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Abstract

Submersed macrophytes are generally found in multispecies beds, with the dominance of individual species varying in both space and time. In estuarine environments, these plants can grow across a range of environmental conditions which may alter species interactions. Three species common to the Chesapeake Bay region, *Vallisneria americana* (wild celery), *Heteranthera dubia* (water stargrass), and *Stuckenia pectinata* (sago pondweed), were planted in a microcosm designed to test their growth and interactions (relative yielding) under a range of conditions of salinity (0, 5, or 10), sediment type (mud or sand), and species combinations. *H. dubia* was most sensitive to elevated salinity, while sediment type impacted only *V. americana,* performing better in mud compared with sand. *V. americana* and *H. dubia* were strong competitors, overyielding in many treatments when grown in mixture, while *S. pectinata* never overyielded and frequently underyielded. Interspecific competition was only strong between *H. dubia* and *S. pectinata* under 0 salinity, regardless of sediment type. *V. americana* on the other hand, showed strong interspecific competition with *S. pectinata* across multiple salinity and sediment types, indicating that this species is able to compete well across a wider range of environmental conditions. Our results suggest that *H. dubia* and *V. americana* are strong candidates for multi-species restoration, as positive interactions were observed when grown together. This measure of complementarity provides evidence for increased mixed bed plant performance under environmental conditions that would typically be more stressful to each growing alone.

Key words: submersed aquatic vegetation; complementarity; competition; restoration; Chesapeake Bay

1. Introduction

Submersed aquatic vegetation (SAV) growing in low-salinity and freshwater systems are typically not found in monotypic communities, but in multispecies beds, with the dominance of individual species varying in both space and time (Moore et al., 2000; Chambers et al., 2008; Orth et al., 2009; Arthaud et al., 2013). This suggests that there is a range of suitable environmental conditions among the diversity of species in these beds. This may allow for greater natural survival or restoration under a wider range of environmental conditions when compared to monotypic communities.

Changing environmental conditions may alter the competitive advantage of one species over another, because each species may have different requirements for their growth or tolerate a different range of conditions. Within an estuarine system such as the Chesapeake Bay, parameters related to light, temperature, nutrients, salinity, and sediment may all play roles in the SAV community dynamics (Kemp et al., 2004). Historically, light availability has been a primary focus when studying SAV habitat requirements (Carter and Rybicki, 1990; Korschgen et al., 1997; Moore et al., 1997; Moore and Wetzel, 2000). Salinity and sediment requirements have not received as much attention, but are likely to be very important in estuarine environments due to their variability in both space and time and their differing effects on individual SAV species.

SAV communities in the Chesapeake Bay are typically distributed by salinity, with *Zostera marina* and *Ruppia maritima* occurring in meso and polyhaline regions, and a variety of freshwater mixed species occurring in oligohaline and tidal fresh regions. Within the oligohaline and tidal fresh regions, over 15 species of SAV have been identified (Moore et al., 2000). Many of these species have been shown to have differing salinity tolerances (Teeter, 1965; Haller et al., 1974; Kantrud, 1990; Twilley and Barko, 1990; French and Moore, 2003; Bergstrom et al., 2006; Frazer et al., 2006) as well as a range of suitable sediment conditions for their growth (Barko and Smart, 1983; Hoover, 1984; Barko and Smart, 1986; Chambers and Prepas, 1990; Batiuk et al., 2000; Jarvis and Moore, 2008).

It is not well understood how different local sediment composition and salinity levels might affect SAV bed growth or how these conditions might affect SAV restoration success when species are planted both singly and in competition with other species. Typically, restoration of SAV has been conducted using a single species approach, while the potential positive interactions of planting multiple species together has generally been overlooked (Halpern et al., 2007). Previous work has determined that there is considerable potential for SAV restoration in the major Chesapeake Bay tributaries including the James River using both whole plants and seeds (Moore and Jarvis, 2007; Moore et al., 2010). It is still poorly known if mixed plantings would be more successful by providing a broader range of bed tolerance when subject to varying environmental conditions. It has been reported that in many regions experiencing re-growth of SAV that *Vallisneria americana* can be found growing in combination with other SAV, including *Hydrilla verticillata, Myriophyllum spicatum, Heteranthera dubia,* and *Ceratophyllum demersum* (Moore et al., 2000; Rybicki and Landwehr, 2007). This suggests that mixed plantings may improve restoration success through complementarity among species in resource utilization.

Plants exhibit positive complementarity when their combined performance is greater than what would be expected from them individually (Loreau et al., 2001). This is due to resource partitioning and facilitative interactions, and has been observed in SAV communities (Salo et al., 2009; Gustafsson and Boström, 2011; Hao et al., 2013). On the other hand, multi-species assemblages may not increase overall productivity, bed resilience or restoration success due to interspecific competition, which has been shown to be strong in both temperate and tropical SAV communities (Titus and Stephens, 1983; Moen and Cohen, 1989; Van et al., 1999; Spencer and Ksander, 2000; Barrat-Segretain and Elger, 2004).

Here we present results from a microcosm that was designed to test the growth and competitive abilities of low-salinity and freshwater SAV under varying conditions of salinity and sediment type. We address the following research questions: a) What effect will different salinity and sediment types have on plants growing separately in monoculture? b) How will the different treatments alter species interactions when plants are grown in combination? Our goals were to examine the degrees of competition and complementarity among three different species exposed to variable environmental conditions, and to improve the site selection criteria and success of restoration efforts of freshwater and low-salinity tolerant SAV.

2. Methods

An outdoor microcosm was used for the experiment which was conducted in the summer and located at the Virginia Institute of Marine Science, Gloucester Point, Virginia (37°14.8’N, 76°30.3’W). 20-liter white translucent containers with a height of 37 cm and diameter of 30 cm were used for each individual experimental unit, and all the containers were housed in a shallow nursery tank approximately 8.5 m x 3 x 0.5 m filled with freshwater to allow for consistent temperatures among the experimental units. Three main treatments were established. Sediment type consisted of two levels (mud and sand), salinity consisted of three levels (0, 5, 10) and species combinations included all combinations of three species (three monocultures, three bicultures, one triculture) for a total of 42 treatments. Each treatment was replicated three times for a total of 126 experimental units. *Heteranthera dubia* (water stargrass) and *Vallisneria americana* (wild celery) plants were taken from adjacent outdoor nursery tanks grown from local Chesapeake Bay stock, and *Stuckenia pectinata* (sago pondweed) was harvested from two outdoor ponds located on the Chesapeake Bay at the University of Maryland Center for Environmental Science Horn Point Laboratory, Cambridge, Maryland (38°35.5'N, 76°08.8'W). These were brought back to Virginia and planted in an outdoor SAV restoration nursery pond next to other ponds containing the other species. Prior to the start of the experiment, oligohaline estuarine sediment was collected from the Chickahominy River, Virginia. Sediments were obtained from two sites where SAV occur, with target organic content of > 8 % for the muddy site (37°17.5'N, 76°51.8'W) and < 2 % for the sandy site (37°15.5'N, 76°52.4'W). At the time of collection, percent organic content was determined through loss on ignition (Erftemeijer and Koch, 2001). NH_4^+ concentrations were determined using a Lachat auto analyzer (Liao, 2001, revised 2002) and PO_4^{3-} concentrations were determined spectrophotometrically at 880nm (VIMS, 1991).

Sediment was homogenized, and each container was filled approximately 10 cm deep with sediment, and then filled with filtered freshwater. Plants were sorted within species to a similar length (*V. americana* 16.8 cm ± 1.2; *H. dubia* 17.6 cm ± 1.4; *S. pectinata* 44.3 cm ± 2.2). A subset of 30 plants from each species was sampled for dry weight measurements of above and belowground biomass (*V. americana* 0.068 gDW; *H. dubia* 0.042 gDW; *S. pectinata* 0.074 gDW per plant). A total of 12 plants were planted in each container in a replacement series design. With this design, the total number of plants in each container was kept constant, but the number of plants per species was altered according to their species combination treatment. For example, in biculture, six plants of each species were used, and in triculture four plants of each species were used. This planting density was chosen based on a literature review of densities of natural plant populations of these species (Moen and Cohen, 1989; Van et al., 1999; Jarvis and Moore, 2008).

After planting, each container was placed in the tank in a randomized design. The tank was filled with freshwater, and a drain pipe ensured the water level in the tank never rose above the rim of the containers. This served as a water bath to help keep temperature constant in the containers. The containers were allowed to sit for two days to allow sediment settlement, and then individual air bubblers and aquarium foam/floss, carbon, and zeolite filters were connected to each container. These filters were routinely rinsed and were replaced halfway through the experiment. Clear plexiglass sheets were placed over each container to minimize evaporation and to protect the containers from rain. A neutral density (50% light reduction) shade cloth was placed over the top of the tank to minimize algal growth and to better mimic natural field light availability.

The experiment started on 17-June and ran for 11 weeks. Plants were kept in freshwater until 10-July, when salinity treatments began, in order to allow the plants to recover from any transplant stress. Salinity was elevated in increments over the course of the next 19 days using Forty Fathoms© Crystal Sea® salt. This was done to parallel rates of salinity change which have been observed under natural field conditions in the region (Shields et al., 2012). The 5 salinity treatments were increased by 1 and the 10 salinity treatments were increased by 2 every 3-4 days during the 19 days until the final concentrations were reached. Salinity was monitored every 3-4 days during this period using a handheld YSI 6000 (Yellow Springs Instrument, Inc.). Additionally, temperature, dissolved oxygen, and pH were also monitored biweekly throughout the experiment.

At the end of the experiment prior to harvesting, sediment was sampled for percent organic content and NH_4^+ and PO_4^{3-}. All plant material was harvested and brought to the lab for measurements of maximum shoot length, shoot density, and above and belowground biomass. Biomass was determined by drying the plants at 60°C until a constant weight was obtained.

2.1. Data Analyses

Relative growth rate (RGR) was determined based on natural logarithm transformed dry weights of total biomass (above and below ground). Initial dry weights were subtracted from final dry weights and divided by the length in days of the experiment (gdw gdw^{-1} day^{-1}). Multivariate analyses of variance (MANOVA) were run for all species separately in monoculture for RGR, density, and length, with salinity and sediment as fixed factors. Where appropriate, univariate ANOVAs were then used to analyze treatment effects on individual response variables. Tukey's HSD tests were run when significant differences were found. Before testing, residual plots and QQ plots were observed to ensure normality and homoscedasticity.

Relative yield (RY) and relative yield totals (RYT) were calculated for RGR based on Hooper (1998) and Engelhardt and Ritchie (2002) in order to analyze the degree of competition and complementarity among species in the different treatments. To calculate an individual RY, the mean RGR of a species in monoculture was calculated individually for all treatments, and this number was used as the expected mean. Next, the RGR of that species in mixture was calculated by accounting for differences in initial planting densities; i.e. biomass in biculture was multiplied by 2, and by 3 in triculture. This number was then divided by the expected mean of the species in monoculture to calculate the RY. Interspecific competition was strong when one species significantly overyielded while another underyielded in mixture. Relative yield totals (RYT) were used to define species complementarity, and were calculated by averaging the RYs of all the species in each treatment. When RYT > 1, species were considered complementary as long as each had an individual RY > 1. One-sided 95% confidence intervals were performed for all RYs and RYTs to test if the value was significantly different from 1. All data analyses were performed in RStudio (R Core Team, 2012).

3. Results

3.1. Environmental Conditions

Temperature, pH and dissolved oxygen remained consistent throughout the experiment with no differences among treatments or planting combinations observed. Mean temperature during the dates measured ranged from 26.3 °C to 28.6 °C, mean pH ranged from 8.40 to 8.75, and mean dissolved oxygen ranged from 7.37 mg l^{-1} to 8.46 mg l^{-1}. Mean salinity concentrations in the containers prior to their increase were constant for all three salinity treatments at 0.23. After the increases were performed, the target concentrations were met, with mean salinity values always within 0.5 of targets. The mud treatments had higher mean organic content, higher NH_4^+ concentrations, and lower

PO_4^{3-} concentrations compared with the sand treatments, both at the beginning and at the end of the experiment (Table 1).

3.2. Individual species response in monocultures

Salinity had significant effects on the performance of *H. dubia*, but not sediment (Fig. 1, Table 2). Salinity impacted both RGR and density, with 0 and 5 treatments greater than 10 for both parameters. Length showed no significant response. *S. pectinata* was not significantly impacted by sediment or salinity (Fig. 1, Table 2). For *V. americana,* sediment showed significant effects (Table 2), with plants growing taller in mud compared with sand, while RGR and density were unaffected (Fig. 1).

3.3. Relative Yield

V. americana and *H. dubia* were the most competitive species, significantly overyielding in 6 and 7, respectively, of the possible 18 treatments, and never underyielding (Fig. 2). *S. pectinata* was a weak competitor, never overyielding and significantly underyielding in 8 of the treatments (Fig. 2).

Interspecific competition was strong in five of the treatments (Fig. 2). With *H. dubia,* significant overyielding paired with significant *S. pectinata* underyielding only occurred in 0 salinity treatments, regardless of sediment type. On the other hand, significant *V. americana* overyielding paired with significant *S. pectinata* underyielding occurred across a variety of salinity and sediment types (Fig. 2). Complementarity occurred in both the *V. americana*/*H. dubia* biculture and triculture grown in sand in 10 salinity. Here, RYT > 1, and the individual RYs for *V. americana* and *H. dubia* were > 1 in both the biculture and triculture. *S. pectinata* remained unchanged in the triculture with a RY = 1.

Salinity appeared to play a different role in the competitive ability of *H. dubia* compared with *V. americana*. *H. dubia* significantly overyielded in mixture primarily in 0 salinity treatments. On the other hand, the majority of cases in which *V. americana* significantly overyielded were in the 10 salinity treatments (Fig. 2).

4. Discussion

The three species studied here demonstrated the wide range of tolerances and competitive abilities which have been found among low-salinity SAV. All survived and grew throughout the summer-long experiment. When each was grown in monoculture, without competition from the other species, there were no interactions observed in the species growth responses to the levels of salinity and sediment tested here. This suggests that the factors of sediment type and salinity may be affecting the plants through different ways. For example, sediment type may be influencing the rates of nutrient uptake (Barko et al., 1991), while salinity levels may be influencing plant respiration or photosynthesis (French and Moore, 2003).

Both *S. pectinata* and *H. dubia* performed equally as well in muddy and sandy sediment types, and *V. americana* and *S. pectinata* grew well across a range of salinities. However, *H. dubia* growth was reduced in the 10 salinity treatment compared to lower salinity levels, and *V. americana* growth was reduced in the sand treatment in comparison to its growth in mud. Morphologically, each species responded differently to these stressful conditions. *H. dubia's* low overall growth rate under high salinity was driven by a decrease in clonal reproduction, with shoot lengths remaining unchanged among treatments. On the other hand, *V. americana's* reduced overall growth in sand was driven by a decline in shoot elongation, while clonal reproduction did not change across sediment type. These changes in growth morphology may have important implications for their competitive abilities or responses to other environmental stressors, such as reduced light availability where an elongated shoot length could be important, or physical disruption where rapid clonal spread may be necessary.

While single species responses to environmental conditions are important, evidence exists for both competition and environmental conditions as drivers for species interactions and distributions in aquatic macrophyte communities (Anderson and Kalff, 1986; Chambers and Prepas, 1990; McCreary, 1991; Gopal and Goel, 1993). Our study showed examples of both, with interspecific competition being the driving force in some cases, and salinity stress in others. Both *V. americana* and *H. dubia* were stronger competitors than *S. pectinata,* though the degree of competition varied with environmental condition. *V. americana* was able to outcompete *S. pectinata* across all sediment and salinity treatments, while *H. dubia* typically only outcompeted in 0 salinity. *S. pectinata* proved to be the least competitive species, as it significantly underyielded in mixtures in many of the multi-species treatments, and never overyielded. Engelhardt and Ritchie (2002) found opposite results in their experiment, where *S. pectinata* was the dominant species, overyielding in all mixed plantings. Their experiment differed from ours in that they did not include *V. americana* or *H. dubia,* which appear to be much stronger competitors than the other species they used (*Potamogeton nodosus, Potamogeton crispus, Zannichellia palustris).* This illustrates the broad range of competitive abilities that may exist among low-salinity SAV communities.

Competitive abilities of plants have been shown to vary along environmental gradients, but how the intensity of competition changes with increasing abiotic stress has proven inconsistent (Gaudet and Keddy, 1995; Greiner La Peyre et al., 2001; Hooper and Dukes, 2004; Elmendorf and Moore, 2007). For *H. dubia,* our results provide evidence that interspecific competition is stronger when abiotic stress is less. This species was typically a strong competitor at 0 salinity, which was the least stressful for this species. As salinity increased, the degree of competition decreased, as the stress of salinity became the driving factor affecting its performance. *V. americana* on the other hand, was able to outcompete *S. pectinata* under a variety of sediment and salinity conditions, indicating that it is able to outcompete weaker competitors under a wider range of conditions than *H. dubia.*

When grown separately in monoculture, *H. dubia* did not perform well in the 10 salinity treatment, and *V. americana* did not perform well in the sand treatment, however when grown together both in biculture and in triculture, these species exhibited positive interactions. They performed relatively better in mixture than they did by themselves, allowing them to perform well in what would otherwise be stressful conditions. This suggests that these two species are complementary in their resource use and under stressful abiotic conditions this allows them to individually access resources, such as light or nutrients, which would be more limiting to each when growing monotypically (Hooper, 1998; Spehn et al., 2000). Morphologically, each species responded differently to these stressful conditions when grown in monoculture, as *H. dubia* decreased clonal reproduction while *V. americana* decreased shoot elongation. When grown in mixture in sand and 10 salinity, *H. dubia's* low shoot density and *V. americana's* stunted shoot height may have worked in complementary ways, allowing maximum resource allocation, though the exact mechanism behind this is beyond the scope of this experiment.

This work was done in an experimental setting in relatively small containers rather than a field setting, in order to control and be able to more precisely manipulate the different treatment combinations and to more accurately measure the species interactions. In these types of confined spaces, interspecific competition may be stronger and positive plant interactions weaker than what would be observed in a natural field setting. Previous studies have indeed demonstrated the importance of spatial scale in aquatic plant communities, with competition dominating at smaller "patch" scales, and positive facilitative interactions dominating at "bed" scales (van de Koppel et al., 2006; Hengst et al., 2010). The fact that positive plant interactions were measured between *H. dubia* and *V. americana* even in a microcosm setting, provides evidence for these interactions perhaps becoming even stronger at the larger bed scale in a natural field setting, and provides a framework for future larger scale studies.

Results from this study can be used to improve restoration techniques for these species and other similar low-salinity SAV in estuarine environments. Here we show that species typically found growing together in multispecies beds respond differently to changing environmental conditions, so using generalized SAV habitat requirements for restoration targets may have limited success in diverse communities. Individual salinity tolerances should especially be considered, and in estuarine areas where higher salinities (5-10) can be expected occasionally, of the species studied here, *V. americana* should be considered as a primary restoration species. All three species tolerated a broad range of sediment conditions, so organic content, for example, may not be as limiting a factor for restoration targets as previously indicated. For example, previous SAV habitat requirement studies (Batiuk et al., 2000; Koch, 2001; Kemp et al., 2004) suggested that sediments for freshwater SAV restoration in the Chesapeake Bay should consist of less than 5% organic matter. While high organic sediments may be deleterious for seagrasses growing under high salinity conditions due to potentially high sediment sulfide concentrations (Borum et al., 2005), this would not be expected to be as great an issue under oligohaline or freshwater conditions. Therefore the sediment habitat requirements for freshwater SAV restoration in some areas may need to be re-evaluated.

Typically, restoration of SAV has been conducted using a single-species approach. This study provides strong support for using *H. dubia* and *V. americana* together in co-plantings when habitat conditions may occur in the ranges of those studied here. When planted together, both species either performed equally as well, or better, than they did when grown by themselves, especially when stressed. This capacity for complementarity is important as restoration efforts are costly, and improvements to the resiliency of restored beds are critical for success, especially in physically variable estuarine habitats.

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Table 1

Mean ± SE for sediment nutrients and organic content for the mud and sand treatments. Initial values were taken in the field at the time of sediment collection, and final values were taken at the time of harvest at the end of the experiment.

	Mud	Sand
Initial		
NH_4^+ (μM)	178.2 ± 18.3	20.5 ± 6.2
PO_4^{3-} (μM)	0.17 ± 0.0	0.45 ± 0.2
Organic (%)	9.8 ± 0.2	0.52 ± 0.1
Final		
NH_4^+ (μM)	74.6 ± 12.6	24.0 ± 2.6
PO_4^{3-} (μM)	0.46 ± 0.0	1.1 ± 0.3
Organic (%)	9.2 ± 0.4	1.0 ± 0.8

Table 2

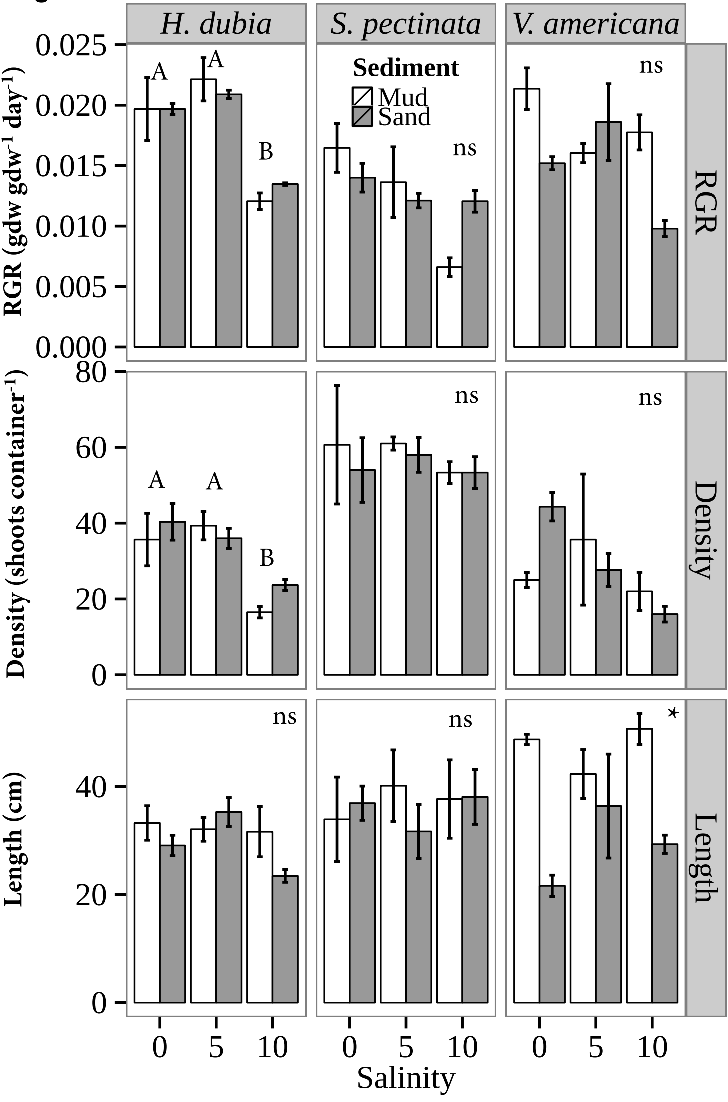
MANOVA results for all response variables (RGR, density, and length) for three species under different sediment and salinity conditions. Significant results are highlighted in bold.

	df	Wilks	F	p value
H. dubia				
Sediment	1	0.77	0.91	0.47
Salinity	2	0.17	4.25	< **0.01**
Sediment x Salinity	2	0.52	1.15	0.37
S. pectinata				
Sediment	1	0.85	0.57	0.65
Salinity	2	0.38	2.08	0.10
Sediment x Salinity	2	0.53	1.25	0.32
V. americana				
Sediment	1	0.34	6.57	**< 0.01**
Salinity	2	0.40	1.92	0.13
Sediment x Salinity	2	0.35	2.29	0.08

Fig. 1. Relative growth rate (RGR), density, and length of all species in monoculture (*Heteranthera dubia* left; *Stuckenia pectinata* middle; *Vallisneria americana* right) across all salinity and sediment treatments. Values are mean ± 1 SE, n = 3. Different letters indicate significant differences among salinity treatments, and the star indicates that the results were significantly different between sediment types. NS = not significant.

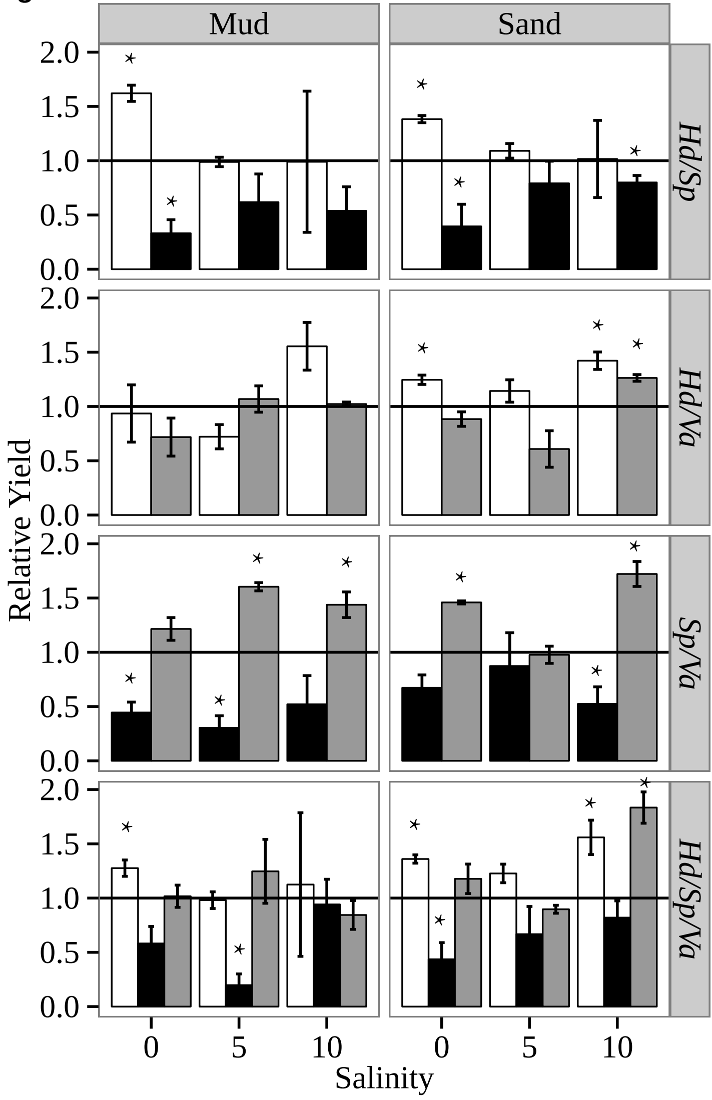
Fig. 2. Relative yield calculated based on relative growth rate for each species in all salinity (x-axis) and sediment (left and right panel) treatments. Species combination treatments are in order from top to bottom: *H. dubia/S. pectinata* biculture; *H. dubia/V. americana* biculture; *S. pectinata/V. americana* biculture; all species in triculture. White is *H. dubia*, black is *S. pectinata,* gray is *V. americana.* Values are mean ± 1 SE, n = 3. A line is drawn across a relative yield of 1 which represents a species performing equally well in mixture compared with monoculture. Stars indicate significant overyielding or underyielding with a 95% confidence interval.

Figure1



H. dubia
S. pectinata
V. americana
Sediment
Mud
Sand
RGR
Density
Length
RGR (gdw gdw-1 day-1)
Density (shoots container-1)
Length (cm)
Salinity
A
A
B
ns
ns
ns
ns
ns
ns
ns
*
0.000
0.005
0.010
0.015
0.020
0.025
0
20
40
60
80
0
20
40
0
5
10

Figure2



Mud
Sand
2.0
1.5
1.0
0.5
0.0
Hd/Sp
Hd/Va
Sp/Va
Hd/Sp/Va
Relative Yield
0
5
10
Salinity