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Influence of environmental factors on *Vallisneria americana* seed germination☆

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Abstract

Over the course of a growing season (April–October) water quality (water temperature, light, salinity, dissolved oxygen) and reproductive phenology (biomass, production of flowering shoots and seed pods, seed bank densities) were quantified in three *Vallisneria americana* beds in Nanjemoy Creek, MD, a tributary to the Chesapeake Bay. Clonal production of *V. americana* biomass increased at all sites when water temperatures rose above 25 °C. Flowering occurred during peak biomass (August–September) and resulted in the production of up to 16,000 seeds m^{-2} at the end of the growing season. However, observed seed bank densities represented <1% of seed production. Laboratory experiments quantified the effects of dissolved oxygen (0.29–8.00 mg l^{-1}), light (0–160 μmol m^2 s^{-1}), temperature (13–29 °C), salinity (0.1–17.4 psu), sediment composition (3–86% sand; 0.9–8.3% sediment organic content), and burial depth (0.2–10 cm) on *V. americana* seed germination. Germination of *V. americana* seeds was enhanced (greater overall germination and shorter time to germination) under oxygenated conditions (8.00 mg l^{-1}), temperatures >22 °C, salinities of <1 psu, and in sediments composed of ≤3% organic content and >40% sand. Light (<160 μmol m^{-2} s^{-1}) and burial depth (0.2–10 cm) had no significant effects on germination. Temperatures most favorable for seed germination (>22 °C) occurred in June, 2 months in the growing season just prior to development of peak vegetative standing stock. Seedlings were therefore at a distinct disadvantage to plants developed from over wintering buds. A lack of viable seed retention and inadequate environmental conditions at critical times in the growing season may be limiting seed germination success and subsequent seedling establishment within *V. americana* beds in the Chesapeake Bay. However, ungerminated seeds were found to maintain high viability, especially at salinities of 10 psu that can have significant negative effects of shoot growth survival. This suggests that seeds may serve as a source of reproductive material for bed recovery after periods of drought or other stressful conditions in estuarine systems.

Keywords: Submerged aquatic vegetation; Seeds; Germination; Viability

1. Introduction

Vallisneria americana Michx. (wild celery) populations are primarily maintained inter-annually through clonal reproduction with a minimal observed contribution from seeds (Sculthorpe, 1967; Stevenson, 1988; McFarland, 2006). However, seeds have been found to provide important functions for submerged macrophyte communities through the establishment of new genotypes in existing populations (Kimber et al., 1995; McFarland, 2006), movement of populations into new regions (Arnold et al., 2000; Figuerola and Green, 2002; Harwell and Orth, 2002), and re-establishment of populations after episodic declines (McMillan and Jewett-Smith, 1988; Titus and Hoover, 1991; Preen et al., 1995). Due to the apparent dominate role of clonal reproduction, there is a lack of information concerning the timing and success of *V. americana* seed germination (Lokker et al., 1997) and the ability of germinated seeds to become established as seedlings under *in situ* conditions (Sculthorpe, 1967).

Influences of environmental factors, specifically temperature and salinity, on sexual reproduction and seed germination have been documented for many submerged macrophyte species (Muenscher, 1936; Barko et al., 1986; Moore et al., 1993). For example, in the Chesapeake Bay flower production of *Zostera marina* is initiated when water temperatures are >14 °C, pollen is released after temperatures reach 16 °C, and seeds are released when temperatures are >25 °C (Silberhorn et al., 1983). In freshwater communities, temperatures of 15 °C

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have been associated with the initiation of germination of *Myriophyllum spicatum* seeds (Hartleb et al., 1993) and temperatures of 23–28 °C initiated seed germination within a week of exposure for *Hydrilla verticillata* (Lal and Gopal, 1993). French and Moore (2003) found that *V. americana* plants exposed to salinities of 10 and 15 psu survived but did not flower, but other effects of increased salinity on seed germination are largely unknown.

Light, oxygen, burial depth, and sediment composition can also affect submerged macrophyte seed germination (Muenscher, 1936; Rybicki and Carter, 1986; Koch, 2001). Kimber et al. (1995) found that *V. americana* seeds collected from the top 5 cm of the substrate germinated when exposed from 2 to 25% of total surface irradiance. Sediment hypoxia (dissolved oxygen (DO) < 1.0 mg l^{-1}; EPA, 2003) has been documented to increase germination of seagrasses *Z. marina* and *Z. capricorni*, seeds (Moore et al., 1993; Brenchley and Probert, 1998). However, high organic content of sediments (>6%), with potentially low DO and negative oxidation–reduction potential (Eh), was found to delay *V. americana* germination 3–5 weeks compared to seeds exposed to less organic sediments (Hoover, 1984). Despite the apparent influence of environmental factors on reproductive phenology and timing and success of submerged macrophyte seed germination, there has been little research on the effects of these factors, especially on many species of freshwater submerged macrophytes (Barko et al., 1986).

In the present study we have quantified environmental conditions and reproductive phenology (biomass, flower production, seed production, seed bank densities) in three established *V. americana* beds over a growing season (April–October) and have quantified the effects of dissolved oxygen (0.29–8.00 mg l^{-1}), light (0–160 μmol m^2 s^{-1}), temperature (13–29 °C), salinity (0.1–17.4 psu), sediment composition (3–86% sand; 0.9–8.3% sediment organic content), and burial depth (0.2–10 cm) on *V. americana* seed germination under controlled laboratory conditions. We hypothesized that seed germination and seedling production in established beds will be highest during the beginning (April–May) of the growing season (providing the longest period for seedling growth), and environmental conditions similar to those observed during that period would result in the greatest overall germination and shortest time to germination under controlled laboratory conditions.

2. Methods

2.1. Field sampling

2.1.1. Site selection

In 2004 three *V. americana* beds were selected for study in Nanjemoy Creek, MD based on historical data and aerial photography. In addition, these sites served as the locations for seed collection for subsequent experiments. Nanjemoy Creek is a 14 mile long tidally influenced tributary to the Potomac River where submerged macrophyte populations have been stable over 15 years (Orth et al., 2004). Site A (38°25.9′N, 77°07.2′W) was located along the western bank of Nanjemoy Creek and contained the largest bed of the three sites. In addition to *V. americana* *Najas* sp., *Myriophyllum spicatum*, and *Hydrilla verticillata* were also observed although they were not considered dominant or co-dominant. All species were found throughout the bed to a maximum depth of 78 cm below mean lower-low water (MLLW). Site B (38°25.9′N, 77°06.4′W) was located along the eastern shoreline of Nanjemoy Creek. In addition to *V. americana*, *Najas* sp. and *H. verticillata* were observed to a maximum depth of 65 cm below MLLW. Site C (38°26.4′N, 77°07.1′W) was the northern most sampling site and contained only *V. americana* distributed to a maximum depth of 48 cm below MLLW. Mean tidal range in the study area during 2004 was approximately 0.45 m (National Oceanic and Atmospheric Administration, National Water Level Observation Network, Silver Spring, MD, USA).

2.1.2. Species description and seed pod collection

A member of the family Hydrocharitaceae, *V. americana*, is a clonal, dioecious, perennial characterized by distinct mid-veined leaves that grow from a basal meristem (Wilder, 1974; Korschgen and Green, 1988; Catling et al., 1994). Adult shoots are able to live in sandy to soft clay substrates (Rybicki and Carter, 1986; Adair et al., 1994), moderate to low light environments (minimums of 5–25% total surface irradiance; Meyer et al., 1943; Carter and Rybicki, 1985; Doyle and Smart, 2001), median temperatures ranging from 19 to 36 °C (Barko et al., 1982; Kraemer et al., 1999) and in moderate salinities (0–5 psu; French and Moore, 2003).

Seed pods of *V. americana* were collected by hand in October 2003 and 2004 in Nanjemoy Creek. Intact pods were transferred to containers, sealed, and stored in de-ionized water at 4–6 °C until analysis and use (Baskin and Baskin, 1998). The total number of seeds within 50 randomly selected seed pods were counted and tested for viability using the tetrazolium staining method (Lakon, 1949; Grabe, 1970; Leist and Krämer, 2003). Seed embryos were removed from their seed coats and soaked in a 1% tetrazolium chloride (tetrazolium) solution for 24 h before examination on a dissecting scope at 10× magnification. Positive tests (live embryos) occurred when >50% of the embryo was stained red (Leist and Krämer, 2003).

2.1.3. Vegetation characterization

To characterize *V. americana* at each site percent cover, shoot density, and flowering shoot density were recorded along one 100 m long transect running perpendicular to the shoreline once a month from April to October 2004. Along each transect a 0.5 m^2 PVC square was randomly tossed within a 2 m^2 area three times every 10 m. Percent cover within the square was estimated visually. Density of shoots, flowering shoots, and seed pods were quantified within a 20 cm diameter ring placed within the PVC square. The number of seeds per seed pod were counted from a subset of seed pods from each site and tested for viability using the tetrazolium method (Lakon, 1949; Grabe, 1970; Leist and Krämer, 2003).

Biomass was quantified at each site using a 22 cm diameter core that was inserted into the sediment to a maximum depth of

20 cm at three random locations dispersed throughout the middle area of each bed. Each core was sieved on site through a 1 cm mesh screen and all plant tissue was collected. All collected material was dried for at 60 °C until a constant dry weight was reached.

2.1.4. Sediment and seed bank characterization

Three sediment cores (11.4 cm in diameter, 20 cm in length) were collected from each site in April 2004, sectioned at 0–2, 2–5, 5–10, 10–15, 15–20 cm, and sieved (63 μm sieve), washing slit and clay fractions into a graduated cylinder. After 24 h, pipette analysis was performed to determine the clay (8 phi) and silt (4 phi) fractions of the sample. Dry weights of the aliquots were compared and percent sand, silt, and clay fractions were determined (modification of Plumb, 1981). All sediment was then classified based on sand/silt/clay ratios (Shepard, 1954). Vertical Eh profiles were measured with a platinum tipped electrode to a depth of 10 cm. The probe was inserted into the top center of each core and Eh was measured every 5 mm. Final readings were temperature corrected relative to the calomel reference electrode (Hinchey and Schaffner, 2005).

Three additional sediment cores were collected from each site in April, June, July, September, and October. All three cores were sectioned as described previously. One half of each section was rinsed through a 0.5 mm sieve. All *V. americana* seeds were collected, counted, and analyzed for viability using the tetrazolium method (Lakon, 1949; Grabe, 1970; Leist and Krämer, 2003). Percent organic matter in the sediment was determined by drying the second half of the sediment subsample at 60 °C until a constant dry weight was reached. After the samples were cooled, the sediment was weighed again and combusted at 500 °C for 5 h. The sample was weighed a final time and percent organic matter was calculated (Erftemeijer and Koch, 2001).

2.1.5. Water column characterization

Water temperature (°C), salinity (psu), and dissolved oxygen (mg l^{-1}), were measured monthly with a Yellow Spring Instruments, Inc. (YSI, Inc., Yellow Spring, OH) model 650 sonde at each site. Photosynthetically available radiation (PAR; 400–700 nm) was measured with a Li-Cor terrestrial sensor (LI-190SA) and down welling light attenuation, or K_d (m^{-1}) was quantified in triplicate by measuring PAR along vertical profiles to the bottom at each site with a Li-Cor underwater sensor (LI-192SA).

2.1.6. Experimental studies

2.1.6.1. Environmental characterization of experimental conditions. Water temperature (°C), DO (mg l^{-1}), and salinity (psu) were measured with a YSI model 650 sonde and recorded daily for all experiments except treatments in sealed bottles. To quantify potential chamber or greenhouse effects on light availability, PAR was measured just above the surface of the water for each treatment with a Li-Cor terrestrial sensor (LI-190SA) and in each tank with a Li-Cor underwater light sensor (LI-192SA).

2.1.6.2. Germination characterization. Seed germination during all experiments was defined as the emergence of the radicle from the seed coat (Leist and Krämer, 2003). To quantify mean time to germination and the total number of germinated seeds, germinated seeds were counted daily. Mean time to germination was analyzed using survival analysis techniques (The SAS® System for Windows, SAS Institute, Inc.). Germination experiments were terminated after 14–16 days if the majority of seeds (>75%) had germinated. If less than the majority of seeds germinated during this period then the experiment was extended to a maximum of 30–34 days (Baskin and Baskin, 1998). The viability of the remaining ungerminated seeds was tested with tetrazolium (Leist and Krämer, 2003). All germination experiments included at least three replicates of 50 seeds or more based on our own preliminary viability tests and Baskin and Baskin (1998).

2.1.6.3. Oxygen and light experiment. To observe the single and interactive effects of oxygen and light on germination of *V. americana* seeds, five replicates of each treatment were placed in 250 ml sealed glass serum bottles in either oxygenated-light, oxygenated-dark, hypoxic-light, or hypoxic-dark conditions. For the hypoxic treatments de-ionized water was bubbled with nitrogen gas until dissolved oxygen levels were below 1 mg l^{-1}. DO levels were measured with a YSI, Inc., DO meter (Model 85). Initial DO concentrations were 8.00 mg l^{-1} for oxygenated treatments and 0.29 mg l^{-1} for the hypoxic treatments. Bottles were filled then sealed with a rubber stopper and metal cap. Air was continuously bubbled into each oxygenated serum bottle throughout the experiment. Serum bottles were randomly placed under diffused light (160 μmol m^2 s^{-1}) in an environmental growth chamber. The atmospheric conditions in the chamber were set to a 12-h photoperiod; temperature of 22 ± 2 °C, and humidity at 12%. Environmental conditions were selected to represent average *in situ* conditions in Nanjemoy Creek in April, the beginning of the growing season. After 16 days the seeds and seedlings were removed and counted. At the end of the DO experiment, water DO levels were not measured. Therefore, a second identical experiment was run and DO levels within the bottles were measured at the end of this second experiment. All germination data represent the results of the first experiment.

2.1.6.4. Temperature experiment. To determine the influence of temperature on germination of *V. americana* seeds, four replicates of each treatment were placed in Petri dishes randomly located in individual 110 l aquaria filled to a depth of 20 cm with de-ionized water. Aquaria were maintained at temperatures of 13, 22, 25, and 29 °C for 14 days. The seeds were covered by a 9.0 cm diameter Whitman GF/F filter and secured. Seeds were secured to ensure equal orientation to the substrate in order to remove any potential orientation bias on germination (Baskin and Baskin, 1998). Tanks were randomly assigned a treatment temperature and placed in the growth chamber. The treatment temperatures were maintained with mini-thermal compact aquaria heaters (50 watts) and monitored daily for seed germination. The chamber photoperiod

was set at 12 h and PAR levels were maintained at 160 μmol m^{-2} s^{-1}.

2.1.6.5. Salinity experiment. The effect of salinity on germination of *V. americana* seeds was tested at salinity levels of >1, 5, 10, and 15 psu (n = 4). Salinity treatments were prepared by diluting 1 μm filtered Chesapeake Bay, York River water (collected at the Virginia Institute of Marine Science, Gloucester Point, VA) to treatment levels with de-ionized water. The seeds were placed in Petri dishes in a design similar to the temperature experiment. One Petri dish with 50 seeds was placed in individual 110 l aquaria filled to a depth of 20 cm with the appropriate salinity water. One aquarium for each treatment was then placed into four 1.22 m × 2.44 m tanks in a randomized block design. All tanks were set up in a greenhouse with a separate recycling water bath system filtered with a Tarpon Lifeguard sand filter system, UV filter, and chilled with a Pacific Coast 1 hp chiller to a temperature between 20 and 23 °C. All treatments received ambient light. Replicates were monitored daily for seed germination for 34 days.

2.1.6.6. Sediment composition and burial depth experiment. - Sediment from Nanjemoy Creek was collected and stored at 7–9 °C until use. To develop a standardized range of sediment composition, sediment was collected from the near shore (NS) region and from the deepest edge (DE) of the bed and then mixed in varying proportions. Sediments were observed to typically grade from coarse to fine continuously along the NS to DE depth gradient. The NS and DE sediments were first sieved through a 0.5 mm sieve to remove any existing seeds and then homogenized separately. The homogenates were then mixed into treatments consisting of 100% NS; 75% NS:25% DE; 50% NS:50% DE; 25% NS:75% DE; and 0% NS. Homogenate mixtures were analyzed for percent organic content and characterized based on sand, silt, clay ratios.

Sediment from each treatment was placed in a 10.2 cm diameter PVC tube (12.0 cm × 10.2 cm) that was capped on the bottom. Fifty seeds were then buried at depths of 0.2, 0.7, 1.5, 2.5, 5.0, or 10.0 cm depending on the treatment. Depths were selected based on the reduction of *V. americana* winter bud germination at from >90% to <25% at depths greater than 10 cm (Rybicki and Carter, 1986). Seeds were not placed on the sediment surface due to water movement in the tanks. Each treatment (sediment type × burial depth) was replicated three times. Vertical Eh profiles were measured for all sediment types (Hinchey and Schaffner, 2005). Cores were placed in one of three tanks (1.2 m × 2.4 m) within a greenhouse in a randomized complete block design (30 cores per tank). Each tank was filled with 550 gal of freshwater that was maintained at a temperature range between 23 and 25 °C and filtered as described previously. All cores received ambient light. Cores were monitored daily for 31 days for seed germination.

2.1.6.7. Statistical analyses. To quantify the effects of time on bed development and sediment characteristics, all data was log transformed, normality was confirmed, and homogeneity of variance verified with Cochran's test (Zar, 1999). The effects of time, site, and burial depth on sediment organic content and redox were determined using a three-way analysis of variance (ANOVA; StatView for Windows, SAS Institute Inc.). The effects of time and site on sediment sand/silt/clay ratios were analyzed using a two-way ANOVA (StatView for Windows, SAS Institute Inc.). Differences in reproductive phenology between sites were analyzed using a one-way ANOVA (StatView for Windows, SAS Institute Inc.). Post hoc comparisons for all analyses were made with Tukey's HSD test. Seed bank characteristics were compared to environmental factors using regression and correlation techniques.

Treatment effects on maximum germination in the oxygen and light, temperature, and salinity experiments were determined using one-way ANOVA (StatView for Windows, SAS Institute Inc.). Post hoc comparisons between treatments were made with Tukey's HSD test. Effects of sediment type and burial depth on maximum germination were determined using a two-way ANOVA (StatView for Windows, SAS Institute Inc.). Prior to analysis, all percent data were arcsine square root transformed. Normality was confirmed and homogeneity of variance verified with Cochran's test. Results are presented as non-transformed means.

Survival analyses using the LIFETEST system were used to test the effects of time to germination data for all experiments (The SAS® System for Windows, SAS Institute Inc.). Survival analysis was selected due to the large amount of right-censored data characteristic of germination experiments (Scott et al., 1984). Seed data was censored if germination did not occur and non-germinated seeds were flagged as censored values prior to analysis.

3. Results

3.1. Field conditions

3.1.1. Water quality conditions

Water temperature at all field sites followed unimodal patterns throughout the growing season. Temperature ranged from a minimum of 12.1 °C in April to a maximum of 31.1 °C in July and decreased again to 18 °C by September (Table 1). DO levels in Nanjemoy Creek fluctuated but never fell below 6.34 mg l^{-1}. Salinity in Nanjemoy Creek never exceeded 4.3 psu and ranged from 1.3 to 4.3 psu. Water clarity, based on light attenuation profiles, varied among sites.

3.1.2. Sediment characteristics

The sediments of sites A and B were both classified as clayey sand, while sediments at site C were sand (Fig. 1). Sand, silt, and clay percentages all varied significantly with site ($p \leq 0.001$, 0.004, <0.001, respectively) but only sand varied significantly with depth ($p = 0.003$). Organic content was significantly different between sites ($p \leq 0.001$) and over time ($p \leq 0.001$), but did not vary significantly with depth ($p = 0.362$). Highest concentrations in organic content for all sites were recorded in August (Fig. 1). Eh was significantly different between sites ($p \leq 0.001$) and through the top 5 cm of sediment ($p \leq 0.001$). Eh decreased with depth (Fig. 1).

Table 1
Water quality data from Nanjemoy Creek collected during the 2004 growing season

Date	Site	K_d (m^{-1})	DO (mg L^{-1})	Salinity (psu)	Water temperature (°C)
12 April 2004	*	–	10.61	1.55	12.7
10 May 2004	*	–	8.67	1.82	19.5
18 June 2004	A	2.3	11.44	1.69	29.3
21 July 2004	A	2.4	9.50	3.84	28.0
19 August 2004	A	0.8	8.93	2.98	27.8
14 September 2004	A	1.7	8.97	3.55	25.6
12 October 2004	A	–	11.21	1.33	18.2
18 June 2004	B	1.5	9.31	1.55	29.5
21 July 2004	B	3.6	11.10	4.29	31.1
19 August 2004	B	2.0	11.83	3.91	29.5
14 September 2004	B	–	8.71	3.37	18.6
12 October 2004	B	–	9.75	1.51	19.2
18 June 2004	C	1.2	6.84	1.54	29.7
21 July 2004	C	2.0	7.83	3.56	26.9
19 August 2004	C	1.4	8.33	2.81	25.6
14 September 2004	C	2.4	8.31	3.48	18.0
12 October 2004	C	2.2	9.43	1.33	–

K_d is light attenuation though the water column.
* Water quality data from Chesapeake Bay Program fixed water quality monitoring station RET 2.3.

3.1.3. Vegetation characteristics

Overall mid-bed cover by *V. americana* was similar in sites A and B during the April–October sampling period with means of 71 ± 7% and 75 ± 7%, respectively. Mid-bed cover at site C was sparse and patchy (34 ± 9%). Mid-bed density (shoots m^{-2}) of *V. americana* increased from June to August at all sites. Site B had the greatest mean density (210 ± 14 shoots m^{-2}), followed by site A (160 ± 14 shoots m^{-2}), and site C (74 ± 19 shoots m^{-2}); however mean density did not vary significantly by site ($p = 0.555$). Total plant biomass of *V. americana* was the greatest at site A with a mean of 195.50 ± 43.22 g DW m^{-2}. Site B produced the second largest amount of biomass with a mean of 171.37 ± 58.16 g DW m^{-2}. Site C produced the smallest amount of biomass with a mean biomass of 148.58 ± 72.17 g DW m^{-2}.

3.1.4. Seed production, abundance and viability

On average, in August and September at site A 33% of the total number of shoots were flowering (72 flowering shoots m^2), 43% were flowering at site B (112 flowering shoots m^2), and 30% were flowering at site C (22 shoots m^2). The mean number of seeds per pod was 168 ± 19. There were no significant differences detected in the percentage of flowering shoots ($p = 0.915$), the number of seed pods ($p = 0.880$), and the number of seeds per pod ($p = 0.820$) among sites (Table 2). On average between 4500 ± 1600 and 16,000 ± 730 seeds m^{-2} were produced in Nanjemoy Creek depending on site (Table 2).

The abundance of seeds in the seed bank did not vary with depth or time at site A or B, therefore all seed abundance data are integrated over depth per square meter of bottom area. There was a significant difference between sites ($p = 0.042$; Table 2). Post hoc analysis with Tukey's HSD test indicated that sites A and B were significantly different than site C (Table 2). This was the result of not finding any viable seeds in the seed bank at site C. Based on tetrazolium staining, 21% of seeds collected from sites A and B in Nanjemoy Creek were viable (Table 2).

4. Experimental results

4.1. Water column conditions

Water temperatures were maintained within the range of 22–25 °C, DO) concentrations at saturated levels, and salinity at <1 psu for all experiments where these conditions were not manipulated (Table 3). For the dissolved oxygen experiment initial DO concentrations were 8.00 mg l^{-1} in the oxygenated treatment and 0.29 mg l^{-1} for the hypoxic treatment. DO levels were not measured at the end of this experiment. However, DO levels dropped to 5.01 mg l^{-1} for the oxygenated treatments and increased to 0.42 mg l^{-1} for the hypoxic treatments at the end of a second similar experiment. Light levels were maintained at approximately 160 μmol m^{-2} s^{-1} during the oxygen and light and temperature experiments and fluctuated with natural during the sediment type, burial depth and salinity experiments (Table 3).

4.2. Seed germination

4.2.1. Dissolved oxygen and light effects

The presence of oxygen significantly increased germination compared to hypoxic treatments ($p = 0.009$), while light/dark treatments had no effect on germination seeds ($p = 0.967$). Due to the lack of a light effect on germination, data were analyzed with the presence/absence of oxygen as a single factor. Total germination increased significantly from 78% under hypoxic conditions to 86% under oxygenated conditions ($p = 0.006$; Fig. 2A). Mean time to germination increased slightly but not significantly in the hypoxic treatments compared to the oxygenated treatments from 11 to 13 days ($p = 0.850$). Viability of the non-germinating seeds in the dissolved oxygen and light experiment was significantly greater ($p = 0.018$) in the hypoxic treatments (37 ± 15%) compared to the oxygenated treatments (6 ± 3%; Table 4).

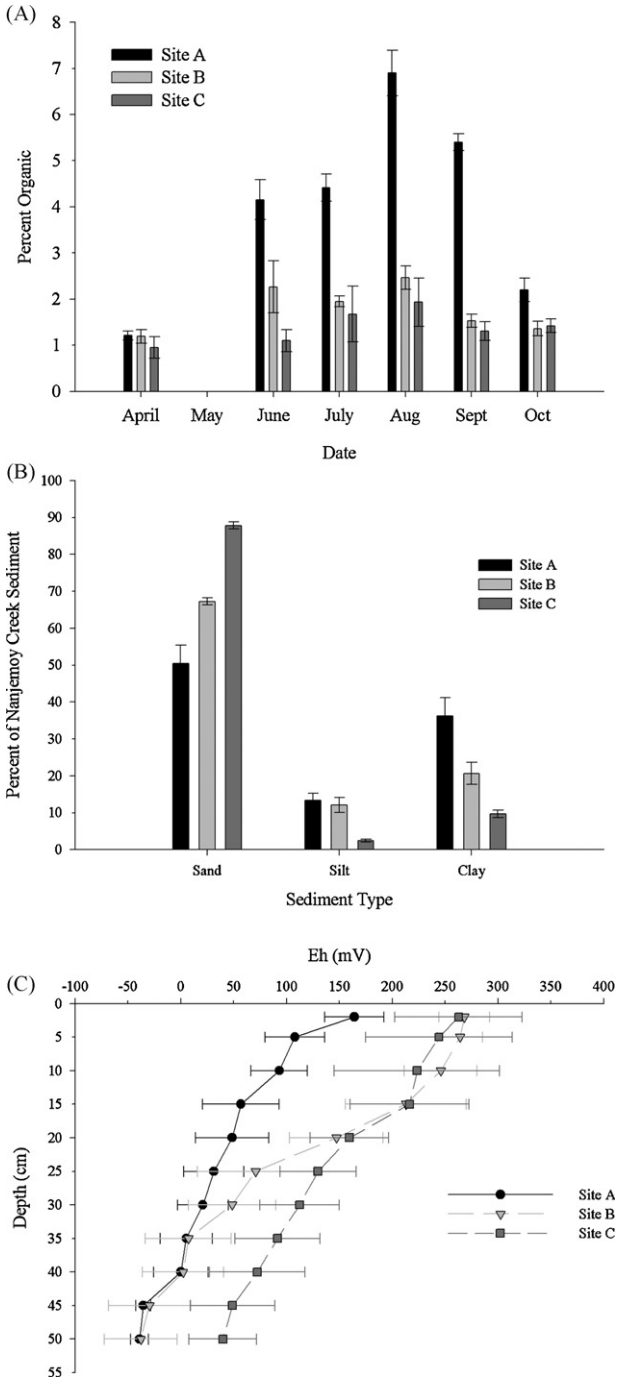

Fig. 1. Characterization of Nanjemoy Creek sediment by site during the 2004 growing season. (A) Mean percent organic content of sediment from all three sites from April to October 2004. No data was collected in May. (B) Mean percent sand, silt, clay for sediment collected from all three sites in Nanjemoy Creek in April 2004. (C) Mean oxygen reduction potential (Eh) measurements from cores collected from all three sites in April 2004. Values for all analyses are mean ± S.E.

4.2.2. Salinity effects

Germination of *V. americana* seeds was significantly influenced by salinity ($p \leq 0.001$; Table 5). Post hoc analysis with Tukey's test revealed that germination was significantly greater at the <1 and 5 psu treatments compared to the 10 and 15 psu treatments (Fig. 2B). Viability of the remaining non-germinated seeds was not significantly different between treatments ($p = 0.1326$; Table 4). Mean time to germination increased significantly with increasing salinity ($r^2 = 0.91$; Fig. 3E). A threshold between 5 and 10 psu is reflected in the decrease in overall final percent germination and increase in mean time to germination (Fig. 3).

4.2.3. Temperature effects

Germination significantly increased with increasing temperature ($p \leq 0.001$; Fig. 3C). Post hoc analysis with Tukey's HSD test revealed that germination at 13 °C was significantly less compared to 22, 25, and 29 °C (Fig. 2C). The proportion of viable but non-germinated seeds at the end of the temperature experiment was significantly different between treatments ($p = 0.035$; Table 4). Post hoc analysis with Tukey's HSD test showed that viability of seeds in the 13 °C treatment were significantly greater than those seeds at the 25 °C treatment (Table 4). Seed viability among the remaining treatments was not significantly different.

Although seeds germinated in all treatments, the small number of germinating seeds in the 13 °C treatment was not sufficient to determine mean time to germination. For the remaining treatments mean time to germination decreased significantly with increasing temperature ($r^2 = 0.90$; Fig. 3F). The significant difference in overall germination and mean time to germination between 13 and the 22, 25, 29 °C treatments suggest a temperature threshold between 13 and 22 °C.

4.2.4. Sediment type and burial depth effects

4.2.4.1. Sediment characteristics. The organic content of the sediment during the sediment type burial depth experiment ranged from 1 to 8%. Organic content increased as the percentage of NS sediment decreased (Fig. 4A). The percentage of sand in the treatment sediments increased proportionally with the amount of NS sediment content within the treatment (Fig. 4B). Based on Shepard's classification (1954) the 0% NS treatment was silty clay, the 25% treatment was mixed sediments, the 50 and 75% treatments were clayey sands, and the 100% NS treatment was sand.

Eh varied significantly among sediment types ($p \leq 0.001$) and depths ($p \leq 0.001$). Post hoc analysis with Tukey's HSD test revealed that the 0% NS treatment was significantly different from the 100%, 75%, and 25% NS sediment treatments. No other sediment treatments were significantly different. Eh measurements at depths >2 cm were significantly different than measurements <2 cm. Eh did vary similarly among all cores with depth with maxima of +200–300 mV at the surface, decreasing to 0 to −40 at depths of 50 mm or greater (Fig. 4C).

4.2.4.2. Seed germination. Sediment type and burial depth both had a significant effect on germination (sediment type: $p \leq 0.001$; burial depth: $p = 0.001$). However, post hoc analysis of sediment type and burial depth with Tukey's HSD test indicated that only two depth comparisons (0.7 cm × 0.50 cm and 0.7 cm × 10.0 cm) were significantly different. Due to the overall lack a burial depth effect on germination, data were analyzed with sediment type as the single factor.

Table 2
Mean sexual reproductive phenology and one-way ANOVA results for *V. americana* shoots in Nanjemoy Creek based on September 2004 transect data

Sexual reproductive output	Site A	Site B	Site C	SS	*p*
# of plants m^{-2}	220 ± 12	260 ± 11	74 ± 35	20.67	0.5551
% flowering shoots m^{-2}	33 ± 22	43 ± 30	30 ± 17	1.56	0.9154
# of pods m^{-2}	71 ± 4	110 ± 5	22 ± 11	0.67	0.8801
# of seeds produced m^{-2}	11,000 ± 590	16,000 ± 730	4500 ± 1600	13,125	0.8200
# of seeds m^{-2} in seed bank after 12 months	590 ab	1100 a	0 b	3.79	0.0426*
# of viable seeds m^{-2} in seed bank after 12 months	118	230	0	0.542	0.245

The number of seeds m^{-2} in the sediment after 12 months and the number of viable seeds m^{-2} in the sediment after 12 months for all sites are counts from September sediment cores. Numbers are mean ± S.E. One-way ANOVA compared differences between sites. Letters indicate results of a Tukey HSD comparison test for each species. Same letters indicate no significant difference between the pairs for $p = 0.05$.
* Significant difference at $p = 0.05$ level.

Table 3
Mean water quality data from all experiments

Experiment	*n*	K_d (m^{-2})	DO (mg L^{-1})	Salinity (psu)	Water temperature (°C)
DO/light	3	Variable	Variable	0.1 ± 0.1	23.0 ± 1.0
Salinity	4	0.4 ± 0.1	7.9 ± 0.1	Variable	24.7 ± 0.3
Temperature	4	0.6 ± 0.2	8.4 ± 0.2	0.2 ± 0.1	Variable
Sediment type burial depth	4	0.2 ± 0.1	8.3 ± 0.1	0.2 ± 0.1	24.7 ± 0.1

Data averaged across all treatments and all dates except for the sediment type burial depth experiment which is averaged across all tanks and dates. K_d is light attenuation though the water column. Values are mean ± S.E.

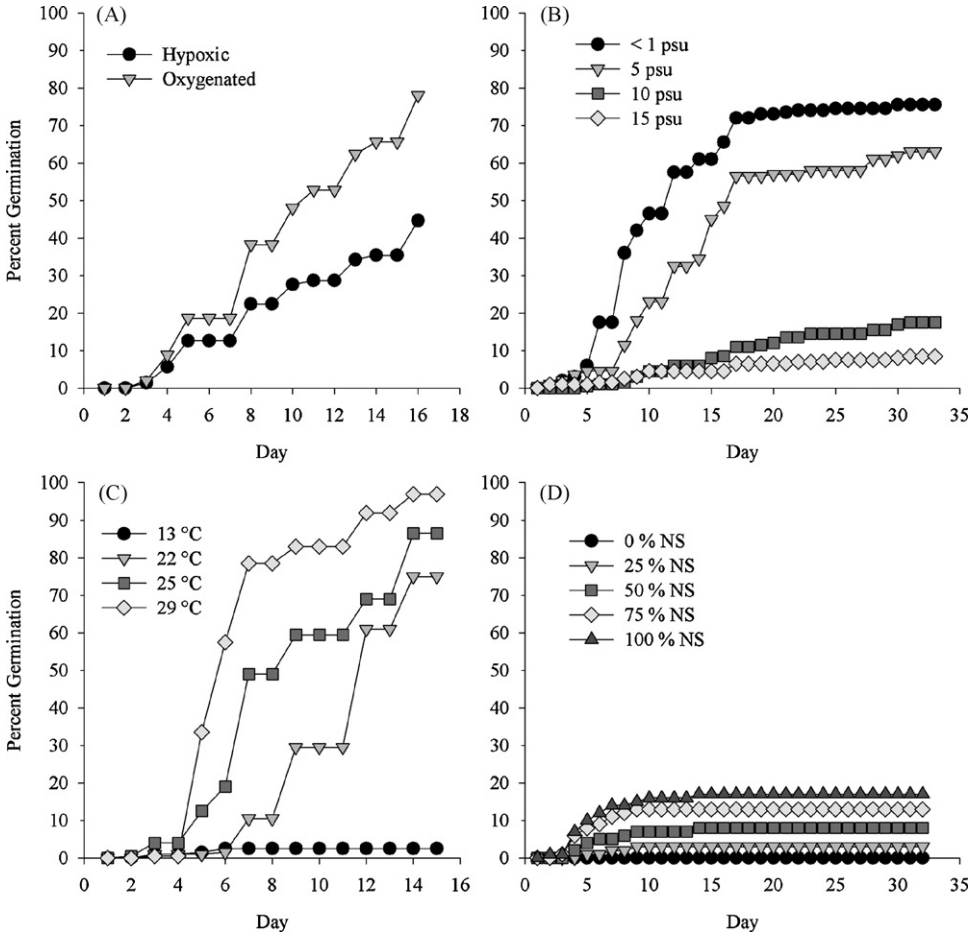

Fig. 2. Cumulative mean percent germination of *V. americana* seeds for all experiments: (A) dissolved oxygen and light; (B) temperature; (C) salinity; (D) sediment type and burial depth.

Table 4
Mean percentage of germinated *V. americana* seeds ($n = 50$) and viability of the remaining non-germinated seeds after completion of all experiments

Experiment	Germinated Seeds % germ	Non-germinated seeds % viable
DO/light		
Oxygenated	45 ± 9 a	6 ± 3 a
Hypoxic	78 ± 6 b	37 ± 15 b
Salinity		
0	76 ± 2 a	21 ± 8 a
5	63 ± 5 a	31 ± 8 a
10	18 ± 3 b	54 ± 4 a
15	9 ± 2 b	53 ± 6 a
Temperature (°C)		
13	3 ± 1 a	89 ± 1 a
22	75 ± 10 b	71 ± 14 ab
25	87 ± 5 bc	32 ± 14 b
29	97 ± 2 c	50 ± 35 ab
Sediment type/burial depth		
0%	<1 ± 0 a	65 ± 4 a
25%	3 ± 1 a	52 ± 6 a
50%	8 ± 1 b	56 ± 6 a
75%	13 ± 2 bc	64 ± 5 a
100%	17 ± 2 c	65 ± 4 a

Sediment type burial depth treatment values are percentages of near shore to deep edge sediments collected from Nanjemoy Creek. Values are reported as mean ± S.E. Letters indicate results of a Tukey HSD comparison test for each treatment. Same letters indicate no significant difference between the pairs for $p = 0.05$.

Germination increased significantly ($p \leq 0.001$) and exponentially ($r^2 = 0.97$) with increasing concentrations of NS sediment resulting in decreasing sediment organic content (Figs. 2D and 3). Final germination was lower in the sediment type and burial depth experiment compared to all other experiments (Fig. 3). The number of viable seeds remaining at the end of the experiment did not significantly differ with treatment ($p = 0.215$). On average 60% of the remaining seeds were viable (Table 4).

Mean time to germination did not decrease significantly with decreasing NS sediment ($p = 0.587$; Fig. 3). Overall mean time to germination for all seeds in the sediment type burial depth experiment was greater than all other experiments by 14–17 days except for the seeds exposed to salinities of 10 and 15 psu (Fig. 3).

5. Discussion

In this paper we demonstrated that germination of *V. americana* seeds was enhanced (greater overall germination and shorter time to germination) under oxygenated conditions (8.00 mg l^{-1}), temperatures >22 °C, salinities <1 psu, and in sediments composed of ≤ 3% organic content and >40% sand. Light (<160 μmol m^{-2} s^{-1}) and burial depth (0.2–10 cm) had no significant effect on germination. When compared to conditions within established *V. americana* beds during the first 2 months of the 2004 growing season salinity, dissolved oxygen, and sediment organic content were comparable to experimental conditions resulting in enhanced germination. However, based on our experimental results, temperature was limiting to seed germination in April and May since water temperature did not increase above 22 °C until June. Therefore, the lack of observed seedlings at the beginning of the growing season may have been constrained by the water temperatures at that time.

In the temperature experiment, an increase of 7 °C from 13 to 22 °C resulted in a 25-fold increase in germination and a 50% reduction of mean time to germination. Therefore, when other environmental factors are constant, temperature is a strong influence on overall *V. americana* seed germination with a threshold temperature between 13 and 22 °C that, when surpassed, initiates germination. Similar effects of temperature on germination have been reported for other species of freshwater SAV. Hartleb et al. (1993) reported that temperatures of 15 °C are required for germination of *M. spicatum* seeds and Lal and Gopal (1993) report that seeds of *H. verticillata* exhibited greater overall germination when temperatures increased from 23 to 28 °C. In April 2004 water temperatures in Nanjemoy Creek increased from 10 to 17 °C. Based on the relationship defined from germination experiments under controlled laboratory conditions, the maximum germination potential of seeds in the seed bank during this time period would be approximately 40%. However, no seedlings were observed in the field during this period, suggesting that other factors were controlling seed germination in these established beds.

Salinity was observed to have a significant negative effect on *V. americana* seed germination. Laboratory results reported here indicate that there may be a threshold effect between 5 and 10 psu that, once surpassed, delays germination. A similar threshold was observed between 5 and 10 psu (for plants receiving 28% of surface irradiance) on overall *V. americana* production (including flowering and winter-bud production) during a season long experiment investigating the effects of salinity on *V. americana* (French and Moore, 2003). During the 2004 growing season a large pulse of salinity occurred between June and July, which increased salinity from 1.55 to 4.29 psu. Despite this pulse in salinity, salinity levels never increased above the 5 psu level and based on our experimental results were not limiting for seed germination in 2004.

In the sediment type and burial depth experiment, seeds in sediments containing >3% organic content had a significantly lower germination rate compared to seeds in sediments with <3% organic content. Therefore, germination of all seeds deposited within the largest bed (site A) would not have been limited by sediment organic content until June due to sediment organic content concentrations of 4.2 ± 0.4% during this time period.

Burial depth up to 10 cm was not a significant factor affecting germination of *V. americana* seeds between treatments in this study. These results support a similar experiment investigating the effects of burial depth on *V. americana* winter-bud survival and germination (Rybicki and Carter, 1986). Survival of *V. americana* winter-buds at depths greater than 25 cm in their study was significantly less than those in ≤10 cm of sediment (<10 cm = 90% survival; >10 cm = 0% survival).

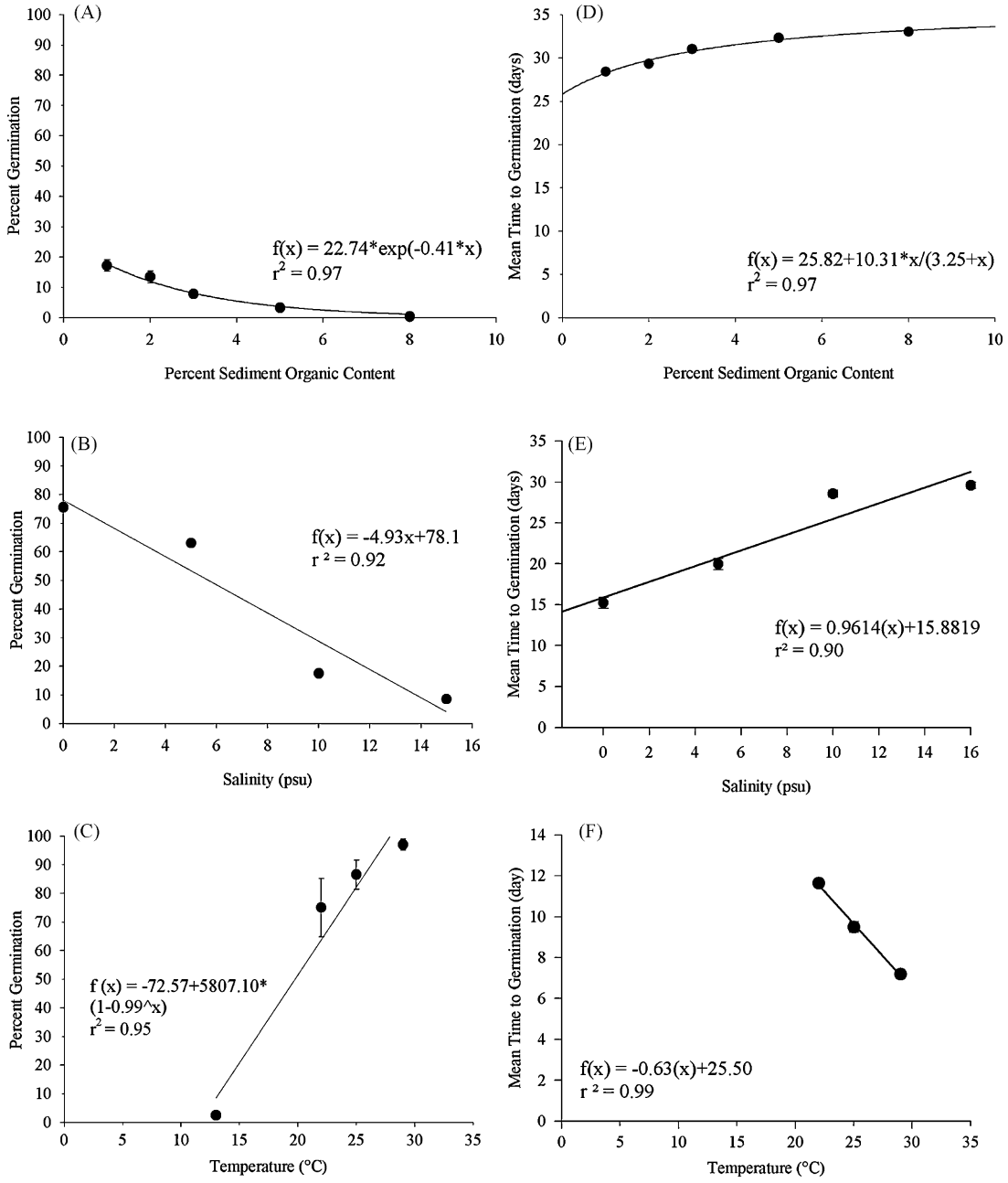

Fig. 3. Final percent germination for all treatments in the (A) salinity, (B) temperature, and (C) sediment type and burial depth experiments. Mean time to germination for all treatments in the (D) salinity, (E) temperature, and (F) sediment type and burial depth experiments. Mean time to germination was not calculated for the 13 °C treatment due to a lack of germination.

If seeds in the Nanjemoy Creek field sites are mixed below the 10 cm threshold the potential for *V. americana* seed germination may decrease significantly and serve as an environmental control on seed germination. Otherwise, all viable seeds within the top 10 cm would comprise the seed bank.

Sediment sand, silt, clay ratios, organic content, and burial depth within the sediment were not able to explain the differences in germination between high sand–low organic content and low sand–high organic content sediments. In addition, these parameters could not fully explain the difference in maximum germination observed when seeds were planted in sediments (17%) compared to seeds observed in water only (63–97%). Alternate hypotheses for germination differences between the treatments and experiments include varying mechanical resistance of the sediment (i.e. hardened particles stuck together with water) inhibiting germination (Hornbaker et al., 1997), bacterial growth within the sediment, or a lack of mycorrhizae development necessary for nutrient uptake and growth necessary in sediments (Wigand and Stevenson, 1997; Zhongqiang et al., 2005).

Laboratory analyses reported here indicate *V. americana* seed germination was enhanced in the presence of oxygen. In contrast, hypoxic conditions are known to enhance seed germination in other SAV species. For example, the presence of

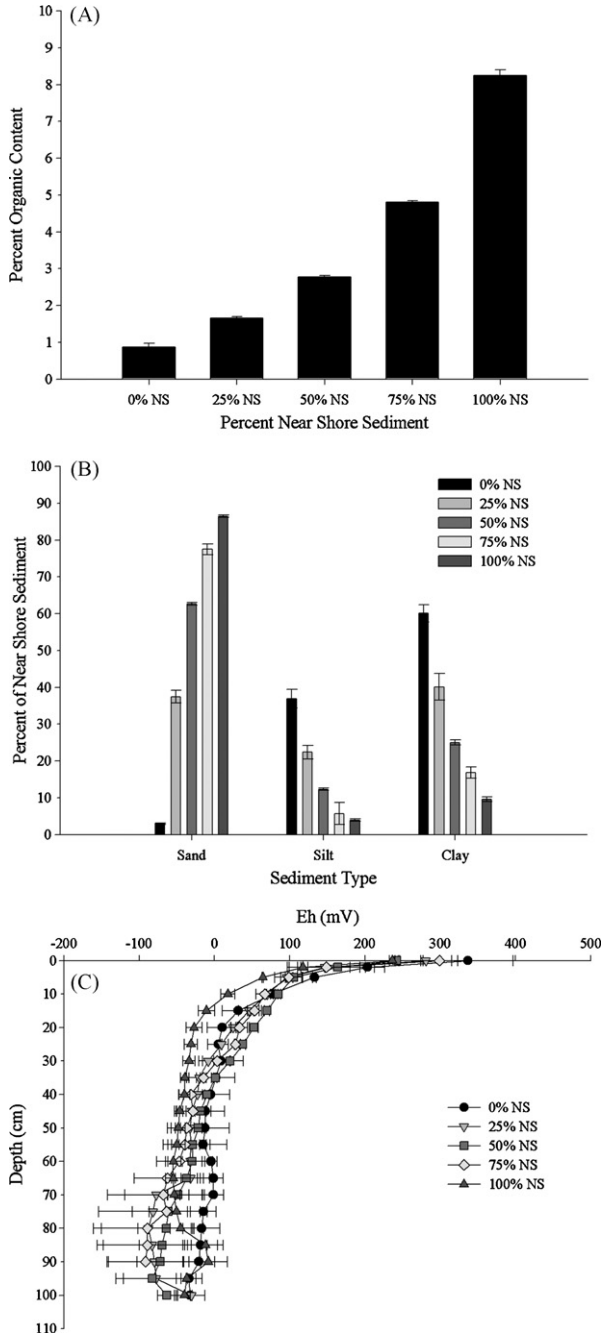

Fig. 4. Characterization of the sediment type burial depth sediment by percent NS treatment. (A) Mean percent organic content of sediment from all treatments. (B) Mean percent sand, silt, clay for all treatments. (C) Mean redox (Eh) measurements from cores collected from all treatments at the beginning of the experiment. Values for all analyses are mean ± S.E.

DO decreases germination in *Z. marina* seeds in Chesapeake Bay, and seeds buried at 5 cm had significantly less germination than seeds buried at 15 and 25 cm (Moore et al., 1993). Additional studies on *Z. marina* seed germination also conclude that hypoxic conditions enhance germination of *Z. marina* from <40% under oxygenated conditions to >90% under hypoxic conditions (Probert and Brenchly, 1999). This suggests varying strategies for submerged aquatics in relation to DO and burial depth.

Light (<160 μmol m^{-2} s^{-1}) had no effect on seed germination under controlled laboratory conditions. These results support the conclusions of Muenscher (1936), that exposure of *V. americana* seeds to low levels of diffuse light does not negatively affect germination. Kimber et al. (1995) also concluded that seeds of *V. americana* do not require light to germinate, as seeds germinated in sediments under all light reduction treatments (2, 5, 9, and 25% available light). Kimber et al. (1995) did find, however, that seedling survival increased significantly under the higher light conditions (9 and 25% surface irradiance) compared to those seedlings exposed to low light conditions (2 and 5% surface irradiance). While adequate light is a requirement for successful *V. americana* bed development and reproduction, in Nanjemoy Creek in 2004 it was not limiting. Since moderate (<160 μmol m^{-2} s^{-1}) light conditions were not found to affect germination of *V. americana* seeds, variations in light due to turbidity or depth should not affect germination of non-buried seeds.

To compound the limitation on seed germination due to environmental factors, <1% of the 16,000 seeds m^{-2} produced were found in the seed bank in Nanjemoy Creek. In the Lake Huron-Erie corridor production of 10 times the number of seeds was observed within the bed than were found in the established seed reserve (Lokker et al., 1997). As with this study, the size of the seed bank was not directly related to the previous season's seed production suggesting that processes after seed production were affecting deposition (Lokker et al., 1997; Howe and Miriti, 2004). Various factors that may influence the difference in seed production compared to seed bank reserves include seed physiology, germination, predation, dispersal, and the surrounding environment (Westcott et al., 1997).

Despite low densities in the sediment, *V. americana* seeds, in our laboratory experiments, retain viability under stressful conditions, including salinities >10 psu. This may serve as a recovery mechanism for the bed after a large scale environmental impact, such as drought which may increase salinities to stressful levels for the shoots (French and Moore, 2003). Of the seeds deposited in the seed bank in Nanjemoy Creek, 21% found were viable. The numbers of viable seeds in the seed bank in Nanjemoy Creek (118–230 seed m^{-2}) were similar to *V. americana* beds in the Great Lakes where the number of seeds deposited ranged from 73.6 ± 22.9 to 878.2 ± 268.6 seeds m^{-2} (Lokker et al., 1997). While the density of seeds necessary for competitive establishment of seedlings with vegetative shoots of *V. americana* is not known, low seed densities combined with low viability within the seed bank may limit seed germination.

Environmental conditions in Nanjemoy Creek were favorable for bed development and growth but they were as not conducive for seed germination. This suggests that seedlings are at a competitive disadvantage to over wintering buds in bed establishment. Experimental results indicate that temperature had a large effect on timing of *V. americana* seeds germination (r^2 = 0.99, Fig. 3). The minimum germination temperature of *V. americana* over wintering buds is similar to that of seeds; however, over wintering buds have greater carbohydrate energy reserves than seeds (Carter et al., 1987). The additional energy

reserve gives shoots emerging from over wintering buds a greater chance at survival and a competitive edge over seedlings. Some seeds in Nanjemoy Creek may have germinated when temperatures increased above 13 °C, but the lower energy reserves of the seedlings may have decreased their ability to compete with vegetative shoots which were well established by June when temperatures were more optimum for seed germination.

While some environmental factors have more control on germination than others, the interactions between all environmental conditions need to be further understood before the effects of any environmental factor can be negated. Light and burial depth did not have a significant effect on seed germination when investigated singularly; however, the interactions between burial depth and additional environmental parameters such as temperature, or salinity were not quantified and should be considered in any future research on *V. americana* seed germination. In addition, not all variability in seed production and germination may be attributable to the parameters investigated here. For example, thousands of seeds per square meter of vegetation were produced in Nanjemoy Creek but only tens of viable seeds per square meter were found in the seed bank. Reasons for this discrepancy are unknown but may include lose of seeds due to grazing of seed pods, dispersal of seeds or seed pods through currents, or decomposition of seeds in the sediment.

Based on our experimental results, environmental conditions at the beginning of the growing season in Nanjemoy Creek were limiting to *V. americana* seed germination. Seed germination may have been suppressed by water temperatures <22 °C and seeds would not contribute to the initial maintenance of existing beds. We observed that seeds were able to remain viable under high salinity conditions (10–15 psu) that are stressful to adult plants (French and Moore, 2003) therefore, seeds may play a significant role in the recovery of beds after large scale declines. However, to fully understand the affects and controls of temperature, salinity, sediment type, etc., on *V. americana* seed germination and subsequent seedling establishment the interactive effects of these conditions need to be further investigated.

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