ABSTRACT

Title of Document: COMPETITION BETWEEN *HYDRILLA VERTICILLATA* AND *VALLISNERIA AMERICANA* IN AN OBSERVATIONAL FIELD STUDY AND GREENHOUSE EXPERIMENT.

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Invasive species continue to have a pervasive influence on biodiversity but it is often unclear how invasive species affect native species. In field observations and greenhouse experiments, I examined the effect of the non-native submersed aquatic plant *Hydrilla verticillata* on the native species *Vallisneria americana*. Field monitoring from 2002 to 2006 showed that coverage of species peaked in 2004 after initial invasion of the estuarine study system in 2002. Substrate characteristics did not limit species distribution. In contrast, substrate and planting density affected plant growth and the outcome of intra- and inter-specific competition in the greenhouse. Although other environmental variables, such as water depth and turbidity, appear to override the effect of substrate in the field, the greenhouse experiment suggests that substrate can be an important driver of submersed aquatic plant community dynamics. Sediment characteristics should therefore be a factor in restoration design and the management of invasive species.

COMPETITION BETWEEN *HYDRILLA VERTICILLATA* AND *VALLISNERIA AMERICANA* IN AN OBSERVATIONAL FIELD STUDY AND GREENHOUSE EXPERIMENT.

By

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Dedication

To my mom, Dee McChesney, who despite not always knowing what I was studying, nevertheless always maintained that it was “really important.”

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Chapter 1: Introduction

The rate of invasive species introductions (both intentional and unintentional) has increased dramatically in the last 40 years due to human population growth, global travel and trade, and environmental alterations (Pimentel et al. 2000). Although some of the roughly 50,000 nonindigenous plant and animal species currently in the United States have proven beneficial financially (e.g., introduced food crops and livestock, which provide more than 98% of the United States' food production and generate approximately $800 billion per year; United States Bureau of the Census 1998), invasive species of plants and animals cost an estimated $137 billion per year in economic and environmental damage within the United States alone (Pimentel et al. 2000), and can negatively affect native species and ecosystems (Flory and Clay 2010).

Of the 1,376 species of plants and animals in the United States that are listed under the Endangered Species Act as either threatened or endangered (United States Fish and Wildlife Service 2010), approximately 400 species of plants and animals are considered to be so designated owing primarily to competition with or predation by invasive species (Wilcove et al. 1998). Globally, the number of endangered species threatened by invasive species may reach 80% in certain regions (Armstrong 1995). Within the United States, there are approximately 17,000 species of native vascular plants (Morin 1995), and an additional 5,000 introduced plant species that have escaped domestic control and now invade natural ecosystems (Morse et al. 1995).

Invasive plant species often grow in monotypic stands (Cross and Fleming 1989), displace native species (Chambers et al. 1993), threaten biodiversity (Adams and Engelhardt 2009), disrupt food webs, nutrient cycles, and biogeochemical and

hydrological processes (Wigand et al. 1997, Farnsworth and Ellis 2001), and interfere with the use of areas by other species (Lodge 1993a, Lodge 1993b, Williamson 1996, Vitousek et al. 1997, Pimentel et al. 2000). Community diversity, environmental variability (Davis et al. 2000, Havel et al. 2005), species traits, abiotic conditions of the habitat, and propagule pressure (Levine 2000, Chadwell and Engelhardt 2008, Jacobs and Macisaac 2009) may all influence the invasibility of an ecosystem (Thomaz et al. 2009). Thus, understanding habitat requirements of invasive species and their interactions with native species is paramount to effectively managing invaded ecosystems.

Submersed Aquatic Vegetation

Although environmental degradation is widespread, estuaries may be some of the most degraded systems because they have been the focus of human colonization for centuries (Edgar et al. 2000, Beck et al. 2001). Within estuaries, submersed aquatic vegetation (SAV) serves as an indicator of local water quality conditions (Dennison et al. 1993), improves water clarity (Ruhl and Rybicki 2010) and stabilizes sediments (Koch and Gust 1999, Orth et al. 2006), reduces water current velocity (Madsen and Warnke 1983, Madsen et al. 2001), takes up nutrients (Wigand et al. 1997, Moore and Wetzel 2000, Dierberg et al. 2002), provides food for migratory and resident waterfowl (Perry and Deller 1996), and provides habitat for economically important species such as crabs and fish (Thayer et al. 1975, Richardson et al. 1998). Thus, declines in SAV coverage have significant ecological and economic consequences (Kahn and Kemp 1985, Waycott et al. 2009).

Major SAV declines occurred in Chesapeake Bay and its tributaries from the mid-1960s through early 1980s (Haramis and Carter 1983; Orth and Moore 1983, Orth and Moore 1984) and all SAV species inhabiting fresh, oligohaline or mesohaline environments were affected by the decline (Orth and Moore 1984). The distribution of appropriate potential habitat is not equal between fresh, oligohaline or mesohaline environments, and freshwater tributaries have proportionally experienced the greatest declines (Moore et al. 2010). For example, within the Patuxent River estuary, approximately 20.9 km^2 capable of supporting SAV (water depths <1m) exist in the mesohaline zone, 5.8 km^2 in the oligohaline zone, and only 2.0 km^2 in the tidal fresh zone (Stankelis et al. 2003). Some studies have found that SAV formerly grew to depths greater than 2m (Moore et al. 1999), whereas 2m is now considered the maximum depth of SAV occurrence in Chesapeake Bay (Kemp et al. 2004). Thus, pre-decline SAV coverage may have been even greater than originally estimated. However, even in the 1950s, when SAV covered more area, only 20-30% of possible SAV habitat within the lower Patuxent River was vegetated (Manning 1957). Thus, restoration goals may be unrealistic if they seek 100% coverage.

In 2009, SAV covered approximately 85,899 acres throughout Chesapeake Bay (Chesapeake Bay Program 2010a). Although SAV coverage has increased in recent years (Carter and Rybicki 1986, Orth et al. 2008), current SAV coverage still only represents approximately 15% of its estimated historic coverage (Moore et al. 2004) of more than 250,000 hectares (Orth et al. 2002). Furthermore, 85% of the increase in biomass and 71% of the increase in area of SAV between 1985 and 1993 was due solely to increases in the species association dominated by *Zostera marina* but where *Ruppia maritima* could

also be present (Moore et al. 2000). Freshwater mixed communities, which were dominated by the invasive species *Myriophyllum spicatum* and *Hydrilla verticillata*, by contrast, only contributed 27% of the total biomass and 16% of total area (Moore et al. 2000). Although this increase in biomass and area implies that SAV within Chesapeake Bay may be recovering, it also demonstrates that freshwater species do not comprise a majority of SAV within Chesapeake Bay. Specifically, between 1985 and 1996, the greatest area covered by freshwater mixed communities of SAV was 4,887 hectares (in 1990), yet this was only 16% of total Chesapeake Bay SAV area for that year (Moore et al. 2000). Furthermore, although coverage of freshwater SAV is increasing, it is not known how much of the increase in biomass or area of freshwater SAV came from invasive species, which may not provide the same ecological benefits as native freshwater species.

Declines in SAV largely reflect changes in the human population within the Chesapeake Bay watershed, which grew from 5 million people in 1900 to approximately 8 million people in 1950 (Chesapeake Bay Program Scientific and Technical Advisory Committee 2002). In 2003, the population was projected to reach 15 million by 2020 (Ernst 2003), but in 2008 alone, 16,883,751 people immigrated into the watershed (Chesapeake Bay Program 2010b). With this growth in the human population have come changes in land use, such as a decrease in the amount of land used for agriculture (85% in 1850 to only 28.5% in 1994; Chesapeake Bay Program 2010a) and a proportional increase in urban and suburban development (Cooper 1995, Costanza and Greer 1995). These trends are also reflected globally, which has led to an increase in the amount of toxins, sediments, and nutrients reaching estuaries worldwide (Short et al. 1996). When

excessive nutrients, especially phosphates and nitrates, reach coastal waters, they can cause eutrophication, wherein the nutrients promote excessive algal growth followed by low oxygen conditions within the water as the algae die and decompose, which in turn leads to the death of other organisms (Art 1993). Pollutants (including excessive nutrients), along with diseases, nonnative and invasive plant species, and natural disturbances, such as hurricanes, have contributed to eutrophication of coastal waters and the subsequent loss of SAV because of poor water quality (Carter et al. 1994, Kemp et al. 2004, Orth et al. 2006, Waycott et al. 2009).

Hydrilla verticillata

In addition to poor water quality and high nutrient loads, native species of SAV are vulnerable to competition from nonnative invasive species such as *Hydrilla verticillata* L. f. (Royle), an invasive submersed aquatic plant native to tropical Asia and introduced via the aquarium trade to Florida in 1958 (Schmitz et al. 1991, Blackburn et al. 1969) as the dioecious variety, and to the Potomac River basin in the early 1980s through a separate introduction of the monoecious variety (Steward et al. 1984). Within the United States, dioecious female *H. verticillata* occurs in Alabama, Arizona, California, Connecticut, Florida, Georgia, Louisiana, Mississippi, Pennsylvania, South Carolina, Tennessee, and Texas, though the dioecious male is not present in any state. The monoecious strain appears to adapt better to more temperate climates, and is now found in Delaware, Maryland, Washington, and the District of Columbia (Netherland 1997, Colangelo 1998). North Carolina and Virginia remain the only states to contain both the dioecious and monoecious varieties (Netherland 1997). Globally, *H. verticillata* is found in Africa, Asia, Australia, Europe, New Zealand, North America, the Pacific

Islands, and South America, in both temperate and tropical regions (Langeland 1996). Although *H. verticillata* occurs as far north as 40°N within the United States, globally it extends north to near 50°N in Poland and the Soviet Union (Langeland 1996), and thus its range may extend farther north in the United States in coming years.

Much of the reason for *H. verticillata*'s prevalence and rapid proliferation comes from its unique biology and physiology, especially in comparison to native SAV species. *H. verticillata* is able to grow from seeds (for the monoecious biotype), vegetative fragments and root crown regeneration, even after exposure to drought (Silveira et al. 2009), tubers (subterranean turions), or axillary turions (Cook and Luond 1982). Almost 50% of *H. verticillata* fragments with only a single whorl of leaves are capable of sprouting and creating a new plant (Langeland and Sutton 1980), and a single subterranean turion can produce over 6000 new turions within one sixteen-week growing season (Sutton et al. 1992) and 2,803 new axillary turions per m^2 (Thullen 1990). Additionally, subterranean turions can remain viable for several days out of water (Basiouny et al. 1978), for over four years in undisturbed sediment (Van and Steward 1990), after ingestion and regurgitation by waterfowl (Joyce et al. 1980), and after herbicide applications (Haller at al. 1990).

Since *H. verticillata* is an exotic species, few native predators are known to control it within the United States. However, as with most SAV, its range and success are delimited by environmental conditions. For *H. verticillata*, temperature (a minimum of 14°C for physiological activity (Haller et al. 1976)) and photoperiod interact to restrict its range to temperate and tropical zones and to affect its reproduction (McFarland and Barko 1990, Miller et al. 1993). After little to no turion production during the summer

months, shorter photoperiods (<12hr light) in September (in the United States) stimulate turion production, which then decreases during the colder winter months of December and January before again increasing in late spring (Miller et al. 1993). Light intensity may also increase turion production (Mitra 1955, Van der Zweerde 1982), though increased *H. verticillata* shoot density inhibits turion production (Miller et al. 1993). Other studies have indicated that *H. verticillata* may be naturally suppressed in certain lakes due to an unidentified inhibitor (possibly a metabolic product of a cellulose-utilizing organism), which suppresses photosynthesis and increases respiration rates (Barltrop and Martin 1983, 1984; Pompey and Martin 1993).

In addition to natural *H. verticillata* inhibitors, several methods have been developed to combat the growing problem of *H. verticillata* expansion, though most are experimental and have had limited success within natural settings. These include, but are not limited to the following: herbicides (usually with copper, diquat, endothall, or fluridone as active ingredients), encouraged predation by other introduced (or occasionally native) species, and mechanical removal. These treatments all include both positive and negative aspects; for instance, although effective, herbicidal treatments often eliminate all SAV (both native and nonnative species) instead of reducing only invasive species, are not species-specific, can require broad scale and repeated applications, and their effectiveness (for fluridone in particular) can vary depending on application rates, contact times, and the timing of application (Langeland 1996).

The introductions of exotic herbivore or fungal species (particularly triploid grass carp (*Ctenopharyngodon idella)* and the leaf-mining fly *Hydrellia pakistanae*) have also been used to reduce *H. verticillata* coverage, though with variable results. For instance,

introduced *C. idella* have an average longevity range of ten to 15 years (Allen and Wattendorf 1987, Sutton and Vandiver 1986), which means that although they may reduce *H. verticillata* to an intermediate level in small impoundments (Cassani et al. 1995, Schramm and Brice 1998), they can potentially be present within and affect an aquatic system for five to nine years after introduction (Kirk and Socha, 2003). Problems exist with using *C. idella* in that they are not species-specific herbivores (and thus all SAV will be reduced); they may emigrate out of the target areas and out of open reservoir systems (Bain 1993); and they have the potential to impact non-target species (including commercial fishes) for a long period of time (Kirk and Socha, 2003). Likewise, although the highly species-specific leaf-mining larvae of *Hydrellia pakistanae* (native to tropical and temperate regions of Asia) have been shown to cause extensive damage to *Hydrilla verticillata* in laboratory experiments (Baloch and San-Ullah 1974, Deonier 1978, Baloch et al. 1980, Buckingham et al. 1989), impacts on *H. verticillata* from introductions to Florida in 1987 have not exceeded one-fifth of the amount deemed necessary for a significant impact (Wheeler and Center 2001). However, Shabana et al. (2003) have found that combining plant pathogenic fungi (specifically *Acremonium sp.* and *Fusarium culmorum*) with *Hydrellia pakistanae* increases the level of damage to *H. verticillata* beyond that produced by either species alone, though these results remain to be tested in the environment.

Finally, efforts have been made to control *Hydrilla verticillata* through mechanical removal, though this too has had varying degrees of success and environmental impacts. Sites manipulated by mechanical removal of *H. verticillata* in the Potomac River (MD) in 1988 showed increased plant biomass after 23 days compared

with unharvested sites, though species-specific tests indicate that harvesting improved habitat for pelagic fish (Serafy et al. 1994). However, cover-oriented fish were negatively impacted and individuals of ten fish species were killed during the mechanical harvesting, which was estimated to represent 11-22% of total fish abundance in the area, whereas the initial (temporary) decrease in *H. verticillata* biomass was only an estimated 4-23% (Serafy et al. 1994). However, Fox et al. (2002) found that repeated clippings of above-ground *H. verticillata* biomass significantly reduced the number of turions produced (most likely due to the plants' inability to form a surface canopy), which could significantly improve long term *H. verticillata* control.

Although several diverse attempts have been made to control, eradicate, and manage *H. verticillata* in its non-native range, most of these have been extremely expensive and retroactive. Because *H. verticillata* has relatively few environmental controls and is very easily transported (with boats, birds, etc), much more effort needs to be made to educate people about the risks of introducing invasive species to minimize future damage. Since invasions of non-native species are one of the world's greatest causes of species extinctions (Sala et al. 2000) and estuaries are among the most vulnerable systems (Wasson et al. 2002), this issue must be addressed before greater ecological damage occurs.

Although *H. verticillata* provides some of the same ecosystem services, or benefits supplied to humans through natural processes (Ehrlich and Ehrlich 1981, Daily 1997), as native SAV species (such as habitat and sediment stabilization), these are often overlooked when *H. verticillata* interferes with human structures and activities such as canals and water use. Due to its canopy forming capabilities, lack of natural predators,

and ability to thrive in environmental conditions where other SAV species cannot, *H. verticillata* is especially problematic in drainage canals, irrigation canals, and utility cooling reservoirs. There it clogs intake pumps and greatly reduces water flow, which can result in flooding and damage to the canal banks (Langeland, 1996). Additionally, *H. verticillata* can interfere with navigation and recreational use of invaded areas, which can lead to financial losses. Although controversial, there is some evidence that *H. verticillata* can adversely affect commercially valuable fish species such as largemouth bass when coverage exceeds 30% (Colle and Shireman 1980), though it is also argued that it might benefit largemouth bass habitat (Tucker 1987). Despite the gravity of financially expensive consequences, the ecological consequences of *H. verticillata* invasions may be even more devastating in that *H. verticillata* can displace already declining native SAV populations, which can have long term effects on ecosystem processes.

However, *H. verticillata* may be beneficial for water quality (and thus other aquatic species), in that it can be used to treat wastewater (Tanaka 2006), remove lead (Gallardo-Williams et al. 2002) and fluoride (Sinha et al. 2000), and improve water clarity (Netherland 2007). This has been shown at my study site, Otter Point Creek, where turbidity levels are high at the beginning of the growing season in May and yet are dramatically reduced when *H. verticillata* is at peak biomass in August/September. For example, in 2004 average Secchi disc depth at Otter Point Creek in late May was 0.35m (n = 109), whereas it was 0.87m in late August (n = 111). *H. verticillata's* ability to inhabit areas of poor water quality and ameliorate natural conditions may potentially

facilitate recolonization by native species, though much more research is needed in this area.

Vallisneria americana

Many of the areas historically dominated by the native species *Vallisneria americana* Michx. (wild celery) are now unvegetated or vegetated with non-native species such as *H. verticillata* or *Myriophyllum spicatum* (Eurasian watermilfoil) (Davis 1985, Moore et al. 2000, Orth et al. 2008). However, *V. americana* has also been known to persist in patches within Chesapeake Bay tributaries even during seasons when *H. verticillata* decreases, such as at low spring temperatures and low available sunshine (Rybicki and Carter 1992). *V. americana* is distributed in North America in Canada (in British Columbia, Manitoba, Ontario, and Quebec) and in all of the United States except for Arkansas, Colorado, Hawaii, Kansas, Montana, Nebraska, Utah, and Wyoming (United States Department of Agriculture 2010), and is also found in Mexico, West Indies, Central America, and Asia (Michaux 1803). In contrast to *H. verticillata* and many other canopy-forming freshwater SAV species, *V. americana* is meadow-forming, in that it grows from a basal meristem (Catling et al. 1994). Although *V. americana* does not form a canopy at the water surface like *H. verticillata* (Titus and Adams 1979, Barko and Smart 1981), it does increase its leaf length and width under low light conditions (French and Moore 2003). It exhibits efficient carbon fixation in low light conditions and acclimates rapidly to increased light availability (Titus and Adams 1979), which enables *V. americana* to survive in low light conditions (Boustany 2010).

Similar to *H. verticillata*, *V. americana* is capable of both sexual and asexual reproduction, with asexual reproduction being the dominant form (Sculthorpe 1967). Unlike *H. verticillata*, *V. americana*'s asexual reproduction occurs through the production of rhizomes and stolons, which create new clonal shoots (McFarland and Shafer 2008). In early fall, when leaf production stops in Chesapeake Bay, *V. americana* is also capable of producing tubers (also known as winter buds) that will give rise to new plants the next spring (Sculthorpe 1967). Sexual reproduction takes place at the water surface when female flowers are pollinated (Sculthorpe 1967, McFarland and Shafer 2008), and flowering occurs from July to August, with fruits maturing by September-October (Moore and Jarvis 2007). Each fruit consists of a seed pod, which contains approximately 100-300 seeds (Lokker et al. 1997).

In addition to other benefits that are characteristic of all SAV species, *V. americana* in particular is an important food source for waterfowl throughout its geographical distribution (Korschgen et al. 1988, Sponberg and Lodge 2005), which is why recent SAV restoration efforts have focused on reestablishing it in areas where it historically thrived (Moore et al. 2010). Unfortunately, this also means that herbivory, even more so than water quality or sediment organic matter, may limit the establishment and expansion of *V. americana* (Moore et al. 2010).

Habitat Requirements of H.verticillata

Successful plant invasions (and subsequent losses of native species) can be due in part to the invader's ability to inhabit areas of sub-optimal environmental conditions or to survive across a wider range of environmental conditions compared with native species

(Poulin et al. 2007, Ren and Zhang 2009). An example of this is *H. verticillata*, an invasive species that can grow and thrive in a wide range of environmental conditions, especially when compared with the environmental restrictions of native SAV. *Hydrilla verticillata* can grow in oligotrophic to eutrophic environments (Cook and Luond 1982), whereas native freshwater SAV typically require less than 15mg/L of total suspended solids (TSS), less than 15µg/L of chlorophyll a (chl-*a*), and less than 0.02mg/L of dissolved inorganic phosphorus (Kemp et al. 2004). In fact, the invasion of exotic plants into either aquatic or terrestrial systems is often an indication of nutrient enrichment (Van et al. 1999, Engelhardt et al. 2009). Additionally, *H. verticillata* can grow in water with up to 7% of the salinity of seawater (Haller et al. 1974), and it tolerates a wide pH range, though it grows best at pH 7 (Steward 1991). Unlike native freshwater species of SAV, which typically require a minimum of >9% light at leaf (PLL), as well as >13% light in the water column (PLW) for growth and survival (Kemp et al. 2004), *H. verticillata* requires only low light levels for photosynthesis (< 1% of full sunlight; Van et al. 1976, Bowes et al. 1977). Due to its low light requirement, *H. verticillata* is able to begin photosynthesis earlier in the morning compared with other species of SAV and to inhabit greater water depths (Langeland 1996). In Brazil, *H. verticillata* has also been found to colonize deep areas and sites highly disturbed by waves (Thomaz et al. 2003), although studies of wave action and water currents affecting SAV within the United States have focused primarily on saltwater species such as *Zostera marina* and *Ruppia maritima* (*see* Wicks et al. 2009, Koch et al. 2010).

In addition to water depth, factors further limiting light availability (and therefore SAV growth) include increased concentrations of total suspended solids and chlorophyll-

a from plankton (which act as physical barriers to light), and dissolved inorganic nitrogen and dissolved inorganic phosphorus, which promote algal growth (Kemp et al. 2004). All of these factors have increased since human settlement of coastal zones (Environmental Protection Agency 1998). *Hydrilla verticillata* can switch from using free carbon dioxide (CO_2) (when available in the surrounding water) to bicarbonate if high pH or high carbonate concentrations exist (Salvucci and Bowes 1983). Bicarbonate uptake is balanced by production and excretion of hydroxyl ions across polarized leaves. This is a common process in SAV beds (Titus and Stone 1982; Madsen and Sand-Jensen 1991) and increases the pH of the water column more than when CO_2 is used as the sole inorganic carbon source. Because the process is energetically expensive, species differ in their capacity to grow at high water pH. For example, *V. americana* shoot growth was suppressed by 54% at pH 9.8-10.0, whereas *Elodea canadensis* and *Stuckenia pectinata* exhibited net shoot and root growth (James 2008). Unfortunately, a direct comparison of bicarbonate uptake has not been done for *V. americana* and *H. verticillata*. Up to 90% of *H. verticillata*'s tissues are composed of water (Van et al. 1976), which allows it to elongate very rapidly (up to an inch per day) until it reaches the surface, where it branches and creates a cover shading other species of SAV (Langeland 1996). Despite all of the information known about *H. verticillata*'s growing tolerances with regard to nutrients and light, little is known about sediment requirements for *H. verticillata* or for native species.

Sediments

Most SAV restoration efforts and experiments have focused on light (Hall et al. 1999), water quality (Dennison et al. 1993), water depth (Arnold et al. 2000, Kemp et al. 2004), available nutrients (Rybicki et al. 2001), and historical distribution of SAV (Stankelis et al. 2003). Sediments have received less attention (Koch 2001). However, growth, morphology, and distribution of SAV, nutrient concentration in sediment interstitial spaces, and sediment density have been shown to be highly correlated (Barko and Smart 1986, Demas et al. 1996, Koch 2001, Livingston et al. 1998, Short 1987, Touchette and Burkholder 2000).

Sedimentation has been implicated in the elimination of SAV in the tidal Potomac River in the late 1930s (Rybicki and Carter 1986). Season in which deposition of sediment occurs is also important, in that SAV seedlings are more susceptible to high burial rates than are established plants (Marba and Duarte 1994, Koch 2001). For instance, deposition of more than 10cm of sediment on top of *Vallisneria americana* tubers has been shown to reduce their chances of becoming mature plants and establishing meadows (Rybicki and Carter 1986).

In addition to sedimentation rates, sediment type can play an important role in freshwater SAV biomass and distribution (Pond 1905, Misra 1938, Barko and Smart 1983, Koch 2001, Moore et al. 2010). For example, Posey et al. (2003) showed that sediments comprised of 40% silt and clay (<63μm) are associated with healthy beds of *H. verticillata* and *V. americana*, whereas beds of *H. verticillata* alone had 58% silt and clay. *Vallisneria americana* has been associated with sediments comprised of 14% silt and 14% clay (Hutchinson 1975). Nutrient availability varies with sediment type, with

coarse sediments generally exhibiting lower nutrient availability than fine sediments (Idestam-Alquist and Kautsy 1995).

SAV beds modify local conditions by attenuating water current velocity and wave action, thus leading to increased sedimentation in addition to accumulation of organic matter from the plants themselves (Koch 2001). Although SAV contributes to sedimentation and organic matter accumulation, it can also be negatively impacted by these processes (Koch 2001). Typically, the growth of SAV is limited to sediments containing less than 5% organic matter (Barko and Smart 1983), although the mechanism behind this limitation is unclear (Koch 2001). However, *H. verticillata* biomass has been shown to be more affected by sediment density than sediment organic matter (up to 20%), and to decrease when grown in inorganic sediments of greater than 75% sand (Barko and Smart 1986). By contrast, sediment organic matter in healthy beds of *V. americana* is typically less than 6.5% (Hutchinson 1975).

Despite the demonstration of sediment deposition, sediment type, and organic matter as important factors in determining the growth, morphology, and distribution of SAV (Livingston et al. 1998, Koch 2001), much of the research has focused on marine species. Very few experiments have been done with freshwater species (but see Barko and Smart 1983, 1986), with little to no research to determine optimal habitat for restoration of individual freshwater species (see Koch 2001, Boustany 2010). Although nutrients, water current velocity, and sediments are all inextricably tied, the scope of my study focused on the role of sediments and their associated nutrients on affecting the biomass of freshwater SAV and their intra- and inter-specific interactions.

Morphological Differences and Nutrient Uptake Abilities of SAV

Declines in native SAV in areas that are invaded by non-native SAV could be due to the different morphologies and nutrient uptake abilities of native and invasive SAV species at low and high nutrient concentrations. For example, Barko (1982) found that whereas nitrogen (N) and phosphorus (P) are readily mobilized from sediments by submersed macrophytes, potassium (K) appears to be taken in by SAV from open water at a much greater rate than through the sediments (especially for *H. verticillata*). Furthermore, *H. verticillata* may rely more on open water than sediments for P, whereas *V. americana* relies almost exclusively on the sediment for P (Wigand et al. 1997). This has important implications for competition between *H. verticillata* and native SAV, especially because phosphorus may be limiting to SAV growth in freshwater sediments (Rattray et al. 1991, Wigand and Stevenson 1994), and potassium may also be limiting to freshwater macrophytes (Anderson and Kalf 1988).

Vallisneria americana has a greater root-to-shoot ratio than *H. verticillata*, which allows *V. americana* to anchor itself within areas of faster water currents and to reach more nutrients within the sediment in times and areas of low nutrient concentrations. *V. americana* has also been shown to oxygenate the surrounding sediment more than *H. verticillata* (Wigand et al., 1997). These effects of a native species on ecosystem processes may facilitate the spread of SAV. In contrast, *H. verticillata* is a canopy former (with most biomass at the surface of the water column) and thus it can shade other meadow-forming species (with most biomass near the sediment surface) in already light-limited environments. Alternatively, owing to its interactions with the water column, *H.*

verticillata may increase water clarity and may therefore facilitate the colonization of other SAV species.

Competitive abilities of different species of SAV at low and high nutrient levels may differ and be affected by sediment grain size. For example, at high sediment nutrient concentrations, *H. verticillata* is a stronger competitor relative to *V. americana*. In contrast, at low sediment nutrient concentrations, *V. americana* is the dominant species (Van et al. 1999). The deep roots of *V. americana* (Wigand et al. 2001) may allow it to anchor itself even within areas of high water current velocities and to reach nutrients beyond the range of *H. verticillata* roots. Additionally, *V. americana* within Chesapeake Bay can have mycorrhizal associations that facilitate phosphorus uptake (Wigand and Stevenson 1994), whereas a literature review shows that *H. verticillata* has never been reported with mycorrhizal associations.

Low nutrient concentrations in the water column stimulate the formation of axillary turions and flowers in *H. verticillata*. In contrast, at high nutrient concentrations in the water column, *H. verticillata* expends most of its energy in increasing biomass, which leads to fragmentation and increased coverage in an area (Pieterse et al. 1984). Since *H. verticillata* is a canopy former, it can produce more biomass than *V. americana* under high nutrient conditions, thereby shading native species and contributing to their decline in a light-limited environment. This results in large mats of *H. verticillata*, which clog boat motors and thus deter recreational use of an area (Langeland 1996) and provide inferior habitat and food for fish, crabs, turtles, and waterfowl (Fields et al. 2003).

Research Site: Otter Point Creek, MD

Conservation and restoration are currently important research and management topics for most of the world's ecosystems and especially for estuaries. Yet, to conserve and restore estuarine habitats, multiple factors, such as the impacts of high nutrient loading and the effects of different environmental conditions on estuarine biota must be better understood. My study was specifically designed to educate managers of Otter Point Creek (OPC), which is part of the Chesapeake Bay National Estuarine Research Reserve (CB-NERR), located in Abingdon, MD USA (39°27'N, 76°16'W). OPC is a shallow tidal freshwater estuary that includes 106 ha of open water (Figure 1), with high nutrient concentrations due to a local waste water treatment plant and an old sewage lagoon. OPC has a maximum depth of approximately 1.5m, with 0.3m average tidal fluctuations. *Hydrilla verticillata* appears to have significantly increased at OPC since its first observed occurrence in 2002 in both distribution and abundance (NERR, *unpublished data*). Not only does *H. verticillata* deter recreational use of the area due to mat formation at the water's surface, but it also may have contributed to the decline of native species of SAV such as *V. americana, Elodea canadensis, Heteranthera dubia, Ceratophyllum demersum,* and *Potamogeton perfoliatus* (NERR, *unpublished data*).

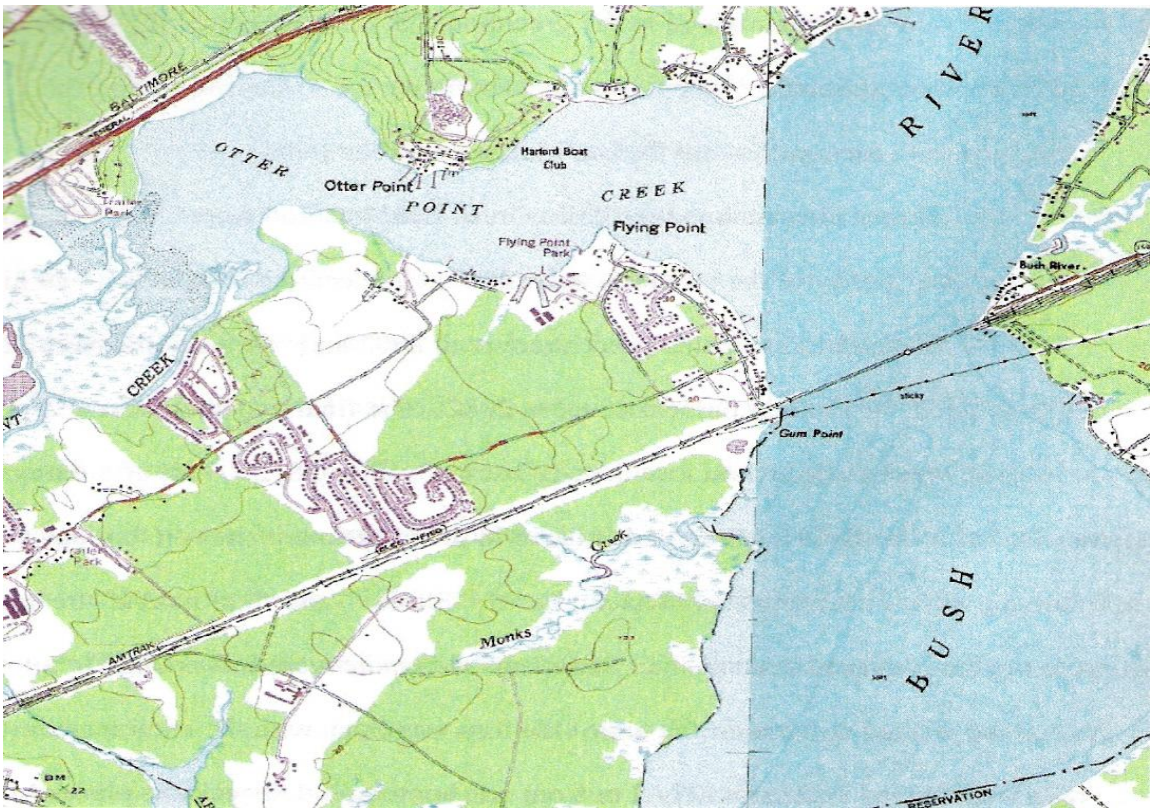

Figure 1: Map of Otter Point Creek study site. Otter Point Creek is located within the Bush River watershed and is a part of the National Estuarine Research Reserve System in Maryland. Source for topographic map: USGS Gunpowder Quadrangle.

Despite the dominance of *H. verticillata* at OPC, a few large patches of one native species (*V. americana*) persist at OPC, although it is unclear why they persist in certain locations. Preliminary observations of water current velocities at high tide within OPC (75 random points taken in March, 2005) using a Marsh-McBirney Flo-Mate 2000 flow meter at 20cm from the sediment and 20cm from the water's surface showed no significant differences in water current velocities within the study area. However, storm events that increase creek discharge may affect water current velocities in different locations.

Although water current velocity can be important in determining SAV distribution (Koch et al. 2010), this study focused on the sediment and nutrient components of the sediment-nutrient-water current interaction. These factors are related in that if all grain

sizes are equally available, then areas with higher water current velocities should contain coarser sediments than areas of slow moving or stagnant water, which should contain finer sediments. Sediment grain size may in turn reflect nutrient concentrations, because sediments will have different amounts of nutrients adsorbed to them dependent on their surface area (Vaze and Chiew 2004). This interaction between water current velocity, sediment size, and nutrients demonstrates part of the complexity of estuarine environments, though many additional factors such as light availability and water depth must also be considered to fully understand the distribution of submersed aquatic vegetation.

Because light availability is a major limiting factor for overall SAV growth (Carter and Rybicki 1990, Duarte 1991, Dunton 1994, Goodman et al. 1995, Masini et al. 1995, Zimmerman et al. 1995), any factors that cause light to be limited to a species (e.g., shading of *V. americana* by *H. verticillata*) can negatively impact biomass accumulation of the light-limited species. Conversely, *H. verticillata* may potentially benefit native species of SAV, in that its biomass throughout the water column may reduce currents (causing suspended materials to settle) and take up nutrients from the water (improving water clarity), allowing light to be transmitted deeper. At the time of *H. verticillata*'s first observation within OPC in 2002, another invasive SAV species, *Myriophyllum spicatum* L., was the dominant species, with smaller populations of *Ceratophyllum demersum* L., *Elodea canadensis* Michx., *Heteranthera dubia* (Jacq.) MacM., *Najas* sp., *Potamogeton crispus* L., *P. perfoliatus* L., *P. pusillus* L., *Stuckenia pectinata* L. Böerner, *V. americana*, and *Zannichellia palustris* L. throughout the estuary (Chadwell and Engelhardt 2008). Of the native species, *V. americana* is the only one that regularly persists throughout the site.

Project Objectives and Hypotheses

To successfully manage *H. verticillata* and to predict future distribution and abundance, the effect of *H. verticillata* on native SAV must be better understood and documented. I therefore quantifed competitive effects between *H. verticillata* and *V. americana* using 3 sediment types ranging from high silt and clay content to high sand content. I also manipulated plant density of both competitors to separate the effects of intra- and inter-specific competition and to make management recommendation for the successful restoration of *V. americana*.

Preliminary field observations of SAV distribution at OPC have led to the hypothesis that patches of native species, such as *V. americana*, persist in the nutrient rich environment at OPC in areas of coarser sediment (see summary chart in Figure 2). *Hydrilla verticillata*, on the other hand, appears to dominate in finer substrates that are composed primarily of silt and clay particles. Because the effects of sediment grain size and water currents cannot be separated in the field, I developed a greenhouse experiment that offered more control over environmental conditions.

Focusing on sediment grain size and associated nutrients, I tested the prediction that 1) finer sediments (with a higher percentage of silt and clay) will have higher nutrient concentrations associated with them than coarser sediments (with a high percentage of sand), and 2) *V. americana* will out-compete (i.e., produce greater biomass) than *H. verticillata* in the greenhouse in mesocosms with coarser sediment grain size compared with mesocosms of fine sediment grain sizes in which *H. verticillata* will out-compete *V. americana*. If nutrients are limited within the mesocosms, I predicted that *V.*

americana will be better able to uptake nutrients from the sediment, therefore producing greater biomass than *H. verticillata*. However, if nutrients are not limited, I predicted that *H. verticillata* will have a higher relative growth rate than *V. americana*.

For plant shoot density, I hypothesized that *H. verticillata* and *V. americana* compete intra- and inter-specifically in the greenhouse, and therefore predicted reduced biomass of both species at higher intra- and inter-specific densities. In addition, since *H. verticillata* appears to dominate at OPC, I predicted that the presence of *H. verticillata* would have a greater negative effect on biomass of *V. americana* than the presence of *V. americana* would have on biomass of *H. verticillata.*

These hypotheses and predictions were generated from observations of SAV communities at OPC, though they also reflect changes in global populations of SAV, which have been dramatically impacted by environmental conditions, anthropogenic effects, and invasive species.

H1	Patches of *V. americana* persist at OPC in areas of coarser sediment.
H2	*Hydrilla verticillata* is found at OPC predominately in finer sediments of silt and clay.
H3	*H. verticillata* and *V. americana* compete intra- and inter-specifically in the greenhouse.
P1	Finer sediments (with a higher percentage of silt and clay) will have higher nutrient concentrations associated with them than coarser sediments (with a high percentage of sand).
P2	*V. americana* will out-compete (i.e., produce greater biomass) than *H. verticillata* in the greenhouse in mesocosms with coarser sediment grain size compared with mesocosms of fine sediment grain sizes in which *H. verticillata* will out-compete *V. americana*.
P3	If nutrients are limited within the mesocosms, *V. americana* will be better able to uptake nutrients from the sediment, therefore producing greater biomass than *H. verticillata*.
P4	If nutrients are not limited, *H. verticillata* will have a higher relative growth rate than *V. americana*.
P5	Both species will have reduced final biomass at higher intra- and inter-specific densities.
P6	The presence of *H. verticillata* will have a greater negative effect on biomass of *V. americana* than the presence of *V. americana* will have on biomass of *H. verticillata.*

Figure 2. Summary of hypotheses (H) and predictions (P).

Chapter 2: Materials and Methods

This project consisted of two related components: an observational field study at Otter Point Creek, MD, and a controlled greenhouse experiment at the University of Maryland Center for Environmental Science's Appalachian Laboratory in Frostburg, MD. The field study component was to determine if *Hydrilla verticillata* and *Vallisneria americana* separate spatially based on different sediment composition, total depth, or Secchi disc depth. The goal of the controlled greenhouse experiment was to examine differences in competitive outcomes among *H. verticillata* and *V. americana* in different sediment types and plant densities in monoculture and biculture.

Field Observations

I monitored the presence and coverage of submersed aquatic plant species from 2004 to 2006 at Otter Point Creek (OPC), one of the National Estuarine Research Reserves in Maryland. I also used data collected in 2002, when *H. verticillata* was first observed at OPC, and 2003. Monitoring in 2002 and 2003 was done by Julie Bortz and Todd Chadwell. Vegetation monitoring occurred at peak biomass in late August in 2002 and 2003. In 2004 until 2006, however, I monitored seasonal as well as annual variation by sampling in mid May, late June, August, and again in early October at the end of the growing season. Sampling locations were selected using a geographic information system (GIS) grid created by Julie Bortz that initially included 64 points in 2002 and was expanded in 2004 to include a total of 101 points (Figure 3). Points were 50m apart within 100m of the shoreline and 100m apart everywhere else.

Figure 3. Sampling points at Otter Point Creek (blue points were part of the initial grid created in 2002; yellow squares are sites added in 2004; red squares are water quality monitoring stations).

Water quality (depth, Secchi disc depth, pH, salinity, dissolved oxygen, and temperature) were measured in the field following standard protocols approximately every two weeks from May to October during the growing season from 2002 through 2005 at six permanent monitoring sites (red squares, Figure 3).

In 2002 when submersed aquatic vegetation (SAV) monitoring began, Julie Bortz evaluated the environmental conditions at OPC and determined that the rake grab method (Jessen and Lound 1962) would be the most feasible in the high turbidity conditions. In

August, when turbidity is much lower, visual observations can be made for coverage. To use the data from 2002 and 2003, I continued to use the rake grab method to ensure comparability between sampling dates. To minimize observer bias, I was trained directly by Julie Bortz in the rake grab technique and visual estimates, and during the training stage we compared our observations to ensure that our estimates of coverage were within five percent of one another.

To sample the SAV at OPC, I threw a six foot long tethered rake with a 46cm wide head and 10cm long prongs four times into the water at different angles from a boat and estimated relative abundance (in percent coverage of the area based on percent coverage of the tongs) of each species by the amount of vegetation that was pulled up by the rake after dragging it along the sediment bottom for approximately 1 meter (see Adair et al. 1994). Although this rake grab method is destructive and may underestimate flat-leaved species such as *Vallisneria americana* (Capers 2000), it has proven to be the only viable method to sample SAV in the high turbidity of OPC. Attempts during the growing season of 2005 to use a view scope, an underwater camera, and snorkeling all failed due to an inability to distinguish species and percent cover in highly turbid conditions.

Rodusky et al. (2005) have compared results of SAV monitoring with a ponar dredge, a rake apparatus, and a $0.5m^2$ PVC quadrat frame deployed by a diver to sample SAV, and found the boat-based rake grab method to be a suitable replacement for the other methods when in-water measurements are not practical (Rodusky et al. 2005). When turbidity is low at OPC late in the season (in August and September), coverage of SAV can be estimated visually, but the rake grab method remains the only possible

method of monitoring at OPC as long as turbid conditions persist. When visual estimates were possible, both visual observations and the rake grab method were used.

I also used the geostatistical technique of kriging (Cressie 1991, Guan et al. 1999) to extrapolate SAV coverage throughout OPC based on observed values at the monitoring points. However, I only used kriging to produce visual depictions of seasonal and annual SAV coverage and not for statistical analysis.

During each monitoring period for SAV, I measured water depth (which I later corrected for tidal fluctuations based on the time of recording) and Secchi depth at each grid point. Total depth was measured by slowly lowering a 2m long, 3cm diameter PVC pipe over the side of the canoe until it reached the sediment and measuring the length of the pipe between the sediment and the surface of the water. Secchi depth was measured using the standard procedure: I lowered a 20cm diameter Secchi disc (a round disc with alternating black and white quadrants attached to a non-stretching rope marked every 10mm) into the water until the distinction between the black and white quadrants was no longer discernible to the naked eye, and recorded the depth. Despite the simplicity of this measurement, it is effective because the light that humans perceive is remarkably similar to the wavelengths plants use for photosynthesis (Carruthers et al. 2001).

Although using a Secchi disc is a cheap and easy way to measure turbidity, it is problematic in two situations: 1) later in the growing season when SAV biomass peaks and the Secchi disc can be physically obstructed by SAV, or 2) when turbidity is low in shallow areas and it is possible to see through the entire water column to the sediment surface. Using a Secchi disc in either one of these conditions could lead to lower estimates of water clarity than what actually exist. To get turbidity measurements at peak

biomass and low turbidity conditions, I took water samples from each grid point during the June and September SAV monitoring periods, and analyzed their turbidity levels within 24 hours using a Hach 2100 P portable turbidimeter. I then performed a linear regression to determine the relationship between Secchi disc and turbidimeter results.

In June 2005, I used a 4cm diameter PVC pipe to take 10 preliminary sediment cores from the top 15cm of the sediment surface to get a representative sample of the sediment within the root zone of SAV. Locations for the sediment cores were selected randomly from SAV sample points throughout OPC to obtain an estimated range of sediment composition at OPC. I placed samples in plastic bags and stored the samples in a cooler for transport. Samples were refrigerated until analysis within two days after sampling. I analyzed the substrate using the hydrometer method of particle size analysis (Gee and Bauder 1986) and determined the percent of sediment organic matter by hydrogen peroxide digestion (Erftemeijer and Koch 2001). In June 2006, I took sediment samples at all 101 grid points and used the same process to analyze them for sediment composition.

Linear regression using field data from June 2006 tested for associations between total SAV coverage, *H. verticillata* coverage, total water depth, Secchi disc depth, and sediment composition. To determine whether spatial autocorrelation influenced the model, I examined a spatial correlogram of the residuals as well as a spatial plot of the residuals. Both confirmed a minimal influence of spatial autocorrelation and thus I proceeded with the use of a linear model. All factors were normally distributed and thus the data did not need to be transformed. Coverage of *V. americana* could not be tested for

associations with sediment composition since it was absent from all sampling points in June 2006.

Greenhouse Experiment

Initial plant size and shoot density may affect competitive abilities of plants (P. Jolliffe, *personal communication*) and must be evaluated to avoid biased results in competition studies (Jolliffe 2000). Resource competition usually creates a negative relationship between individual size and density, since the size of the individuals must be reduced to accommodate a limited resource in a fixed area (Damuth 1981, Silvertown and Charlesworth 2001). However, plant shoot and root density may have other effects besides reduced individual size. For instance, plant density may increase fruit production but decrease plant growth rate at high density (Jolliffe and Gaye 1995); it can facilitate biomass production at intermediate densities in a high-stress environment (Chu et al. 2008); and it may affect survivorship rates of SAV plants transplanted into "nursery beds" of another established species (Hengst et al. 2010).

Replacement series, in which combined initial density of the species is held constant in a set of monocultures and bicultures, have been used more widely than any other experimental structure for competition and interference studies (Trenbath 1974, Cousens 1991, Gibson et al. 1999). However, a complete binary factorial structure, in which the densities of the species are varied independently of one another (Mead 1979, Snaydon 1991), can produce more accurate and comprehensive results regarding competition between two species. Additionally, measurements of initial biomass must be taken to compare with final biomass in order to determine which species truly produces

greater biomass during a competition experiment (Connolly et al. 2001). Using these methods, plant interactions can effectively be studied to inform future restoration efforts.

The traditional method of conducting a greenhouse experiment to evaluate plant competition and interactions has been to use the simplest experimental design (the replacement series), and to harvest, dry, and weigh all above ground biomass produced during the experiment (Keddy and Shipley 1989, Goldberg and Barton 1992, Gibson et al. 1999). This method ignores initial biomass differences within and between species, below-ground biomass production, and density-dependence. Thus, the data cannot be used to estimate the rate of growth of the study species, and can lead to improper interpretations of competitive abilities (Connolly and Wayne 2005). Despite the fact that some experimental biologists have recognized that "most competition experiments are invalidated if initial plant sizes are not equal," (Cousens 2000), little effort is made to start competitive experiments with equal plant sizes or to at least evaluate and document initial plant size. Although biomass is important, rate of growth and potential for future growth are equally, if not more so, important, since they indicate how a species will grow over time, and how its growth will be affected by another species and changes in the environment. I analyzed biomass from a greenhouse experiment using two methods (final biomass and rate of growth) in a complete binary factorial structure.

In the fall of 2005, I collected sediments at OPC in an area that was dominated by *H. verticillata* to be used in a controlled greenhouse experiment at the University of Maryland Center for Environmental Science's Appalachian Laboratory in Frostburg, MD. The sediments were air dried, homogenized, sterilized, and analyzed for grain size and organic content. Although drying the soils may change its biogeochemistry, it was

necessary to minimize variability between treatments. I then mixed the OPC sediments with different amounts of commercially available sand to create three sediment treatments that differed in their proportions of sand, silt, and clay. Sediment treatment 1 ("coarse") consisted of 85% sand and 15% silt/clay; sediment treatment 2 ("medium") of 50% sand and 50% silt/clay; and sediment treatment 3 ("fine") of 15% sand and 85% silt/clay. These compositions were chosen to reflect the range of sediment composition at OPC, so that results of the experiment could be applied to field conditions at OPC.

In June 2006, I filled 144 clean mesocosms (27 cm diameter, 40 cm height) 7 cm high with sediment (n=48 per sediment treatment). I then filled the experimental mesocosms with dechlorinized tap water that was diluted 50% with reverse osmosis water to minimize build-up of nutrients within the mesocosms over time. The temperature within the greenhouse was controlled at 24°C. I arranged the mesocosms randomly within the greenhouse to eliminate the effects of position and shading. Re-randomization during the experiment was not possible owing to the size of the experiment, weight of the mesocosms, and the potential of disturbing the mesocosms during transport. Epiphytes were frequently scraped off the mesocosm walls using a sterilized piece of mesh nylon to minimize the build-up of algae that could potentially compete with the rooted macrophytes for nutrients.

On June 9, 2006, I harvested *H. verticillata* from OPC and used 10 cm long fragments with roots as my study subjects. Because *V. americana* is relatively rare at the study site, I purchased *V. americana* from a nursery in Wisconsin (Kester's Wild Game Food Nurseries, Inc.). *V. americana* plants were approximately 10 cm long and rooted. Winter buds of *V. americana* typically germinate when water temperatures reach 10 to

14°C (Zamuda 1976; Rybicki and Carter 2002), and can germinate in light or dark conditions (Korschgen and Green 1988). By contrast, optimum germination temperatures for *H. verticillata* tubers and turions are between 15 and 35°C (Haller et al. 1976), and therefore *H. verticillata* may germinate slightly later in OPC than *V. americana*, with *V. americana* starting to germinate in late March to April, and *H. verticillata* germinating in mid April (*personal observation*). Although *V. americana* maygerminate earlier than *H. verticillata* (based on temperature), *H. verticillata* is able to elongate more rapidly than *V. americana*, and thus in late May and early June, individuals of both species at Otter Point Creek are roughly the same size, depending upon environmental conditions (*personal observation*). The size of the plants used in the greenhouse experiment was thus roughly equivalent to the size of plants *in situ* at OPC at the start of the growing season. I estimated initial biomass of each species using fifteen random specimens of each species that were dried and weighed. Initial biomass can then be compared to final biomass to determine which species gained the most biomass during the experiment (Connolly et al. 2001).

On June 10, 2006, I planted *H. verticillata* and *V. americana* at four shoot densities (0, 3, 7, and 10 ramets (individual members of a clone) per mesocosm) per species in a full factorial design so that 16 initial shoot density treatments were created ranging from 0 to 20 individuals per mesocosm. Some mesocosms were therefore monocultures of either *H. verticillata* or *V. americana* or bicultures of the two species. I replaced any plants that died during the first two weeks. The 16 plant shoot density treatments were crossed with the 3 sediment treatments such that each density*sediment treatment was replicated three times. I chose to use an additive design with a complete

binary factorial structure to explore competition between the two study species comprehensively. Although more complex than a replacement series design (Jolliffe 2000), the complete binary factorial structure allows for analysis of intra- and inter-specific competition, since both sediment composition and initial plant shoot density are studied (P. Joliffe, *pers. comm.*).

To examine possible differences among sediment types and examine potential mechanisms of competition among species, I measured nutrient concentration in the sediment pore water and water column water of the monocultures at the beginning of the experiment (before plants were introduced), in the middle (6 weeks into the experiment), and at the end (immediately before harvest) using handmade ceramic lysimeters and sterile syringes. I also measured temperature and pH (using a probe in the greenhouse) and turbidity (using a Hach 2100P Portable Turbidimeter) at each sampling period to determine if different environmental conditions existed between the mesocosms and changed through time. Turbidity served as an indicator for the amount of particles in the water column. Since the substrate was not disturbed during the experiment, particles came from algae. Although chlorophyll-a would have been a more direct measure of water column algae, turbidity was a more cost-effective measure.

All water samples were analyzed for phosphate, nitrate, nitrite, calcium, and potassium concentrations. After realizing its importance as a nutrient for aquatic plants, I also analyzed ammonium at the second and third sampling periods and created three additional mesocosms of each sediment type to get an average initial ammonium concentration. Phosphate, ammonium, nitrite, and nitrate were measured on a Lachat QuikChem 8000 FIA Automated Ion Analyzer, and calcium and potassium were

measured using flame atomic absorption (Flame AA) spectrometry. Flame AA samples were preserved with nitric acid and treated with lanthenum to bind to any phosphate present in the sample and allow cations to be detected. All samples were refrigerated until analyzed.

Nutrient data at each sampling period were screened for outliers and normality, transformed if necessary, and analyzed in SPSS using Multiple Analysis of Variance (MANOVA). I then sorted the data and analyzed by level (monocultures or bicultures), species, sediment type, and number of plants.

I harvested all plant matter at peak biomass and before plant senescence between September 4 and 7, 2006, for a total experiment time of 12.5 weeks. Harvesting was done by manually pulling up all aboveground and attached belowground biomass. Gently pulling on the aboveground portions of the plants while loosening the sediment ensured that the majority of roots remained intact. I also sieved the sediments to extract any roots that had been broken from the aboveground plant matter and all turions in each mesocosm. Samples were refrigerated until the harvested plant material could be sorted, dried, and weighed within the next week. The majority of roots remained attached to the above-ground biomass and was therefore easily identified by species during sorting. Since root morphology of the two species is not distinctive, any roots that detached from the above-ground biomass during harvesting were collected and weighed separately from roots known to be from a particular species to ensure that their addition would not have been significant to either species.

Plant biomass for each species was based on total plant biomass produced per experimental unit and per capita biomass (total biomass divided by the number of

individuals planted per species). Initial biomass was subtracted from ending biomass to account for initial differences in biomass among species (Connolly et al. 2001). I also analyzed total and per capita above ground, root, tuber, and total below ground weight, and total root to shoot ratio. Independent variables were sediment type (coarse, medium and fine), initial number of *H. verticillata* plants, initial number of *V. americana* plants, and total number of initial plants.

To evaluate group differences caused by multiple factors, I used a univariate factorial ANOVA with a 4x4x3 design (representing four densities for *H. verticillata*, four densities for *V. americana*, and three sediment types). I prescreened the data for outliers within each group, removed outliers outside of the 95^{th} percentile confidence interval for the mean of each treatment group, and tested for normality and homogeneity of variance. In almost all cases, removal of outliers caused the data within each group to be normally distributed according to the Kolmogorov-Smirnov test for normality, although there were several instances of non-homogeneity of variance, as measured by Levene's Test of Equality of Variances. However, since the histograms for normality looked relatively normal and ANOVA is robust to slightly non-normal distributions as long as group sizes are adequate (Mertler and Vannatta 2010), data were not transformed.

Chapter 3: Results

Otter Point Creek

Hydrilla verticillata was first observed at Otter Point Creek (OPC) in 2002, at which time surveying of submersed aquatic vegetation (SAV) began to monitor the spread of this invasive species and its impacts on the native SAV community. Presence of both *H. verticillata* and *V. americana* have fluctuated annually since 2002 (Figure 4). In 2002, *H. verticillata* was present at approximately half of the 64 sampling points and covered up to 75 percent of the area at the individual sampling points (Figure 5). In 2003, *H. verticillata* had spread and was present at every single sampling point, and had increased in coverage so that the species covered 100 percent of the area at some sampling points (Figure 4). *H. verticillata* coverage peaked in 2004, when it was present at every point of the expanded grid (101 points) and covered between 50 and 100 percent of the area at all but 8 points (Figure 4). *H. verticillata* coverage decreased in 2005 and the species was absent at 8 points (Figure 4). By late summer 2006, *H. verticillata* was absent at 45 points, but at 100% coverage at other points (Figure 5).

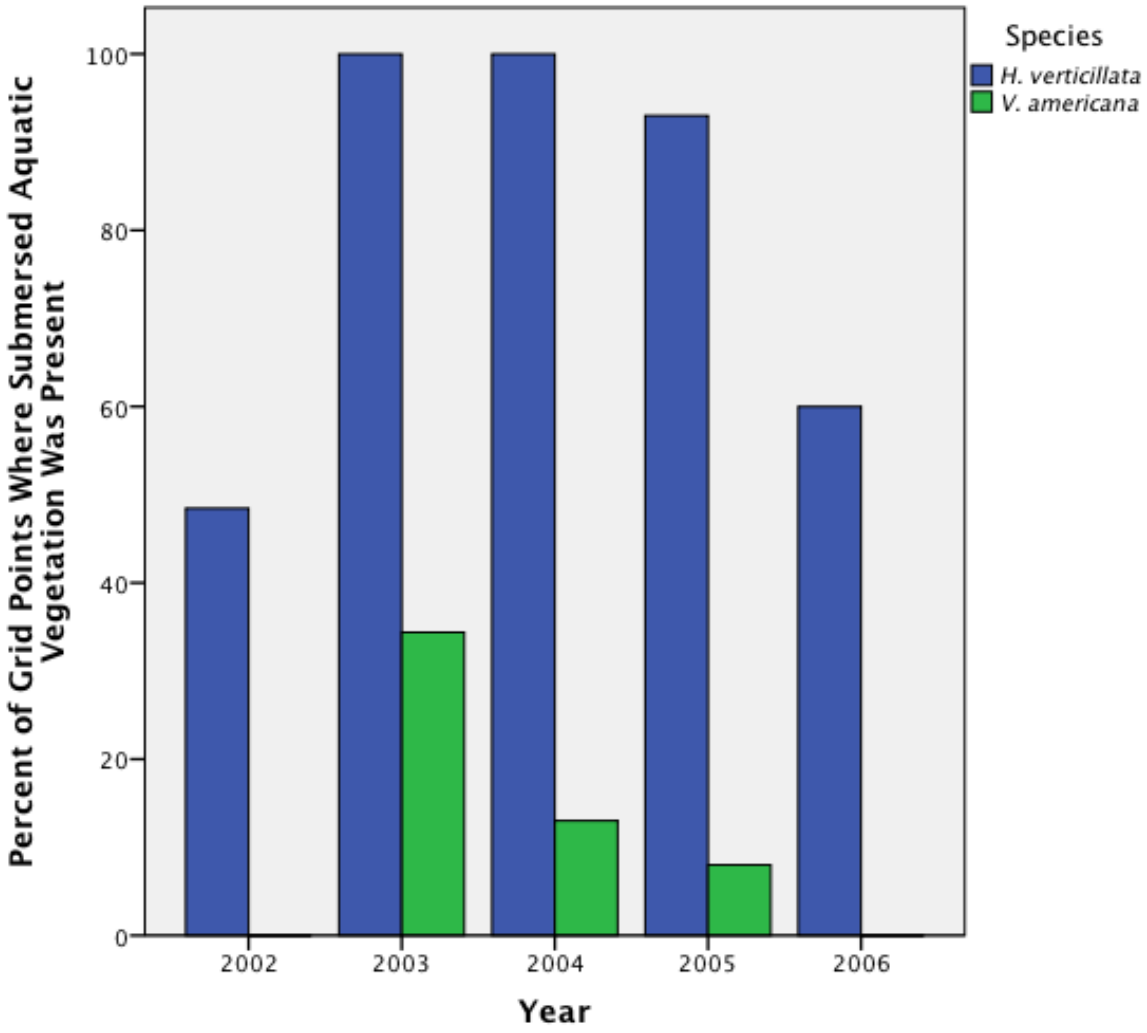

Figure 4. Annual presence of *H. verticillata* and *V. americana* at grid points in OPC.

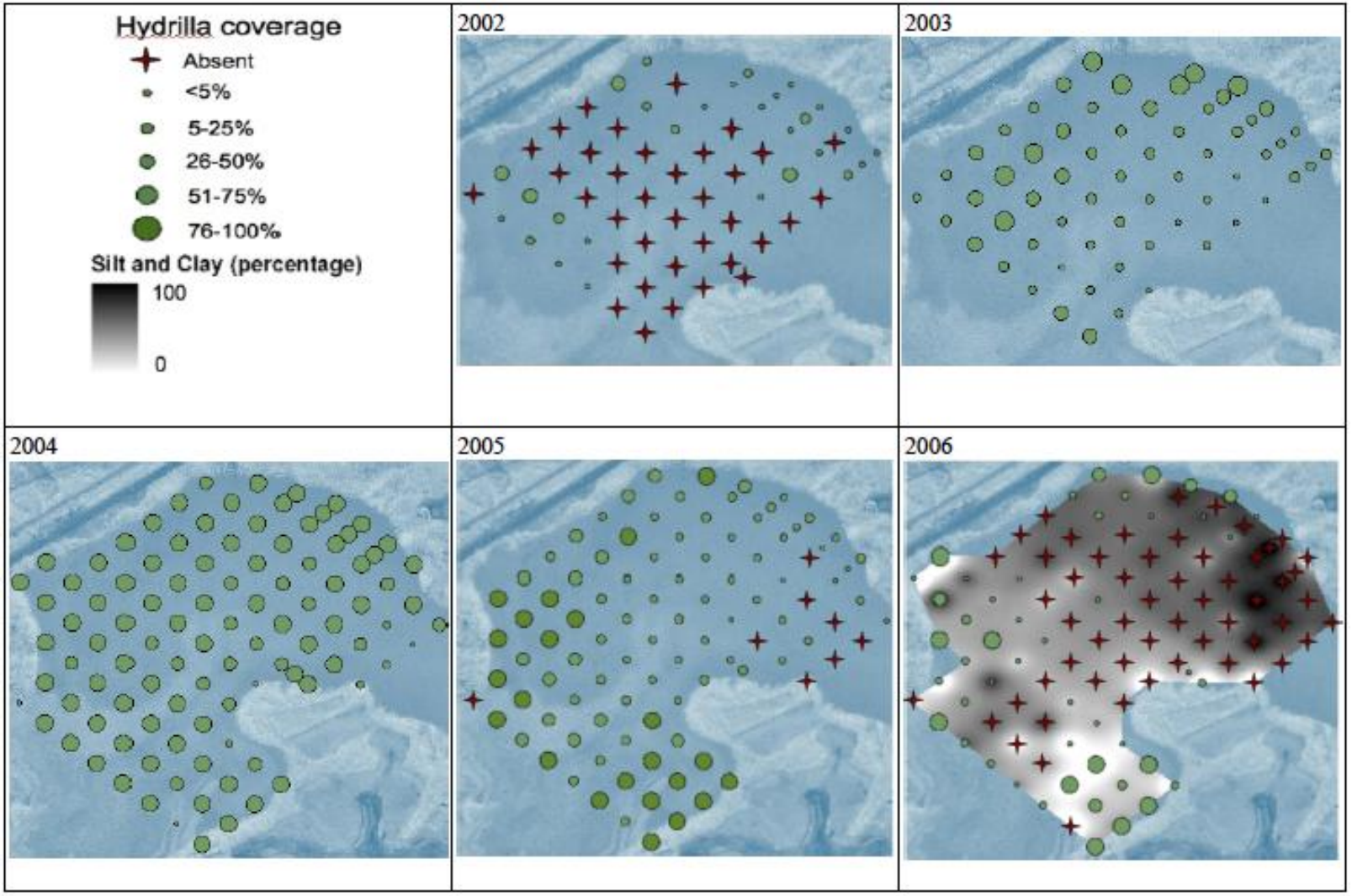

Figure 5: *H. verticillata* coverage (%) at OPC, 2002-2006, and sediment composition in 2006 from kriging. Source for photo layer: Department of Natural Resources.

Because *H. verticillata* has been found at every sampling point within OPC (Figure 5), its presence does not appear to be limited by any sediment composition or water depth variances within the estuary (Figure 5). However, multiple linear regression of water depth, Secchi depth, sediment composition (percent silt and clay), and percent organic matter with *H. verticillata* coverage shows that *H. verticillata* coverage was negatively correlated with total depth (R^2_{adj} = 0.183, n=98,P = <0.001; (model); r = -0.401, P < 0.001). In contrast, Secchi disc depth (r = -0.088, P = 0.057), percent of silt and clay (r = -0.069, P = 0.557), and percent organic matter (r = -0.018, P = 0.213) were unimportant variables in predicting *H. verticillata* coverage. As previously mentioned, both a spatial correlogram of the residuals and a spatial plot of the residuals confirmed a minimal influence of spatial autocorrelation and thus we proceeded with the interpretation of the linear model.

Hydrilla verticillata coverage varied both annually (Figure 5), and seasonally, with hotspots of high plant shoot density (determined by a rake grab) or coverage (determined from visual observations when water clarity allowed) changing within a single season (Figure 6). Although rake grabs were predominately used prior to peak biomass (May through July) whereas coverage could be estimated visually later in the growing season (August through September), both methods were used for comparative purposes 150 times (10.6%). Within that subset of 150 samples, 112 sites were vegetated. Estimates of vegetative cover were only different between the two methods ten times cumulatively at six locations, and only for *Ceratophyllum demersum* and *Hydrilla verticillata*. *Ceratophyllum demersum* was observed visually but not by the rake grab method six times; in all instances, it was estimated to have only trace coverage (meaning occupying less than 0.01% of the sampling area). In contrast, the rake grab method showed *Hydrilla verticillata*

coverage four times when it was undetected by visual observations; estimated coverage based on the rake grabs at those four sites was estimated at 12, 20, 20, and 40 percent, respectively. *Myriophyllum spicatum* was the only other SAV species present at the sites where both rake grabs and visual observations were used, and was detected and estimated by both methods equally all 56 times it was present.

In 2004 and 2005, *H. verticillata* first appeared near the access point closest to the Anita C. Leight Estuary Center and marina and then proceeded to expand to the rest of the estuary. *H. verticillata* coverage in spring 2006 was comparable with coverage in other years, although by early summer it showed reduced coverage (Figure 7).

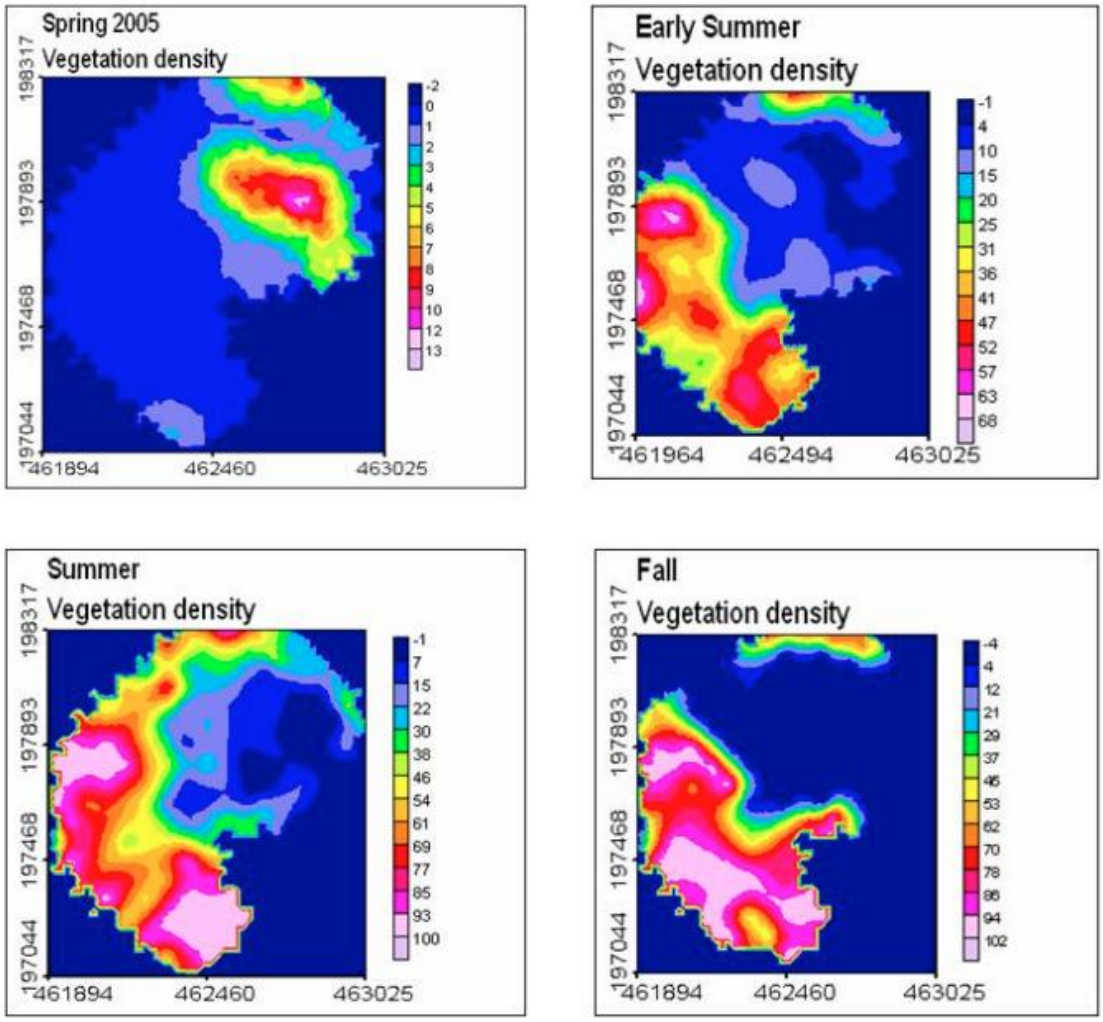

Figure 6. Seasonal changes in *H. verticillata* coverage at Otter Point Creek, 2005. Axis labels are Universal Transverse Mercator (UTM) coordinates.

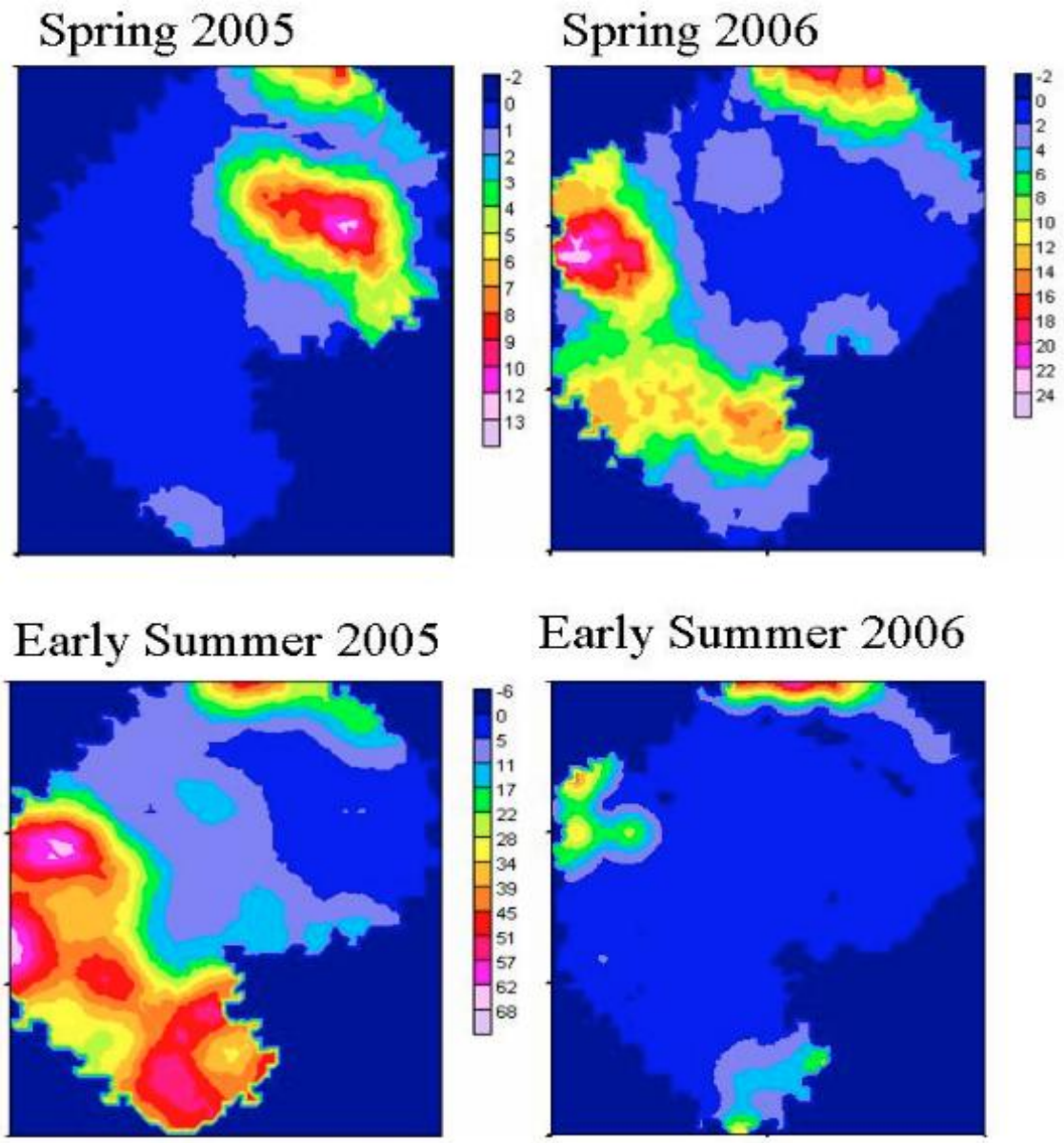

Figure 7. Comparison of early season (spring and early summer) *H. verticillata* coverage at Otter Point Creek in 2005 and 2006.

By contrast, *V. americana* was completely absent from OPC in the August sampling of 2002 and 2006. In 2003, *V. americana* was present at 19 sampling points, with percent coverage up to 26%. *V. americana* was present at only 10 points in 2004, though coverage was greater than the previous year. As with *H. verticillata*, cover of *V. americana* was reduced in 2005. Although *V. verticillata* has never dominated a majority of sampling sites

in a single monitoring period, it has been observed at a total of 31 of the 101 grid points between 2002 and 2006, in all sediment compositions (Figure 8).

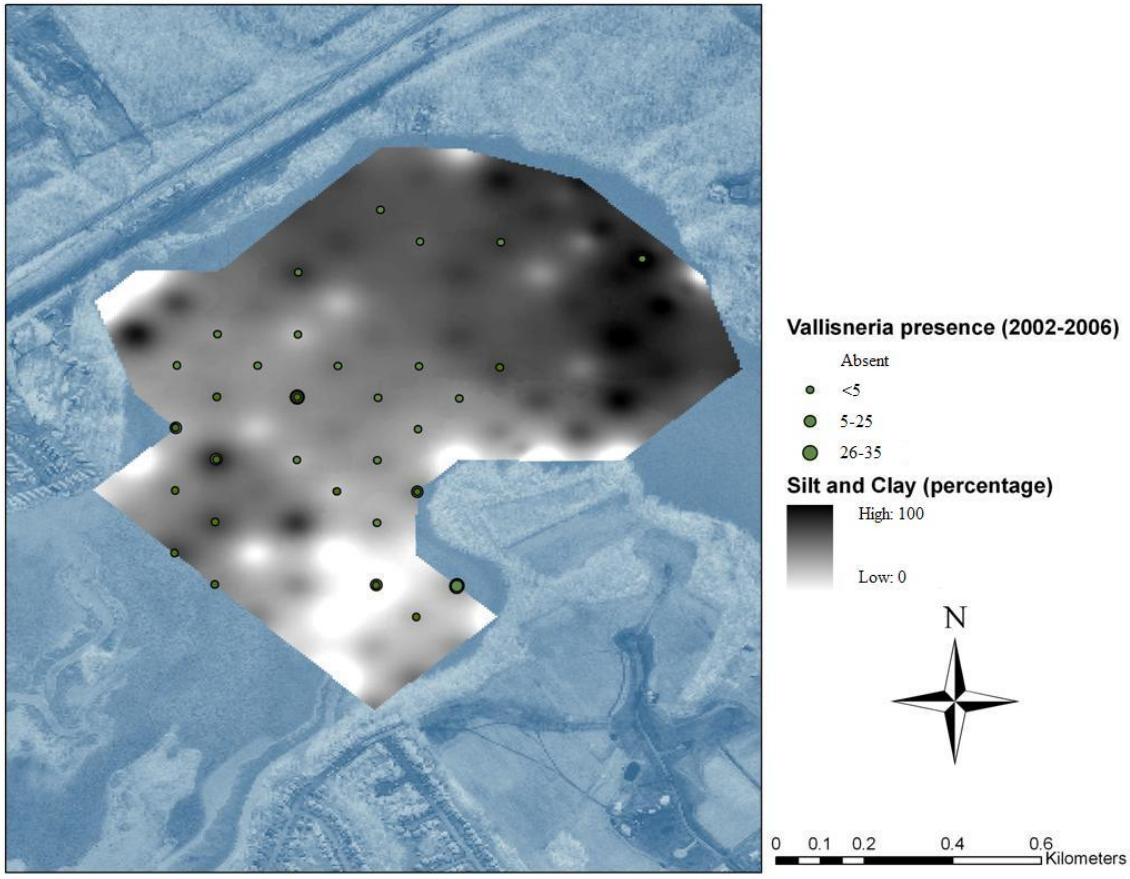

Figure 8. Locations of observed *V. americana* at Otter Point Creek, 2002-2006, and sediment composition in 2006. Source for photo layer: Department of Natural Resources.

All variables measured at the permanent water quality monitoring sites varied during the growing season but remained relatively constant on an annual basis, although Secchi disc depth was particularly low in 2005 (Table 1). The Marina and TPN stations had the greatest average water depth (3.00m and 1.42m, respectively), and the Marina station was never vegetated.

Table 1. Water quality parameters at permanent monitoring sites in OPC, 2002-2005. Measurements were taken every two weeks from May to October of each year at 6 stations.

Variable	**Mean(+/-SE)**	**Minimum**	**Maximum**
Water Depth (m)			
2002	1.07 (0.18)	0.27	3.00
2003	1.29 (0.08)	0.34	3.20
2004	1.40 (0.15)	0.50	3.10
2005	1.35 (0.09)	0.53	3.10
Secchi Disc Depth (m)			
2002	0.40 (0.03)	0.24	0.68
2003	0.43 (0.02)	0.11	1.14
2004	0.57 (0.03)	0.20	1.65
2005	0.34 (0.01)	0.14	0.69
pH			
2002	7.71 (0.25)	6.06	8.80
2003	7.95 (0.08)	7.00	10.00
2004	8.32 (0.08)	7.00	10.10
2005	7.84 (0.10)	6.50	9.48
Salinity			
2002	1.09 (0.24)	0.30	3.20
2003	0.95 (0.00)	0.00	0.10
2004	0.10 (0.00)	0.10	0.20
2005	0.38 (0.06)	0.10	2.10
Dissolved Oxygen (mg/L)			
2002	8.53 (0.40)	6.80	11.24
2003	6.99 (0.22)	3.60	12.39
2004	7.79 (0.21)	5.49	12.34
2005	7.79 (0.23)	3.84	12.31
Temperature °C			
2002	28.09 (0.46)	25.40	31.20
2003	23.42 (0.64)	2.60	30.00
2004	24.12 (0.52)	12.10	31.30
2005	25.27 (0.49)	17.20	31.60

Similarly, water depth, Secchi depth, and sediment composition varied throughout the vegetation monitoring sites (Table 2), with the greatest water depth and highest percentage of silt and clay at the mouth of OPC near the marina (Figure 9). Total depth, Secchi depth, percent silt and clay, and percent sediment organic matter are correlated, except for percent sediment organic matter and Secchi depth ($P = 0.638$); total depth is

positively correlated with Secchi depth and percentage of silt and clay (r = 0.139, P < 0.001; r = 0.398, P < 0.001, respectively) and negatively correlated with percent sediment organic matter (r = 0.247, P = 0.012), whereas Secchi depth is negatively correlated with percentage of silt and clay (r = -0.286, P = 0.004) and percent sediment organic matter (r = -0.331, P = 0.001). All but 8 of the grid points had sediment organic values under 5%.

Table 2. Water depth, Secchi depth, percent silt and clay, and percent organic matter at submersed aquatic vegetation monitoring sites in OPC in 2006, when all variables were measured at 101 sites.

Variable	**Mean (+/- SE)**	**Minimum**	**Maximum**
Water Depth (m)	1.00 (0.02)	0.08	2.00
Secchi Disc Depth (m)	0.33 (0.01)	0.08	0.61
Sediment Silt and Clay (%)	76.10 (2.28)	3.59	100.00
Sediment Organic Matter (%)	1.27 (0.23)	0.00	12.20

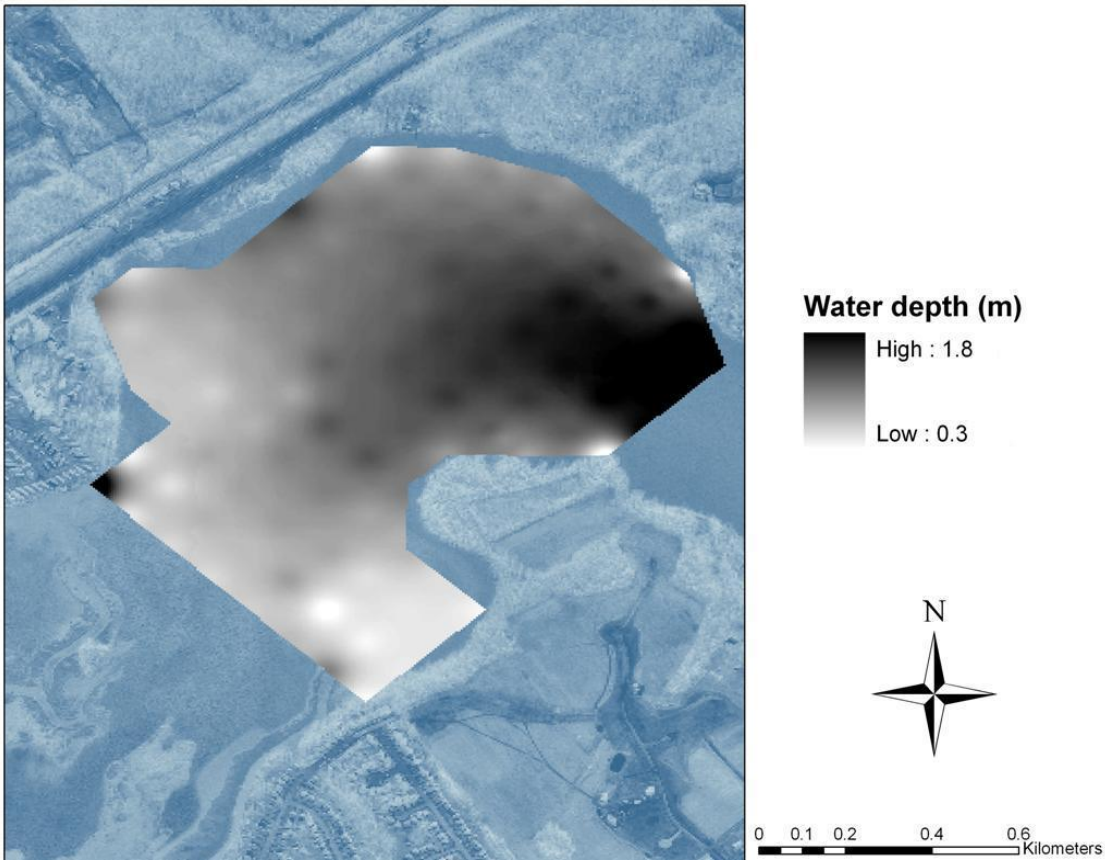

Figure 9. Water depth at OPC in 2006. Source for photo layer: Department of Natural Resources.

To get a better estimate of turbidity at peak biomass and low turbidity conditions, when Secchi disk measurements become censored, I analyzed water samples with a turbidimeter. A linear regression between Secchi depth (m) and log turbidity (NTU) from every SAV monitoring location in May, June, July, and August of 2005 and June 2006 (n = 404) generated an r^2 of 0.71 (Figure 10).

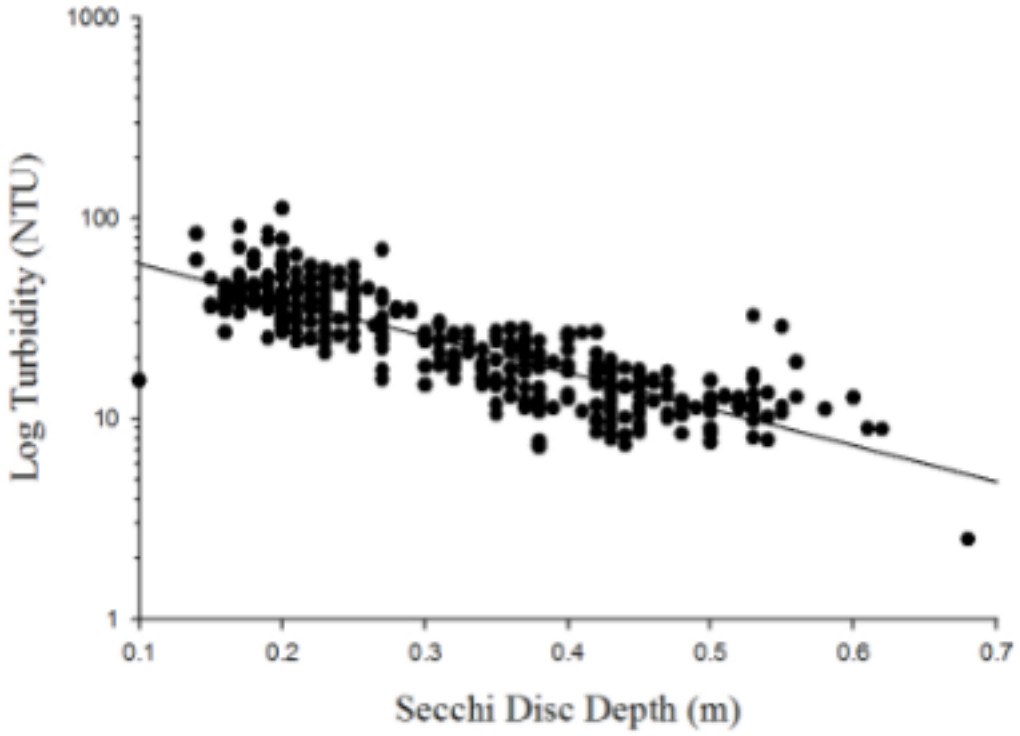

Figure 10. Linear regression of Secchi disc depth (m) and log turbidity (NTU).

Greenhouse Experiment

The greenhouse experiment tested for effects of sediment type (3 levels – 15% ("coarse"), 50% ("medium"), and 85% ("fine") silt&clay), initial density of *V. americana* shoots (4 levels – 0, 3, 7, and 10 individuals), and initial density of *H. verticillata* shoots (4 levels – 0, 3, 7, and 10 individuals) on above- and below- ground biomass production of *H. verticillata* and *V. americana*. I also tested for differences in nutrient levels within the water column and sediment pore water among sediment, species, and initial plant shoot density treatments to examine potential nutrient limitations and mechanisms of competitive interactions.

Initial conditions

Although plants of both species were similar in length (10 cm) at the beginning of the greenhouse experiment, initial dry mass was 10-fold higher for *V. americana* plants (0.0713 g/individual) than for *H. verticillata* plants (0.0076 g/individual). Over the 12.5-week experiment, each *V. americana* plant gained on average 0.7409g of dry biomass, for a 10-fold increase in total biomass. In contrast, each *H. verticillata* plant gained on average 0.6293g of dry biomass, for an 80-fold increase in total biomass. Average above-ground biomass across all treatments at the end of the experiment did not differ between *H. verticillata* and *V. americana* plants when either initial biomass was accounted for (t-test; n=108, P=.609) or when it was not (t-test; n=108, P=.629). However, total biomass (above- and below-ground) of *V. americana* was 1.25 times higher than *H. verticillata*, owing to greater root and tuber biomass production in *V. americana* (Figure 11).

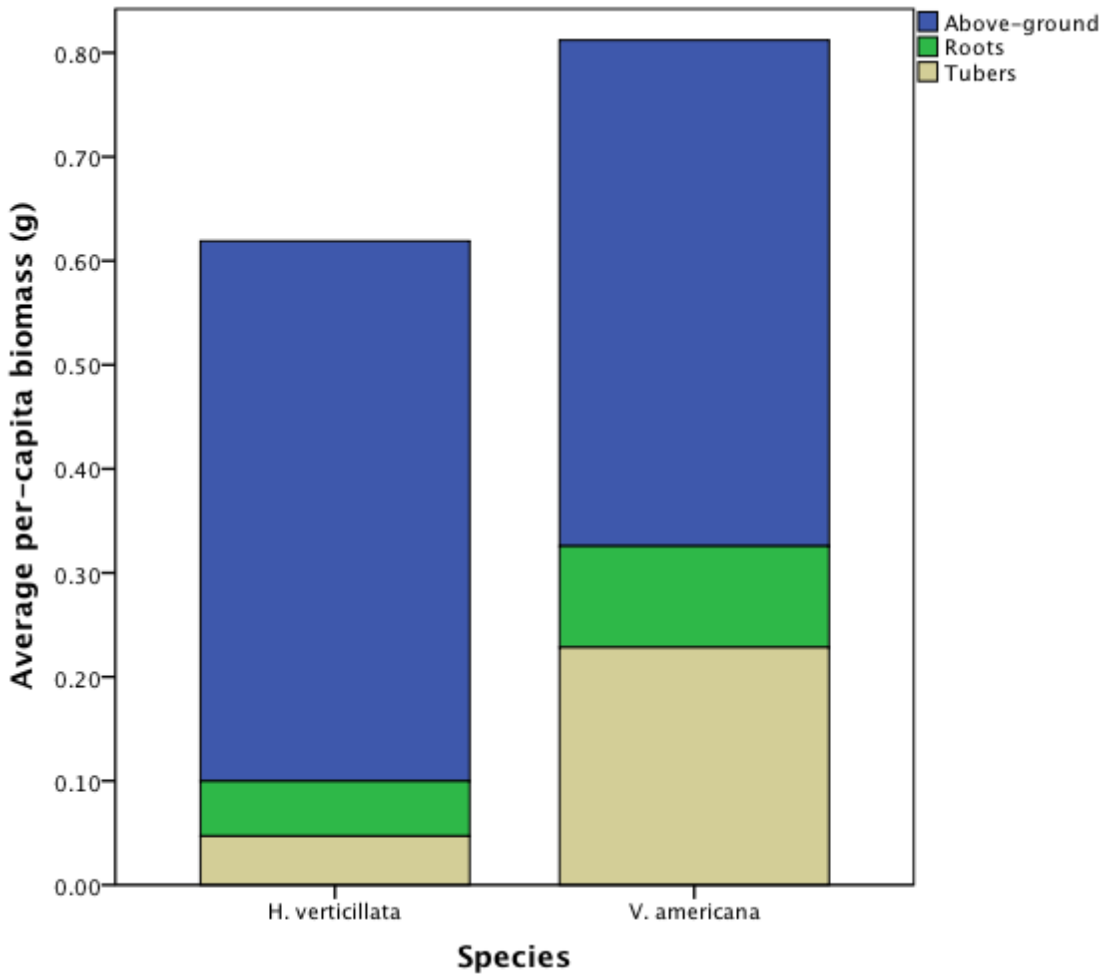

Figure 11. Final Above-ground, root, and tuber biomass of individual *H. verticillata* and *V. americana* plants, all treatments combined.

Concentration of PO_4, NO_2, NO_3, Ca and K, pH, and turbidity in the water column and in pore water at the beginning of the experiment differed between sediment types for every variable except PO_4 concentration within the water column and NO_3 concentration within the sediment pore water (Table 3). Nitrogen concentration in the water column and pore water was dominated by NH_4. In both cases, NH_4 concentration decreased with higher amounts of silt and clay in the substrate. Similarly, pH decreased in value with increasing % silt and clay in the substrate. In contrast, concentration of Ca and K, and turbidity in the water column increased with higher amounts of silt and clay in the

substrate. Similarly, concentration of Ca, K, and PO_4 in the pore water was lowest in the low silt and clay treatment. Calcium had especially high values, with high variance, both within the water column and in pore water samples (Table 3).

Table 3. Water chemistry parameters for the water sampled from the water column and pore water in experimental mesocosms at the beginning of the experiment. Water chemistry data for three sediment treatments (15%, 50%, and 85% silt and clay) are compared (n = 18, df = 2); all units except for pH and turbidity (Nephelometric Turbidity Units) are mg/L.

Variable	**Mean (+/- SE)**			**F**	**P**
	15%	**50%**	**85%**		
Water Column					
PO_4	0.010 (0.005)	0.014 (0.014)	0.012 (0.016)	0.21	0.814
NH_4	2.402 (0.421)	1.225 (0.189)	0.419 (0.006)	NA^+	NA^+
NO_2	0.003 (0.001)	0.031 (0.041)	0.010 (0.007)	11.53	<0.001
NO_3	0.062 (0.104)	0.191 (0.208)	0.036 (0.023)	11.30	<0.001
Ca	16.736 (4.249)	21.114 (5.688)	27.890 (6.961)	17.18	<0.001
K	1.985 (0.546)	3.854 (0.620)	5.669 (0.776)	137.78	<0.001
pH*	8.670 (0.498)	8.197 (0.659)	8.034 (0.605)	5.88	0.005
Turbidity*	8.384 (6.347)	32.551 (22.850)	46.648 (26.006)	23.39	<0.001
Pore Water					
PO_4	0.016 (0.006)	0.037 (0.033)	0.033 (0.024)	4.03	0.024
NH_4	7.720 (1.260)	6.892 (1.734)	4.296 (0.247)	NA^+	NA^+
NO_2	0.015 (0.006)	0.047 (0.042)	0.051 (0.033)	7.30	0.002
NO_3	-0.001 (0.005)	0.002 (0.010)	0.003 (0.006)	1.71	0.192
Ca	60.535 (16.962)	205.924 (94.128)	140.896 (40.609)	25.87	<0.001
K	14.410 (3.635)	14.411 (3.635)	16.767 (3.721)	58.64	<0.001

* n = 45

$^+$ NH_4 samples were not taken during the initial sampling period. During the experiment, I created three additional mesocosms of each sediment type (without plants) estimate initial NH_4. I did not perform statistical analysis on initial NH_4 because of the small sample size (n = 3 for each sediment type).

Monocultures

I focused first on monocultures of *H. verticillata* and *V. americana* to test for differences in species responses to sediments and initial plant shoot density without having to account for the confounding effects of inter-specific interactions in bicultures.

H. verticillata and *V. americana* did not differ in individual above-ground biomass or individual above- and below-ground biomass, but they did differ in total above- and below-ground biomass produced at the end of the experiment (Table 4). For example, *H. verticillata* and *V. americana* produced 4.89±0.66g and 7.23±0.69g total biomass, and 0.94±0.17g and 1.27±0.18g total individual biomass, respectively. However, *V. americana* produced more below-ground biomass (2.75±0.27g) than *H. verticillata* (0.79±0.12g), which significantly influenced total biomass production. Sediment composition did not affect total above-ground biomass production, total above- and below-ground biomass production, or individual above- and below-ground biomass production. Similarly, initial plant shoot density did not affect total above- and below-ground biomass production. However, individual plants of both species produced more than 2.5 times as much above-ground biomass and more than twice as much total biomass when planted in low density (3 plants) than in higher density (7 and 10 plants; Table 4, Figure 12).

Table 4: ANOVA results for testing for differences in plant biomass among sediments, species, and total initial plant shoot density treatments in monocultures (n = 54).

Variable	**df**	**F**	**P**	**R^2**
Above-ground Individual Plant Weight	17	2.600	.008	.551
Sediment	2	.740	.484	
Species	1	.148	.703	
Total Density	2	10.769	<0.001	
Total Plant Weight (Above and below)	17	2.029	.037	.489
Sediment	2	.991	.381	
Species	1	7.341	0.010	
Total Density	2	1.323	.279	
Total Individual Plant Weight (Above and below)	17	2.725	.006	.563
Sediment	2	.683	.512	
Species	1	2.509	.122	
Total Density	2	11.210	<.001	

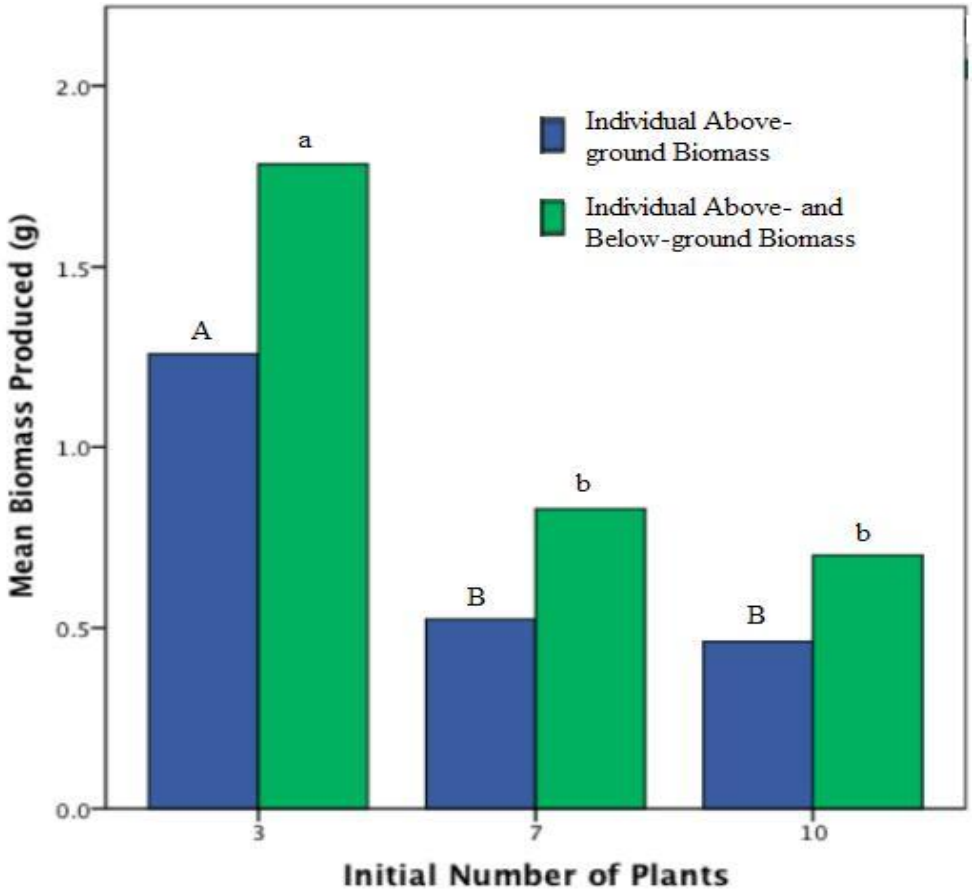

Figure 12. Individual plant biomass (g) in each plant density treatment within monocultures. Different letters denote significance at α=0.05. Upper and lower case letters show the results of two separate ANOVAs on individual above-ground biomass (upper case) and individual total biomass (lower case).

Differences in water column and pore water nutrient concentrations, and water column pH and turbidity were consistent across the 3 substrate treatments mid-way through and at the end of the experiment. Substrate composition was generally associated with greater nutrient concentration differences within the pore water than within the water column and explained most of the variation in pore water concentration to the exclusion of other factors such as species identity and initial plant shoot density. One

exception was pore water K concentration, during the middle of the experiment, where *H. verticillata* monocultures supported lower K concentrations (6.45 g ±0.78 SE) than *V. americana* (9.11 g ±1.35 SE). In contrast, water column variables were frequently affected by initial plant shoot density (PO_4, K, pH, and turbidity) and species (Ca, K). With the exception of Ca, these differences were observed mid-way through the experiment and disappeared by the end of the experiment. Higher initial plant shoot density was associated with lower concentration of PO_4 and K, lower turbidity, and slightly elevated pH (10.04 to 10.20). Ca was higher in *V. americana* monocultures (27.79 mg/L ±1.36 SE) than in *H. verticillata* monocultures (22.22 mg/L ±1.27 SE). In contrast to pore water K concentration, K concentration in the water column was lower in *V. americana* monocultures (1.62 mg/L ±0.30 SE) than *H. verticillata* monocultures (3.37 mg/L ±0.53 SE).

Total biomass produced by the end of the experiment was negatively correlated with most of the water column variables (PO_4, NH_3, K and turbidity) and positively correlated with water pH, but not with pore water variables. In contrast, root biomass was positively correlated with water column variables as well as negatively correlated with PO_4, NH_3 and NO_2 concentration in the pore water. Nutrient concentration in the water column and pore water, as well as water column pH and turbidity, were significantly correlated. For example, turbidity was positively correlated with water column PO_4, NO_2, and K, and pore water PO_4, NH_3, NO_2, and NO_3. Turbidity was negatively correlated with water column pH.

Root biomass and root-to-shoot ratio of species in monoculture were negatively correlated with nutrient concentration in the pore water (Table 5), but these correlations were determined by the presence of *H. verticillata* and not by *V. americana*.

Table 5. Correlations between root to shoot ratio, root biomass, and nutrients in monocultures. NS = Non Significant at $\alpha = 0.05$.

	Root to Shoot Ratio				Root Biomass			
	H. verticillata		*V. americana*		*H. verticillata*		*V. americana*	
Water Column								
PO_4	-0.48	0.02	NS	NS	-0.57	0	NS	NS
NH_3	NS	NS	NS	NS	NS	NS	NS	NS
NO_2	-0.5	0.01	NS	NS	-0.65	<0.001	NS	NS
NO_3	NS	NS	NS	NS	NS	NS	NS	NS
Ca	NS	NS	NS	NS	-0.43	0.03	NS	NS
K	NS	NS	NS	NS	-0.49	0.01	NS	NS
pH	0.42	0.04	NS	NS	0.63	0	NS	NS
Turbidity	-0.42	0.04	NS	NS	-0.51	0.01	NS	NS
Pore Water								
PO_4	-0.65	0	NS	NS	-0.71	<.001	NS	NS
NH_3	-0.52	0.01	NS	NS	-0.62	0	NS	NS
NO_2	-0.61	0	NS	NS	-0.67	<0.001	NS	NS
NO_3	0.62	0	NS	NS	0.66	<.001	NS	NS
Ca	-0.58	0	NS	NS	-0.52	0.01	NS	NS
K	-0.62	0	NS	NS	-0.73	<0.001	NS	NS

Competition Results

Above-ground Biomass

H. verticillata above-ground biomass in the finest (85% silt and clay) substrate was half that compared to the other two sediments. Initial *H. verticillata* plant shoot density did not affect ending *H. verticillata* above-ground biomass, especially in finer sediments where variability was high and many plants did not grow well. *V. americana*

initial plant shoot density, on the other hand, negatively affected *H. verticillata* above-ground biomass especially when 7 and 10 *V. americana* plants were initially planted (Figure 13). This effect was diminished in finer sediments.

In contrast, *V. americana* above-ground biomass was twice as high in the medium (50% silt and clay) and fine (85% silt and clay) substrate than in the coarse substrate (15% silt and clay). This effect was especially pronounced at higher initial plant shoot density of *V. americana* (Figure 13). *H. verticillata* initial plant shoot density decreased *V. americana* above-ground biomass, especially at high total densities and in the coarse sediment.

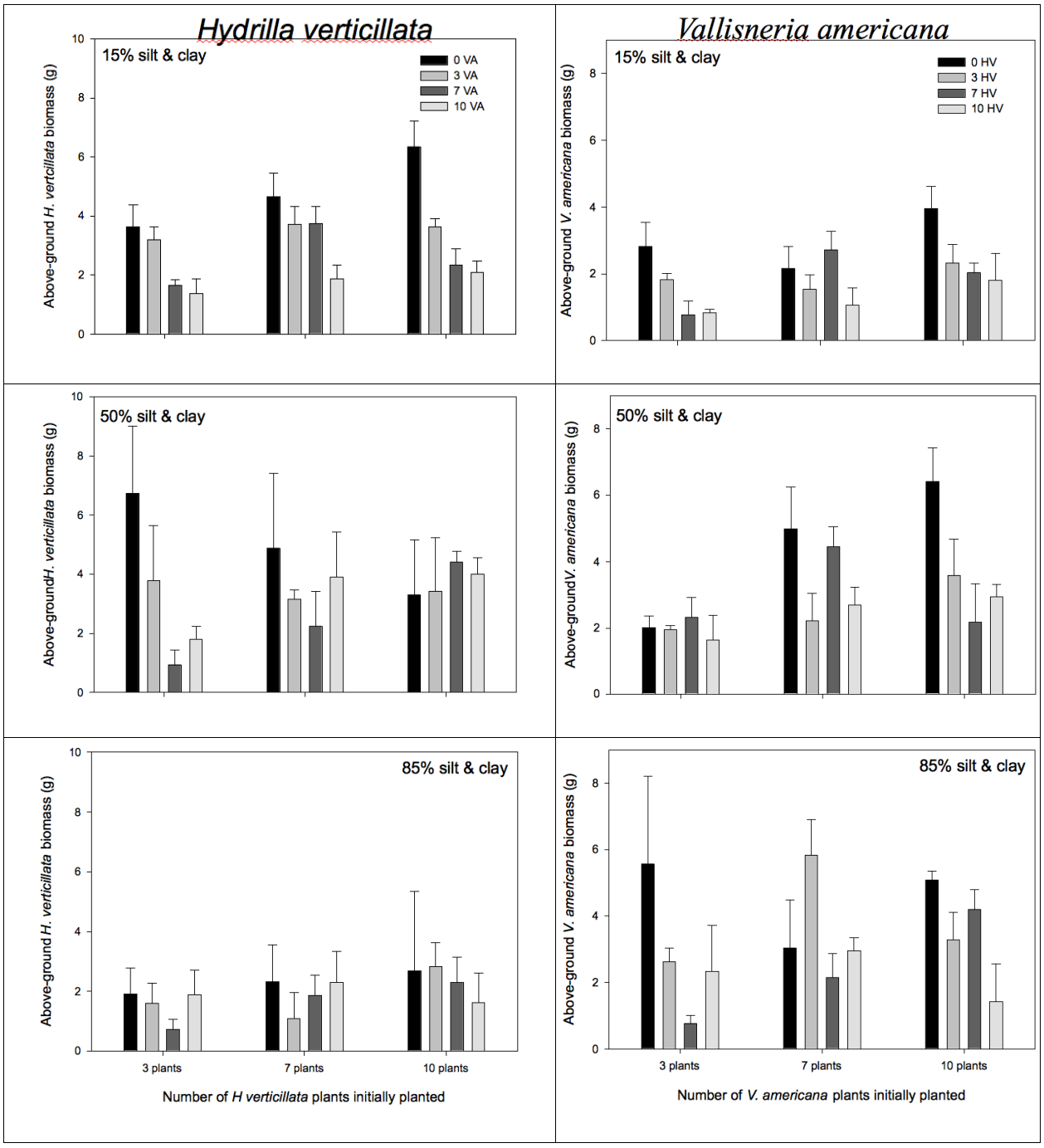

Figure 13. *H. verticillata* and *V. americana* above-ground *b*iomass in different sediment and density treatments. Note: Scaling of the Y-axes is not the same between the two species.

Root Biomass

Root biomass of *H. verticillata* in the coarse substrate (15 % silt and clay) was two times higher than in the medium sediment and eight times higher than in the fine sediment (Table 6; Figure 14). Initial plant shoot density of *H. verticillata* was only a significant factor for *H. verticillata* root biomass in the coarse sediment, where root biomass was approximately 1.5 times higher when 7 or 10 plants were planted than when 3 plants were planted (Figure 14). Likewise, *V. americana* initial plant shoot density affected *H. verticillata* root biomass the most in the coarse sediment where *H. verticillata* root biomass was twice to 4 times as high in monoculture than when *V. americana* was present. Effects of *V. americana* were most noticeable when initial *H. verticillata* plant shoot density was low. In contrast, once *V. americana* was present, additional plants had relatively little impact on *H. verticillata* biomass. Because effects of initial planting density on *H. verticillata* were the strongest in the coarse substrate, a significant density by substrate interactions was observed for both species (*H. verticillatta*, $F=2.54$, $P=0.05$; *V. americana*, $F=7.07$, $P<0.001$).

In contrast to *H. verticillata* root biomass, root biomass of *V. americana* doubled in finer sediments, but only when initial *V. americana* plant shoot density was low (Table 6). Variance in the root biomass data also increased substantially in finer sediments. Initial *H. verticillata* plant shoot density decreased *V. americana* root mass especially at higher planting density (Figure 14). NS = Non Significant at $\alpha = 0.05$.

Table 6. Competition results for *H. verticillata* and *V. americana* biomass categories with different plant densities and substrates. The two numbers in the df column denote numerator and denominator degrees of freedom.

	Model			# of *H. verticillata*		# of *V. americana*		Substrate	
H. verticillata	F	df	P	F	P	F	P	F	P
Above-ground biomass	5.96	5, 102	<0.001	NS	NS	5.15	0.002	7.17	0.001
Root biomass	10.47	17, 90	<0.001	3.62	0.03	12.04	<0.001	41.09	<0.001
# of Tubers	15.52	2, 102	<0.001	NS	NS	NS	NS	15.52	<0.001
V. americana									
Above-ground biomass	6.88	7, 100	<0.001	8.12	<0.001	5.02	0.008	6.89	0.002
Root biomass	6.94	7, 100	<0.001	6.62	<0.001	4.11	0.02	10.26	<0.001
# of Tubers	8.65	13, 94	<0.001	11.73	<0.001	13.22	<0.001	18.46	<0.001

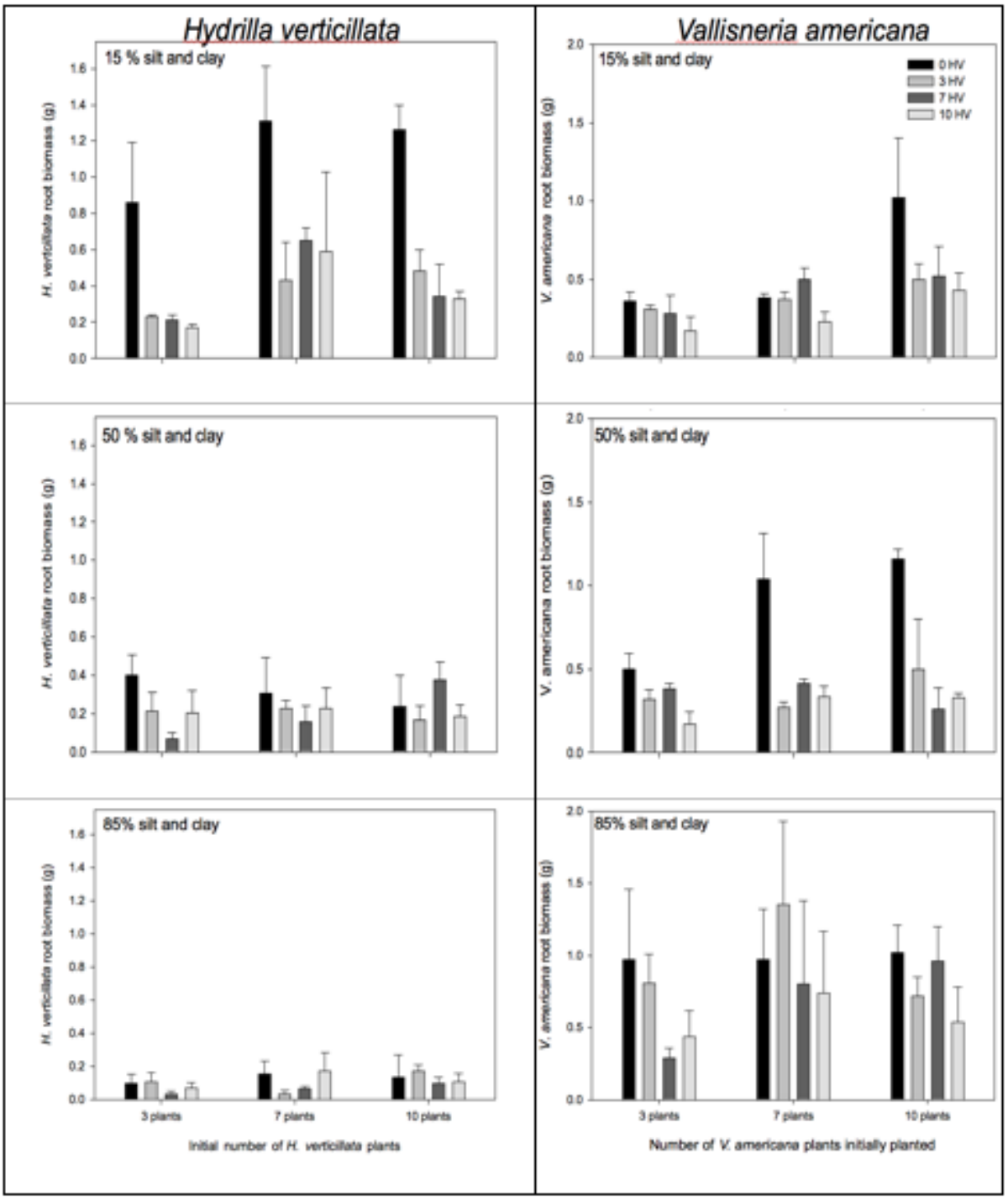

Figure 14. *H. verticillata* and *V. americana* root biomass in different sediment and density treatments. Note: Scaling of the Y-axes is not the same between the two species.

Tubers

Both species produced underground storage organs as starchy tubers. *V. americana* had produced approximately 10 times more tubers (in numbers) than *H. verticillata* at the time of harvesting. Both *H. verticillata* and *V. americana* produced fewer tubers in the coarse sediment and 2 to 3 times more tubers in the medium and fine sediment. Initial plant shoot density of either species had no effect on *H. verticillata* tuber production.

In contrast, *V. americana* tuber production was affected by both *H. verticillata* and *V. americana* initial plant shoot density (Figure 15). Specifically, initial *V. americana* plant shoot density in monoculture had no effect on *V. americana* tuber production in coarse substrate, however, in medium and fine substrate, tuber production approximately doubled at planting densities of 7 and 10 plants, respectively, compared to the lowest planting density (3 plants). On the other hand, initial *H. verticillata* plant shoot density decreased *V. americana* tuber production and this effect was especially pronounced in low initial densities of *V. americana* and in coarse sediment.

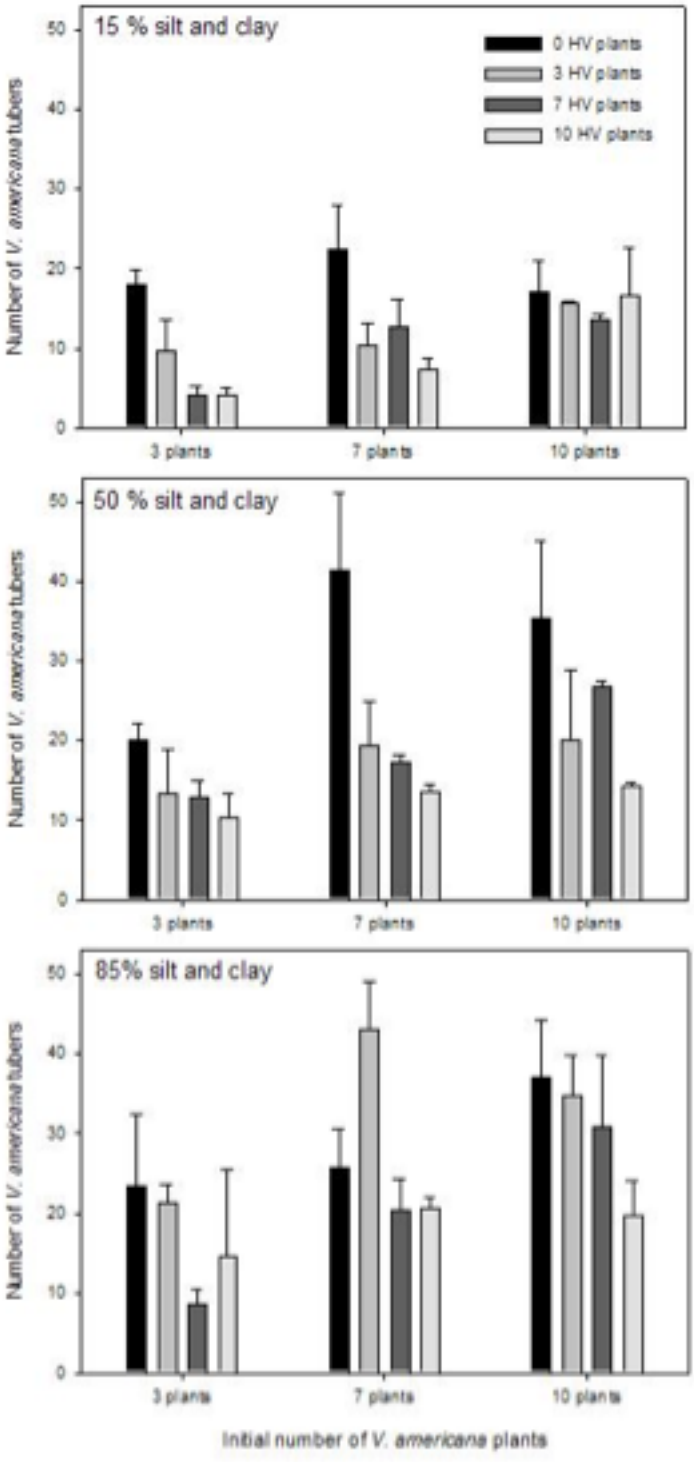

Figure 15. *V. americana* tuber production in different sediment and density treatments.

Chapter 4: Discussion

This project tested for species-specific responses of a submersed aquatic plant invader, *Hydrilla verticillata*, and a common native plant, *Vallisneria americana*, to initial plant shoot density and sediment composition using complementary field observations and greenhouse experiments. The field survey spanned 5 years, starting the year that *H. verticillata* was first observed in the study system and including subsequent years of expansion and population decline. The field data show that *H. verticillata* and *V. americana* population densities and distributions were not limited by substrate characteristics. However, water depth was predictive of *H. verticillata* population density, suggesting that the high turbidity of the system is limiting light availability, a factor that is commonly associated with submersed aquatic vegetation (SAV) population decline and slow recovery in estuaries (Hall et al. 1999, Kemp et al. 2004) or that another factor associated with water depth limited SAV growth. One possibility is water current velocity, which was not evaluated in this study.

In contrast to water depth, Secchi disc depth was not predictive of *H. verticillata* population density (which would more directly indicate light limitation); this may be an inaccurate conclusion due to the constraints of the Secchi disc method (particularly in low turbidity conditions and times of peak SAV biomass) or the effect of averaging all Secchi disc depths during the growing season instead of differentiating between sampling periods. Given this and the strong relationship between Secchi disc depth and NTUs measured by a

turbidimeter, future studies might benefit from less reliance on Secchi disc measurements and greater use of turbidimeter analyses, as well as analysis of water current velocities.

Unlike conditions at Otter Point Creek (OPC), where it is unclear whether, and if so, when, light was limiting, light was not limiting in the greenhouse experiment, and sediment characteristics, in combination with initial plant shoot density, had a strong effect on nutrient availability, inter-specific interactions, and biomass production. Thus, initial plant density and sediment composition can be key considerations of submersed aquatic community dynamics in systems where light and other factors, such as herbivory, are not limiting populations.

Although *H. verticillata* quickly spread throughout OPC after its first observation in 2002, its rate of spread appears to have reached a peak in 2004, after which *H. verticillata* coverage diminished. This mirrors the invasion pattern of *H. verticillata* within the Potomac River, although the reason for the subsequent decline remains unknown (Carter and Rybicki 1986). One unexplored hypothesis is that *H. verticillata* limits its own growth by growing rapidly and lowering nutrient availability within the water column and interstitial pore spaces. For example, in the greenhouse, *H. verticillata* significantly decreased Ca in the water column and K in the interstitial pore water compared to *V. americana*. However, N and P were unaffected.

At OPC, *H. verticillata* distribution was not affected by sediment composition, as was initially predicted. Nor was it affected by sediment type or percent of organic matter, despite the occurrence of 8 monitoring sites with sediment organic values greater than 5%,

which has been shown to limit SAV growth (Barko and Smart 1983). However, *H. verticillata* coverage was negatively correlated with water depth, which is critical in turbid systems, such as OPC. In estuarine environments, SAV is predominantly, but not exclusively (Koch 2001), limited by light attenuation through the water column (Dennison et al. 1993; Stevenson et al. 1993), which influences the depth distribution for SAV (Meyer et al. 1943; Chambers & Kalff 1985). For example, in the freshwater tidal Potomac River, the abundance of SAV populations (Carter & Rybicki 1986, 1990; Carter et al. 1994), the survival of *Vallisneria americana* transplants (Carter & Rybicki 1985; Carter et al. 1996), and the maximum depth that *V. americana* can occur (Carter & Rybicki 1990) has been attributed to light availability in the water column. At OPC, average Secchi depth ranged from 0.29 m in 2005 to 0.58 m in 2004; thus, light availability fluctuates annually with consequences for submersed aquatic vegetation growth.

H. verticillata also varied seasonally in location and coverage, with a general trend towards greatest coverage in late summer. The exception to this trend was early summer 2006, when *H. verticillata* coverage decreased in comparison to its coverage in spring. This may have been due to spring storm events (which have been shown to affect SAV coverage) or to three unintentional discharges from a nearby wastewater treatment plant that occurred on July 6, 15, and 22 of 2006 that dumped nearly 1.4 million gallons total of untreated sewage into tributaries of the Bush River.

Since 2003, the presence and coverage of *V. americana* at OPC have decreased, although it is not clear whether this is due to the increased presence of *H. verticillata* or to

environmental conditions. Most areas are shallow enough to allow growth of either species, and it appears that SAV has been able to grow at all of the sampling sites. Thus, similar to *H. verticillata*, sediment composition does not appear to be a significant limiting factor for *V. americana* in Otter Point Creek. It appears that another factor, possibly light limitation (as posited by Koch 2001 as the main limiting factor to SAV growth) and high turbidity or eutrophication (caused by sedimentation, runoff, storms, or pollutants) is limiting SAV growth and recovery at OPC. For example, Rybicki and Landwehr (2007) show that native species coverage increased with *H. verticillata* species coverage under improving water quality conditions in the Potomac River. Thus, native and exotic SAV appear to respond to the same environmental conditions – eutrophication and low light.

Though length of initial plants planted in the greenhouse experiment was equal, initial biomass of *H. verticillata* plants was 10 times lower; that is, *H. verticillata* is approximately 10 times the size of *V. americana* per unit biomass and can therefore occupy more water column space. In the field, this may translate into 10 times more coverage of *H. verticillata* than *V. americana* despite both species attaining the same amount of biomass. This also means that the rake grab method may overestimate *H. verticillata* because the rake is 10 times more likely to grab hold of a *H. verticillata* shoot than a *V. americana* shoot. In the greenhouse, *H. verticillata* grew 8 times faster than *V. americana* such that total above-ground biomass produced by the end of the season was equal between the two species. This corroborates previous studies that have shown that *H. verticillata* contains more water and less matter in its tissues, which allows the species to expand rapidly and

occupy more space in the water column (Van et al. 1976). *V. americana*, on the other hand, produces more structural tissue and therefore occupies less space per unit biomass. These differences in water content and structural tissues between the two species have significant ramifications for the invasiveness of the two species and, hence, community assembly. Because *H. verticillata* occupies more space per unit biomass, it can pre-empt resources (light and nutrients) in the water column, thereby potentially outcompeting *V. americana* in cases where water column resources are limiting.

Alternatively, *V. americana* has been described as being tolerant of low light conditions (Meyer et al. 1943, Titus and Adams 1979, Twilley and Barko 1990, Harley and Findlay 1994, Blanch et al. 1998, French and Moore 2003), although Carter et al. 1996 found that transplants of *V. americana* into the tidal Potomac River were light limited. It is possible that light limitation for *V. americana* is caused more by epiphytic growth or suspended particles within the water column than by physical shading from competitors such as *H. verticillata*. Boustany et al. (2010) found that light and *V. americana* growth in a mesocosm experiment were not correlated, and shading appeared to reduce algal growth and enhance *V. americana* tolerance and survival to salinity exposure. Although this has not been tested in the field, the presence of *H. verticillata* at OPC may facilitate *V. americana* growth through shading and nutrient uptake that, in turn, limits epiphytic growth.

In the greenhouse experiment, *V. americana* produced greater overall biomass than *H. verticillata* (Figure 10), which was mainly due to *V. americana*'s higher below-ground

biomass production of roots and tubers (Figure 10). Higher root biomass could lead to greater nutrient uptake, thus leading to a competitive advantage over *H. verticillata* in nutrient-limited conditions. Root biomass and root-to-shoot ratio of species in monoculture were negatively correlated with nutrient concentration in the pore water (Table 5), but these correlations were determined by the presence of *H. verticillata* and not at all by *V. americana*. In addition, comparison of the two species in monoculture did not show a greater capacity of *V. americana* to decrease nutrients in the pore water, as would be expected by a superior competitor (Tilman 1982). Thus, it appears that despite its higher root biomass, *V. americana* does not draw down nutrients to lower levels. Focusing on the three sediment types separately (Figure 14) shows that *H. verticillata* is capable of plastically responding to the coarse substrate by producing higher root biomass than in fine substrates. This plasticity may also provide *H. verticillata* a competitive edge and is a characteristic trait of an invader (Engelhardt et al. 2009).

V. americana produced more tubers by the end of the 12-week experiment than *H. verticillata*, which may confer a competitive advantage in future generations, a hypothesis that I could not test within the duration of the experiment. Tubers are starchy storage organs that allow a plant to overwinter when all other tissues senesce, which is generally the case for both *H. verticillata* and *V. americana* at the latitude of the study site. Tubers allow a plant to grow rapidly and expand in the spring, whereas plants from seeds are less likely to survive and grow rapidly initially. *V. americana* produced on average 5 times more tubers than *H. verticillata* (19.32 and 3.85 tubers, respectively), suggesting that its

population density at the onset of the next growing season would be greater, thereby potentially pre-empting resources (but see below). Alternatively, *H. verticillata* tuber production may have been underestimated if *H. verticillata* produces turions later in the fall than *V. americana*.

Per-capita biomass of *H. verticillata* and *V. americana* was greatest in the lowest initial plant shoot density treatments, thereby confirming the Law of Constant Final Yield (Farazdaghi and Harris 1968, Drew and Flewelling 1977). Thus, as expected, density-independent growth was generally most strong in low initial plant shoot density treatments whereas both species were limited at higher initial plant shoot densities by density-dependent growth (or by nutrient or carbon limitations). If nutrients and carbon were not limiting, density-independent responses would show an increase in biomass production with increasing initial plant shoot density, where 7 planted individuals would be expected to produce twice the amount of biomass than in treatments planted with only 3 individuals. Likewise, treatments planted with 10 individuals would support approximately 3 times the biomass than the low initial plant shoot density treatment when growth is completely density independent. That this was never realized shows that results of the study are not simply a reflection of different growth responses to the environment, but were also a reflection of intra- and inter-specific interactions.

In contrast to density-independent growth, density-dependent growth would show a decrease in per-capita biomass production with higher initial plant shoot density. No differences in total biomass production among density treatments would suggest complete

density-dependence, as is the case for total *V. americana* above-ground biomass and tuber production in the coarse sediment and for *V. americana* root biomass in the fine sediment.

For *H. verticillata*, its own initial plant shoot density was a significant factor for growth of the above ground biomass, roots, and turions of the individual plants for all treatments combined, monocultures, and bicultures, respectively. This implies that management strategies focused on keeping *H. verticillata* plant shoot density low at invaded sites may not be effective for overall control of *H. verticillata*, since lower densities will allow for greater biomass production and presumably greater turion production, fragmentation, and colonization of *H. verticillata*. Although perhaps counterintuitive, this is similar to the results of Serafy et al. (1994), who found that sites manipulated by mechanical removal of *H. verticillata* in the Potomac River in 1988 showed increased plant biomass after 23 days compared with unharvested sites. However, Fox et al. (2002) found that repeated clippings of above ground *H. verticillata* biomass significantly reduced the number of turions produced (most likely due to the plants' inability to form a surface canopy), which could significantly improve long term *H. verticillata* control. Given the fast growth rate of *H. verticillata*, it is unlikely that repeated clippings of all *H. verticillata* during the entire growing season at invaded sites will be a viable or cost-effective control mechanism for most managers, and thus other strategies should be investigated.

As with *H. verticillata*, *V. americana* also had the highest biomass in the lowest initial plant shoot density treatments. *V. americana* initial plant shoot density was a

significant factor in the growth of *V. americana* above-ground biomass, roots, and tubers for all treatments combined and for bicultures. For monocultures, none of the factors studied affected the amount of either above-ground or root biomass produced during the experiment. In contrast to *H. verticillata* control efforts that might not succeed if attempts are made only to minimize plant shoot density, restoring *V. americana* to its native habitat might benefit from planting low density patches in places were other competitors, such as *H. verticillata*, are absent. In cases where competitors are present, however, low planting density allows the competitor to establish a foot-hold in the community during density-independent growth, which may not always be desirable, as in the case of *H. verticillata* establishment. For example, *V. americana* in monoculture produced the same amount of tubers (20) in coarse sediment irrespective of initial planting density. However, in the lower initial *V. americana* shoot density treatments, *V. americana* tuber production was significantly lower when *H. verticillata* was present and this competitive effect was especially strong at higher initial *H. verticillata* shoot density. In contrast, when 10 *V. americana* plants were planted, *H. verticillata* presence and initial shoot density had no effect on tuber production. It is unclear from the results of this study whether this is due to nutrient limitations, space constraints, or some other factor. However, these results clearly indicate that effective restoration efforts of *V. americana* at OPC will require higher density plantings so that *V. americana* has a better chance of overcoming competition from *H. verticillata*. They also suggest that restoration efforts should focus first on areas with lower *H. verticillata* density to minimize competition. However, this strategy might run

into issues of environmental conditions; when conditions are not conducive for *H. verticillata* growth, they may be sub-optimal for *V. americana* growth as well, except in areas that have fine sediments.

Both species were clearly limited by intra- and inter-species competition, though *H. verticillata* and *V. americana* were juxtaposed in how they grew in the different sediment compositions. *H. verticillata*, contrary to the original hypothesis, did not grow well in the fine substrate and, in a few cases, survival to the end of the experiment was low. However, *H. verticillata* created the most biomass in the sandy sediment. In contrast, *V. americana* produced the most biomass in the fine sediment. Since the 85% silt+clay treatment, in which *V. americana* produced the most biomass, was composed entirely of sediment taken from OPC, it can be assumed that *V. americana* would grow well in OPC sediment in the field, if water quality could be improved. Given that the highest abundance of native and invasive species at OPC occurred in 2004, it is unlikely that the presence of *H. verticillata* is the limiting factor for native SAV growth at OPC. Instead, growth of native (and invasive) species at OPC has most likely been deterred by other factors such as high turbidity (and thus low light availability) caused by suspended sediment or nutrients, high nutrient levels, physical disturbance by invasive carp, or intense storm events in the early spring. However, since *H. verticillata* is a canopy former, it may not be as affected by low light availability as native SAV species. In addition to the environmental issues mentioned, several sewage spills in July 2006 and other potential sources of pollution may have had deleterious effects upon SAV growth at OPC.

As often occurs with plant competition studies, which species is the better competitor is still unclear; both species significantly affected each other's biomass in biculture. Competitive effects of and responses to a competitor (Goldberg and Barton 1992) appear to be generally equal. Both nitrate and carbon may have been limiting nutrients, as indicated by the low nitrate levels within the water column and pore water samples throughout the experiment and the relatively high pH values, respectively. However, ammonium, which is considered the most important source of nitrogen for aquatic plants, was adequate, and both *H. verticillata* and *V. americana* are capable of switching to bicarbonate if carbon dioxide is limited, although it is not known if they can do so with the same efficiency.

Policy recommendations generated by this study are for scientists to be extremely cautious of analyzing results from incomplete studies, and for restoration efforts of *Vallisneria americana* to target areas of fine soils (silt and clay), with individuals planted in low densities, away from *H. verticillata* plants, or in high initial plant density when competitors are present. Although planting *V. americana* in sub-optimal conditions should not damage the ecosystem, it could waste much effort, resources, and time, and hinder the recovery of this native species and the ecosystem functions it provides. Invasions of non-native species are one of the world's greatest causes of species extinctions (Sala et al. 2000) and estuaries are among the most vulnerable systems (Wasson et al. 2002). Thus identifying approaches that effectively conserve and restore habitats are currently important global research and management topics.

Three important results for *H. verticillata* management and native SAV restoration efforts at OPC have come out of this project, with the caveat that both nutrients and carbon may have been limiting by the end of the greenhouse experiment, and experimental results must be validated with *in situ* studies. First, both *H. verticillata* and *V. americana* produce more biomass at low initial shoot densities. This means that simply reducing the plant density of *H. verticillata* will not be enough to control its spread or abundance, and in fact may lead to greater biomass. It also means that restoration efforts using *V. americana* should potentially focus more effort on patch area and protection rather than planting in high plant density. Second, it is important to note that although *H. verticillata* spread very quickly in location and coverage after the initial observation of it at OPC in 2002, it also appears to have peaked and has now declined in both coverage and abundance. Unfortunately, native species of SAV appear to have declined as well, which implies that another environmental factor besides the presence of *H. verticillata* may be impacting the growth of SAV at OPC. However, this may also indicate that *H. verticillata* spread so quickly that it limited nutrients and carbon within OPC, thereby decreasing the amount of turions produced in 2004 and the amount of biomass in subsequent years.

Finally, that *V. americana* produced the most biomass in the 85% silt+clay treatment should be encouraging news for restoration efforts at OPC, since the sediments used in my experiment were taken directly from OPC. Further studies may show that *V. americana* is capable of tolerating even finer sediments and greater amounts of organic matter. Results of this study imply that if other limiting factors for SAV growth at OPC are

determined and ameliorated, *V. americana* should be able to persist at OPC again in the future. Whether or not this actually happens will most likely depend on how the estuary is treated as a whole, since planted or recruited SAV at OPC will still be vulnerable to poor water quality and other environmental factors.

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