR 4/4	224 ± 8	198 ± 23			

Table B.1. (cont'd) Morphological descriptions of <2mm and >2mm fractions of randomly selected microcosm substrates based on treatment.

Trtmnt	csm	Matrix	Redox	Redox (mv)		>2mm fraction – summarized per treatment		
		Color	Color	Shallow	Deep	gL^{-1} or g/cosm	$\%L^{-1}$	Description
Kent	61	2.5Y 6/2	2.5Y 6/3	162 ± 53	121 ± 54	9.5 ± 1.5[a]	0.47 ± 0.1[a]	sc, p, g, sh, b
Narrows	62	2.5Y 3/2		379 ± 29	299 ± 113			
	63	2.5Y 4/2		222 ± 37	260 ± 30			
	65	2.5Y 4/1	10YR 4/6	606 ± 34	548 ± 78			
	70	2.5Y 4/1	10YR 5/8	335 ± 12	279 ± 12			

* bioturbation evidence = b, oxidized rhizospheres = or, few to no redox features = na
shell = sh, limestone = l, plant matter = p, gravel = g sc = sediment concretions
**letters indicate highly significant differences at the .05 level.

Appendix 3. Stem length as an indicator for occurrence of inflorescences

Average stem lengths for six different substrate treatments with Sherwood Forest or Kent Narrows Turions (some cosm data missing: For Sherwood Forest turions: n=21/24. Kent Narrows turions N=21/24). Data for all stems is based on 0.0269 m^2. Final turion growth data was separated for visualization but not statistically significantly different.

Sherwood Forest Plants: Stem Lengths, Substrate, and Absence/Presence of Inflorescences

*Substrates →	Sand		soil/sand		oyster/peat		oyster		Kent Narrows		Sherwood Forest	
Stem data ↓	A**	P**	A	P	A	P	A	P	A	P	A	P
ave stem length (cm)	7.52	0	10.93	45	11.61	37.13	14.09	46.20	15.32	44.13	19.01	54.71
std error +/-	0.38	0	0.66	0	0.47	6.31	0.85	5.97	0.20	2.30	1.27	4.65
No. stems	147	0	143	1	211	6	123	10	139	8	116	17
total stems	147	147	144	144	217	217	133	133	147	147	133	133
% stems	100	0	99.31	0.69	97.24	2.76	92.48	7.52	94.56	5.44	87.22	12.78
No. of cosms in analysis	4	0	4	1	4	2	4	4	2	2	3	3

*Turions were planted in one of six substrate treatments.
**A= Absence (reduced possibility) of flowers on stems (<23.5 cm) P = Presence (increased possibility) of flowers (≥ 23.5 cm for any individual stem)

Kent Narrows Plants: Stem Lengths, Substrate, and Absence/Presence of Inflorescences

*Substrates →	Sand		soil/sand		oyster		oyster/peat		Kent Narrows		Sherwood Forest	
Stem data	A**	P**	A	P	A	P	A	P	A	P	A	P
ave stem length (cm)	9.14	0	11.74	38.25	16.62	33	17.98	45.4	16.37	46.21	17.20	68.61
std error +/-	0.88	0	0.65	1.97	1.43	0	0.90	2.80	0.85	4.13	1.22	6.67
No. stems	87	0	97	4	98	1	107	5	190	19	119	19
total stems	87	87	101	101	99	99	112	112	209	209	138	138
% stems	100	0	96.04	3.96	98.99	1.01	95.54	4.46	90.91	9.09	86.23	13.77
No. of cosms in analysis	4	0	3	2	3	1	3	2	4	3	4	3

*Turions were planted in one of six substrate treatments.
**A= Absence (reduced possibility) of flowers on stems (<23.5 cm) P = Presence (increased possibility) of inflorescences (≥ 23.5 cm for any individual stem)

Appendix 4. Footnotes to Table 4.2 providing calculations and rationale for energy required to restore 16m^2 SAV bed by the hand transplant method. That method entails hand-planting sods from four propagation trays, thereby covering 36% of each m^{-2}.

Source	Calculation	Units
Renewable Sources		
1/ Sunlight		
Propagation tank area:		
(2.59 m)(2.59 m) = 6.712m^2		
*Insolation: 5.5845E9 Jm^{-2}yr^{-1}		
**Albedo: 0.1		
Energy input per year:		
(6.712 m^2)(5.5845E9 Jm^{-2}yr^{-1})(1-0.1) =	3.37348476E+10	Jyr^{-1} system total
Energy input per 1m^2: (3.37348476E+10 Jyr^{-1})		
/ (6.712 m^2) =	5.026050000E+09	Jyr^{-1}m^2 system area
Energy input per m^2 SAV bed restored =		
(64 propagation trays/4 trays each m^2 SAV restored)=	16	m^2 total restored
(3.37348476E+10 Jyr^{-1}) / (16 m^2) =	**2.108427975E+09**	**Jyr^{-1} / m^2 SAV bed restored**
Total Cost Sunlight USD$:	0	USD$ total and per m^2 SAV bed restored
*(NREL 2012) **(Holman 1997)		
2/ Rain, chemical potential		
Propagation tank area:		
(2.59 m)(2.59 m) = 6.71 m^2		
*Estimated Maryland Annual rainfall:	1.2	myr^{-1} annual rainfall
Density of water:	1.00E+06	gm^{-3} density of water
Gibbs Free Energy (GFE) of water:	4.94	Jg^{-1}
Energy (J) input avg. precipitation per year=		
(area)(rainfall)(Density H_20)(GFE) =		
(6.712 m^2)(1.2 myr^{-1})(1.00E+06 gm^{-3})(4.94 Jg^{-1}) =		
Total System Precipitation Energy input:	3.9788736E+07	Jyr^{-1} system total
Precipitation Energy input per m^2 = (3.979E+07)/(6.712m^2)	5.928E+06	Jyr^{-1}m^2
Precipitation Energy input per m^2 SAV bed restored:		
(3.9788736E+07 Jyr^{-1})/(16 m^2) =	**2.486796E+06**	**Jyr^{-1} rain E per m^2 SAV bed restored**
Total Cost Precipitation USD$	0	USD$ total and per m^2 restored
*0.09m/mo for 8 weeks propagation season/year		
(NOAA/National Weather Service/BWI		
Airport precipitation data)		
3/ Evaporation		
Propagation tank area:		
(2.59m)(2.59m) = 6.71m^2		
Estimated Maryland mean Annual		
pan evaporation:*1.3myr^{-1}		
Density of water: 1.00E+06 gm^{-3}		
Gibbs Free Energy of water: 4.94 Jg^{-1}		
Evaporation energy (E) =		
(evap)(H_2O density)(GFE)(prop tank area)		
EvapE=		
(1.3 myr^{-1})(1.00E+06 gm^{-3})(4.94 Jg^{-1})(6.712m^2)=		
Evaporative Energy Input:	4.3104464E+07	Jyr^{-1} system total
Evaporative Energy Input per m^2:		
(4.31044E+07 Jyr^{-1})/(6.712m^2) =	6.422E+06	Jyr^{-1}m^2
(4.3104464E+07 Jyr^{-1})/(16 m^2) =	**2.694029E+06**	**Jyr^{-1} per m^2 SAV bed restored**
Total Cost Evaporation USD	0	USD$ total and per m^2 SAV bed restored
*0.13m/mo for 8 weeks propagation season/year		
(Farnsworth and Thompson 1982)		

4a/ Water/Irrigation:

Propagation tank volume filled by farm/municipal water: Filled once (259cm)(259cm)(60cm) = 4.024860E+06 cm^3.		
Topped off four times, every other week for 4 weeks: (259cm)(259cm)(6 cm)(4) = 1.609944E+06 cm^3		
Total irrigation water: (4.024860E+06 cm^3) + (1.609944E+06 cm^3) = 5.634804E+06 cm^3 = 5.634804 m^3		
Annual energy of municipal water = (Volume water)(density of water)(Gibbs free Energy of water) = (5.634804 m^3)(1.0E+03 k/m^3) (4990 J/kg*) =	2.8117672E+07	Jyr^{-1}
Energy Input of water per m^2 = (2.8117672E+07 Jyr^{-1}) / (6.71 m^2) =	4.190413E+06	$Jyr^{-1}m^2$
Energy Input of irrigation water for restoration: (2.8117672E+07 Jyr^{-1}) / (16 m^2) =	**1.757355E+06**	**Jyr^{-1} per m^2 SAV bed restored**
*(Maradi et al. 2014)		

4b/ Water/Irrigation USD$ Basis:

(1,488.56 gal)(3785.41ml/gal) = 5.634804E+06 cm^3 @ $3.20 cost* for 1000 gallons (3.785E+06 cm^3); for 1,488.56 gallons (5.634804E+06 cm^3)	4.78	USD$ System Total annually
USD$ input for irrigation per m^2 of prop. system = (4.78USD$)/(6.71$m^2$) =	0.71	USD$ per m^2 of the system
USD$ input for restoration (m^2)= (USD$ 4.78) / (16 m^2 for restoration)	0.30	USD$ total per m^2 SAV bed restored
(Source: Washington Suburban Sanitary Commission Cost figures per 1,000 gallons https://www.wsscwater.com/rates)		

5a/ Pressure treated lumber (for tank)

($22.97 each)(24 timbers) – 90 lbs each: (40.8kg or 40.823E+03g)(1 (one) propagation tank)		
Dimensions for each tank: (2.59m)(2.59m)(0.76m) = 5.098 m^3		
Mass = (979.2 kg)(1000g/kg)=979,200g for Pressure-treated lumber for each tank (24 timbers)		
Replacement period: 20 years (979,200g)/(20 yrs)=	48,960	g/yr for total system
Per unit area = 6.71m^2		
Annual use: 979,200g/20yrs=(48,960g/yr)/6.71 m^2) Pressure-treated wood use/unit area=	7,296.57	g/m^2/yr lumber for propagation system
Per m^2 restoration (64 total trays/4 trays each m^2) 48,960/(16 m^2) =	**3060**	**g lumber/m^2/yr of SAV bed restored**

5b/ Pressure treated lumber (for tank) – dollar basis

($22.97 each)(24 timbers)	551.28	$USD for system
Life of lumber: 10 years $551.28 / 10 years =	55.128	$USD pressure treated lumber annually
Cost per m^2 system= (55.128USD$)/(6.71 m^2) =	8.22	$USD per m^2 propagation system
Cost per m^2 restoration= ($55.128)/(16 m^2) =	3.446	$USD pressure treated lumber per m^2 of SAV bed restored/yr

6a/ 1 x 6 x 8 Cedar Plank (for top of tank)

(4 timbers @ 2.46 kg each) (for 1 propagation tank)		
Dimensions for each board: (0.75in or 1.9cm)(5.5in or 13.97cm)(96in or 243.8cm)		
(mass of 4 boards – (Home Depot stock)= 9.84kg/9,840g		
Replacement period: 10 years Annual use: 9,840g/10 years	9.84E+02	g/yr for system
Dimensions for each tank: 6.71 m^2 Cedar plank use/unit m^2 propagation area = (984g/yr)/(6.71 m^2)=	1.47E+02	g/m^2/yr
Cedar plank required for Restoration m^2= (984 g/yr)/(16 m^2) =	**61.5**	**g cedar plank/yr per m^2 SAV bed restored**

6b/ Cedar plank USD$ Basis

(1x6x8) Cedar Plank (for top of tank)		
($11.52 each)(4 timbers)=	46.08	USD$ Cedar plank for Prop. System Total
Replacement period:	10	years
Annual cedar plank used (46.08)/(10 years)=	4.608	Cedar plank USD$ per year
Per unit area: 6.71m^2=	0.686	Cedar plank $USD per m^2 of system
Per m^2 of SAV bed restored	16	m^2 SAV bed restored
Total Cedar plank ($4.608)/(16 m^2) =	0.288	USD$ cedar plank/m^2 SAV bed restored/yr

7a/ Hardware

20, 9-inch		
Steel Hex bolts, $25.58 for		
(453g) (1lb) for each		
(10 pack)(2 packs) =	906	g hex bolts for system
5 lb (2.267E+03g box of nails) =	2.267E+03	g box of nails
Hardware Total mass=	3,173	g
Replacement period=	20	years
Per unit area=	6.71	m^2
Annual use: (3,173g)/(20yrs)=	158.65	gyr^{-1}
Hardware use/unit area:		
(158.65 gyr^{-1})/(6.71 m^2)=	23.64	$gm^{-2}yr^{-1}$
Hardware use/restored area:		
(158.65gyr^{-1})/(16 m^2) =	**9.92**	**g steel per m^2 of SAV bed restored/yr**

7b/ Hardware – $USD Basis

Steel Hex bolts, ($25.58 for		
each 10 pack)(2 packs) = ($51.16) +		
(5 lb box of nails @ $15.96) =	67.12	USD$ for system total
Life of hardware:	20	yrs
($67.12)/(20 yrs)=	3.36	USD$ hardware/yr for propagation system
(3.36 USD$ hardware/yr)/(prop area: 6.71m^2) =	0.50	USD$ per m^2 for prop. system
($3.36)/(16 m^2 SAV bed restored) =	0.21	USD$ per m^2 of SAV bed restored/yr

8a/ Pond liner (EPDM)

10ft x 15ft liner = 1.5545E+03 m^2		
Mass: (105 lbs)/(2.205 kg/lb) = 47.627 kg		
or 4.7627E+04 g		
Replacement: 20 years		
Liner use/unit on an annual basis:		
= (4.7627E+04 g)/(20 years) =	2.381E+03	g/year EPDM for System Total
Liner use per m^2 for system:		
(2.381E+03gyr^{-1})/(6.71 m^2) =	3.548E+02	g/m^2/yr
Liner use/restored SAV bed (m^2)		
Bed: (2.381E+03gyr^{-1})/ (16 m^2) =	**148.8**	**g per m^2 of SAV bed restored/yr**

8b/ Pond liner (EPDM) - USD Basis

USD$ basis $149.00 =	149.00	$USD for system total
$149.00 / 20 years =	7.45	$USD/year for system total
($7.45)/(6.71 m^2) =	1.11	$USD/year/ m^2 of system
($7.45)/(16 m^2) =	**0.47**	**$USD per m^2 of SAV bed restored/yr**

9a/ PVC for plumbing

Propagation tank		
Bulkhead strainer and pvc fitting		
Plumbing (0.52 lbs) 236 g		
Three 3.81cm diam (1.5 in)x3.048m (10ft) (pvc pipes - 3)		
pvc (1.5 inch) pipe $5.84		
5.247lb (2.238kg) or (2,238g)(3 pipes)= 6,714 g		
Sum PVC plumbing: 236 g + 6,714 g=	6,950	g for system total before replacement
6,950g/20 yrs life of PVC=	347.5	g pvc per yr system total after depreciation
*Replacement rate: 25% of pipe every five		
years (assume 4 replacements in 20 years)		
(4x(0.25x347.5))/20 yrs =	17.38	g PVC replaced over 20 year lifespan
Total annual mass input of		
PVC/yr: (347.5g pvc + 17.38 g pvc) =	364.88	g pvc per yr for system total
Mass / yr / area of tank: (364.88)/(6.71 m^2) =	54.38	g pvc per year per m^2 of built tank system
Mass /year / area restored = 364.875 / (16 m^2) =	**22.81**	**g pvc/yr per m^2 of SAV bed restored**

9b/ PVC for plumbing USD basis:

Propagation tank		
Bulkhead strainer /pvc fitting ($10.00)		
Three (3) 3.048m (1.5 inch) x (10 ft) pvc pipe $5.84		
($5.84)(3 pipes)= $17.52		
Sum PVC plumbing $USD: ($17.52) + ($10.00)	27.52	$USD system total without depreciation
Replacements: Life of PVC: 20 years		
Total costs of PVC parts/yr: ($27.52)/20 years =	1.38	USD/yr
Replacement rate: 25% of pipe every five years		
(assume 4 replacements in 20 years)=		
(4x(0.25x$1.38))/20 yrs = 0.069 PVC replaced/yr		
PVC/yr: ($1.38 pvc)+(0.069 pvc)=	1.45	$USD pvc used per year
$USD/yr/area of tank: ($1.45)/(6.71 m^2) =	0.21	$USD per year/ m^2 for tanks system
$USD/year ($1.45) / (16 m^2):	**0.09**	**$USD pvc used per m^2 of SAV bed restored/yr**
(THD 2016, Ciotala et al. 2011)		

10a/ Purchased Potting soil (Topsoil J/yr basis)

Caloric energy of soil: 5.4E+03 kcal/kg (Odum 1996)		
Annual energy of soil purchased for input =		
(3.911 kg per tray)*(64 trays)*(5.4E+03 kcal)		
*(4,186 J/kcal) =		
Annual energy (J) in topsoil system total=	5.6566E+09	J/year for system total
Annual energy (J) in topsoil in prop system/m^2 =		
(5.6566E+09J/yr)/(6.71m^2)=	8.4301E+08	J/m^2/yr for system total
Units topsoil/yr required to restore 1 m^2 SAV bed=		
(5.6566E+09J/yr)/(16 m^2) =	**3.53537500E+08**	**J energy per yr/m^2 SAV restoration**

10b/ Potting soil gram basis (40 lb bag (18.14kg) -

For (3.911 kg. of soil per Tray)*(64 trays) =	250.30	kg soil used per tank prop. system total
(amnt soil used)(1000g/kg)/(lifespan (1yr))=	2.50E+05	g/yr soil used for propagation system
For m^2 of propagation system:		
(2.50E+05 g/yr)/(6.71 m^2)	3.7258E+04	g/yr/m^2 soil used for propagation system
Topsoil used/ to restore each m^2 SAV		
(2.50E+05 g/yr)/(16m^2) =	**1.5625E+04**	**g topsoil for each m^2 of SAV restoration**

10c/ Potting soil USD basis: (40 lb bag (18.18kg))@$1.65

Total bags required: (250.30kg)/(18.18kg) =	14	bags purchased (13.7)
(14)($1.65) =	23.10	USD$ for bags purchased
Price per kg: ($1.65)/18.18 kg)	0.091	USD$ cost per kg soil
Price for one propagation tray (mass=3.911 kg)=		
($0.09/kg)(3.911 kg. of soil per tray)=	0.35	USD$/tray in propagation system
USD$ cost for soil for propagation system:		
(USD$ 0.35/tray)(64 trays) =	22.77	USD$ Total soil used annual input
USD$/$m^2$ of propagation system:		
(22.77 USD$)/(6.71 m^2)=	3.39	USD m^2 total SAV propagation system
($22.77)/(16 m^2)=	**1.42**	**USD$ for each m^2 of SAV restoration**

11a/ Sand - gram basis

Amount used: (4.53 kg for one tray) x		
(64 trays) = 289.92 kg sand =	2.89920 E+05	g sand for propagation system total
For m^2 of propagation system:		
(2.89920E+05 g/1yr)/(6.71 m^2)=	4.3207E+04	g/yr/m^2 sand in propagation system
Sand used/ to restore 1m^2 SAV:		
(2.89920E+05)/(16m^2)=	**1.8120E+04**	**g/yr/m^2 sand required to restore 1m^2 SAV**

11b/ Sand USD$ basis

$4.05 for 50lb bag:(0.45kg/lb)(50lb)=	22.5	kg
(22.675 kg)/($4.05)=	0.18	USD$ per kg
Amount used per tray: (4.53 kg)($0.18)	0.82	USD$ / each tray in system
($0.82)(64) trays =	52.48	USD$ System total each year
USD sand for total propagation system:		
($52.48)/(6.71 m^2)=	7.82	USD$ sand/m^2 for propagation system
USD Sand used/ to restore 1m^2 SAV:		
($52.48)/(16$m^2$)=	3.28	$USD sand required to restore 1m^2 SAV

12a/ Turions

No. turions placed in each trays=	8	**turions for each tray**
No. propagation trays:	64	trays
(8 turions)(64 trays)=	512	turions total for propagation system

Fresh weight (FW) calculations used to derive DW equiv.

Each Turion weighs (FW) on average	*0.3*	*g fresh weight (FW) each turion*
(0.3g FW)(8 turions per tray)=	*2.4*	***g FW per tray for each m^2 restored***
(2.4 g FW)(64 trays)=	*153.6*	*g FW turions for entire propagation system*
**Est. Energy in each turion:*		
Kcal in 1 gram turion FW equivalent	*0.32*	*kcal/g FW, using bean sprouts as proxy**
(0.32kcal/g FW)(0.3g FW)=	*0.09*	*kcal FW each turion*
*Turions dry weight equivalent		
Turions are ~90% water, So DW is ~10%;		
(0.3g FW each turion)(0.10) =	0.03	gDW (Dry Weight) each turion
8 turions in each tray =		
(0.03gDW each turion)(8 turions per tray)=	0.24	g DW turions for each tray
(0.246g DW)(64 trays) =	15.36	g DW for entire propagation system
total lifetime:	1 year	
*Est. Energy in each turion:	0.33	kcal/g FW, using bean sprouts as proxy*
3.0 kcal/gram turion DW (organic matter)		
(3.0 kcal/g)(0.03 gDW for one turion)=	0.09	kcalories in each turion
(0.09 kcal/turion)(8 turions in one tray)	0.72	kcalories/tray
(0.72 kcal)(64 trays)	46.08	kcalories/g turions for system total
J:kcal conversion: (46.08 kcal/g)(4,184 J/kcal)=	1.92891E+05	J of turion energy in propagation system
Turion energy system per m^2 for propagation:		
(1.92891E+05 J)/(6.71 m^2)=	2.8747E+04	J/ m^2 turion energy for propagation system
Turion energy required to restore one sq. meter SAV bed:		
(1.92891E+05 J)/(16 m^2)=	**1.2056E+04**	**J/yr for each m^2 of SAV restoration**
*kcal in FW bean sprouts: 0.3 kcal/g		
http://www.weightlossresources.co.uk/calories-in-food/veg/bean-sprouts.htm		

12b/ Turions USD$ basis:

**Priced at ($0.95 each turion)*		
(8 turions each tray)(0.95)=	7.60	USD$ turions/tray in propagation tray
(7.6 USD$)*(64 trays) =	486.4	USD$ System Total per year
Turions in USD$ per m^2 for propagation:		
(486.4)/(6.71 m^2)=	72.48	USD$ turion cost per m^2 propagation area
(486.4)/(16 m^2) =	30.4	USD$ turion cost per m^2 SAV bed restored

**Propagule sources for turions (prices may vary between $0.85-0.95):
Kester Wild Game Food Nurseries, Inc.
http://www.kestersnursery.com/Wetland%20Plant's.htm
Aquascapes Unlimited:
http://www.aquascapesunlimited.com/Native-Wetland-Plants

13a/ Plastic: Propagation/growout trays

Amount of plastic for each propagation tray	21	g
(21g)(64 propagation trays)=	1,344	g system total before replacement rate
/ replacement rate=	5	years
(1344 g)/(5 yr life of plastic trays)	2.688E+02	g/yr system total
268.8g/yr /(6.71m^2)=	4.01E+01	g/yr for each m^2 of propagation system
268.8g/yr /(16 m^2)=	**16.8**	**g/yr for each m^2 of SAV bed restored**

13b/ Plastic USD basis: Propagation/growout trays

($3.00 each)(64 total)=	192	USD
/ five years life of plastic trays	38.4	USD$ plastic for propagation system/year
(38.4)/(6.71 m^2)=	5.72	USD$/yr plastic per m^2 of system
(38.4)/(16 m^2) =	**2.4**	**USD$/yr plastic per m^2 of restoration**

Purchased services

14a / Labor for Building of propagation tank J basis

2 staff x 5 days per week	10	days
Kcal per human Conversion =	2500	kcal/person/day
10 days x 2500 kcal/person / day =	25000	kcal/day
Kcal to Joules conversion:	4186	J in one Kcal
(Kcal/day system total)(4186 joules in kcal)=		
(25000)(4186) =	104650000	J for system total
(J/ system total)/(prop system area)=		
(104650000 J/yr)/(6.71 m^2) =	1.5596125E+07	J/yr/m^2 labor energy for propagation system
(J/ system total)/(SAV bed restored)=		
(104650000)/(16 m^2) =	**6.540625E+06**	**J/m^2/yr labor energy for SAV restoration**

14b / Labor for building of propagation tank USD basis

Labor USD$		
(10 days)*(8hrs/day)*($10/hr) =	800	USD$
800 USD / 20 yr life of tank	40.00	USD$/yr
(40USD/yr)/(6.71 m^2)	5.96	USD$/ m^2/yr for area of one tank system
(40USD/yr)/(16 m^2) =	2.5	USD$ labor /m^2/yr SAV bed restoration

15a/ Labor for Propagation

Propagation,		
2 people x 3 days =	6	days
+ growing and maintenance: 1person x		
8 weeks x 2 days per week (6 days+16 days)=	22	days/yr
(propagation)+(maintenance)=		
Total days for propagation=	28	days/year
Kcal/human conversion	2500	kcal/person/day
(2500kcal/day)(28 days)=	70000	kcal total for the system
Kcal to Joules conversion:	4186	J in one Kcal
(4186J)(70000kcal)=	293020000	J/year system total
(J/year system total)/(m^2 propagation area)=		
(293020000)/(6.71 m^2) =	43669151	J/m^2 SAV bed restored
(J/year system total)/(area restored)=		
(293020000)/(16 m^2) =	**18313750**	**J/m^2 SAV bed restored**

15b/ Labor for Propagation USD basis

Labor USD (22 days)*(8hr)*($10USD) =	1,760	$USD/yr for total propagation
($1760)/(6.71 m^2) =	262.3	$USD labor/$m^2$ /yr for propagation area
($1760)/(16 m^2) =	27.5	$USD labor/$m^2$ /yrof SAV bed restored

16/ Shipping UPS Ground 20$	2.0E+01	$USD/year Total costs shipping for system
Shipping costs/unit area=		
Total system shipping costs /area of system		
($USD20.00)/(6.71$m^2$) =	2.98	$USD/year per m^2 total costs shipping prop.
System SAV Bed restored: (USD20.00)/(16m^2) =	**1.25**	**$USD/$m^2$ SAV bed restored**

***Emergy of Goods* and *Services* / *Total Emergy Inputs* (R+F)**

17a/ Total Output = Results of Emergy Inputs
(Dry Weight (DW) basis

*****Empirical Dry Weight (DW) m^{-2} (summed aboveground and belowground biomass grown in soil sand substrate in microcosms from Table 4.1	0.47	$gDWm^{-2}day^{-1}$
Area ea propagation tray (0.26mx0.35m = 0.09m^2) with	0.09	m^2
NPP for tray/day: (0.47 $gDWm^{-2}day^{-1}$)(0.09)=	0.0423	gDW/day^{-1} each propagation tray total
8 week *P. perfoliatus* biomass growout:	56	days (8 weeks) propagation prep
(0.0423 gDWday-1)(56 days propagation time)=	2.37	gDW total biomass per tray for 8 weeks
Four (4) trays required for each m^2 SAV bed=		
(2.37 gDW/tray)(4 trays m^2 SAV bed)=	9.5	g DW as input to restore one m^2 SAV bed
No trays total (64)	64	trays
(64 trays)/(4 trays/m^2)=	16	m^2 total output from propagation
(Total meters restored)(gDW one m^2 restored)=		
(16 m^2)(9.5 g DW each m^2)=	152	gDW for annual yield input
Total Energy Output of System (based on DW) =		
(152 g DW)(**3.5kcal/g) =	532	kcal tot DW to restore 16m^2 SAV bed
Joules conversion: (532 kcal)(4186J/kcal) =	2.226952E+06	J/system total to restore 16 m^2 SAV bed
For J/m^2 of propagation system and restoration site:		
(2.226952E+06 J)/(6.71m^2) =	331886	J/m^2 for propagation system biomass/m^2
For J/m^2 to restore SAV bed:		
(2.226952E+06 J)/(16m^2) =	**1.39184E+05**	**J/m^2 DW to restore each m^2 SAV bed**

17b/ Total Cost of USD Restoration Based on inputs

Total Budgetary Initial investment:		
Total cost of SAV system restored per year:		
The sum of all inputs in tank system:	372.09	$USD for each m^2 of the tank prop. system
Similar to Yield, the sum of all inputs yielded/		
m^2 SAV bed restored (added inputs):	73.55	$USD to restore each m^2 SAV bed
Total cost of SAV system annually:	2,496.83	$USD operating cost annually
Total initial expenditure/startup for first year:	4,179.76	$USD init. invest+1st year propagation

***Table 4.1 NPP growth rate for Soil Sand substrate**
= (0.47 $gDWm^{-2}day^{-1}$)

*****Energy in grown plants: Ave. Kcal for seaweed: 3.5 kcal/g***
Calorie count (11/11/2016):https://www.caloriecount.com/calories-eden-foods-nori-10-sheets-i104332

Appendix 5. Footnotes to Table 4.3 providing calculations and rationale for energy required to restore 16m^2 SAV bed by the hand transplant method for an initial coverage from 8 weeks of propagation (initially covering 36% of each m^{-2} and then in-situ growout).

Source and calculation	Value	Units
Renewable Sources		
1/ Sunlight		
Restoration **Area (A)**:(4 m)(4 m) =	16	m^2
***Insolation (I)** (April):	5.0	kWh $m^{-2}day^{-1}$
Insolation conversion to J (I_J): 1kWh =	3.6E+06	J
(5.0kWh $m^{-2}day^{-1}$)(3.6E+06 J)=	1.8E+07	J m^{-2} day^{-1}
****Albedo (**α) **/Albedo Correction** (1-α): (1-0.1)=	0.9	
(I) x (1-α)=		
(1.8E+07J m^{-2} day^{-1})(0.9)	1.62E+07	J m^{-2} day^{-1}
Energy input= [(**I_J**) x (1-α)]x(A)(365 days)		
(1.62E+07 J m^{-2} day^{-1})(16 m^2)(365 days/yr) =	9.4068E+10	Jyr^{-1} system total
Energy input per 1m^2:		
(9.4068E+10 Jyr^{-1})/(16 m^2) =	**5.879E+09**	**J/m2/ yr^{-1} SAV bed restored**
Total Cost Sunlight USD$:	0	USD$ total and per m^2 SAV bed restored
*(NREL 2012) http://www.nrel.gov/gis/images/map_pv_us_april_dec2008.jpg		
**(Holman 1997)		
2/ Tides		
Restoration **Area**:(4m)(4 m) =	16	m^2
Tidal Range	0.45	m
Density of water, (15 psu):	1005.9	kgm^{-3}
Gravity	9.8	ms^{-2}
Tides per year	730	yr
Tidal Energy (J) absorbed per year=		
(area elevated)(0.5, center of gravity)(tides/yr)		
(height^2)(density)(gravity)=		
(16m^2)(0.5)(730 tides/yr)(0.45m/tide)^2(1005.858 kgm^{-3})		
(9.8 ms^{-2})=	1.1657371E+07	J/yr
Tidal energy per m^2 SAV bed restored:		
(1.1657371E+07J/yr)/(16 m^2)=	**7.28586E+05**	**J/yr for each m^2 SAV bed restored**
Total Cost Tidal Energy USD$	0	USD$ total and per m^2 restored
3/ River channel velocity/flow		
Restoration Site Base Parameters		
Restoration area=(4m)(4m)	16	m^2
Restoration Site Depth=	1	m
Restoration Site Width=	4	m
Site Cross sectional channel area (**A**)=(1m)(4m)	4	m^2
Wetted Perimeter (wp)=(1m)+(4m)+(1m)	6	m
Pre-restoration Velocity (*u*) / Flow (Q)		
Avg site velocity (*u*) pre-restoration:		
u=[(*k*)($Rh^{2/3}$)($S^{1/2}$)]/[n], where		
k –SI (int'l system) unit conversion factor for metric	1	(m)
Rh - hydraulic radius=cross sectional area (A)/		
Wetted perimeter (wp): Rh = (**A**/*wp*)		
Rh=(4m/6m)	0.67	
S* – slope	0.0006	m/m
*n*** –Manning's roughness coefficient		
for mud bottom	0.03	unitless
Avg Site velocity=		
u=[(*k*)($Rh^{2/3}$)($S^{1/2}$)]/[n]= [(1)(0.67$^{(2/3)}$)(0.0006$^{(1/2)}$)]		
/ [0.03] =	0.625	m/s
Flow rate (Q, volume) pre-restoration=		
Q = (*u*)(A) = (0.625m/s)(4m^2)=	2.5	m^3/s system total
Q_{annual} = (Q)(Number of seconds in one year) =		
Q_{annual} = (2.5 m^3/s)(3.15569E+07 seconds/yr) =	7.8892250E+07	m^3/yr system total
Q_{annual/m^3} = (Q_{annual})/(restoration area) =		
Q_{annual/m^3} = (7.8892250E+07m^3/yr)/(16m^2)=	4.930766E+06	m^3/yr/per one m^3 pre-restoration

Post-restoration Velocity (*u*) / Flow (Q) Ranges based on manning's n varying with vegetation density

Avg site velocity (*u*) pre-restoration:		
$u=[(k)(Rh^{2/3})(S^{1/2})]/[n]$, where		
k –SI (int'l system) unit conversion factor for metric	1	(m/s)
Rh - hydraulic radius=cross sectional area (A)/		
Wetted perimeter (wp): Rh = (A/*wp*)		
Rh=(4/6) =	0.67	m
S* slope	0.0006	m/m
*n****Manning's roughness coefficient:		
n for low biomass =	0.03-0.04	unitless
n for high biomass =	0.25-2.25	unitless
Avg Site velocity=		
(low biomass) $u_{L1}=[(k)(Rh^{2/3})(S^{1/2})]/[n]=$		
$[(1)(0.67^{(2/3)}(0.0006^{(1/2)})]$ /[**0.03**]=	0.625	m/s
(low biomass) $u_{L2}=[(k)(Rh^{2/3})(S^{1/2})]/[n]=$		
$[(1)(0.67^{(2/3)}(0.0006^{(1/2)})]$ / [**0.04**]	0.469	m/s
(high biomass) $u_{H1}=[(k)(Rh^{2/3})(S^{1/2})]/[n]=$		
$[(1)(0.67^{(2/3)}(0.0006^{(1/2)})]$/ [**0.25**]=	0.075	m/s
(high biomass) $u_{H2}=[(k)(Rh^{2/3})(S^{1/2})]/[n]=$		
$[(1)(0.67^{(2/3)}(0.0006^{(1/2)})]$/ [**2.25**]=	0.0264	m/s
Flow rate (Q, volume) post-restoration		
(low biomass):		
$Q_{L1} = (u)(A)= (0.625m/s)(4m^2)=$	2.5	m^3/s system total
$Q_{L2} = (u)(A)= (0.469m/s)(4m^2)=$	1.876	m^3/s system total
$Q_{L1annual}=(2.5\ m^3/s)(3.15569E+07\ s/yr)=$	7.8892E+07	m^3/yr system total
$Q_{L2annual}=(1.876\ m^3/s)(3.15569E+07\ s/yr)=$	5.92007444E+07	m^3/yr system total
$Q_{H1} = (u)(A)= (0.075m/s)(4m^2)=$	0.3	m^3/s system total
$Q_{H2} = (u)(A)= (0.0264m/s)(4m^2)=$	0.1056	m^3/s system total
$Q_{H1annual}=(0.3\ m^3/s)(3.15569E+07\ s/yr)=$	9.467070E+06	m^3/yr system total
$Q_{LH2annual}=(0.1056\ m^3/s)(3.15569E+07\ s/yr)=$	3.332408E+06	m^3/yr system total
$Q_{annual/m^3} = (Q_{annual})/$(restoration area) =		
$Q_{annualL1/m^3} = (7.8892E+07m^3/yr)/(16m^2)=$	4.937000E+06	m^3/yr/per one m^3 post-restoration
$Q_{annualL2/m^3}= (5.920E+07m^3/yr)/(16m^2)=$	3.700000E+06	m^3/yr/per one m^3 post -restoration
$Q_{annualH1/m^3} = (9.467E+06m^3/yr)/(16m^2)=$	5.91688E+05	m^3/yr/per one m^3 post -restoration
$Q_{annualH2/m^3} = (3.332E+06m^3/yr)/(16m^2)=$	2.08250E+05	m^3/yr/per one m^3 post -restoration
Mass of water per year		
Before SAV restoration		
H_2O mass (m_{H2O}) =		
(YearlyFlowRate)(Density H_2O)=		
$(Q_{annual})(\rho_{H2Obrackish})=$		
H_2O mass (m_{H2O}) =		
$(7.8892250E+07m^3/yr)(1005.858\ kgm^{-3})=$	7.9354E+10	kg/yr
Kinetic Energy (KE) pre-restoration		
KE=1/2 $(m_{H2O})(u^2)=$		
$KE=0.5(7.935E+10\ kg/yr)(0.625\ m/s)^2=$	1.549804688E+10	J/yr
Mass of water per year		
After SAV restoration		
H_2O mass (m_{H2O}) =		
YearlyFlowRate(Q_{annual})(Density H_2O $\rho_{H2Obrackish}$)=		
H_2O mass (m_{H2O}) =		
$Q_{L1annual=}$ $(7.8892E+07m^3/yr)(1005.858\ kgm^{-3})=$	7.9354E+10	kg/yr
$Q_{L2annual}= (5.92007444E+07\ m^3/yr)(1005.858\ kgm^{-3})=$	5.954754196E+10	kg/yr
$Q_{H1annual}= (9.467070E+06\ m^3/yr)(1005.858\ kgm^{-3})=$	9.522528096E+09	kg/yr
$Q_{LH2annual}= (3.332408E+06m^3/yr)(1005.858\ kgm^{-3})=$	1.015735526E+09	kg/yr
Kinetic Energy (KE) post-restoration		
KE=1/2 $(m_{H2O})(u^2)=$		
$KE(u_{L1})=0.5(7.935E+10\ kg/yr)(\ 0.625\ m/s)^2=$	1.549804688E+10	J/yr
$KE(u_{L2})=0.5(5.955E+10\ kg/yr)(\ 0.469\ m/s)^2=$	6.549068439E+09	J/yr
$KE(u_{H1})=0.5(9.52258E+09\ kg/yr)(0.075\ m/s)^2=$	2.6782110E+07	J/yr
$KE(u_{H2})=0.5(1.016E+09\ kg/yr)(\ 0.0264\ m/s)^2=$	3.5405568E+05	J/yr
$KE_{(abs)}$ absorbed by restoration vegetation		
At different densities=		
($KE_{(pre\text{-}rest)}$ of flow entering site) –		
($KE_{(post\text{-}rest)}$) of flow leaving the site) =		

$KE_{(abs)}$= ($KE_{(pre\text{-}rest)}$) - ($KE_{(post\text{-}rest)}$) =		
$KE_{(pre\text{-}rest)}$: (1.549804688E+10 J/yr) –		
($KE_{(post\text{-}rest\ uL1)}$): (1.549804688E+10 J/yr) =	0	J/yr
($KE_{(post\text{-}rest\ uL2)}$): (6.549068439E+09 J/yr) =	8.948978441E+09	J/yr
($KE_{(post\text{-}rest\ uH1)}$): (2.6782110E+07 J/yr) =	1.5471264770E+10	J/yr
($KE_{(post\text{-}rest\ u_{H2})}$): (3.5405568E+05 J/yr) =	1.549769282E+10	J/yr
Energy per unit area= ($KE_{(abs)}$)/A		
$KE_{(abs\ -\ uL1)}$: (0)/(16m^2) =	0	J/m^2/yr
$KE_{(abs\ -\ uL2)}$: (8.948978441E+09 J/yr**)/16**m^2)=	5.59311152E+08	J/m^2/yr
$KE_{(abs\ -\ uH1)}$: (1.5471264770E+10 J/yr) / (16m^2) =	9.66954048E+08	J/m^2/yr
$KE_{(abs\ -\ uH2)}$: (1.549769282E+10 J/yr) / (16m^2) =	**9.68605801E+08**	**J/m^2/yr**
Total Cost $USD	0	$USD total and per m^2 SAV bed restored

*http://www.charts.noaa.gov/InteractiveCatalog/nrnc.shtml?rnc=12270
**http://www.engineeringtoolbox.com/mannings-roughness-d_799.html
***Champion and Tanner 2000

4a/ Plastic construction fencing 8.5lbs/100ft		
(g basis)		
1 roll = (3855.54 g)/(30.48m)=	126.49	g/m
Perimeter=4+4+4+4=	16	m linear required
Amount fencing required (grams per meter)(perim.)=		
(126.49g/m)(16m)=	2023.62	g fencing for system total system
Replacement period – life of fencing=	5	yrs
Total use per yr=(mass)/(life of fence)=		
(2023.6g)/(5 yrs)=	404.72	g fencing cost/year over 5 years
Plastic per m^2 SAV bed restored		
Per year = Total use per yr /		
Area restored = (404.72g)/(16m^2)	**25.30**	**g/yr for each m^2 of SAV bed restored**
4b/ Plastic, orange, construction fencing (USD basis):		
Total USD fencing req. for restoration=		
($29.97ea roll, measures 30.48m) =($29.97)/(30.48m)=	0.983	$USD per linear meter cost of fencing
Perimeter=4+4+4+4 =	16	m linear required
(16 m)(0.98$/m)=	15.68	$USD for system total
Replacement period – life of fencing=	5	yrs
Total $ cost per yr= ($USD)/(replacement period)=		
($15.68)/(five years) =	3.14	$USD fencing cost per year
Plastic cost per m^2 SAV bed restored		
Per year = Total cost per yr /		
Area restored = ($3.14)/(16$m^2$)=	0.20	$USD/yr for each m^2 of SAV bed restored
5a/ Steel fence T-posts:		
Four (4) Uprights for construction fencing (6.484 pounds or		
2941.09 g) = (4)(2941.09g)=	11764.36	g system total without depreciation
Replacement period – life of steel posts = 20 yrs		
(System total steel) / (20 years)=		
(11764.36g)/(20 yrs) =	588.22	g steel per year
Mass/yr/area restored: (588.22g)/(16 m^2) =	**36.76**	**g steel per year per m^2 SAV bed restored**
5b/ Steel fence T-posts $USD basis:		
Four (4) Steel fence posts @ $3.67each=	7.02	$USD System total cost of steel fence posts
Replacements: Life of Steel posts:	20	years
Total system costs of steel posts)/yr: ($14.68)/(20 years)=	0.734	$USD cost of steel per year
$USD/year ($0.734)/(16 m^2):	0.046	$USD steel; used per m^2 of SAV bed
(THD 2016, Ciotala et al. 2011)		restored/yr
6a/ Machinery and Equipment		
Truck for transporting staff, propagules and equipment		
to restoration site average miles drive each year: 13476*		
Life of vehicle @ cost USD 200,000 miles /13476 =	15	years replacement period of vehicle
Mass of vehicle =	1859727	g
Yearly (g) use = Mass of vehicle/replacement period of vehicle=		
1859727 g / 15 years=	123981.8	g used per year for vehicle transport
Annual vehicle mass (g) in system/m^2 total		
123981.8g/16 m^2 =	**7748.86**	**g/m^2/yr/m^2 SAV bed restored**

6b/ Machinery and Equipment (USD Basis)

For transporting staff, propagules and equipment to restoration site, purchased:		
Toyota Tacoma USD basis:	30,000.00	USD$
Life of vehicle	15	years
USD$ of Tacoma Truck / Annual use of vehicle =	2000	USD$ of truck per year
USD$ of Tacoma Truck for each m^2 of SAV = Annual use of vehicle USD / area restored=		
2000 USD / 16 m^2 =	125.00	USD$ per m^2/yr SAV bed restored
*Average mileage driven per year:		
*http://www.fhwa.dot.gov/ohim/onh00/bar8.htm		

7a/ Fuel, gasoline

Total gasoline input to travel 50 miles:	2.47	gallons/year
Energy density	124,340	Btu/gallon
Total BTUs annually	307,120	Btu/year
BTUs to Joules conversion=	1055.06	J/Btu
Joules per year=(Btu/yr)(J/Btu)=:		
(307,119.8 Btu/yr)(1055.06J/Btu)=	3.24029816E+08	J/yr annual use of fuel for restoration
Fuel consumed per m^2 SAV bed restored		
(3.24029816E+08 J/yr)/(16m^2)=	**2.0251864E+07**	**J/m^2/yr fuel use per m^2 SAV bed restored**

7b/ Fuel, gasoline, USD basis

Total input to travel 50 miles:	2.47	gallons/year
(2.47gal.)(*$USD 2.35/gal)=	5.80	$USD / year round trip site travel
$USD expenditure per unit area=		
($USD 5.80)/(16 m^2) =	0.36	$USD per each m^2 SAV bed restored
eia.gov, U.S. Energy Information Administration		

8a/ Propagules

Total Input of Propagule to restoration site		
Area of each tray:	0.09	m^2
Number of trays deployed each meter sq. =	4	trays
Biomass deployed = final output from propagation site:		
64 Trays with 8 week *P. perfoliatus* biomass growout:		
Empirical avg. estimated DW per tray (Area=0.09 m^2)	2.37	g/tray DW from 8 weeks growth
Fresh Weight (fw) = (2.37g DW/0.14)=	16.93	g/tray FW biomass
Four trays deployed each meter sq=		
(2.37gDW per tray)(4 trays each m^2) =	**9.5**	**g DW/m^2 SAV bed restored**
Total trays deployed to site:	64	trays
Total area restored: (64)/(4 trays ea m^2)	16	m^2 restored at site
Total starting biomass deployed to site:		
(64 trays)(2.37 g DW/tray)=	152	g DW initially deployed to 16 m^2 site

8b/ Propagule est. cost USD$ basis

From previous propagation system:		
(Table 4.2, Appendix 4) Est Cost/m^2:	73.69	$USD/$m^2$
Total cost to restore 16 m^2 SAV=		
(73.69$/$m^2$)(16 m^2) =	1179.04	$USD cost annually for 64 trays propagules

9a/ Labor for Restoration planting and installation

Total labor input:		
4 people x 2 days =	8	days
Kcal per human Conversion =	2500	kcal/person/day
(8 days)(2500 kcal Conversion)=	20000	kcal/day for system total
(Kcal/day system total)(4186 joules in kcal)=	83720000	J/day for system total
(J/day system total)/(area restored)=		
83720000/16 m^2 =	**5232500**	**J/day/m^2 SAV bed restored**

9b/ Labor for restoration planting and installation USD basis

Total labor input=		
Labor USD$		
(8 days)*(8hrs/day)*($20/hr) =	1280	USD$/yr for restored system
USD / m^2 = 1280/16 m^2	80	USD$/$m^2$/yr for the restored area

10a/ Total Output = Restoration Results of Emergy Inputs

64 Trays with 8 week *P. perfoliatus* biomass growout:		
Initial Inputs based on growth rate of:	0.47	gDW/m^2day^{-1} (see Table 4.1**)
Empirical ave Dry weight/tray after 8 weeks growout	2.37	gDW/0.09m^2 ea tray (App. A, 17a/)

Total initial site input **(x_0)** gDW/m^2=(2.37gDW)(4 trays m^2):	9.5	g DW/m^2 initial biomass input to rest. site
Growing season duration **(t)**(input June 1st – September 31):	120	days (additional to 8 weeks propagation)
Area/tray= (0.26m)(0.35m)=	0.09	m^2
Total area (no of trays) input for each m^2:		
(0.09 m^2)(4) = **(F)** (fraction of m^2) occupied by propagated sods:	0.36	Fraction biomass /m^2 planted at site
Final biomass input/m^2 from restoration + 1st season growth=		
NPP_{rs1} (Net Primary Productivity $_{\text{restoration season one}}$) =		
x_0+[(**g*****t**)(**F**)]= [(**0.47gDW/m^2/day)(120 days)*0.36]=	20.30	gDW growth each m^2 for 4mo growth
x_0 = 9.5 gDW/m^2 (56 day growth site input) + 20.30 gDW=	**29.80**	**gDW each m^2 at restoration site 1st season**
Total restoration area=	16	m^2
Total output for area restored after season one=		
(29.80 gDW/m^2)(16 m^2)=	476.8	gDW per 16m^2 of SAV bed restored
Final energy input per m^2=	3.5	g/kcal in organic matter
J/kcal conversion:	4186	J/kcal
(29.80gDW/m^2)(3.5 g/kcal for seagrass)(4186J/kcal)=	**436600**	**J/m^2 biomass output 1st season restoration**
Total ENERGY for entire SAV area restored=		
(436600 J/m^2)(16 m^2)=	6985600	J SAV plant energy in restored SAV bed system after first season

** Table 4.1, NPP for Soil Sand treatments Zinecker (CH2)

10b/ Total Output USD: Cost of Restoration Based on $Inputs

Similar to Yield, the sum of all inputs yielded/ m^2 SAV bed restored:	279.30	$USD to restore each m^2 SAV bed
Total cost of SAV system restored per year:	4468.67	$USD/yr to restore 16 m^2 SAV habitat
Total cost of restoration start-up +1st season	32484.21	$USD total to fund 1st restoration

Energy in grown plants:
Ave. Kcal for seaweed: 3.5 kcal/g
From Calorie count:
(2)https://www.caloriecount.com/calories-eden-foods-nori-10-sheets-i104332
accessed 11/11/2016

Appendix 6. Footnotes to Table 4.4: SAV propagation/preparation for restoration deployment using PHA biodegradable pots.

Source and calculation	Value	Units
Renewable Resources		
1a/ Sunlight		
Propagation area:		
(0.26 m x 0.35 m)(64 trays) =	5.82	m^2
Insolation: 5.5845E9 $Jm^{-2}yr^{-1}$ (NREL 2012)		
Albedo: 0.1 (Holman 1997)		
Energy input per year:		
(propagation area) x (insolation) x (1-albedo) =		
(5.82 m^2) x (5.5845E9 $Jm^{-2}yr^{-1}$) x (1-0.1) =	2.9251611E+10	Jyr^{-1} system total/propagation area
Energy input per year for each m^2 of propagation area:		
(2.9251611E+10 Jyr^{-1})/(5.82 m^2)=	5.03E+09	J/m^2 /yr sunlight for propagation area
Energy input per year to create 1 m^2 restored SAV bed:		
(Energy input)/(Total SAV bed area restored)=		
(2.9251611E+10 Jyr^{-1})/(512 m^2 total restored area)=	**5.7132052E+07**	**J/m^2 /yr sunlight required for propagation prep for each m^2 of restored SAV bed**
1b/ Sunlight $USD basis		
Total Cost Sunlight USD	0	USD$ total and per m^2 SAV bed restored
2a/ Rain, chemical		
Propagation tank area:		
(0.26 m x 0.35 m)(64 propagation trays) =	5.82	m^2
*Estimated Maryland Annual rainfall: 1.2 myr^{-1} (from NOAA/National Weather Service/BWI Airport precip data)		
Density of water:	1.00E+06	gm^{-3}
Gibbs Free Energy (GFE) of water:	4.94	Jg^{-1}
Energy (J) input avg. precipitation per year=		
(area)(rainfall)(Density H_20)(GFE) =		
(5.82 m^2)(1.2 myr^{-1})(1.00E+06 gm^{-3})(4.94 Jg^{-1}) =		
Precipitation Energy (E) input:	3.4500960E+07	Jyr^{-1} system total/propagation area
Energy input per year for each m^2 of propagation area:		
(3.4500960 E+07 Jyr^{-1})/(5.82 m^2)=	5.928000E+06	J/m^2/yr sunlight for propagation area
(Precipitation E input)/(Total SAV bed area restored)=		
(3.4500960 E+07 Jyr^{-1})/(512 m^2 total restored area)=	**6.7384E+04**	**J/m^2/yr rain per m^2 restored SAV bed**
2b/ Rain, chemical		
Total Cost Rain, chemical USD	0	USD$ total and per m^2 SAV bed restored
3a/ Evaporation		
Propagation area:		
Measurement of each tray: (0.26 m x 0.35 m) =	0.091	m^2
x 64 trays =	5.82	m^2
*Estimated Maryland mean Annual pan evaporation:	1.3	myr^{-1}
Density of water:	1.00E+06	gm^{-3}
Gibbs free energy of water:	4.94	Jg^{-1}
Evaporation energy=		
(evap)x(H_2O density)x(GFE)x(prop tray area)		
EvapE=(1.3 myr^{-1})(1.00E+06 gm^{-3})(4.94 Jg^{-1})(5.82 m^2)=		
Evaporative Energy Input:	3.7401728E+07	Jyr^{-1} system total
Evaporative Energy Input per m^2:		
(3.7401728E+07 Jyr^{-1} system total)/(5.82 m^2) =	6.426413E+06	$Jyr^{-1}m^2$ for growout system
(3.7401728E+07 Jyr^{-1} system total)/(512 m^2) =	**7.3050E+04**	**Jyr^{-1} per m^2 SAV bed restored**
(Farnsworth and Thompson 1982)		
3b/ Evaporation		
Total Cost Evaporation USD	0	USD$ total and per m^2 SAV bed restored
* x 0.13m/mo for 1 week propagation season/year (Farnsworth and Thompson 1982)		

4a/ Irrigation well/water

Deployment tray volume filled		
(25cm x 34 cm x 2 cm) =	1.7E+03	g (ml) each tray
(1.7E+03g)(64 trays) =	1.088E+05	g for PHA-seed propagation growout system
Irrigation (g) for each m^2 of system:		
(1.088E+05 g)/(5.82 m^2) =	1.8694E+04	g per m^2 growout system
Irrigation (g) for each m^2 SAV bed restored:		
(1.088E+05g)/(512 m^2)=	**2.13E+02**	**g irrigation water required per m^2 SAV bed restored**

4b/ Water/Irrigation USD$ Basis:

*WSSC cost for 1000 gallons =	3.20	$USD
1000 US gallon =	3.785E+06	cm^3 (ml)
Cost of one ml water:		
(USD$3.20)/(3.785E+06 ml)=	8.45E-07	$USD/ml
SAV plant propagation/deployment tray volume:		
(26cm x 33 cm x 2 cm) =	1.7E +03	g, ml, or cm^3
Cost of water for system:		
(One tray vol)(No. trays)(cost water USD$/ml)=		
(1.7E+03ml)(64)(8.45E-07$/ml)=	9.19E-02	$USD irrigation for propagation system
USD$ input for irrigation per m^2 for system		
(9.19E-02 $USD)/(5.82$m^2$) =	1.58E-02	USD$ to pay irrigation for total area of propagation system
Irrigation USD$ for each m^2 SAV bed restored:		
(9.19E-02 $USD)/(512 m^2)=	1.80E-04	USD$ total per m^2 SAV bed restored

*(Source: Washington Suburban Sanitary Commission Cost figures per 1,000 gallons https://www.wsscwater.com/rates)

Semi-Non-renewable Resources

5a/SAV Bed Sediment

Amount required:	4	cm^3 in each pot
*Density of SAV Bed sediment	1.1	g/cm^3
/lifespan	1	yr
Annual amount require per PHA pot:		
(Amt)(density)/(lifespan)=	4.40	g/yr/PHA pot
Amount required for system:		
(4.40 g/yr/PHA pot)(40 pots/tray)	176	g/yr growout tray
(176 g/yr/tray)(64 trays)=	1.1264E+04	g/yr sediment required for system
Sediment required (g) for each m^2 of system:		
(1.1264E+04 g/yr)/(5.82 m^2)=	1.935E+03	g/yr sediment required per m^2 of system
SAV bed sediment req'd for each m^2 SAV bed restored=		
(1.1264E+04 g/yr)/(512 m^2)=	**2.2E+01**	**g/yr required per m^2 SAV bed restored**

*Brady and Weil 2002, Fig. 4.14

5b/ SAV Bed sediment $USD basis

Total Cost Bed Sediment $USD	0	USD$ total and per m^2 SAV bed restored

6a/ SAV Bed Sediment bacteria (inoculant)

*Density of soil bacteria=	1.0E+10	cells per one g soil
**mass (g) of average bacteria=	1.0E-12	g for one cell
(No cells)(mass of one cell)=		
mass in one g soil = (1.0E+10)(1.0E-12)=	1.0E-02	g cells for one gram of soil
SAV bed sediment bacteria in one PHA pot:		
(g bacterial cells/gram soil)(mass (g) soil in one PHA pot):		
(1.0E-02)(4.40g) =	4.4E-02	g cells (inoculant) for each PHA pot
Mass bacteria in system=		
(g cells in 1 PHA pot)(No. pots in each tray)(no. plastic trays):		
(4.4E-02 g inoc)(40 PHA pots)(64 trays) =	1.126E+02	g cells inoculant in PHA pots in system
Inoculant (g) required for each m^2 in propagation system=		
(1.126E+02 g cells)/(5.82 m^2)=	1.94E+01	g cells inoculant per m^2 in system
Inoculant(g) required for each m^2 SAV bed restored=		
(1.126E+02 g cells)/(512 m^2)=	**2.2E-01**	**g cells inoculant per m^2 SAV bed restored**

*Raynaud and Nunan (2014) **Davis et al. (1973)

6b/ SAV Bed sediment bacteria $USD basis

Total Cost Bed Sediment bacteria $USD	0	USD$ total and per m^2 SAV bed restored

Purchased Goods

7a/ Oyster shell $7.19/5lb bag

Or 2.268E+03 g		
Amount of oyster shell:	4	cm^3
x Density of oyster shell	0.949	g/cm^3
(1mm diam. estimated,		
0.5 cm diam. = 0.849*)		
/ lifespan	1	
Oyster shell energy 1 pot:	3.796	g/yr per one PHA pot
(40 pots)=	1.518 E+02	g/yr per all pots in plastic transport tray
(64 trays)=	9.718 E+03	g/yr system total (req'd for restoration site)
g/yr oyster shell per m^2 of system:		
(9.718 E+03 g/yr)/(5.82 m^2)=	1.669E+03	g/yr/oyster shell per m^2 propagation system
g/yr oyster shell required for each m^2 SAV bed restored=		
(9.718 E+03 g/yr)/(512 m^2)=	**1.898E+01**	**g/yr required per m^2 SAV bed restored**

*Engineering Toolbox: http://www.engineeringtoolbox.com/density-materials-d_1652.html

7b/ Oyster shell USD basis

One 5 pound bag =	7.19	$USD cost of 5 pound bag
1 pound = 453.592 grams		
(5 pounds)(453.592 g)=	2267.96	g for 5 pound bag of shell
Per gram price of oyster shell		
($USD 7.19)/(2267.96g)=	3.17E-03	$USD per gram oyster shell
Amount of oyster shell (7a/ above)	3.796	g/yr oyster shell per one PHA pot
Price oyster shell for one PHA pot:		
(Amt oyster shell g)($ cost per gram)=		
(3.796 g/yr)($USD 3.17E-03)=	0.01	$USD/yr ea pot with oyster shell
Price oyster shell for entire system (pots)(trays):		
($USD 0.01 each pot)(40 pots)(64 trays)=	30.81	$USD/yr oyster shell for propagation system
Price oyster shell per m^2 of system:		
($USD 30.81)/(5.82 m^2)=	5.29	$USD/$m^2$ oyster shell in system
Price oyster shell required for each m^2 SAV bed restored=		
($USD 30.81)/(512 m^2)=	6.02E-02	$USD oyster shell required per m^2 SAV bed restored

8a/ Peat moss ($10.47 bag wt 65 lbs)

Amount required for one pot:	4	cm^3
(*Density of peat)	0.160	g/cm^3
/ (lifespan of one year)	1	yr
Peat moss mass 1 pot (4)(0.160)=	0.64	g/yr for one pot
x(40 pots in each tray):	2.56E+01	g/yr for 40 pots
x(64 trays):	1.638 E+03	g/yr peat moss system total
Peat moss g/yr per m^2 of system:		
(1.638E+03 g/yr)/(5.82 m^2) =	2.81E+02	g/m^2/yr peat moss propagation system
Peat moss req'd for each m^2 SAV bed restored=		
(1.638E+03 g/yr)/(512 m^2)=	**3.2**	**g/m^2/yr peat moss SAV bed restored**

* Source: Engineering Toolbox: http://www.engineeringtoolbox.com/density-materials-d_1652.html

8b/ Peat moss USD basis

One 65 lb bag =	10.47	$USD cost of 65 pound bag
1 lb = 453.592 grams		
(65 lb)(453.592 g/lb)=	29483.48	g/65 pound bag of peat moss
Per gram price of peat		
($USD 10.47)/(29483.48g)=	3.55E-04	$USD ea gram peat moss
Amount of peat moss (7a/ above)	0.64	g/yr peat moss per one PHA pot
Price peat for one PHA pot:		
(Amt peat g)($ cost per gram)=		
(0.64 g/yr/pot)($USD 3.55E-04)=	2.27E-04	$USD / yr /pot filled with peat moss
Price peat moss for entire system (pots)(trays):		
($USD 2.27E-04 each pot)(40 pots)(64 trays)=	5.81632E-01	$USD /yr of peat moss for propagation
Price peat moss per m^2 of system:		
($USD 0.5816)/(5.82 m^2)=	9.994E-02	$USD peat moss per m^2 for propagation
Price peat moss required for each m^2 SAV bed restored=		
($USD 0.5816)/(512 m^2)=	1.14E-03	$USD peat moss/$m^2$ SAV bed restored

9a/ *Seeds (mass or J basis) (1 lb bag wrack + seeds and water		
(0.45 kg or 450g) @ $5/bag, only 50% of bag is seeds)		
(0.5)(450g)=	225	g of seeds/$5USD bag
(2g seeds per PHA pot - approx. 7 cm long bead		
of seeds 2 mm wide to fit one PHA pot =	~35	seeds (or 2 gFW) each PHA pot
No bags required: (g/pot)(no pots)(no trays):		
(2 g FW/PHA pot)(40 pots)(64 trays):	5,120	g FW seeds
Total bags required: (5120 g seed needed)/225 g seeds ea bag=	22.7	bags of seed /year
No of pots total: (40 pots ea tray)(64 trays)=	2,560	PHA pots in system
No seeds in system (~35 seeds/pot)(2560 pha pots)=	89600	seeds input into system
Seeds per m^2 of system: (89600)/(5.82 m^2 prop area)=	15395.2	seeds input/m^2 of prop. system
Seeds per m^2 SAV bed restored: (89600)/(512 m^2)=	175	seeds input/m^2 of SAV bed restoration
*Average empirical P. Perfoliatus individual seed mass (DW):	0.0028	g D W each seed
Mass seeds in one pot: (35seeds)(0.0028g)	0.098	g DW each PHA pot
Mass (gDW seeds in each m^2) SAV bed restored:		
Mass seeds in five pots: (0.098 gDW)(5 PHA pots)=	**0.49**	**g DW for each m^2 SAV bed restored**
Total input of seed mass gDW into propagation system:		
(mass g DW/pot)(no pots/propagation tray)(no. of prop. trays)=		
(0.098gDW)(40 PHA pots)(64 plastic propagation trays)=	250.88	g DW seeds input into propagation system
Seeds/m^2 input into propagation system=		
(250.88 gDW seeds system total)/(5.82m^2)=	43.11	gDW seeds input/m^2 of prop. system
**Average kcal in each seed:	0.01	kcalories/seed
Kcal in each gram of seed = (0.01kcal/seed)/(0.0028gDW)=	4.0	kcal/g seed
Kilocaries each PHA pot = (0.01)(35)=	0.35	kcal/ of seeds each PHA pot
Energy in one pot: (0.35)(4186j/kcal)=	1,465	J seeds in each PHA pot
No of pots in each m^2:	5	pots each m^2 SAV bed restored
Joules each m^2 restored: (1465J seed energy)(5 PHA pots)=	**7325**	**J/m^2 seed energy each SAV bed restored**
No of pots/tray	40	PHA pots in each plastic propagation
No of trays total for propagation system	64	plastic propagation trays in system
Energy calculations for seed energy input into system:		
(1465 Joules/pot)(40 pots per tray)(64 trays)=	3750400	J seed energy input into system
Energy/m^2 in propagation system= (J for system)/(area)=		
(3750400 J)/(5.82m^2) =	644398	J/ m^2 for seed biomass input to system
(40)/(5 pots for each m^2 restored) =	8	m^2 of SAV bed restored for each tray
(8m^2 of SAV bed for each tray)(64 trays) =	512	m^2 of SAV bed restored for system

*Zinecker (2009) unpublished
** Beck et al., 2001

9b/ *Seeds (USD basis) (1lb bag wrack + seeds and water		
(0.45 kg FW or 450g) @ $5/bag**))		
~50% of bag is seed, thus: cost = 225g @ 5$ USD		
Total grams seed needed for system:	5,120	g seed
(5120)/(225 g/bag) = 22.75	~23	bags system total, or 22.75 bags
(5$USD)/(225g)=	2.2E-02	USD$ price of one gram of seed
35 seeds weighing	2	g FW each pot
(0.022 USD)(2 g/ seeds per pot)=	0.044	USD$ price of seeds, (2g) in one PHA pot
Price for system pots: (one pot $)(no. pots)(no trays):		
(0.044 USD)(40 pots)(64 trays)=	115	USD$ / system for 23 450g bags seed
(or round up to 23 bags seed		
Cost per m^2 for propagation system:		
(115 USD)/(5.82 m^2)=	19.75	USD$/$m^2$ for seeds for the system
Cost/m^2 for SAV bed restoration:		
(115 USD)/(512 m^2)=	0.225	USD$ per m^2 restored

Various other values for seeds:
Seeds g amnt per year 3.45E+03g
(sej/unit)=1.11E+09
Fogelberg 2005 sej/yr E12: 3.8

*Propagule sources for seeds (prices may vary):
Kester Wild Game Food Nurseries, Inc.
http://www.kestersnursery.com/Wetland%20Plant's.htm
**Recommendation for broadcast seeding
2.25-4.5 kg (Per 0.405 ha=1 acre)

10a/ Plastic: Propagation/growout trays		
(grams plastic for one tray)	21	g plastic for one tray
(64 total trays) =	1,344	g system total including replacement period
/ replacement period=	5	yr
(1344g system total)/(5 years)=	2.688E+02	g/yr system total

Plastic required per m^2 for system:		
268.8g/yr/(5.82m^2) =	4.62E+01	g/yr for each m^2 of propagation system
Plastic required per m^2 SAV bed restored:		
268.8g/yr/(512 m^2) =	**5.25E-01**	**g/yr for each m^2 of SAV bed restored**
10b/ Plastic USD basis: Propagation/growout trays		
($3.00 each)(64 total)=	192	$USD for trays for system
/ five years life of plastic trays	38.4	$USD/year
For each m^2 of propagation system		
$USD 38.4/(5.82 m^2) =	6.60	$USD/yr plastic for each m^2 of system
For each m^2 of SAV restored		
$USD 38.4/(512 m^2) =	**0.075**	**USD/yr plastic tray for each m^2 restored**
11a/ *PHA Plastic pots –		
Mass of 1 pot **=**	3.75	g
For 1 m^2 restoration=	5	PHA pots each 1m^2 at restoration site
(3.75g)(5 pots for 1m^2 restoration)=	**1.875E+01**	**g of PHA pots for each m^2 SAV bed restored**
Total mass (g) PHA pots for system:		
(3.75g)(40 pots in ea tray)(64 trays total)=	9.6E+03	g/yr for propagation system total
Total mass (g) PHA pots per m^2 for system:		
(9.6E+03g)/(5.82m^2)=	1.649E+03	g/yr for each m^2 of propagation system
11b/ PHA Plastic pots $USD basis		
Price per 1 pound PHA resin:	4.00	$USD per pound PHA resin
1 lb=	453.592	g
PHA resin price per gram=	8.8E-03	$USD per g
1 PHA pot**=**	3.75	g
Price 1 PHA pot=		
(3.75g)(8.8E-03)=	3.3E-02	$USD resin ea PHA pot (3.3 cents per pot)
PHA resin price system total:		
($USD 0.033)(40 pots/tray)(64 trays) =	84.48	$USD PHA pots for system/yr & tot input
PHA resin price per m^2 for propagation system area:		
($USD 84.48)/(5.82 m^2)=	14.52	$USD resin cost/$m^2$ for propagation area
($USD 84.48)/(512 m^2)=	0.165	$USD resin cost/$m^2$ for SAV bed restored
**PHA pot mold manufacturing costs:		
Cost of mold:	4000.00	$USD for mold for total pot production
Lifespan of mold:	20	years
(Cost of mold life)(cost of mold 1 yr)=		
($USD 4000)/20 years life=	200.00	$USD mold cost/yr propagation system
($200)/(5.82 m^2 prop. area)=	34.36	$USD / m^2 mold cost for propagation area
($200)/(512 m^2 SAV area restored)=	0.391	$USD / m^2 SAV bed restored
PHA pot injection molding costs:		
Cost of set-up (flat fee from company):	450.00	$USD /yr for pot production in prop. system
Mold run price/unit	1.00	$USD for each pot
Total mold run cost for pots=		
(40 pots)(64 trays)($1.00 ea pot)=	2560.00	$USD mold run costs
Total pot production and output $USD Basis=		
(2560+450)=	3010.00	$USD mold run / flat fee total system inputs
($3010)/(5.82 m^2 prop. area)=	517.18	$USD / m^2 mold cost for propagation area
($3010)/(512 m^2 SAV area restored)=	5.87	$USD / m^2 SAV bed restored
Total costs of PHA pot USD basis:		
(resin)+(cost of mold)+(mold injection/production costs)=		
($84.48)+($4000)+($3010)=	7094.48	$USD for total costs/startup plus first year
Total cost first year		
(84.48)+(200.00)+(3010)=	3294.48	$USD cost for 1st yr total prop. system
(3294.48)/(5.82 m^2)=	566.06	$USD cost for each m^2 of prop. system
Cost per m^2 for SAV bed area		
($3294.48)/(512$m^2$)=	**6.43**	**$USD PHA cost per m^2 SAV bed restored**

*no need for replacement as pots biodegrade after four months

**Mold manufacture and injection expertise provided by T. Walworth and N. Shannahan, personal conversations

Purchased services

12a/ Labor for planting PHA pots

Total labor input:

6 days (8 hour days) =	6	days
Kcal per human Conversion/day =	2500	kcal/person/day
(6 days)(2500 kcal Conversion)=	15000	kcal/day for system total
(Kcal/day system total)(4186 joules in kcal)=	62790000	J/day for system total
(J/day system total)/(propagation area)=		
(62790000 J/day)/(5.82 m^2)=	1.08E+07	J/day/ Labor energy for each m^2 for propagation
(J/day system total)/(area restored)=		
62790000/512 m^2 =	**1.22637E+05**	**J/m^2/year Labor to restore SAV bed**

12b/ Labor for planting PHA pots
USD basis

Total labor input:		
(8 hours)(6 days)(1 employee) =	48	hrs
(48hrs)($20/hr) =	960.00	USD$ for system
Labor input per m^2 of area restored		
(960USD$)/(5.82 m^2) =	165.00	USD$/$m^2$/yr labor cost for the propagation
Labor input per m^2 of area restored		
(960USD$)/(512 m^2) =	**1.88**	**USD$/$m^2$/yr for the SAV bed restored**

13/ Shipping

UPS Ground	40.00	$USD/year Total costs shipping for system
Shipping costs/unit area=		
Total system shipping costs /area of system		
($USD40.00)/(5.82$m^2$) =	6.87	$USD/$m^2$/yr shipping seeds for system
SAV Bed restored:		
($USD40.00)/(512$m^2$) =	**0.078**	**$USD/$m^2$ SAV bed restored**

14a/ Total Output = Results of Emergy Inputs
(Dry Weight (DW) basis for seed inputs
ENERGY

Average empirical P. Perfoliatus individual seed mass (DW):	0.0028	g DW each seed
*Average kcal in each seed:	0.01	kcalories ea seed
Kcal in each gram of seed = (0.01kcal/seed)/(0.0028gDW)=	3.57	kcal per 1 gDW of seed
Approx. No. *P. perfoliatus* seeds in each PHA pot:	35	seeds
Kilocalories each PHA pot = (0.01)(35)=	0.35	kcal/ of seeds each PHA pot
Energy in each PHA pot – $kcalJ^{-1}$ conversion:	4186	kcal/joule
(0.35 kcal /one pha pot)(4186kcal/J)=	1465.1	J seed energy each PHA pot
Total J in propagation system:		
(1465 J seed E/pot)(40 PHA pots/tray)(64 trays)=	**3750656**	**J seed energy in propagation system**
Empirical DW seeds per PHA pot (0.0028g/seed)(35seeds)=	0.098	g/seeds DW each PHA pot
Mass of seeds each m^2 restored SAV bed:		
PHA pots deployed for each m^2 restored SAV bed=	5	PHA pots
(0.098 g/pot)(5 PHA pots)=	**0.49**	**g/DW seeds per m^2 restored SAV bed**
Total input of seed mass gDW into propagation system:		
(mass g DW/pot)(no pots/propagation tray)(no. of prop. trays)=		
(0.098gDW)(40 PHA pots)(64 plastic propagation trays)=	**250.88**	**g DW seeds input in propagation system**
Area of Propagation:	5.82	m^2
Energy/m^2 in propagation system=(J for system)/(area)=		
(3750656 J)/(5.82m^2) =	64442.6	J/m^2 DW seed energy for propagation sys.
Energy output per m^2 restoration area:		
(3750656 J)/(512 m^2) =	7325.5	**J/m^2 DW to restore each m^2 SAV bed**

*Calorie King: Yellow Mustard seeds, quinoa, flax seed:
Compare 4-5 kcal per one g, or 0.01-0.015 kcal/seed

14b/ Total Cost of USD PHA pot propagation phase Based on inputs

Total Budgetary Initial investment:		
Total cost of SAV system restored per year:		
Similar to Yield, the sum of all inputs yielded/		
m^2 SAV bed restored (added inputs):	8.75	$USD to restore each m^2 SAV bed
Cost/ m^2 propagation trays area:	769.69	$USD m^2 cost
Total cost of SAV system annually:	4479.29	$USD operating cost annually
Total initial expenditure/startup for first year:	8442.85	$USD init. invest+1st year propagation

Appendix 7. Footnotes to Table 4.5: SAV restoration deployment using PHA biodegradable pot method.

Source and calculation	Value	Units
Renewable Sources		
1/ Sunlight		
Restoration **Area (A)**:(8 m)(64 m) =	512	m^2
***Insolation (I)** (April):	5.0	kWh $m^{-2}day^{-1}$
Insolation conversion to J (I_J): 1kWh = 3.6E+06J		
(5.0kWh $m^{-2}day^{-1}$)(3.6E+06 J)=	1.8E+07	J m^{-2} day^{-1}
****Albedo (**α**) /Albedo Correction**(1-α): (1-0.1)=	0.9	
(I) x (1-α)=		
(1.8E+07J m^{-2} day^{-1})(0.9)	1.62E+07	J m^{-2} day^{-1}
Energy input= [(I_J) x (1-α)]x(A)(365 days)		
(1.62E+07)(512)(365) =	3.02756E+12	Jyr^{-1} system total
Energy input per $1m^2$: (3.02756E+12Jyr^{-1})		
/ (512 m^2) =	**5.913E+09**	**Jyr^{-1} / m^2 SAV bed restored**
Total Cost Sunlight USD$:	0	USD$ total and per m^2 SAV bed restored
*(NREL 2012) http://www.nrel.gov/gis/images/map_pv_us_april_dec2008.jpg		
**(Holman 1997)		
2/ Tides		
Restoration **Area**:(8 m)(64 m) =	512	m^2
Tidal Range	0.45	m
Density of water, (15 psu) :	1005.9	kgm^{-3}
Gravity	9.8	ms^{-2}
Tides per year	730	yr
Tidal Energy (J) absorbed per year=		
(area elevated)(0.5, center of gravity)(tides/yr)		
(height^2)(density)(gravity)=		
($512m^2$)(0.5)(730 tides/yr)(0.45m/tide)^2(1005.858 kg/m^3)		
(9.8 ms^{-2})=	3.73035878E+08	J/yr
(3.73035878E+08J/yr)/(512 m^2)=	**7.28585E+05**	**J/m^2/yr SAV bed restored**
Total Cost Precipitation USD$	0	USD$ total and per m^2 restored
3/ River channel velocity/flow		
Restoration Site Base Parameters		
Restoration area (A)=(8m)(64m)	512	m^2
Restoration Site Depth=	1	m
Restoration Site Width=	8	m
Site Cross sectional channel area (A)=(1m)(8m)	8	m^2
Wetted Perimeter (wp)=(1m)+(8m)+(1m)	10	m
Pre-restoration Velocity (*u*) / Flow (Q)		
Avg site velocity (*u*) pre-restoration:		
u=[(*k*)($Rh^{2/3}$)($S^{1/2}$)]/[n], where		
k –SI (int'l system) unit conversion factor for metric	1	(m)
Rh - hydraulic radius=cross sectional area (A)/		
Wetted perimeter (wp): Rh = (A/*wp*)		
Rh=(8m/10m)	0.8	slope
S* – slope	0.0006	m/m
*n***–Manning's roughness coefficient		
for mud bottom	0.03	unitless
Avg Site velocity=		
u=[(*k*)($Rh^{2/3}$)($S^{1/2}$)]/[n]= [(1)($0.8^{(2/3)}$)($0.0006^{(1/2)}$)]		
/ [0.03] =	0.704	m/s
Flow rate (Q, volume) pre-restoration=		
Q = (*u*)(A) = (0.704m/s)($8m^2$)=	5.629	m^3/s system total
Q_{annual} = (Q)(Number of seconds in one year) =		
Q_{annual} = (5.63 m^3/s)(3.15569E+07 seconds/yr) =	1.77665347E+08	m^3/yr system total
Q_{annual/m^3} = (Q_{annual})/(restoration area) =		
Q_{annual/m^3} = (7.8892250E+07m^3/yr)/($512m^2$)=	3.47003E+05	m^3/yr/per one m^3 pre-restoration

Post-restoration Velocity (*u*) / Flow (Q) Ranges based on manning's n varying with vegetation density

Avg site velocity (*u*) pre-restoration:		
$u=[(k)(Rh^{2/3})(S^{1/2})]/[n]$, where		
k –SI (int'l system) unit conversion factor for metric	1	(m/s)
Rh - hydraulic radius=cross sectional area (A)/		
Wetted perimeter (wp): Rh = (A/*wp*)		
Rh=(8m/10m) =	0.8	m
S* – slope	0.0006	m/m
*n****–Manning's roughness coefficient:		
n for low biomass =	**0.03-0.04**	unitless
n for high biomass =	**0.25-2.25**	unitless
Avg Site velocity=		
(low biomass) u_{L1}=$[(k)(Rh^{2/3})(S^{1/2})]/[n]$=		
$[(1)(0.8^{(2/3)}(0.0006^{(1/2)})]$ /[**0.03**]=	0.704	m/s
(low biomass) u_{L2}=$[(k)(Rh^{2/3})(S^{1/2})]/[n]$=		
$[(1)(0.8^{(2/3)}(0.0006^{(1/2)})]$ / [**0.04**]=	0.5277	m/s
(high biomass) u_{H1}=$[(k)(Rh^{2/3})(S^{1/2})]/[n]$=		
$[(1)(0.8^{(2/3)}(0.0006^{(1/2)})]$/ [**0.25**]=	0.0844	m/s
(high biomass) u_{H2}=$[(k)(Rh^{2/3})(S^{1/2})]/[n]$=		
$[(1)(0.8^{(2/3)}(0.0006^{(1/2)})]$/ [**2.25**]=	0.0094	m/s
Flow rate (Q, volume) post-restoration		
(low biomass):		
$Q_{L1} = (u)(A)= (0.7036m/s)(8m^2)=$	5.63	m^3/s system total
$Q_{L2} = (u)(A)= (0.5277m/s)(8m^2)=$	4.2215	m^3/s system total
$Q_{L1annual}=(5.63\ m^3/s)(3.15569E+07\ s/yr)=$	1.77627478E+08	m^3/yr system total
$Q_{L2annual}=(4.2216\ m^3/s)(3.15569E+07\ s/yr)=$	1.33220609E+08	m^3/yr system total
$Q_{H1} = (u)(A)= (0.0844m/s)(8m^2)=$	0.6752	m^3/s system total
$Q_{H2} = (u)(A)= (0.0094m/s)(8m^2)=$	0.0752	m^3/s system total
$Q_{H1annual}=(0.6752m^3/s)(3.15569E+07\ s/yr)=$	2.1307218E+07	m^3/yr system total
$Q_{LH2annual}=(0.0752\ m^3/s)(3.15569E+07\ s/yr)=$	2.373078E+06	m^3/yr system total
$Q_{annual/m^3} = (Q_{annual})/$(restoration area) =		
$Q_{annualL1/m^3} = (1.77627478E+08m^3/yr)/(512m^2)=$	3.46929E+05	m^3/yr/per one m^3 post-restoration
$Q_{annualL2/m^3}= (1.33220609E+08m^3/yr)/(512m^2)=$	2.60197E+05	m^3/yr/per one m^3 post-restoration
$Q_{annualH1/m^3} = (2.1307218E+07m^3/yr)/(512m^2)=$	4.1615E+04	m^3/yr/per one m^3 post-restoration
$Q_{annualH2/m^3} = (2.373078E+06m^3/yr)/(512m^2)=$	4.635E+03	m^3/yr/per one m^3 post-restoration
Mass of water per year		
Before SAV restoration		
H_2O mass (m_{H2O}) =		
(YearlyFlowRate)(Density H_2O)=		
$(Q_{annual})(\rho_{H2Obrackish})=$		
H_2O mass (m_{H2O}) =		
$(1.77665347E+08m^3/yr)(1005.858\ kgm^{-3})=$	1.787061106E+11	kg/yr
Kinetic Energy (KE) pre-restoration		
KE=1/2 $(m_{H2O})(u^2)=$		
$KE=0.5(1.787E+11\ kg/yr)(0.704\ m/s)^2=$	6.29024E+10	J/yr
Mass of water per year		
After SAV restoration		
H_2O mass (m_{H2O}) =		
YearlyFlowRate(Q_{annual})(Density H_2O $\rho_{H2Obrackish}$)=		
H_2O mass (m_{H2O}) =		
$Q_{L1annual=}$ $(1.77627478E+08m^3/yr)(1005.858\ kgm^{-3})=$	1.78668E+11	kg/yr
$Q_{L2annual}= (1.33220609E+08\ m^3/yr)(1005.858\ kgm^{-3})=$	1.340010153E+11	kg/yr
$Q_{H1annual}= (2.1307218E+07\ m^3/yr)(1005.858\ kgm^{-3})=$	2.143203568E+10	kg/yr
$Q_{LH2annual}= (2.373078E+06m^3/yr)(1005.858\ kgm^{-3})=$	2.38697491E+09	kg/yr
Kinetic Energy (KE) post-restoration		
KE=1/2 $(m_{H2O})(u^2)=$		
KE(**u_{L1}**)=$0.5(1.78668E+11\ kg/yr)(0.704\ m/s)^2=$	6.2891136E+10	J/yr
KE(**u_{L2}**)=$0.5(1.340010153E+11\ kg/yr)(0.5277\ m/s)^2=$	3.535616789E+10	J/yr
KE(**u_{H1}**)=$0.5(2.143203568E+10\ kg/yr)(0.0844\ m/s)^2=$	9.04431905E+08	J/yr
KE(**u_{H2}**)=$0.5(2.38697491E+09\ kg/yr)(\ 0.0094\ m/s)^2=$	1.1218782E+07	J/yr
$KE_{(abs)}$ absorbed by restoration vegetation		
At different densities=		
($KE_{(pre\text{-}rest)}$ of flow entering site) –		
($KE_{(post\text{-}rest)}$) of flow leaving the site) =		

$KE_{(abs)}$= ($KE_{(pre\text{-}rest)}$) - ($KE_{(post\text{-}rest)}$) =		
$KE_{(pre\text{-}rest)}$: (6.29024E+10 J/yr) –		
($KE_{(post\text{-}rest\ uL1)}$): (6.2891136E+10 J/yr) =	~0	J/yr
($KE_{(post\text{-}rest\ uL2)}$): (3.535616789E+10 J/yr) =	2.754623211E+10	J/yr
($KE_{(post\text{-}rest\ uH1)}$): (9.04431905E+08 J/yr) =	6.19979681E+10	J/yr
($KE_{(post\text{-}rest\ u_{H2})}$): (1.1218782E+07 J/yr) =	6.289118122E+10	J/yr
Energy per unit area= ($KE_{(abs)}$)/A		
$KE_{(abs\ -\ uL1)}$: (0)/(512m^2) =	0	J/m^2/yr
$KE_{(abs\ -\ uL2)}$: (2.754623211E+10 J/yr**) / (**512m^2)=	5.3801235E+07	J/m^2/yr
$KE_{(abs\ -\ uH1)}$: (6.19979681E+10 J/yr) / (512m^2) =	1.21089781E+08	J/m^2/yr
$KE_{(abs\ -\ uH2)}$: (6.289118122E+10 J/yr) / (512m^2) =	**1.22834338E+08**	**J/m^2/yr**
Total Cost USD	0	USD$ total and per m^2 SAV bed restored

*http://www.charts.noaa.gov/InteractiveCatalog/nrnc.shtml?rnc=12270
**http://www.engineeringtoolbox.com/mannings-roughness-d_799.html
***Champion and Tanner 2000

Semi-Non-renewable Resources

4a/ *Seeds/PHA pot system (mass or J basis)		
Total Input of seeds and pots to restoration site:		
From Table 4.4, Item No. 14, System Output		
Seeds in each PHA pot:	35	seeds
Approximate g DW seeds each pot:	0.098	gDW each pot
No pots each m^2=	5	PHA pots + seeds to restore ea m^2 SAV bed
No seeds total each m^2 SAV bed restored:	175	seeds/m^2 SAV bed restored
***(5 pots)(0.098 - mass of 35 seeds per pot)=**	**0.49**	**gDW/m^2 SAV bed restored**
No. plastic propagation trays available:	64	plastic propagation trays
No. pots fitting in each tray	40	
(40)/(5 pots for each m^2 restored) =	8	m^2 of SAV bed restored per propagation tray
(8m^2 of SAV bed for each tray)(64 trays) =	512	m^2 of SAV bed restored for system
For total gDWseeds in deployed to site:		
(0.098 gDW each pot)(40 pots each tray)(64 trays)=	2.51E+02	gDW seeds deployed to restoration site
* See Appendix 6, Zinecker 2009 unpublished, mass ave. each seed: 0.0028g		
4b/ Seed/PHA pot system (USD basis)		
From previous seed/pha pot propagation system:		
(Table 4.5, Appendix 6), Est. cost/m^2	8.75	$USD/$m^2$ restored SAV bed
Total annual cost for system	769.69	$USD/ m^2 propagation area (5.82 m^2)
Total Cost to Restore 512m^2 SAV=		
(8.75$/$m^2$)(512 m^2) =	4479.36	$USD cost annually for SAV restoration
5a/ PVC for fencing uprights		
¾ in pvc OD: 1.05 in (2.667 cm), ea 2.183lbs (990.19 g)		
Five (5) ten ft (304.8cm) pvc poles= (5)(990.19g)=	4950	g for system total without depreciation
(4,950 g)/(20 yrs) life of PVC=	247.5	g pvc per year without depreciation
*Replacement rate: 25% of pipe every five years (assume 4 replacements in 20 years)		
(4x(0.25 x 247.5))/20 yrs =	12.38	g PVC replaced in 20 years
Total annual mass input of PVC/yr: (247.5g pvc + 12.38 g pvc) =	259.88	g pvc per year
(Mass (g) / year) / (area restored) = (259.88)/(512 m^2):	**0.508**	**g pvc /m^2/yr SAV bed restored**
5b/ PVC for fencing uprights USD basis:		
¾ inch pvc 10 ft. ($2.67)		
Five (5) 3.048m (1.05 inch OD) x (10 ft) pvc pipe ($2.67)(5 pipes)=	13.35	$USD system total without depreciation
Life of PVC:	20	years
Costs of PVC parts/yr= (Totl PVC cost)/(20)= ($13.35)/(20 years)=	0.66	$USD/PVC used per year with depreciation
Replacement rate: 25% of pipe every five years (assume 4 replacements in 20 years)		
(4x(0.25x$0.66))/20 yrs =	0.03	$USD/year PVC replaced
Total dollar annual input of PVC/yr: ($USD PVC/year)+($USD PVC replaced)= ($0.66)+($0.03)=	0.69	$USD total per year
($USD/yr)/(area restored) =		

$USD/year ($0.69)/(512 m^2):	1.3E-03	$USD pvc/$m^2$ of SAV bed restored/yr
(PVC Specifications from www.homedepot.com)		
6a/ Plastic construction fencing 8.5lbs/100ft		
(g basis):		
One roll: (3855.54 g)/(30.48m)=126.49 g/m		
Perimeter=8+8+64+64=	144	m linear required
Amount fencing required (grams per meter)(perim)=		
(126.49g/m)(144m)=	18214.14	g fencing for system total system
Replacement period – life of fencing=	5	yrs
Total use per yr=(mass)/(replacement period)=		
(18214.14g)/(5 yrs)=	3642.83	g fencing per year
Plastic per unit area restored		
Per year = Total use per yr /		
Area restored = (3642.83g)/(512 m^2)=	**7.11**	**g/yr for each m^2 of SAV bed restored**
6b/ Plastic, orange, construction fencing (USD basis):		
Total USD exclosure fencing req. for restoration=		
($29.97ea roll, measures 30.48m) =($29.97)/(30.48m)=	0.983	$USD per linear meter cost of fencing
Perimeter=8+8+64+64=	144	m linear required
(144m)(0.98$/m)=	141.55	$USD for system total
Replacement period – life of fencing=	5	yrs
Total $ cost per yr= ($USD)/(replacement period)=		
($141.55)/(five years) =	28.31	$USD fencing cost per year
Plastic cost per m^2 SAV bed restored		
Per year = Total cost per yr /		
Area restored = ($28.31)/(512$m^2$)=	0.06	$USD/yr for each m^2 of SAV bed restored
7a/ Steel fence T-posts (gram basis):		
Ten (10) Uprights for construction fencing (6.484 pounds or		
2941.09 g) = (10)(2941.09)=	29410.90	g system total without depreciation
Replacement period – life of steel posts = 20 yrs		
(System total steel) / (20 years)=		
(29410.9 g)/(20 yrs) =	1470.55	g steel per year
Mass/yr/area restored: (1470.55)/(512 m^2) =	**2.87**	**g steel per year per m^2 SAV bed restored**
7b/ Steel fence T-posts (USD$ Basis):		
Ten (10) Steel fence posts @ $3.67each=	36.7	$USD System total cost of steel fence posts
Replacements: Life of Steel posts:	20	years
Total system costs of steel posts)/yr: ($36.7)/(20 years)=	1.835	$USD cost of steel per year
$USD/year ($1.84) / (512 m^2):	0.0036	$USD steel; used per m^2 of SAV bed
(THD 2016, Ciotala et al. 2011)		restored/yr
8a/ Machinery and Equipment		
Truck for transporting staff, propagules and equipment		
to restoration site average miles drive each year: 13476*		
Life of vehicle @ cost USD 200000 miles / 13476 =	15	years replacement period of vehicle
Mass of vehicle =	1859727	g
Yearly (g) use = Mass of vehicle/replacement period of vehicle=		
1859727 g / 15 years=	123981.8	g used per year for vehicle transport
Annual vehicle mass (g) in system/m^2 total		
123981.8g / 512 m^2 =	**242.15**	**g/m^2/yr machinery-equipment per m^2 SAV bed restored**
8b/ Machinery and Equipment		
For transporting staff, propagules and equipment		
to restoration site, purchased:		
Toyota Tacoma USD basis:	30000.00	USD$
Life of vehicle	15	years
USD$ of Tacoma Truck / Annual use of vehicle =	2000	USD$ of truck per year
USD$ of Tacoma Truck for each m^2 of SAV =		
Annual use of vehicle USD / area restored=		
2000 USD / 512 m^2 =	3.906	USD$ per m^2/yr SAV bed restored
*Average mileage driven per year:		
*http://www.fhwa.dot.gov/ohim/onh00/bar8.htm		
9a/ Fuel, gasoline		
Total gasoline input to travel 50 miles:	2.47	gallons/year
Energy density	124,340	Btu/gallon
Total BTUs annually	341,593	Btu/year

BTUs to Joules conversion=	1055.05585	J/BTU
Yearly Use	360,399,693	J/yr
Fuel consumed per m^2 SAV bed restored		
(360,399,693)/(512 m^2)=	**7.03906E+05**	**J/ m^2/yr fuel use per m^2 SAV bed restored**
9b/ Fuel, gasoline, USD basis		
Total input to travel 50 miles:	2.47	gallons/year
(2.47gal.)(*$USD 2.35/gal)=	5.80	$USD / year round trip site travel
$USD expenditure per unit area=		
($USD 5.80)/(512 m^2) =	0.01	$USD per each m^2 SAV bed restored
10a/ Labor for Restoration planting and installation		
Total labor input:		
4 people x 2 days =	8	days
Kcal per human Conversion =	2500	kcal/person/day
(8 days)(2500 kcal Conversion)=	20000	kcal/day for system total
(Kcal/day system total)(4186 joules in kcal)=	83720000	J/day for system total
(J/day system total)/(area restored)=		
83720000 / 512 m^2 =	**163515.6**	**J/day/m^2 SAV bed restored**
10b/ Labor for restoration planting and installation USD basis		
Total labor input		
Labor USD$		
(8 days)*(8hrs/day)*($20/hr) =	1280	USD$ for restored system
USD / m^2 = 1280 / 512 m^2	2.5	USD$/ m^2/yr for the restored area
11a/ Total Output = Restoration Results of Emergy Inputs		
64 trays, each containing 40 pots with 35 seeds each:	175	seeds/m^2, occupy ~ 40% of m^2 plot in 2 mo.
Initial input: **(x_0)** gDW/m^2=(0.098gDW seeds)(5 pots m^2):	0.49	g DW/m^2 initial biomass input to rest. site
*Estimated growth rate/day/m^2 **(g)** of input propagules:	0.92	g/m^2/day for biomass growth
**Contribution of input biomass to initial fraction of plot	0.20	fraction of plot occupied within first 60 days
Growing season duration **(t)**(input April 15 – Sept 31):	150	days
Final biomass input/m^2 from restoration + 1st season growth=		
NPP_{rs1} (Net Primary Productivity $_{\text{restoration season one}}$) =		
x_0+[(**g*****t**)]= [(0.61gDW/m^2/day)(150days)=	**91.5**	**gDW/m^2 4.5 mo growth SAV restoration**
Total restoration area=	512	m^2
Total output for area restored after season one=		
(91.5 gDW/m^2)(512 m^2)=	46848	gDW biomass total of SAV bed restored
Final energy input per m^2=	3.5	kcal/g in organic matter
J/kcal conversion:	4186	J/kcal
(91.5gDW)(3.5 kcal/g for seagrass)(4186J/kcal)=	**1340567**	**J/m^2 biomass output 1st season restoration**
Total ENERGY for entire SAV area restored=		
(1340567 J/m^2)(512 m^2)=	686370048	J/total biomass energy at restoration site

*Wetzel and Penhale 1983
**Empirical Evidence from Zinecker (CH3) indicates just 4 seeds able
To occupy 20% of one m^2 in two months at growth rate of 0.61 gDW/m^2/day

11b/ Total Output USD: Cost of Restoration Based on $ Inputs		
Similar to Yield, the sum of all inputs yielded/		
m^2 SAV bed restored:	15.23	$USD to restore each m^2 SAV bed
Total cost of SAV system restored per year:	7,795.99	$USD/yr to restore 512 m^2 SAV habitat
Total cost of restoration start-up +1st season	39,920.25	$USD to fund startup+1st restoration

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