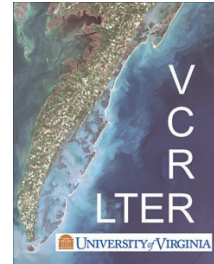




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THEME SECTION

# Eelgrass recovery induces state changes in a coastal bay system

Editors: Robert J. Orth, Karen J. McGlathery, Kenneth L. Heck Jr.

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## INTRODUCTION

# Eelgrass recovery in the coastal bays of the Virginia Coast Reserve, USA

Robert J. Orth<sup>1,\*</sup>, Karen J. McGlathery<sup>2,\*\*</sup>

<sup>1</sup>Virginia Institute of Marine Science, School of Marine Science, 1208 Greate Road, College of William and Mary, Gloucester Point, Virginia 23062, USA

<sup>2</sup>Department of Environmental Sciences, University of Virginia, PO Box 400123, Charlottesville, Virginia 22903, USA

**ABSTRACT:** Coastal bay systems are prominent features of coastlines on nearly all continents and are vulnerable to long-term environmental changes related to climate and nutrient over-enrichment. Eelgrass *Zostera marina* disappeared in the 1930s from the coastal bays of the Virginia Coast Reserve, USA, primarily due to a wasting disease and the effects of a hurricane. It has been re-established recently as a result of a large-scale seeding and restoration effort. The contributions to this Theme Section provide the most comprehensive account available of large-scale recovery of an eelgrass ecosystem, the consequences of the state change from a bare-sediment system to eelgrass dominance, and projections of meadow resilience to future climate change scenarios.

**KEY WORDS:** *Zostera marina* · Coastal restoration · State change · Regime shift

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Coastal bay systems are prominent features of coastlines on nearly all continents, and although their watersheds are small relative to large river-fed estuaries, they are as vulnerable to long-term environmental changes related to climate and nutrient over-enrichment (McGlathery et al. 2007). In these shallow-water systems, where most of the seafloor is in the photic zone, seagrass and benthic algae dominate metabolism and nutrient cycling, play a critical role in stabilizing the coastline, and provide habitat for important components of the fauna. Long-term trends in seagrass abundance and distribution indicate that the rate of habitat loss is accelerating worldwide, due to degraded water quality, disturbance and disease; this is accompanied by a loss of ecosystem services (Orth et al. 2006a, Waycott et al. 2009, Short et al. 2011). There has been some success in

large-scale restoration efforts to mitigate these losses, but not enough to reverse the declining trends globally (Waycott et al. 2009).

### The Virginia Coast Reserve

The coastal bays of the Virginia Coast Reserve (VCR) are renowned for their local, regional, and global value to migratory birds (Watts & Truitt 2001) and diverse marine life, as well as for historically supporting fisheries of significant commercial value (Barnes & Truitt 1998). The VCR coastal bays suffered a catastrophic ecosystem state change in the 20th century, primarily due to a wasting disease that devastated *Zostera marina* (eelgrass) and a hurricane in 1933 that likely eliminated remaining populations.

\*Email: jjorth@vims.edu

\*\*Email: kjm4k@virginia.edu

The state change from *Z. marina* to an 'unvegetated' bottom dominated by benthic algae resulted in the loss of critical ecosystem services, including the provision of food and nursery habitat for numerous avian and marine species such as the bay scallop *Argopecten irradians*. While *Z. marina* eventually rebounded from the pandemic decline in the Chesapeake Bay and in many coastal bays along the eastern seaboard of the United States (Cottam & Munro 1954), there are no records of *Z. marina* in the VCR coastal bays until the mid-1990s (Orth et al. 2006b).

What makes the coastal bays of Virginia unique, and what has been important to the restoration of *Zostera marina*, is that they represent systems that receive relatively little impact from human activities. The VCR is a Long Term Ecological Research (LTER) site that is part of a network of 26 sites representing diverse marine and terrestrial ecosystems ([www.lternet.edu](http://www.lternet.edu)). At the VCR, ongoing studies for 2 decades examining physical, biogeochemical and biological aspects of the coastal bays in the absence of *Z. marina* (e.g. McGlathery et al. 2001, Anderson et al. 2003, Tyler et al. 2003) set the stage for understanding the consequences of the state change back to eelgrass dominance from the unvegetated state. Long-term data from the VCR LTER show that watershed nutrient loading to the coastal bays is low and that water quality with respect to nutrients and chlorophyll has remained high for the last 2 decades ([www1.vcr.lter.virginia.edu/home1/?q=data\\_wq](http://www1.vcr.lter.virginia.edu/home1/?q=data_wq)). In 1970, the VCR was established as a reserve by The Nature Conservancy and later was recognized as a Man and the Biosphere Reserve, creating a legacy of conservation and stewardship. The VCR is both a model system for understanding the dynamics of *Z. marina* recovery where habitat quality is high, and an important reference point for the more heavily-impacted systems that are typical of coastal regions (Kennish & Paerl 2010).

### Return of a foundation species

Restoration of *Zostera marina* with seeds in this system has been very successful since the late 1990s. Approximately 1700 ha of bottom in 4 lagoons that had abundant *Z. marina* prior to its demise again support robust populations (Fig. 1). While there has been no definitive answer as to why *Z. marina* never recovered from the 1930s demise, recent work provides strong support that recruitment limitation (primarily seeds), and not increased turbidity following

the loss of *Z. marina* (Peterson & Lipcius 2003), was the primary reason why *Z. marina* did not recover. This conclusion is supported by the recent success of restoration by seeding, monitoring that revealed good water quality, physical modeling of sediment suspension and water column light attenuation (Lawson et al. 2007), and by a more complete understanding of the biology and ecology of this species (Moore & Short 2006), especially its dispersal dynamics (Harwell & Orth 2002).

The change from an unvegetated state to one with dense and extensive *Zostera marina* populations has provided a unique opportunity to understand the central role of *Z. marina* as a foundation species in temperate shallow coastal systems. Throughout this theme section we use the term 'restoration' in the broadest sense. While this term has generally been applied to systems that have been altered due to anthropogenic activities, we adopt this term for our work here based on the comment in Elliott et al.

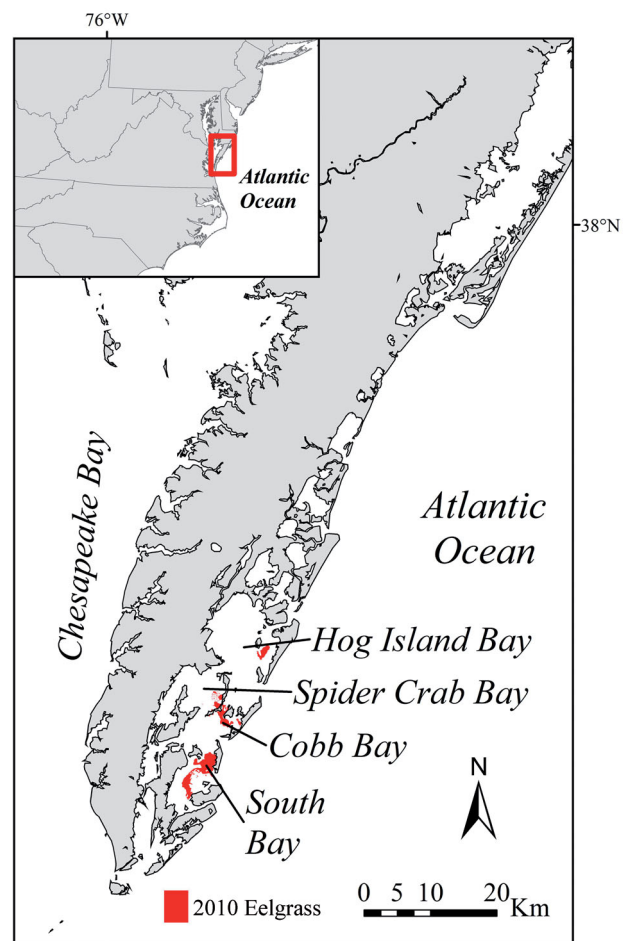


Fig. 1. Delmarva Peninsula and sites of eelgrass restoration (red)

(2007, p 357) 'we recommend that producing new habitat can be termed creation or enhancement whereas re-creating habitat that was present within historical records, no matter how old, should be termed restoration.'

### Contributions to this Theme Section

The studies in this Theme Section cover a broad and comprehensive range of topics. Orth et al. (2012) describe the rapid changes in *Zostera marina* distribution initiated by seeding and by natural recruitment processes that have facilitated recovery. By continuously monitoring water quality, they show how one meadow modulated water clarity and altered the sediment as it developed and expanded. Marion & Orth (2012) investigate the processes leading to the low initial seedling establishment noted in Orth et al. (2012) through a manipulative field experiment that addressed the relative importance of germination failure and seedling loss during the winter. They show that some of the key processes in recruitment and restoration of *Z. marina* involve physical sediment–seedling interactions rather than seed germination. McGlathery et al. (2012) compare sites from 0 (unvegetated) to 9 yr after seeding and show the restoration of key ecosystem services such as primary productivity, carbon and nutrient sequestration, and sediment deposition. However, their results indicate that none of the parameters monitored reached an asymptote after 9 yr, indicating that more time is required for full restoration of these ecosystem services. They also identify the depth limit (1.6 m) for eelgrass populations in the coastal bays. Using micro-satellite markers, Reynolds et al. (2012) show that the high genetic diversity in donor beds from Chesapeake Bay is maintained in meadows restored by seeding in the Virginia coastal bays. Cole & McGlathery (2012) found that nitrogen fixation increased as the meadows aged, with older beds fixing almost 3 times more nitrogen than younger meadows and 30 times more than bare sediment. Moore et al. (2012) compare water quality conditions associated with *Z. marina* populations in the coastal bays and in nearby areas in the lower Chesapeake Bay, where *Z. marina* has either declined or remained static over the same time period. Their results indicate that lower summertime water temperatures and lower light attenuation interact to both increase the proportion of light available for *Z. marina* photosynthesis and decrease *Z. marina* community light requirements at the coastal bay sites. They suggest that the greater tidal range and

proximity of the coastal bays to cooler ocean waters ameliorates the stress from high air temperature periods during the summer. Using controlled microcosm experiments, Lawson et al. (2012) show that at low shoot densities *Z. marina* increases sediment suspension due to the horizontal deflection of flow around eelgrass shoots, but that past a threshold density, eelgrass reduces sediment suspension. In field studies, Hansen & Reidenbach (2012) quantify the relative effects of meadow structure on tidal currents, waves, and near-bed turbulence, and the resulting sediment suspension: expansion of *Z. marina* within the coastal bays has shifted the seafloor from an erosional to depositional environment, leading to enhanced light penetration through the water column and creating a positive feedback for *Z. marina* growth. Carr et al. (2012) build this positive feedback into a coupled vegetation-growth hydrodynamic model to investigate *Z. marina* stability and leading indicators of ecosystem shift under future scenarios of sea level rise and warmer sea temperatures. Their model identifies the emergence of alternative stable states (vegetated vs. barren bottom) with a maximum depth threshold based on water clarity and growth conditions. Their results also indicate that *Z. marina* meadows in these coastal bays have limited resilience to increases in water temperature predicted from current climate change models, and agrees with field evidence presented by Moore et al. (2012).

### Perspectives

The contributions to this Theme Section provide the most comprehensive account available of large-scale recovery of a seagrass ecosystem, the consequences of the state change from a bare-sediment system to eelgrass dominance, and projections of meadow resilience to future climate change scenarios. This is a model system to understand state change dynamics in shallow coastal bays, and future work will include trophic dynamics, restoration of bay scallops, and landscape analysis.

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# Seed addition facilitates eelgrass recovery in a coastal bay system

Robert J. Orth\*, Kenneth A. Moore, Scott R. Marion, David J. Wilcox,  
David B. Parrish

Virginia Institute of Marine Science, School of Marine Science, 1208 Greate Road, College of William and Mary,  
Gloucester Point, Virginia 23062, USA

**ABSTRACT:** Eleven years of eelgrass *Zostera marina* seed additions conducted in a coastal bay system where *Z. marina* had not been reported since 1933 have resulted in rapid *Z. marina* expansion beyond the initially seeded plots. From 1999 through 2010, 37.8 million viable seeds were added to 369 individual plots ranging in size from 0.01 to 2 ha totaling 125.2 ha in 4 coastal bays. Subsequent expansion from these initial plots to approximately 1700 ha of bay bottom populated with *Z. marina* through 2010 is attributable to seed export from the original plots and subsequent generations of seedlings originating from those exports. Estimates of annual patch vegetative expansion showed mean estimated diameter increasing at varying rates from 10 to 36 cm yr<sup>-1</sup>, consistent with rhizome elongation rates reported for *Z. marina*. Water quality data collected over 7 yr by spatially intensive sampling, as well as fixed-location continuous monitoring, document conditions in all 4 bays that are adequate to support *Z. marina* growth. In particular, median chlorophyll levels for the entire sampling period were between 5 and 6 µg l<sup>-1</sup> for each of the bays, and median turbidity levels, while exhibiting seasonal differences, were between 8 and 9 NTU. The recovery of *Z. marina* initiated in this coastal bay system may be unique in seagrass recovery studies because of how the recovery was initiated (seeds rather than adult plants), how rapidly it occurred (years rather than decades), and the explicit demonstration of how one meadow modulated water clarity and altered sediments as it developed and expanded. Our results offer a new perspective on the role seeds can play in recovery dynamics at large spatial scales.

**KEY WORDS:** Seeds · Germination · Seedling establishment · *Zostera marina* · Virginia coastal bays

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## INTRODUCTION

Seagrasses are in decline worldwide as a result of human perturbations, most notably declining water quality (Orth et al. 2006a). Global declines in seagrass abundance have accelerated, with rates of loss increasing from <1% yr<sup>-1</sup> before 1940 to 5% yr<sup>-1</sup> after 1980 (Waycott et al. 2009). Efforts to restore seagrasses have been successful for some regions and species, but have generally failed to alter the declining trajectories (Fonseca et al. 1998, Paling et al. 2009, Waycott et al. 2009). Recently, the study of dispersal dynamics and in particular the role propagule

supply plays in bed dynamics is improving our understanding of how seagrasses respond to change (Kendrick et al. 2012).

One of the most dramatic changes in a seagrass species in the last century was the rapid decline of populations of eelgrass *Zostera marina* across the North Atlantic basin in the 1930s (Rasmussen 1977). While the decline was attributed to the 'wasting disease' caused by *Labyrinthula*, environmental factors such as temperature changes during this period may have also played a significant role (Rasmussen 1977). Regardless of the cause, the distribution of *Z. marina* populations throughout the North Atlantic was sig-

\*Email: jjorth@vims.edu

nificantly and rapidly altered (Cottam & Munro 1954, Rasmussen 1977). Populations in regions with lower salinities appeared to be less affected, possibly as a result of the *Labyrinthula*'s intolerance to reduced salinities (Pokorny 1967). These unaffected populations apparently provided a propagule source for recovery of *Z. marina* in many locations. In one of the first extensive surveys of *Z. marina* populations along the Atlantic Coast of North America, Cottam & Munro (1954) noted significant improvement in many, but not all, populations by the early 1950s, commenting that recovery was complete or nearly complete in a few areas. In Chesapeake Bay, where aerial photography from 1937 documents low to moderate levels of *Z. marina* abundance throughout its range, full recovery was apparent within 30 yr (Cottam & Munro 1954, Orth & Moore 1983, 1984).

In contrast, *Zostera marina* populations in many high-salinity coastal bays were more susceptible to complete elimination by the disease. The coastal bays of the Delmarva Peninsula, USA, comprise one such region that lost *Z. marina*. While the northern bays (e.g. Chincoteague, Sinepuxent, Isle of Wight, and Assawoman Bays) recovered, possibly due to *Z. marina* either being transplanted from Chesapeake Bay sources or from surviving stock in the 1 bay that did have some low-salinity areas (Orth et al. 2006b), no recovery was recorded in the southern bays (e.g. South, Cobb, Spider Crab, and Hog Island Bays) almost 7 decades after the 1930s demise (Orth et al. 2006b). There was no within-bay source of seeds, as all populations of *Z. marina* had been eliminated by 1933, and seed banks, which are transient, would have been depleted in a year (Harwell & Orth 2002b, Jarvis & Moore 2010). The coastal bays in this region are relatively isolated from the nearest populations of surviving *Z. marina* 75 km to the north in Chincoteague Bay, or 50 km around the southern end of the peninsula into Chesapeake Bay. There are no accurate estimates of the total area historically occupied by *Z. marina* in these southern bays, as aerial photographs were not available until 1937. However, records of substantial bay scallop *Aequipectin irradians* harvests from these beds until 1932 indicate broadly abundant *Z. marina*, as bay scallops are generally found in *Z. marina* (Thayer & Stuart 1974, Orth et al. 2006b). In addition, anecdotal reports from residents, especially those that harvested bay scallops, suggest these bays had an abundance of *Z. marina*.

In 1997, 2 small (ca. 1 m<sup>2</sup>) patches of *Zostera marina* were discovered in South Bay, one of the southern coastal bays. The patches were of

unknown origin, and their reproductive shoot density suggested they were at least 2 yr old. Ground surveys at this time revealed no other patches in this region. In order to assess the suitability of this bay to support sustained *Z. marina* growth, we transplanted adult plants in 1998 in several small test plots near these patches, first in South Bay, and in subsequent years in Cobb, Spider Crab, and Hog Island Bays (Orth et al. 2006b). While the 2 patches were covered by sand from a migrating spit within a few years of discovery, survival of the test plots provided additional evidence that the region could sustain *Z. marina*. We subsequently began an attempt to restore *Z. marina* to the coastal lagoons with seeds. In 1999, we initiated large-scale (>100 m<sup>2</sup> areas) seed introductions using millions of seeds starting in South Bay and in later years expanding to include a total of 4 adjacent sub-basins where the relative isolation from the nearest seed-producing beds may have historically resulted in rare, low-density seedling recruitment.

Our objectives in this paper were to (1) highlight the rapid rate of expansion of *Zostera marina* following seed additions in this coastal bay system; (2) demonstrate that the rapid spread documented by aerial mapping was attributable to seed dispersal from patches initially produced from our seed additions; (3) highlight water quality conditions that have allowed *Z. marina* to survive and spread, documented by data collected through both spatially-sampled and continuously-monitored techniques over 7 yr; and (4) evaluate how the expansion of *Z. marina* influenced water clarity and sediments.

## MATERIALS AND METHODS

### Study sites

Seed addition studies were conducted in 4 adjacent sub-basins along the lower Delmarva Peninsula: South Bay, Cobb Bay, Spider Crab Bay, and Hog Island Bay (Fig. 1). The coastal bays are part of the Virginia Coast Reserve Long-Term Ecological Research site. We initiated large-scale *Zostera marina* restoration with seeds in South Bay in 1999, Cobb Bay in 2001, Spider Crab Bay in 2003, and Hog Island Bay in 2006 following at least 1 yr survival of test plots in each bay (Orth et al. 2006c). Water depths at the seeded sites ranged from approximately 0.9 to 1.6 m at Mean Low Water (MLW), and the tidal range was approximately 1.2 m.



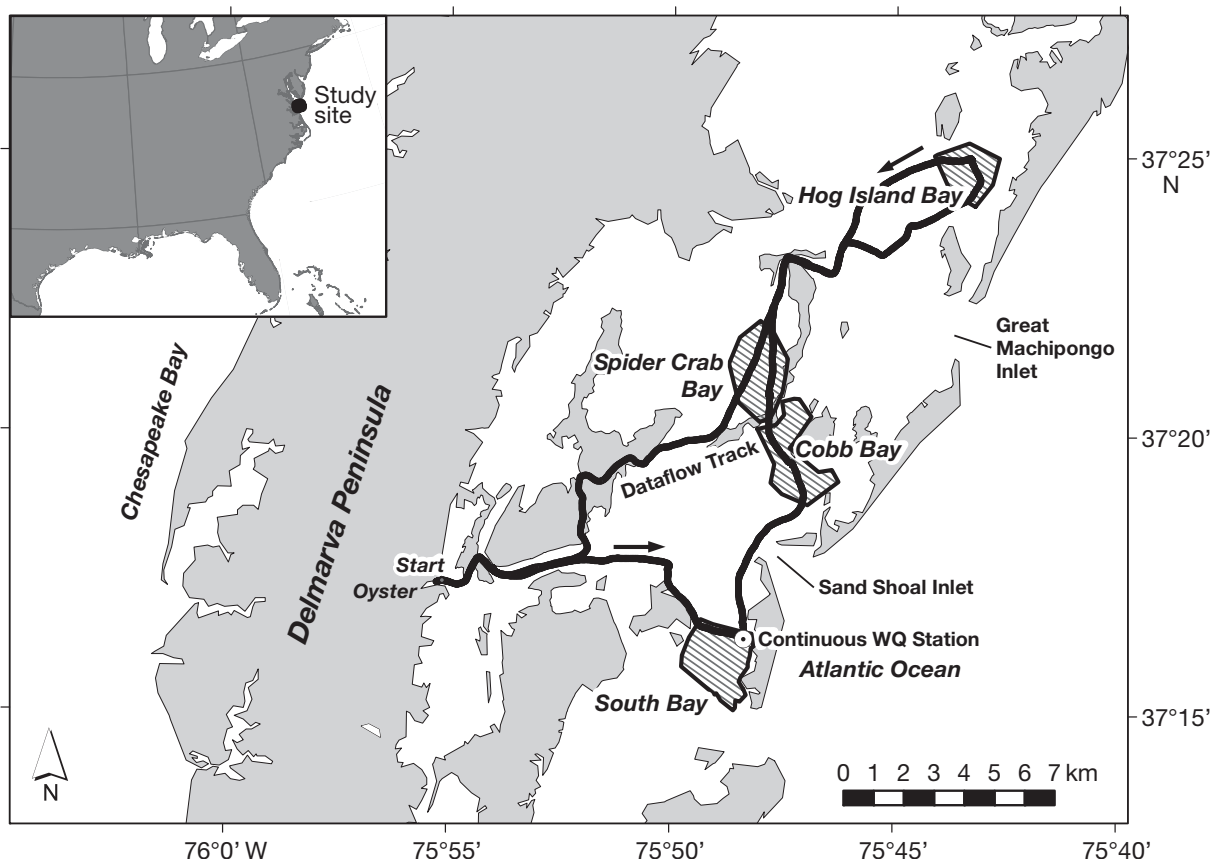


Fig. 1. Study region in the lower Virginia coastal bays, USA. Hatched polygons represent *Zostera marina* seed distribution regions. The solid line across all 4 bays represents the boat track for continuous underway water quality (WQ) sampling (Dataflow) cruises. The open circle in South Bay is the site of the continuous WQ monitoring station

### Seed collection and distribution

*Zostera marina* flowering shoots with maturing seeds were harvested either by hand or by mechanical harvester in May/June and stored in aerated, flow-through tanks until seed release following procedures described by Marion & Orth (2010a). Seeds were collected from Chesapeake Bay donor beds until 2007. After that time, we began collecting seeds from a 10 ha region of the expanding bed in South Bay. Seeds were separated from the senescing shoots and held in recirculating seawater tanks until distribution in October/November, just prior to the normal period of seed germination in this region (Moore et al. 1993). The proportion of viable seeds was determined just before distribution by individually assessing firmness and fall velocity of seeds in subsamples as detailed by Marion & Orth (2010a). Batches of seeds with targeted numbers of viable seeds for individual restoration plots were measured volumetrically, and all seed numbers reported here refer to viable seeds.

Each fall from 1999 through 2010, *Zostera marina* seeds were hand broadcast from a boat into unvegetated plots. Fall broadcasting has been shown to maximize establishment rates (Marion & Orth 2010a), partly by reducing losses to seed predators (Fishman & Orth 1996). Plot size ranged from 0.01 to 2 ha, and seed density ranged from 6 to 1000 seeds  $m^{-2}$  (Table 1). A large majority of plots were either 0.2 or 0.4 ha, and were seeded at 25, 37, or 50 seeds  $m^{-2}$ . In spring 2004, we deployed reproductive shoots holding developing seeds using an adaptation of the Buoy-Deployed Seeding method (Pickerell et al. 2005) into 0.8 to 2.0 ha plots in Spider Crab Bay. Plots distributed in different years were interspersed across each bay's suitable depth range (usually  $<1.5$  m MLW), allowing us to distinguish the relative effectiveness of different years' seedings and to compare the success of deep and shallow plots across years (McGlathery et al. 2012, this Theme Section). Seeded plots were separated by at least 60 to 100 m of unvegetated bottom, though patterns varied among bays and years. Additional small-scale seed experi-

Table 1. *Zostera marina*. Summary of seed distributions for each of 4 coastal bays: number of viable seeds distributed ( $\times 10^6$ ), total area seeded (ha), plot size (ha) and number of plots seeded

Year	South Bay				Cobb Bay				Spider Crab Bay				Hog Island Bay			
	Seeds	Area	Plot size	n plots	Seeds	Area	Plot size	n plots	Seeds	Area	Plot size	n plots	Seeds	Area	Plot size	n plots
1999	0.3	1.2	0.6	2	–	–	–	–	–	–	–	–	–	–	–	–
2000	0.6	0.1	0.01	9	–	–	–	–	–	–	–	–	–	–	–	–
2001	3.6	9.7	0.4	24	0.6	1.6	0.4	4	–	–	–	–	–	–	–	–
2002	1.8	9.7	0.4	24	–	–	–	–	–	–	–	–	–	–	–	–
2003	–	–	–	–	1.1	4.9	0.2	24	0.5	2.2	0.2	11	–	–	–	–
2004	0.7	2.0	2.0	1	–	–	–	–	0.6	1.6	0.2	8	–	–	–	–
2004	–	–	–	–	–	–	–	–	5.9	11.8	0.8–2.0 <sup>a</sup>	7	–	–	–	–
2005	0.5	1.6	0.2	8	–	–	–	–	1.0	2.8	0.2	14	–	–	–	–
2006	–	–	–	–	–	–	–	–	0.5	2.4	0.2	12	0.6	2.8	0.2	14
2006	–	–	–	–	–	–	–	–	–	–	–	–	1.2	5.7	0.4	14
2007	–	–	–	–	–	–	–	–	1.5	6.1	0.2	30	0.5	2.4	0.2	12
2007	–	–	–	–	–	–	–	–	–	–	–	–	0.9	4.9	0.4	12
2008	–	–	–	–	–	–	–	–	1.2	4.7	0.2	23	0.6	2.4	0.4	6
2009	–	–	–	–	2.3	6.1	0.4	15	6.0	16.2	0.4	40	–	–	–	–
2010	–	–	–	–	–	–	–	–	5.5	22.3	0.4	55	–	–	–	–
Total	7.5	24.3	–	68	4.0	12.5	–	43	22.7	70.1	–	200	3.7	18.2	–	58

<sup>a</sup>In 2004, Spider Crab Bay had a range of plot sizes

ments covering 1 to 4 m<sup>2</sup> were conducted in all 4 bays between 1999 and 2008, but these represented <1% of the total seeds broadcast over the 11 yr time period (Orth et al. 2003, Marion & Orth 2010b, R. J. Orth unpubl. data). Plot sizes, numbers, and seeding densities used each year were generally determined by seed availability and the design of individual experiments conducted as part of the restoration effort (e.g. see McGlathery et al. 2012). From 1999 through 2010, 37.8 million viable *Z. marina* seeds were distributed into the 4 bays, with 7.5, 4.0, 22.7, and 3.7 million seeds distributed into total seeded areas of 24.3, 12.5, 70.1, and 18.2 ha in South Bay, Cobb Bay, Spider Crab Bay, and Hog Island Bay, respectively (Table 1).

Germination rates of seeds collected each year were estimated by planting replicate batches of either 10 or 20 seeds at approximately 5 to 7 mm depth in sandy sediments (generally >95% sand and <1% organic matter; Moore et al. 1993) in chilled, recirculating seawater inside a greenhouse. Water temperatures were adjusted to follow ambient water temperatures in the field. Germination was considered successful with the emergence of the cotyledon and first leaf.

Field assessment of seedling establishment was made in April, 6 mo after broadcast. Since seeds become rapidly incorporated into the sediment and do not move far from where they settle to the bottom (Orth et al. 1994), we were able to accurately assess establishment rates in seeded plots. Seeds typically

germinate in early to late November in this region (Moore et al. 1993) and grow slowly during the winter months when water temperatures range from 0 to 5°C. There is no evidence of a multi-year seed bank for *Zostera marina* in this region (Harwell & Orth 2002a, Jarvis & Moore 2010), and we assumed that no viable, ungerminated seeds from the previous year remained in the sediment by April. For large plots (>100 m<sup>2</sup>), seedlings were counted in 1 m line transects along the diagonals of each plot, and initial seedling establishment rates were calculated. Seedling establishment was assessed in smaller-scale experimental plots by complete counts of seedlings (Orth et al. 2003, Marion & Orth 2010b, R. J. Orth unpubl. data). Data from both scales was used to assess seedling success for each bay.

## Eelgrass assessment

### Broad scale

*Zostera marina* bed areas were delineated from aerial photography acquired annually from 2001 through 2010, except for 2005. Black and white photography was acquired at a scale of 1:24 000 from an altitude of 3658 m with a mapping camera, following acquisition timing guidelines that optimize visibility of *Z. marina* beds (Orth et al. 2010). Acquisition timing rules specified tidal stage ( $\pm 90$  min of low tide), plant growth season (peak biomass), sun angle

(between 20 and 40°), atmospheric transparency (cloud cover <10%), water turbidity (edge of grassbeds should be visible), and wind (less than 10 knots; Dobson et al. 1995). Images incorporated 60% flight-line overlap and 20% side lap. Two flight lines were flown each year covering all shorelines and adjacent shoal areas of the 4 bays where the seed addition work was conducted. Aerial photography was scanned from negatives at a 1 m resolution and orthorectified using ERDAS LPS image-processing software. *Z. marina* bed boundaries were then directly photo-interpreted on-screen while maintaining a fixed scale using ESRI ArcMap GIS software (Orth et al. 2010). *Z. marina* beds were categorized as very sparse (1–10% cover), sparse (10–40% cover), moderate (40–70% cover), or dense (70–100% cover) based on a visual estimate of the percent cover on the photograph (Orth et al. 2010). For broad-scale distribution assessments in this study, we collapsed the 4 categories into 2: very sparse to sparse (1–40%) and moderate to dense (40–100%). Ground surveys were conducted in the bays each year to confirm the occurrence of *Z. marina* identified in the photography within and outside the boundaries of the seeded plots.

#### Patch scale

Three studies were conducted at a finer spatial scale in order to document *Zostera marina* recovery at the level of individual eelgrass patches. The first 2 evaluated additional high-resolution imagery of the South Bay study site to document seedling spread along a photographic transect and vegetative growth by tracking patches within a fixed area, while the third study used field sampling to document the spatial spread of seedlings across large distances outside of seeded plots.

The first study focused on a photographic transect, a 10 × 280 m region in South Bay selected to document the rate of spread of *Zostera marina* from seeded plots into the nearby unvegetated area. The transect started along a linear plot seeded in 1999 and extended westward where no seagrass had been identified. High-resolution imagery was acquired for South Bay from 2001 to 2009 with 3 different imaging systems, all following the acquisition guidelines detailed above but flown at lower altitudes. Natural color aerial photography was acquired and scanned with a 7 cm resolution in June 2001, November 2003, and November 2004. Four-band multispectral imagery with a 30 cm resolution was captured in November 2003. Natural color digital imagery with a

15 cm resolution was captured in December 2006, June 2007, January 2008, June 2008, February 2009, and December 2009. This resolution allowed us to follow the development of individual patches emerging from seedlings that originated from the nearby seeded plots. For each year that the higher-resolution imagery was available, the images were visually analyzed along the transect, and *Z. marina* cover within each 10 m section was classified using the 4 cover classes described above (very sparse, sparse, moderate, or dense). Potential nearby sources of *Z. marina* seeds included the 1999 plot at the eastern end, a 2001 plot 160 m to the SW, a 2002 plot 95 m to the NW, a 2005 plot 29 m to the N, and a 2005 plot 63 m to the S.

We examined the role of vegetative growth in bed development in a separate, 67 × 81 m study area that was more isolated from the directly seeded plots (200 m distant from a 2005 plot), allowing us to track the growth of individual patches without interference from additional nearby seedlings. We analyzed *Zostera marina* patches using 4 years of late fall/winter high-resolution imagery (November 2004, December 2006, January 2008, and February 2009) where individual patches had not coalesced on the 2009 imagery. By late fall, *Z. marina* vegetative growth slows in this region, and patches generally persist through the winter with sparser cover than the mid-summer maximum due to typical late-summer senescence. This permits the use of photographs acquired during the relatively less turbid fall–winter conditions to assess the extent of patches during the preceding growing season (March through November). *Z. marina* patches were delineated using eCognition (Trimble GeoSpatial) object-oriented classification for each year. Patch polygons for all years were then merged across the years, following Wilcox et al. (2000), so that their growth history could be analyzed. Patches that did not persist were interpreted as ephemeral patches of macroalgae and eliminated from the analysis ( $n = 28$ ), leaving 100 persistent patches. For each polygon, we measured the area and used the diameter of a circle of equal area as an estimate of the patch diameter to determine linear growth rates. This estimated diameter is slightly shorter than the actual patch maximum dimension since many patches were somewhat elongated, but provides a consistent linear metric for comparison of growth rates.

In addition to these 2 areas captured by aerial imagery, diver transects in 2006 were conducted to help assess the spread of *Zostera marina* across a broad zone in South Bay south of our targeted

restoration plots. The 3 transects (1 m wide) were surveyed by divers in June 2006. The transects originated at edges of 0.4 ha plots established with seeds in 2002 and extended 650 m across regions where no seeds were broadcast. Divers recorded the presence or absence of *Z. marina* in every 1 m<sup>2</sup> segment along each transect.

### Plant characteristics

Reproductive output and bed characteristics were quantified in a region of restored *Zostera marina* in South Bay that had developed into a dense and continuous bed by 2006. Vegetative and reproductive shoot density was measured within 20 cm diameter rings haphazardly tossed by divers during the spring seed collection period in a portion of the bed in South Bay in early June each year from 2007 and 2010. The sampling spanned an area of approximately 10 ha, and coincided with the portion of the bed selected for seed collections in those years. In some years, the region was subdivided into sub-regions to examine spatial variability in shoot density and seed production. In addition, in 2009 and 2010, we assessed seed production by holding a representative sample of reproductive shoots in mesh bags in seawater tanks, and counting the number of viable seeds released.

### Sediments

Sediment samples were collected from unvegetated areas in South Bay in 2000 and 2002 (n = 12) when there was only limited, sparse vegetation in the larger region. Sediments were sampled again in the same general region in 2010 after South Bay became continuously vegetated (n = 10). Samples were taken using a 20 cm deep, 5 cm diameter tube. The top 2 cm of the sample were analyzed for percent sand, silt, clay fractions and total organic matter. Sand fractions (2000 to 62.5 microns) were analyzed by a rapid sediment analyzer, while silts and clays (<62.5 microns) were analyzed by the pipette method (Folk 1980). Total organic matter (percent ash free dry weight) was measured by drying and combusting a 10 g sample for 24 h at 550°C.

### Water quality

Two complementary approaches to documenting water quality conditions were undertaken. Broad

spatial patterns in water quality were documented using continuous underway sampling (hereafter referred to as Dataflow) between 2003 and 2009. In addition, temporal patterns in water quality were documented through sensor deployments at 1 fixed station in South Bay.

The Dataflow cruise track (Fig. 1) traversed restoration areas in South Bay, Cobb Bay, Spider Crab Bay, and Hog Island Bay. Cruise tracks were expanded from the initial track in 2003 over South Bay as successive bays were added to the restoration effort. By 2005, the cruise track covered all 4 major bays and remained similar through 2009. Cruises were generally conducted monthly throughout the *Zostera marina* growing season, from March through November, although the total number of cruises varied annually (2003: 6; 2004: 4; 2005: 7; 2006: 8; 2007: 9; 2008: 9; 2009: 5). The length of cruise tracks in vegetated and unvegetated areas varied annually as the *Z. marina* beds developed and expanded. In South Bay in 2004, the cruise track (Fig. 1) was 0.7 and 1.2 km in the vegetated and unvegetated areas, respectively. In 2009 when the *Z. marina* bed had greatly expanded (see below), the length of the track was 1.5 and 0.40 km in the vegetated and unvegetated areas, respectively. The Dataflow sampling technique inherently caused some imbalances in the sampling size among the groups we analyzed (e.g. inside/outside beds) and between sampling dates, but not to the degree that would degrade the validity of our statistical comparisons.

The Dataflow underway sampler recorded *in vivo* measurements of surface water quality taken at 2 to 3 s intervals (0.25 m depth below surface; approximately every 50 m) along each cruise track. Measurements included turbidity, chlorophyll fluorescence, temperature, salinity, pH, dissolved oxygen, GPS location, and depth using a YSI 6600 EDS sensor array (synchronized with a Garmin GPSMAP 169 Sounder). All sensors on the YSI 6600 EDS were both pre-cruise calibrated and post-cruise checked according to YSI standard procedures. In addition to the continuous underway sensor measurements, 8 calibration and verification stations were sampled at discrete locations spaced along each cruise track for total suspended solids (TSS), extracted pigment chlorophyll, and light attenuation profiles. TSS were determined by filtration of a known volume of seawater on pre-combusted Gelman, Type A/E filters, rinsing with fresh water, and drying at 60°C. Chlorophyll *a* (chl *a*) was collected on Whatman GF/F glass fiber filters, extracted in a solvent mixture of acetone, dimethyl sulfoxide, and 1% diethylamine (45:45:10

by volume) and determined fluorometrically (Shoaf & Lium 1976). Chlorophyll concentrations were uncorrected for phaeopigments. Chlorophyll fluorescence measurements were converted to extracted chlorophyll equivalents by developing a regression between extracted and fluoresced chlorophyll using the extracted chlorophyll and fluoresced samples taken simultaneously at each verification station for the entire study period. Diffuse downwelling attenuation of photosynthetically available radiation (PAR) was determined by triplicate water column measurements of downwelling photosynthetic photon flux density measured with a LI-COR, LIO-192, underwater cosine corrected sensor taken every 25 cm from 10 cm below the surface to 25 cm above the bottom. Similar to the YSI chlorophyll measurements, YSI turbidity measurements were converted to light attenuation equivalents using regression analysis relating turbidity to downwelling light attenuation coefficients ( $K_d$ ) using all simultaneously measured light profiles and turbidities taken at the verification stations over the course of the study.

In order to capture temporally intensive water quality information at 1 site, a YSI 6600 EDS identical to that used in the Dataflow was deployed at a fixed monitoring station in South Bay (Fig. 1). Two-week deployments of the YSI sensor were undertaken at 2 mo intervals concurrent with every other Dataflow cruise. Water quality was measured 0.25 m above the bottom at 15 min intervals throughout each 2 wk deployment. Four 2 to 3 wk sampling periods were conducted each year between 2003 and 2009, except 2003 and 2005 when only three 2 wk periods were assessed. In 2009, the YSI sensor was deployed continuously from 28 July through 18 December. Similarly, in 2010, the YSI was continuously deployed starting on 8 March and continued through the end of 2010. As with the Dataflow array, all sensors were pre-deployment calibrated and post-deployment checked for each deployment.

### Analyses

Sediment data (percent organics and percent silt-clay) from the period of sparse *Zostera marina* and the period of dense *Z. marina* were compared using *t*-tests. Spatial water quality was compared between vegetated and non-vegetated areas in each bay, within each bay over time, and among bays for each sampling year by first converting each spatial dataset to a congruent 10 m grid format using GIS. The grids were then combined to classify each year's Dataflow

measurements according to permanent bay boundaries, and according to the year-specific *Z. marina* bed boundaries for each bay delineated from aerial photography (Fig. 1). Non-parametric Kruskal-Wallis rank sum tests were used to compare Dataflow measurements of water quality among bays, within bays over time, and between vegetated areas and non-vegetated areas within each bay using R statistical software (R Foundation for Statistical Computing). Paired post hoc comparisons followed procedures originally published by Siegel & Castellan (1988). Similarly, water quality measurements made at the fixed continuous-monitoring station in South Bay during the early summer deployment periods (when the *Z. marina* bed biomass and cover was typically the greatest annually) were compared among years using Kruskal-Wallis rank sum tests. Regression analysis was used to develop conversions between *in vivo* turbidity (nephelometric turbidity units, NTU) and underwater light attenuation ( $K_d = 0.088 \times \text{NTU} + 0.559$ ;  $R^2 = 0.716$ ), *in vivo* chlorophyll fluorescence and extracted chlorophyll (Extracted chl =  $1.386 \times \text{In vivo chl} + 1.765$ ;  $R^2 = 0.730$ ), and *in vivo* turbidity (NTU) and TSS ( $\text{mg l}^{-1}$ ) ( $\text{TSS} = 2.592 \times \text{NTU}$ ;  $R^2 = 0.532$ ) sampled simultaneously at each calibration and verification station sampling.

## RESULTS

### *Zostera marina* seedling establishment

Seeding was successful each year, but seedling establishment rates varied among individual plots, bays, and years (Fig. 2). The median seedling establishment rate for all 202 evaluated plots was 6.1%, with median rates of 7.0, 2.0, 6.3, and 6.4% recorded at South, Cobb, Spider Crab, and Hog Island Bays, respectively (Fig. 2). Laboratory germination rates of seeds previously assessed as viable were always greater than 80%, confirming that the seeds we dispersed were largely viable seeds, and that differences between years in field seedling establishment were not explained by differences in viability of the seeds used.

### Meadow expansion and development

Seed dispersal from plants in the restored plots to nearby unvegetated areas has resulted in an estimated 1714 ha containing *Zostera marina* by 2010 in these 4 bays, almost 14 times the originally seeded area.

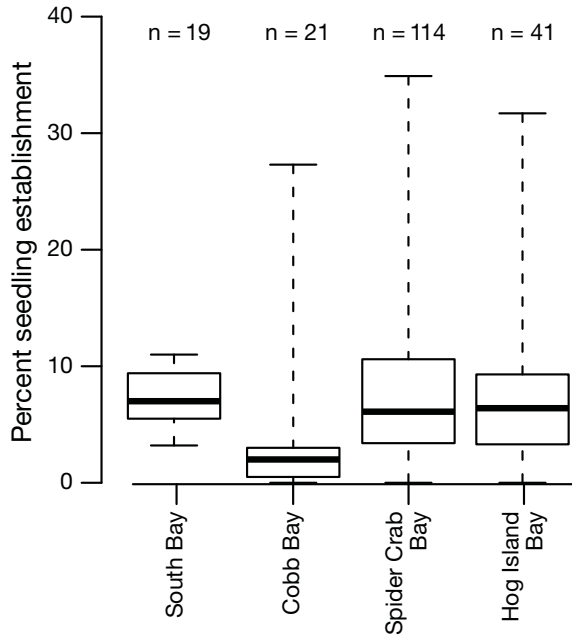


Fig. 2. *Zostera marina*. Seedling establishment rates observed across multiple years. Seedlings were assessed in April following seed distribution the previous fall

South Bay, where seeding began in 1999, showed the greatest spread and increase in coverage of the 4 bays. Eelgrass was first mapped for this bay in 2001, when 15.7 ha were recorded, all having sparse eelgrass cover (Fig. 3). This increased to 200 ha, also sparsely covered, in 2006. By 2010, 1020 ha were mapped, a 410 % increase from 2006, with 71 % classified as moderate to dense cover. Aerial photographs from 2001 to 2010 show the rapid spread of *Zostera marina* in South Bay especially between 2006 and 2010 (Fig. 4).

Seed distribution in Cobb Bay began in 2001. Eelgrass was first mapped for this bay in 2003 when 3.9 ha, all sparse cover, were recorded (Fig. 3). By 2006, 41 ha were mapped with 11% considered moderate to dense cover. By 2010, *Zostera marina* coverage increased to 349 ha, with 27 % classified as moderate to dense cover.

Seed distribution in Spider Crab Bay began in 2003. Eelgrass was first mapped in this bay in 2004 when only 0.3 ha, all sparse cover, was recorded (Fig. 3). In 2006, 1.6 ha were mapped as all sparse cover. By 2010, 163 ha were mapped, with 39% considered moderate to dense cover.

Seed distribution in Hog Island Bay began in 2006. Eelgrass was first mapped in this bay in 2007 when 25.5 ha, all sparse cover, were recorded (Fig. 3). By 2010, 182 ha were mapped, with 52% considered moderate to dense cover.

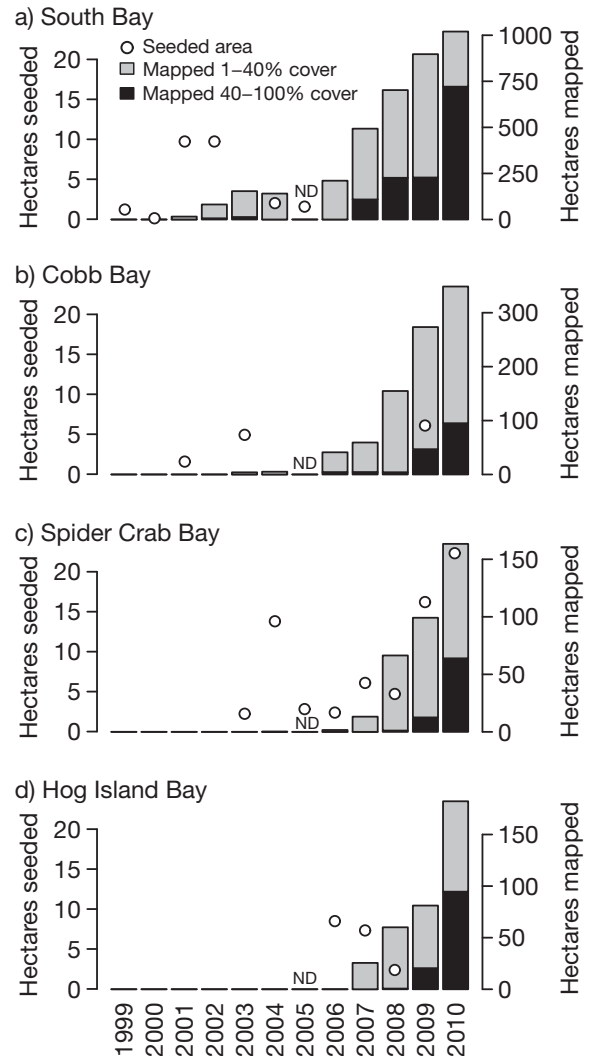


Fig. 3. *Zostera marina*. Area of seeding in each of 4 bays (circles, left y-axis), and area mapped in 2 density classes by aerial photography each year (bars, right y-axis). ND: no mapping data for 2005

**Patch-scale investigations**

The yearly photographic transect in South Bay documented a progression from a small number of eelgrass patches in 2001 to dense cover in 2009 (Fig. 5). In 2001, only 4 cells at the eastern end of the transect contained *Zostera marina*. By 2006, all cells had *Z. marina*, and the easternmost cell was dense. In 2009, all cells had at least sparse cover, and 64 % of the transect had dense coverage.

The South Bay patch-tracking study area had *Zostera marina* patches that ranged in size from 0.03 to 3.85 m<sup>2</sup> over the 4 photo acquisition dates (Table 2). The mean patch area for each cohort (patches first appearing on each of the 4 dates)

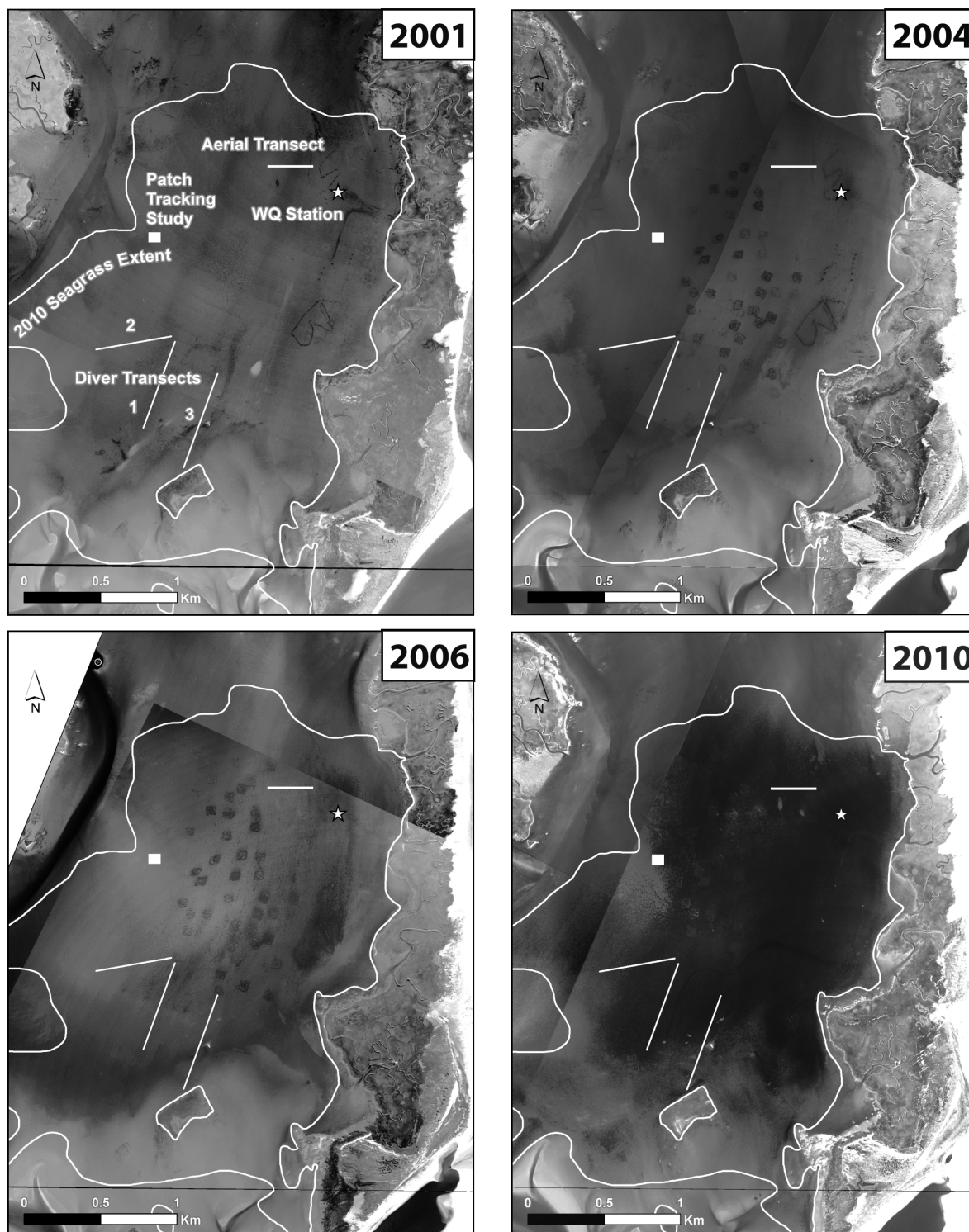


Fig. 4. *Zostera marina*. Aerial photo-mosaics from 2001, 2004, 2006, and 2010, showing meadow development in South Bay. Dark squares in the 2004 and 2006 photo-mosaic are the 0.4 ha seed plots from seed additions in 2001 and 2002. These plots have coalesced and spread and the dark area in the 2010 photo-mosaic is one large continuous bed. The white line around the bed is the extent of the bed as mapped in 2010. Lines indicate a 280 m photographic transect where detailed analysis of aerial photography was conducted for 2001 to 2009, and the location of three 650 m diver transects conducted in 2006. Box indicates area where patches were tracked to study vegetative growth. Star indicates fixed-location water quality station. Linear artifacts are photographic boundaries within the mosaics

increased in each subsequent observation (Table 2), with the mean estimated diameter increasing at varying rates from 10 to 36 cm yr<sup>-1</sup> (Table 3). Patch area totaled 15.45 m<sup>2</sup> in November 2004 and expanded to 115.40 m<sup>2</sup> in February 2009. The contribution of new patches to the total area on each subsequent date was 45% in December 2006, 29% in January 2008, and 33% in February 2009. Analysis of imagery from December 2009 documents a dramatic increase to 2670.50 m<sup>2</sup> for the entire area, compared to 140.79 m<sup>2</sup> for the entire area in February 2009, including the 28 excluded patches.

The 2006 diver transects in South Bay (Fig. 4) showed *Zostera marina* presence for hundreds of meters outside the plots seeded in 2002 (Fig. 6). Careful inspection of aerial photography of the region acquired from 2001 and 2004 revealed that several small patches present in 2001 contributed to the spread of seedlings in this area, in combination with the directly seeded plots to the north. The origin of these plots is unknown, but their size in 2001 suggests they could have been second-generation seedlings originating from our early seed dispersals in 1999 and 2000 or from the initial 1998 and 1999 adult plant test plots.

**Plant characteristics**

Vegetative and reproductive shoot densities in South Bay varied among the years, with vegetative shoot densities decreasing from 2007 to 2010 (Table 4). Reproductive shoots also varied among years, repre-

Table 2. *Zostera marina*. Mean ± SD area (m<sup>2</sup>) of patches repeatedly measured from aerial photographs. Patches first observed on each date were each re-measured on subsequent dates

First observed	No. of patches	Nov 2004	Dec 2006	Jan 2008	Feb 2009
Nov 2004	11	0.12 ± 0.10	0.63 ± 0.50	0.99 ± 0.54	1.84 ± 1.01
Dec 2006	22		0.39 ± 0.29	0.58 ± 0.26	1.33 ± 0.56
Jan 2008	35			0.38 ± 0.19	1.13 ± 0.52
Feb 2009	42				0.90 ± 0.47

Table 3. *Zostera marina*. Mean ± SD estimated diameter (cm) and change from previous observation for patches repeatedly measured from aerial photographs on 4 dates. Estimated diameter is the diameter of a circle equal in area to the measured patch

First observed	No. of patches	Nov 2004	Dec 2006	Change	Jan 2008	Change	Feb 2009	Change
Nov 2004	11	21 ± 08	47 ± 19	27 ± 19	61 ± 18	14 ± 18	97 ± 20	36 ± 20
Dec 2006	22		37 ± 13		47 ± 12	10 ± 12	79 ± 14	32 ± 14
Jan 2008	35				38 ± 09		68 ± 13	30 ± 13
Feb 2009	42						59 ± 14	

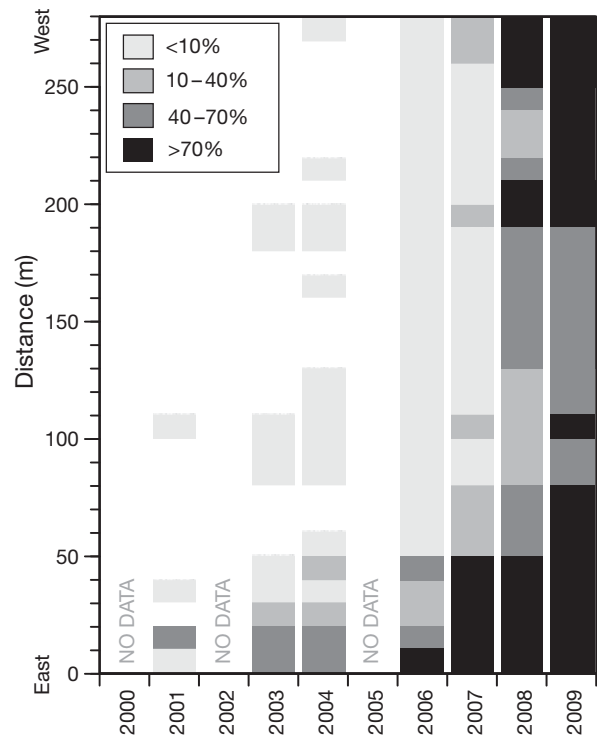


Fig. 5. *Zostera marina*. Changes in percent cover estimates in 10 m segments across a 280 m aerial photographic transect from 2001 to 2009

sented 8 to 41% of the total number of shoots. There was substantial spatial variation among sub-regions of the bed in vegetative and reproductive shoot density, despite very few perceived environmental gradients across the sampled region. Reproductive shoots sampled in 2009 and 2010 produced an average of 50 and 43 viable seeds shoot<sup>-1</sup>, respectively.

**Sediments**

There was a significant increase in the percent silt-clay fraction and percent organics (*t*-tests, *p* < 0.001) from 2000 and 2002 when *Zostera marina* was very patchy to 2010 when



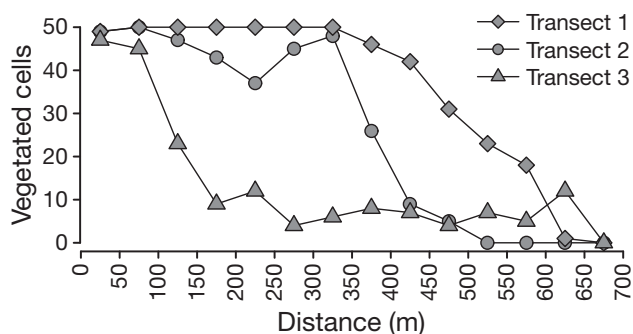


Fig. 6. *Zostera marina*. Diver observations of *Z. marina* presence in three 1 × 650 m transects assessed in June 2006. Data are the number of 1 m<sup>2</sup> cells containing *Z. marina* within each 50 m transect segment. Transects started adjacent to 0.4 ha plots seeded in 2002 and extended to the southwest as indicated in Fig. 4

the bed had become dense and continuous (see photographs in Fig. 4). The mean silt-clay fraction more than doubled from  $14.5 \pm 4.9$  (SD) to  $34.6 \pm 4.4\%$  while the organic content almost tripled from  $1.0 \pm 0.2$  to  $2.8 \pm 0.4\%$ .

### Water quality

Dataflow provided a characterization of each bay's conditions over short spatial scales as evidenced from 1 cruise in July 2009 (Fig. 7). Salinity demonstrated consistent levels among the various bay restoration sites, with concentrations typically within 1 to 2 PSU. Temperature, especially during the summer months, showed some variability, with lower summertime temperatures in the vicinity of the inlets. Dissolved oxygen and pH concentrations were generally lower near the western shore compared to the restoration

Table 4. *Zostera marina*. Mean  $\pm$  SD density of vegetative and reproductive shoots (no. m<sup>-2</sup>) assessed in early June between 2007 and 2010 in an area of South Bay with high percent cover. Multiple rows within years reflect sampling in sub-regions separated by 100 to 200 m

Year	n plots	Vegetative	Reproductive
2007	17	675 $\pm$ 236	471 $\pm$ 111
2008	40	583 $\pm$ 162	51 $\pm$ 38
	40	497 $\pm$ 194	41 $\pm$ 38
	40	665 $\pm$ 210	76 $\pm$ 60
2009	36	395 $\pm$ 134	73 $\pm$ 51
	30	707 $\pm$ 264	140 $\pm$ 92
	30	337 $\pm$ 108	102 $\pm$ 60
2010	21	404 $\pm$ 118	54 $\pm$ 32
	42	395 $\pm$ 115	45 $\pm$ 29

sites. Turbidity levels varied over short distances as much as 20 to 30 NTU, with higher levels near the channels, while chlorophyll levels usually varied by 5 to 10  $\mu\text{g l}^{-1}$  across the transect.

Integrated monthly water quality conditions measured using Dataflow across each bay for all years combined (2003 to 2009) showed that salinities ranged between 28 and 34 PSU, with median levels between 31 and 32 PSU (Table 5). Temperatures during the March to November *Zostera marina* growing season ranged from  $<5$  to  $>25^\circ\text{C}$  with medians near  $20^\circ\text{C}$  (Table 5). Dissolved oxygen was always high, with median levels between 7 and 8  $\text{mg l}^{-1}$ , while pH was well buffered at around 8.0 (Table 5). Turbidity levels were generally low, with median levels at 10 NTU or less (Table 5). Occasionally, high turbidity levels of up to 50 NTU were observed; however, approximately 75% of the time, turbidity levels were less than 15 NTU (K. A. Moore unpubl. data). Chlorophyll concentrations were also low, with median levels less than 6  $\mu\text{g l}^{-1}$ . The results show significant differences among the bays, although the differences are slight and the magnitude and direction of the differences are not consistent. Hog Island Bay had slightly lower median turbidity and slightly higher salinity, dissolved oxygen, pH, and chlorophyll than the other 3 bays.

Integrated growing season turbidity levels for all cruises within each study year and bay showed significant yearly variation with 2005 demonstrating the highest turbidities across all bays (Table 6). Similarly, chlorophyll levels were highest in 2005, but no systematic temporal trends were observed across the 7 yr time period for any of the bays (Table 7).

In South Bay, monthly variability in median turbidity for cruises from May to September (the time period with the most complete data) was observed, with lowest levels in May and September (Table 8). Turbidity levels within the vegetated areas were significantly lower than levels outside of the vegetated areas from June through September. Similarly, chlorophyll concentrations inside the beds were lower than levels outside the beds during July and August when chlorophyll concentrations were highest (Table 9).

Turbidity measurements obtained from the continuous monitoring station during deployments in the June to July periods each year when *Zostera marina* biomass was greatest demonstrated significant ( $p < 0.05$ ) decreases over time (Fig. 8a). The median levels decreased from approximately 20 to  $<5$  NTU, and the pulses of high turbidity also decreased over time, showing a consistent improvement in water clarity during this period of the growing season.

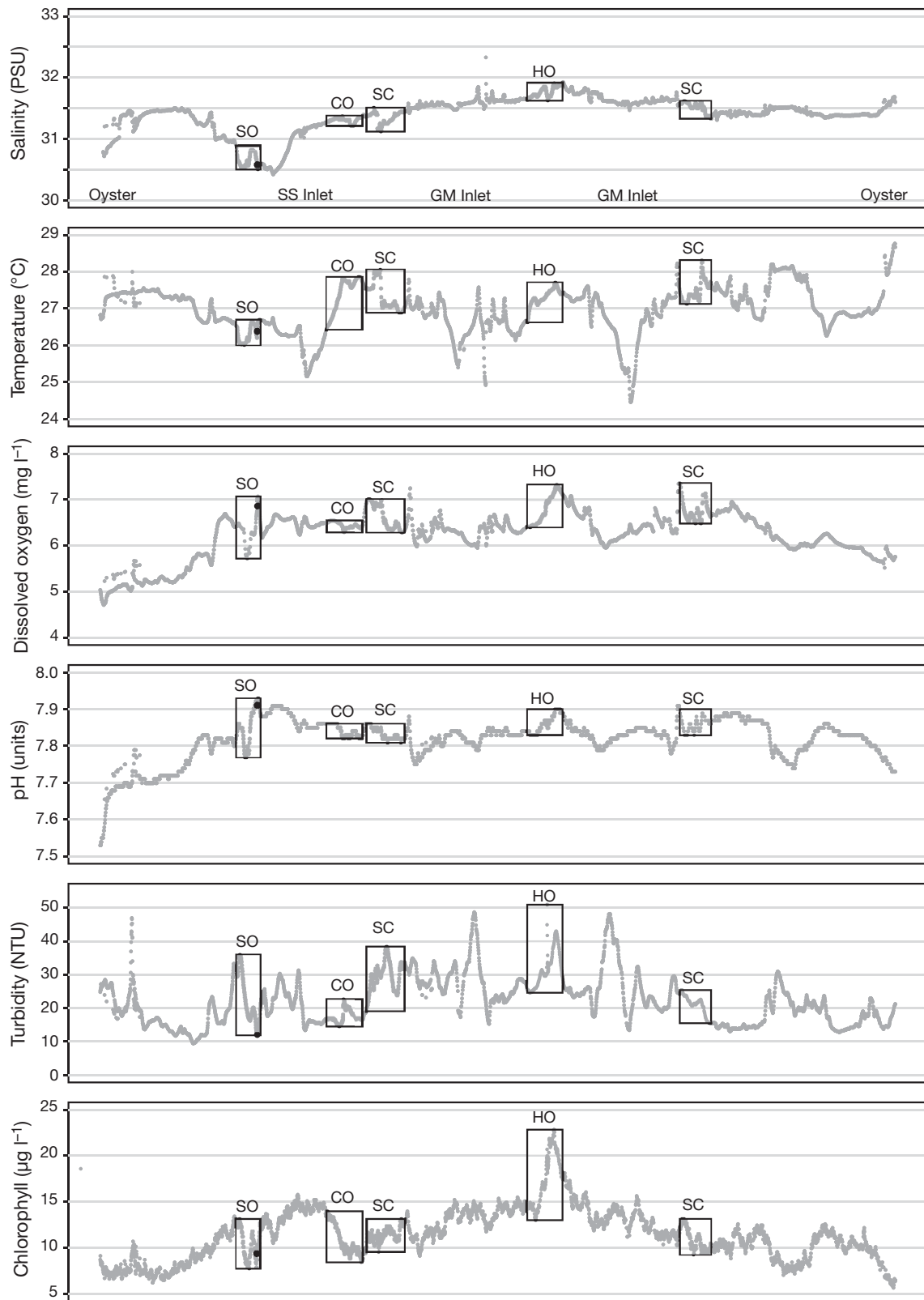


Fig. 7. Water quality parameters measured along the Dataflow track beginning and ending at Oyster, Virginia, as recorded on 28 July 2009. Boxes around data indicate the locations along the track of target restoration areas shown in Fig. 1. SO: South Bay, CO: Cobb Island, SC: Spider Crab Bay, HO: Hogg Island Bay, SS Inlet: Sand Shoal Inlet, GM Inlet: Great Machipongo Inlet. Black dot shows the South Bay continuous monitoring station

Table 5. Median water quality parameters measured by Dataflow for all cruises combined (March through November, 2003 to 2009) within each bay. Identical superscripts indicate no significant differences in individual water quality parameters among the bays. DO: dissolved oxygen

Parameter	Cobb	Hog	South	Spider
Salinity (PSU)	31.46 <sup>a</sup>	31.50 <sup>b</sup>	31.62 <sup>c</sup>	31.68 <sup>a</sup>
Temperature (°C)	20.55 <sup>a</sup>	20.57 <sup>b</sup>	20.61 <sup>b</sup>	20.43 <sup>c</sup>
DO (mg l <sup>-1</sup> )	7.20 <sup>a</sup>	7.43 <sup>b</sup>	7.37 <sup>c</sup>	7.71 <sup>d</sup>
pH (units)	7.90 <sup>a</sup>	7.90 <sup>b</sup>	7.90 <sup>c</sup>	7.92 <sup>a</sup>
Turbidity (NTU)	9.93 <sup>a</sup>	9.94 <sup>b</sup>	9.49 <sup>c</sup>	8.02 <sup>d</sup>
Chlorophyll (µg l <sup>-1</sup> )	5.37 <sup>a</sup>	5.51 <sup>b</sup>	5.64 <sup>a</sup>	5.06 <sup>c</sup>

Table 6. Median turbidity (NTU) measured by Dataflow within each bay for March through November each year. Identical superscripts indicate no significant differences in turbidity among the years within each bay. ND: no data

Bay	2003	2004	2005	2006	2007	2008	2009
South	9.90 <sup>a</sup>	7.80 <sup>b</sup>	16.90 <sup>c</sup>	13.30 <sup>d</sup>	8.20 <sup>e</sup>	9.30 <sup>a</sup>	12.05 <sup>f</sup>
Cobb	12.25 <sup>a</sup>	9.20 <sup>b</sup>	13.60 <sup>c</sup>	10.60 <sup>d</sup>	7.00 <sup>e</sup>	9.30 <sup>a</sup>	7.70 <sup>d</sup>
Spider	9.55 <sup>a</sup>	8.80 <sup>a</sup>	14.10 <sup>b</sup>	10.70 <sup>a</sup>	7.70 <sup>c</sup>	9.30 <sup>a</sup>	7.70 <sup>a</sup>
Hog	ND	ND	ND	11.41 <sup>a</sup>	5.20 <sup>b</sup>	8.50 <sup>ac</sup>	8.40 <sup>c</sup>

Table 7. Median chlorophyll (µg l<sup>-1</sup>) measured by Dataflow within each bay for March through November each year. Identical superscripts indicate no significant differences in chlorophyll among the years within each bay. ND: no data

Bay	2003	2004	2005	2006	2007	2008	2009
South	3.84 <sup>a</sup>	4.81 <sup>b</sup>	6.89 <sup>c</sup>	6.20 <sup>d</sup>	5.79 <sup>e</sup>	5.09 <sup>be</sup>	5.23 <sup>d</sup>
Cobb	4.81 <sup>a</sup>	5.37 <sup>b</sup>	6.75 <sup>c</sup>	6.34 <sup>b</sup>	5.09 <sup>a</sup>	4.90 <sup>d</sup>	5.61 <sup>b</sup>
Spider	4.26 <sup>a</sup>	5.09 <sup>bd</sup>	6.86 <sup>c</sup>	5.79 <sup>b</sup>	5.51 <sup>a</sup>	5.23 <sup>d</sup>	5.65 <sup>d</sup>
Hog	ND	ND	ND	6.75 <sup>a</sup>	4.81 <sup>b</sup>	6.82 <sup>a</sup>	7.03 <sup>a</sup>

Table 8. Median turbidity (NTU) measured by Dataflow inside and outside of *Zostera marina* beds in South Bay for all cruises combined (2003–2009) for each month. Identical superscripts indicate no significant differences in turbidity measured inside or outside of the vegetated areas for that month

Month	Inside	Outside
May	7.24 <sup>a</sup>	7.5 <sup>a</sup>
June	10.6 <sup>a</sup>	14.6 <sup>b</sup>
July	15.6 <sup>a</sup>	24.5 <sup>b</sup>
August	13.5 <sup>a</sup>	13.8 <sup>b</sup>
September	8.1 <sup>a</sup>	10.7 <sup>b</sup>

Table 9. Median chlorophyll measured by Dataflow inside and outside of *Zostera marina* beds areas in South Bay for all cruises combined (2003–2009) for each month. Identical superscripts indicate no significant differences in chlorophyll measured inside or outside of the vegetated areas for that month

Month	Inside	Outside
May	4.67 <sup>a</sup>	4.26 <sup>b</sup>
June	5.51 <sup>a</sup>	5.65 <sup>a</sup>
July	8.97 <sup>a</sup>	9.25 <sup>b</sup>
August	8.14 <sup>a</sup>	8.56 <sup>b</sup>
September	6.34 <sup>a</sup>	7.03 <sup>a</sup>

Chlorophyll levels measured at the continuous monitoring station demonstrated a pattern of significant ( $p < 0.05$ ) decline over time (Fig. 8b) similar to that observed for turbidity. A marked decline from the highest levels in 2003 was followed by a continuing decrease. Not only did the median levels decrease from approximately 17 to  $<6 \mu\text{g l}^{-1}$ , but the variability decreased markedly, especially the pulses of high chlorophyll.

## DISCUSSION

Eleven years of *Zostera marina* seed additions conducted in 4 coastal bays where *Z. marina* had been absent since 1933 have resulted in the successful establishment and subsequent rapid expansion of *Z. marina* beyond the initially seeded plots. The success of this seeding in establishing expanding meadows in these 4 coastal bays suggests that seed supply was the recruitment bottleneck preventing re-establishment of *Z. marina* during its almost 70 yr absence. The expansion in these bays outside the originally seeded plots is attributable to multiple generations of seedlings derived from the large number of seeds produced in and dispersed from these plots, rather than to vegetative growth from seeds we directly distributed. The development of these beds has influenced their ecosystems by altering sediment grain size, total organic matter, and water clarity.

### *Zostera marina* bed development

The spatial and temporal patterns of change in *Zostera marina* we have observed in these coastal bays over the decade following initial seeding is a product of this species' reproductive phenology, seed production yields, dispersal mechanisms, and vege-

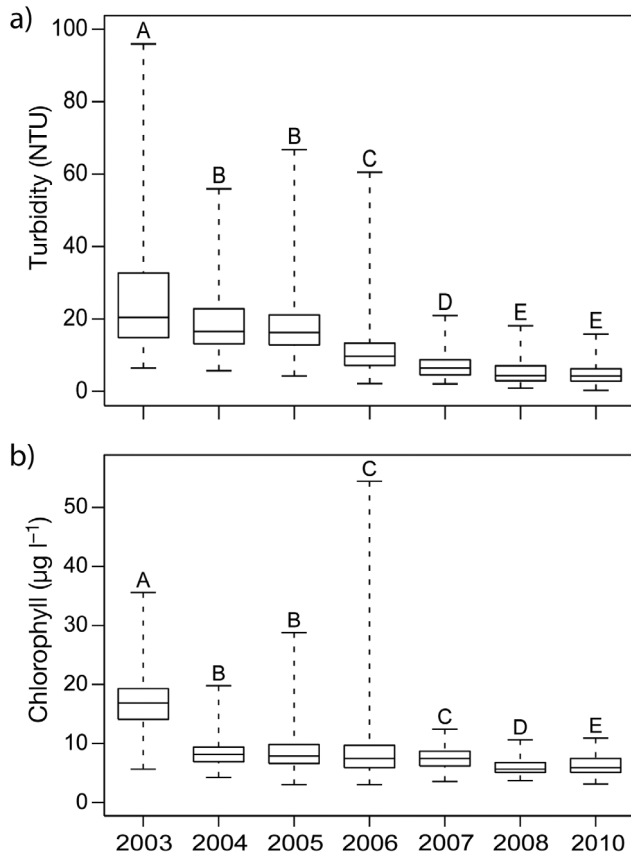


Fig. 8. (a) Turbidity and (b) chlorophyll (median, 25th and 75th percentiles, and the minimum and maximum of the lower 99% of the data). Data are from June and July from the South Bay monitoring station (see Fig. 1 for its location). Data are 15 min observations recorded during multiple 2 wk deployments. Identical letters indicate no significant differences ( $p < 0.05$ ) among medians

tative growth in our region. Seedlings in our region generally flower and produce seeds in their second year (Setchell 1929), and in each subsequent year. They add new recruits either close to the parent plant via floating seeds (Churchill et al. 1985) or distant from the meadow via buoyant flowering shoots with viable seeds (Harwell & Orth 2002b, Källström et al. 2008). Flowering shoots, once they become negatively buoyant, are also transported along the bottom (Harwell & Orth 2002b) and can be retained on benthic features, such as tubes of the onuphid polychaete *Diopatra cuprea* (Harwell & Orth 2001), which are abundant in these bays. Once on the sediment surface, seeds are retained close to their settlement point by topographic complexities of the bottom (Orth et al. 1994) and are rapidly buried. These dispersal processes result in both outward expansion of the bed and increasing seedling density within the bed. Sparse patches developed and expanded outside

the seeded plots from seeds generated by the initial plants within plots. Simultaneously, the seeded plots themselves became denser, not only by vegetative growth, but also by the production of seeds by newly developed reproductive shoots. These natural recruitment processes can be important for bed persistence, especially after diebacks due to stressful events such as unusually high summertime temperatures (Jarvis & Moore 2010), high turbidity events due to phytoplankton blooms (Dennison et al. 1989), or storms (Williams 1988). We have discounted dispersal and establishment of vegetative fragments in developing new patches, as there is little evidence that these fragments can successfully establish (Ewanchuk & Williams 1996, Di Carlo et al. 2005, Hall et al. 2006).

The relatively low rates of seedling establishment at 6 mo (medians of 2 to 7% of viable seeds distributed) in our unvegetated seed plots are largely a result of wintertime losses of early-stage, shallow seedlings which, without a substantial root system, are easily removed by wave action and currents that can erode sediments to several centimeters (Marion & Orth 2012, this Theme Section). In addition, losses could have been exacerbated by drifting macroalgae which can dislodge newly emerging, or even well-developed, seedlings in less cohesive sediments (Valdemarsen et al. 2010). In the spring, we often observed uprooted seedlings anchored only by a few root hairs, and these seedlings sometimes had entangling masses of filamentous algae attached. This mechanism may help explain differences between years in seedling establishment as a product of different wind patterns and macroalgal abundances. The largest variability in seedling establishment was found between plots, but we did not attempt to correlate plot performance with plot-specific exposure to currents and wave action. Regardless, the within-year plot variability demonstrates that seed viability was not a substantial source of variation in seedling establishment in the restoration plots. Seedling establishment rates for seeds subsequently produced by patches we established are unknown, but would likely balance summer seed mortality, which we expect to be higher than for seeds stored in the lab, with potentially higher seedling retention rates due to physical protection provided by nearby pre-existing *Zostera marina* plants.

While we have highlighted the role seeds played in bed expansion rates, our estimates of patch vegetative expansion suggest that once a seed establishes, rhizome elongation is an important component in the early in-filling of the meadow. In our patch-expansion study area, the growth in estimated patch diameter

from the low-level aerial imagery (27 cm for 2 yr; 14, 10, 36, 32, and 30 cm for a single year; Table 3) is consistent with the 26 cm average annual rhizome elongation rates reported for *Zostera marina* (Marbà & Duarte 1998). Areal spread of different cohorts of patches demonstrated variable growth rates, especially in their first year's growth (0.12 to 0.90 m<sup>2</sup>) likely due to different environmental conditions between years. The longest-tracked patches attained an areal coverage of almost 3 m<sup>2</sup> in 5 yr, illustrating the potential for vegetative growth to contribute substantially to internal meadow development through coalescence of adjacent seedling patches. However, by December 2009, the total area covered by new seedlings emerging in the unvegetated area between patches dwarfed the accumulated area resulting from vegetative growth of existing patches.

#### Eelgrass effects on water quality and sediments

Numerous studies have demonstrated the ability of seagrasses to influence their ecosystems by altering hydrodynamics and consequently sediment characteristics, increasing habitat complexity for fauna, altering predator-prey dynamics, or enhancing primary production (Larkum et al. 2006, van der Heide et al. 2011). The influence of eelgrass *Zostera marina* on 2 key characteristics, water quality and sediment composition, was documented in South Bay where *Z. marina* spread over the entire region by 2007. Water column turbidity was altered in South Bay between 2003 and 2010, with substantial reductions in both the range and the median value. There was a strong relationship between median June to July turbidity levels in South Bay and bed development (Fig. 9). Although these data are correlative, we regard the reduction in turbidity to be a consequence of the expanding bed, as similar reductions in turbidity over time were not seen in the other bays where *Z. marina* was much less extensive. This relationship is also consistent with our spatial comparisons of turbidity inside and outside of beds in all bays, which shows significantly lower turbidity within beds (Table 8). Median turbidities below 10 NTU were found only after the bed area exceeded 200 ha. At this point, bed expansion accelerated and turbidities continued to decrease to median levels of approximately 4 to 5 NTU in 2008 and 2010. In Chesapeake Bay, Moore (2004) found that the effects of *Z. marina* vegetation on water column suspended sediments, chlorophyll, and light attenuation increased with bed development. The effects of increasing *Z. marina* abundance on

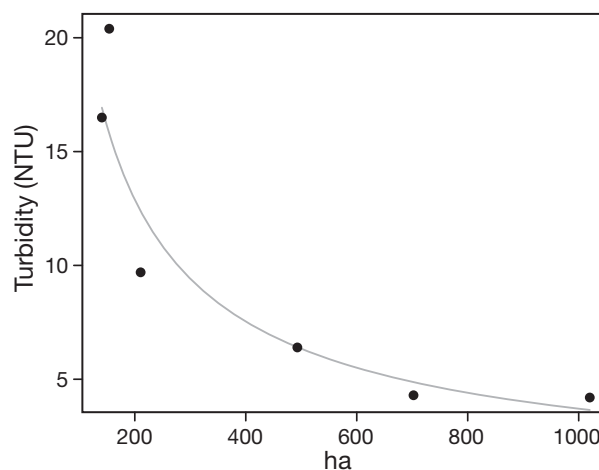


Fig. 9. *Zostera marina*. Relationship between yearly June to July South Bay median turbidity and yearly abundance (ha). The power regression function is  $y = 780.65x^{-0.775}$ , where  $R^2 = 0.9287$

suppressing resuspension and enhancing deposition that he observed were also apparent here, as both bed percent cover and area in the South Bay restoration site increased over time. One concurrent study in South Bay found near-bed mean velocity reduced by 70 to 90%, and wave heights 45 to 70% compared to an adjacent unvegetated region shifting the seafloor from an erosional to depositional environment (Hansen & Reidenbach 2012, this Theme Section). Similarly, chlorophyll concentrations also declined over time as the *Z. marina* beds developed, although the effects were not as consistent as with turbidity. In part this may be due to the relatively low levels of chlorophyll present in the bay even before the *Z. marina* expansion (see [www1.vcrilr.virginia.edu/home1/?q=data\\_wq](http://www1.vcrilr.virginia.edu/home1/?q=data_wq)), as well as other factors including South Bay's short residence time (Oertel 2001). The differences observed between the vegetated areas and adjacent unvegetated areas within these bays suggest that the *Z. marina* recovery has improved local water quality conditions.

Turbidities of 10 NTU were found to be equivalent to a light attenuation coefficient ( $K_d$ ) of approximately 1.5 m<sup>-1</sup> or approximately 22% of surface light reaching the bottom at a depth of 1 m. In Chesapeake Bay, these light attenuation levels have been associated with shallow water areas where *Zostera marina* has been found growing to depths of 1 m at MLW or less (Dennison et al. 1993, Moore et al. 1996). Potentially stressful median June to July levels of >10 NTU were observed in the early years of the restoration project, but these summer levels decreased significantly in later years (Fig. 8a). Higher levels (>10 to 15 NTU) have been found closer to the mainland

(Fig. 7), where there is more effect of watershed loadings (Stanhope et al. 2009, Giordano et al. 2011) that contribute to the gradient of organic and inorganic constituents observed in the water column (Anderson et al. 2010). Higher turbidity levels were also found near the channels separating the different bays. These elevated levels may be caused by the local resuspension of sediments due to high currents or wave action in the channel areas. Previous field and modeling results of wind-driven sediment suspension and feedbacks on light availability in Hog Island Bay also show that >60% of the bay may be suitable for *Z. marina* growth (Lawson et al. 2007).

Low water column chlorophyll levels were typical of the coastal bays throughout the entire study period, with concentrations typically below 5 to 10  $\mu\text{g l}^{-1}$ . Chlorophyll levels of 15  $\mu\text{g l}^{-1}$  or greater have been associated with submersed aquatic vegetation (SAV) habitats that are under stress or in decline (Dennison et al. 1993). These low phytoplankton concentrations may be related to the short residence time of water within much of the eastern regions of these coastal lagoon areas (Oertel 2001).

Water quality conditions in the Virginia coastal bays studied here are largely related to the loadings that are affected by land use along the adjacent Delmarva Peninsula (Giordano et al. 2011). Here the watersheds are dominated by agriculture and forests, resulting in relatively low nutrient loadings (Anderson et al. 2010), in contrast to other coastal bays in Maryland and Delaware, where extensive development and intensive agriculture contribute to elevated loadings (Giordano et al. 2011) and enriched conditions (Boyn-ton et al. 1996). Similarly, in coastal bay systems including Pamlico Sound, North Carolina, to the south, human activities have resulted in nutrient enrichment and sedimentation (Paerl et al. 2010), and in Barnegat Bay, New Jersey, to the north, increased loadings have been linked to seagrass declines (Kennish et al. 2010).

The percentages of silt-clay and total organics in the sediments increased significantly between 2002 and 2010. Because seagrass alters the hydrodynamic environment, with leaves baffling waves and currents, and roots and rhizomes stabilizing the sediments, fine-grained material is deposited and retained within the bed rather than remaining suspended in the water column (Ward et al. 1984, Koch 2001, Moore 2004, Koch et al. 2006, Gruber & Kemp 2010). This increase in organic content in the sediments may provide increased nutrients for continued seagrass growth providing a positive feedback for continued bed development. Shoot densities in a portion of the South Bay

meadow that had coalesced by 2007 generally declined from 2007 to 2010 despite sustained low turbidity. This potentially highlights the complex relationships between sediments and seagrass bed condition (Koch 2001), and indicates that further study on shoot dynamics is warranted as these beds develop over time.

### Restoration implications

Our restoration effort over the course of 11 yr used seeds rather than adult plants because of their availability in significant quantities annually (10s of millions per hectare), their ease of harvesting, and their adequate germination and establishment rates at our sites. Our seed distributions incorporated several different seed delivery techniques, seed densities, plot sizes, and plot configurations, an approach intended to allow optimization of future restoration efforts. Relative efficiencies of several seed collection and distribution techniques utilized here were discussed by Marion & Orth (2010a), and detailed photographic analyses of plot performance relative to size, year, location, and seeding density are underway and are outside the scope of this paper. However, in general we found that plot size and seeding density were much less consequential to the eventual success of the restoration effort than simply the time allowed for subsequent generations of seedlings to spread from our initial distributions. Plots seeded at low densities (12 seeds  $\text{m}^{-2}$ ) remained very sparse for several years, but the eventual consolidation of plots occurred regardless of initial density. In addition, the sizes of our plots, though comparatively large as restoration plots, were much smaller than the distance across which seeds were exported. Therefore, for restoration at the scale of these coastal bays over a decadal time frame, the most important design considerations seem to be how widely the earliest plots are distributed across the landscape (determining how much total area will be within reach of subsequently exported seeds), and the number of seeds distributed (determining how many plants will initiate reproductive spread).

### CONCLUSIONS

The successful re-establishment of *Zostera marina* in these 4 unvegetated coastal bays following the introduction of a large number of seeds suggests that propagule limitation may have been the ultimate factor that prevented *Z. marina* recovery following

its disappearance in the 1930s, rather than a state change characterized by altered water quality conditions limiting re-growth (Peterson & Lipcius 2003). While we do not have water quality data following the disappearance of *Z. marina*, anecdotal accounts during the period following the decline make no reference to possible changes in water quality. Given that water quality has shown no trends over the last 2 decades (see [www1.vcrllter.virginia.edu/home1/?q=data\\_wq](http://www1.vcrllter.virginia.edu/home1/?q=data_wq)) in one of the coastal bay systems (Hog Island Bay), we suggest that water quality was likely not an impediment to recovery. While these bays are within the potential colonization distance of 2 source populations (Harwell & Orth 2002a), the geography and isolation of these bays may have made seed delivery events rare. Currents along this coast flow from the north (Epifanio & Garvine 2001), and Chincoteague Bay, the nearest northern source 75 km away, supported very little *Z. marina* until the 1990s (Orth et al. 2006b). Recent genetic evidence from small natural patches of *Z. marina* observed in Hog Island Bay in 2004 linked these plants to Chincoteague Bay (Reynolds et al. 2012, this Theme Section, L. K. Reynolds unpubl. data). Given these genetic data, we believe the original patches we observed in South Bay in 1997 were also derived from Chincoteague Bay stock.

To illustrate the mechanism by which our seed additions facilitated the process of *Zostera marina* recovery, especially in South Bay, we present a simple synthetic model of the relationship we hypothesize between regional *Z. marina* abundance and seedling recruitment rate for an initially empty bay with suitable conditions for growth and reproduction (Fig. 10). A critical feature of the model is the rapid rise in seedling recruitment rate as the local population grows; this exponential increase results from subsequent generations of seedlings in turn flowering and releasing their own seeds. The effect of our seed additions was to mimic the seed production of a large local population, but at a stage of development with ample open space for seedlings to develop without competition from nearby adults. Placement of restoration plots across the extent of each targeted region helped accelerate the development of broad-scale continuous coverage, compared with a strategy of concentrating recruitment in a core region that would later expand outward.

The 2 ends of the curve represent areas where additional research would be fruitful. The initial period of slow increase in recruitment rate is the result of limited seed production from early colonists and the time required for subsequent generations of

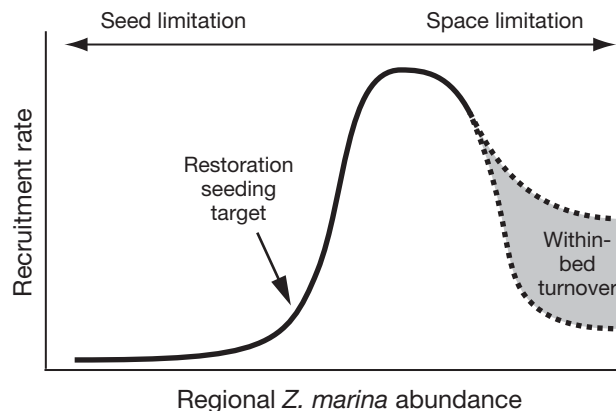


Fig. 10. *Zostera marina*. Hypothesized relationship of seedling recruitment rate to regional abundance

seedlings to spread across the region. However, an important question is whether additional factors may act to maintain low recruitment during this period. Such factors might include high seed predation on small numbers of seeds, disturbance of plants by foraging crabs or rays, macroalgal attachment causing the uprooting of isolated seedlings, and low pollination success for sparse, isolated plants. Our high-density seed additions may have provided a critical number of seedlings to escape the constraints that might otherwise limit or kill natural colonists. The decrease in recruitment at the right end of the curve reflects limited seedling establishment within a high-density bed of relatively continuous coverage. The actual scale of this reduction is poorly understood because seedling success rates are most easily documented in unvegetated sand, while their contribution to the maintenance of existing beds is not well understood (Olesen 1999), except in the case of recovery of gaps after a disturbance (Plus et al. 2003, Greve et al. 2005, Jarvis & Moore 2010).

The recovery of *Zostera marina* initiated in this coastal bay system may be unique in seagrass recovery studies because of how the recovery was initiated (seeds rather than adult plants), how rapidly it occurred (years rather than decades), and the explicit demonstration of how one meadow modulated water clarity as it developed and expanded. The success we observed here was enabled by species traits (high seed yield, regional seed dispersal, adequate germination, and rapid vegetative growth) and site traits (semi-enclosed bays with suitable depth, bottom type, and water quality) that may be found in only certain species and regions. For example, *Z. marina* in the northeast United States may recover at a slower rate because of slower patch recruitment rates and slower vegetative growth (Neckles et al. 2005).

Regardless of these potentially unique conditions our results offer a new perspective on the role seeds can play in recovery dynamics at large scales and may explain, in part, the rapid rate of recovery of *Z. marina* following its 1930s demise (Cottam & Munro 1954) in areas that retained a regional source of seeds.

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# Seedling establishment in eelgrass: seed burial effects on winter losses of developing seedlings

Scott R. Marion\*, Robert J. Orth

Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, Virginia 23062, USA

**ABSTRACT:** Constraints on the transition of seeds to seedlings have the potential to control plant dispersal and persistence. We investigated the processes leading to low initial seedling establishment in eelgrass *Zostera marina* through a manipulative field experiment assessing the relative importance of germination failure and seedling loss during the winter. Seed plots were established in October at 3 unvegetated sites in the Chesapeake Bay (USA) region, with seeds either at the sediment surface or buried at 2 to 3 cm. Emerging seedlings were monitored at 6 wk intervals between December and April using a video camera, and seed germination was tracked in separate destructively-sampled plots. Sediment height change was measured, and sediment disturbance depth was estimated by deploying cores layered with tracer particles and examining tracer loss upon core retrieval. We found a low rate of seedling establishment 6 mo after seeding (1.2, 3.8, and 2.8 % for surface seeds at the 3 sites) that was largely due to seed and seedling loss rather than to germination failure, with 90 % of seeds retrieved after December having germinated. Seed burial significantly enhanced seedling establishment at 2 of 3 sites (40.4, 16.8, and 10.3 % establishment for buried seeds). Seed loss occurred mostly within the first month of the experiment, and was most severe for seeds at the sediment surface. Indicator core results showed widespread disturbance of sediments to depths that could have dislodged early seedlings developing from surface seeds, and to a lesser degree seedlings from buried seeds. Our findings help identify the nature and timing of a substantial *Z. marina* seedling establishment bottleneck in our region, and show that some of the key processes pivotal to *Z. marina* recruitment dynamics and optimal restoration strategies involve physical sediment–seedling interactions rather than seed germination.

**KEY WORDS:** Seagrass · Recruitment limitation · SAV restoration · Sediment disturbance · Wave energy · Germination · Seed burial

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## INTRODUCTION

The transition of seeds to successfully established seedlings is a complex ecological process that has the potential to control plant dispersal and persistence (Harper 1977). For prolific seed-producing plants with high dispersal potential such as the seagrass *Zostera marina* (eelgrass), this transition can be a crucial but poorly understood stage. For many plants, predation, pathogens, and inadequate nutrient reserves contribute to high rates of seed loss, with few seeds surviving to the seedling stage (Clark et al. 2007). Once seeds germinate, early-stage seedlings

are subject to additional abiotic stresses such as extremes in temperature, moisture, or nutrients. In aqueous environments, waves and currents can erode sediments and dislodge emerging seedlings or smother seedlings under sediment deposits (Koch et al. 2006, 2010), and sediment characteristics can also limit developing rhizomes (Handley & Davy 2002). Identifying the factors limiting the seed to seedling transition is critical to understanding plant demographic processes and developing seed-based restoration techniques.

Seagrasses, marine angiosperms, are unique among the flowering plants because they live entirely sub-

\*Email: smarion@vims.edu

mersed, pollination is hydrophilous, and there are no obvious pollinators (Ackerman 2006). Seagrasses are also threatened around the world because of their susceptibility to anthropogenic changes in water quality, with reported declines rivaling marshes and mangroves (Waycott et al. 2009). As conservation efforts increasingly direct attention toward achieving adequate water quality for healthy seagrass, more attention is focusing on restoring seagrass (Paling et al. 2009). With interest in restoration shifting toward use of seeds in appropriate areas, a fundamental knowledge of the processes influencing seagrass seed survival and seedling establishment is essential.

Eelgrass *Zostera marina* is widely distributed in the North Atlantic and Pacific Oceans (Green & Short 2003). Flowering and seed production are related to increasing springtime water temperatures (Setchell 1929, Silberhorn et al. 1983), and seeds are released either directly from the plant to the sediment surface or can be dispersed meters to kilometers from the parent plant on floating air bubbles or on detached flowering shoots (Churchill et al. 1985, Harwell & Orth 2002). Most seeds do not move far from where they initially settle (Orth et al. 1994), but beyond that fact, the processes controlling subsequent seedling establishment are relatively poorly understood.

In the US mid-Atlantic region, *Zostera marina* seeds have been used to study dispersal patterns and processes (Orth et al. 1994, Luckenbach & Orth 1999, Harwell & Orth 2001) and have been used to initiate recovery of *Z. marina* in coastal lagoons (Orth et al. 2012, this Theme Section [TS]) that were devoid of *Z. marina* since the 1930s 'wasting disease' pandemic. These studies identified a consistently low rate of seedling establishment, finding that generally less than 10% of seeds deployed in September or October transitioned to seedlings surviving 6 mo (Orth et al. 2003, Marion & Orth 2010a). In contrast, laboratory experiments have demonstrated a much higher potential recruitment, with 80 to 95% of seeds typically germinating (R. Orth unpubl. data). Very few field observations of seedlings have been made between germination in November or December, and April, when rapid seedling growth in the warming waters allows easier observation than is possible for small seedlings (less than 5 cm) during the cold of winter. Churchill (1983) reported on a progressive loss of *Z. marina* seedlings in containers under field conditions from the time of germination in the fall to late spring but did not document the processes leading to their loss. Moore et al. (1993) documented high wintertime germination of most *Z. marina* seeds contained in above-ground enclosures, but no direct observations

of germination rates (as opposed to seedling establishment rates) for unprotected seeds in sediment under field conditions are available. The proportion of seeds that might remain in sediments without germinating (germination failure) was unknown.

We hypothesized that sediment disturbance at sandy sites with low sediment cohesion could be an important mechanism of loss of early-stage seedlings, and sought to document sediment dynamics (accumulation/erosion events and depth of disturbance) associated with seedling development at multiple field sites. The work presented here addressed the processes leading to low seedling establishment in unvegetated sediments through a field manipulative experiment during the winter, a period largely ignored in *Zostera marina* ecology. Specifically, we addressed the following 4 questions: (1) Does germination failure limit initial seedling establishment? (2) What proportion of developing seedlings is lost during the winter? (3) Is seedling loss associated with measurable sediment re-suspension or net changes in sediment height? (4) Does seed burial reduce the subsequent loss of developing seedlings?

## MATERIALS AND METHODS

### Study sites

The study was conducted at 3 sites in the US mid-Atlantic region that had a history of *Zostera marina* presence. Two were within the lower Chesapeake Bay (Piankatank River, 37.503°N, 76.329°W and Mumfort Island in the York River, 37.268°N, 76.515°W) and a third was located in Spider Crab Bay (37.358°N, 75.800°W), one of the coastal bays that are part of the Virginia Coast Reserve Long-Term Ecological Research site. The 2 Chesapeake Bay sites have been devoid of *Z. marina* since a bay-wide dieback after Hurricane Agnes in 1972 (Orth & Moore 1983). The coastal bay site lost *Z. marina* in the early 1930s in a North-Atlantic-wide pandemic wasting disease outbreak (Rasmussen 1977), but is now slowly recovering as a result of a large-scale re-seeding project (Orth et al. 2012). All 3 sites have been used in previous seed experiments (Orth et al. 2003, Marion & Orth 2010a), with Spider Crab Bay generally showing higher seedling establishment rates than the 2 Chesapeake Bay sites. All sites were between 0.5 and 0.9 m water depth (mean low water, MLW), with the Spider Crab site the deepest at 0.9 m. Tidal range was approximately 0.4 m at the Piankatank River

site, 0.7 m at the Mumfort Island site, and 1.2 m at the Spider Crab Bay site. Maximum near-bottom currents at the Mumfort Island site are approximately  $20 \text{ cm s}^{-1}$  (Orth et al. 1994), and spring-tide currents at the Spider Crab Bay site have been measured at similar velocities (S. Marion unpubl. data). Currents at the Piankatank River site were unmeasured, but diver observations over multiple years suggest substantially lower maximum currents than at the other 2 sites.

### Germination failure for surface and buried seeds

To assess the contribution of seed germination failure to low seedling establishment rates, we established 32 seed plots at each site on 4, 22, and 25 October 2007 at the Mumfort Island, Piankatank River, and Spider Crab Bay sites, respectively. The 20 cm diameter circular plots ( $0.03 \text{ m}^2$ ) were separated by 10 m and marked by PVC stakes to allow precise relocation and seed retrieval. Stakes were offset by 0.5 m, and protruded only 5 cm from the sediment. At each site, 16 surface-seed plots were established by placing 50 seeds on the sediment surface and gently pressing them into the sediment with a 1 mm sieve, covering them with approximately 1 to 2 mm of sand. This technique was intended to prevent immediate horizontal transport of the seeds by currents during the following tidal cycle. Sixteen additional plots were created with 50 seeds buried at a target depth of 2 to 3 cm by injecting seeds into the sand using a handheld plastic pipette with the opening cut to approximately 3 mm diameter. Groups of 10 to 15 seeds were drawn into the pipette, then slowly injected into the sediment with the pipette tip inserted to a calibrated depth at an angle of  $30^\circ$ , while the pipette was pulled at approximately  $2 \text{ cm s}^{-1}$  through the sediment maintaining the target planting depth. This process was repeated until all 50 seeds were planted. Preliminary trials during the development of this technique showed reliable planting in the target 2 to 3 cm depth range. All seeds used were individually assessed as viable seeds exhibiting an intact seed coat, high density, and rapid fall velocity in seawater (Marion & Orth 2010b). Plots were placed in 2 rows along a  $210 \text{ m} \times 10 \text{ m}$  constant-depth region parallel to the shoreline at each site, and were divided into 4 blocks for sampling. We interspersed surface-seed and buried-seed treatments along each row to ensure equal exposure to any undetected along-shore gradients in energetic regime or sediment conditions.

At 4 approximately 45 d intervals after planting, corresponding to mid-day low tide periods, 1 surface-seed plot and 1 buried-seed plot were destructively sampled from each block. Sampling dates were 4, 10, and 11 December 2007; 18, 22, and 23 January 2008; 3, 4, and 6 March 2008; and 11, 16, and 18 April 2008. Water temperatures during the 4 sampling periods were  $8\text{--}10^\circ\text{C}$ ,  $3\text{--}5^\circ\text{C}$ ,  $7\text{--}10^\circ\text{C}$ , and  $14\text{--}15^\circ\text{C}$ , respectively. Sediments were removed to a depth of 15 cm in a square  $0.9 \text{ m} \times 0.9 \text{ m}$  area incorporating the original  $0.03 \text{ m}^2$  plot, and were sieved through a 1 mm sieve to retrieve all seeds. We used a 30 cm wide square-sided scoop to remove sediment, and separately processed sediment from the central  $0.09 \text{ m}^2$  area (a single, central scoop) in order to detect whether any seeds had moved from the original plot into the adjacent area. We chose to use a scoop slightly larger than the original plot area to allow for any imprecision in relocating or sampling the central area. Divers also surveyed a 1 m wide area around each plot for any seedlings. Recovered seeds were counted and classified as germinated (split seed coat and obvious hypocotyl extension) or ungerminated. Two-factor analyses of variance (ANOVAs) were used to assess the effects of month and depth on seed recovery for each site separately. Following results indicating a possible month  $\times$  depth interaction ( $p < 0.15$ ), separate 1-way ANOVAs were run assessing the effect of month at each depth.

### Seedling loss for surface and buried seeds

To quantify loss rates through the winter for developing seedlings, we created 12 plots at each site that were repeatedly, non-destructively sampled at the same intervals described above. Each  $0.25 \text{ m}^2$  plot received 200 seeds, either at the surface (6 plots) or buried (6 plots) as described above. Plots were placed in a single row parallel to the germination plot rows, with surface and buried plots alternating along the row to ensure that the 2 treatments had similar exposure to any undetected gradients in wave energy or sediment dynamics. Fixed PVC stakes protruding less than 5 cm from the sediment surface, offset from the corners of the seed plots by 10 cm, allowed precise repositioning of a quadrat with  $25 \text{ cm}^2$  cells. A video camera was moved systematically over the grid, recording the position of all seedlings. In the lab, the tape was reviewed to produce a map of seedlings. The use of video reduced the inaccuracies related to direct recording of data by divers struggling with cold water tempera-

tures, and allowed detailed matching of individual seedling positions between sampling periods by reference to the previous video tape if necessary. By April, many seedlings had developed multiple shoots, making it impossible to distinguish individual seedlings in the video footage. Therefore, we gently removed the sediment above the rhizome layer and counted seedlings directly in the field. Divers also surveyed a 1 m wide area around each plot for any seedlings.

Repeated-measures ANOVAs were used to assess the effects of month and depth on seedling abundance for each site separately. Data for December were omitted from analyses, as seedlings had not yet emerged from most plots.

### **Sediment disturbance depth**

Sediment disturbance depth was estimated by deploying tracer particles at known depths concurrently with seed distributions, and later examining the depth distribution of recovered tracer particles. Layered cores containing tracer particles at 1 cm intervals ('indicator cores') were created in an 8 cm diameter, 15 cm tall plastic tube using sediments collected from each experimental site. Tracer particles were flat plastic sequins with unique shapes at each discrete depth layer (e.g. circles at 1 cm, squares at 2 cm). The tracer particles were intended to wash away if sediments were momentarily re-suspended by storm waves, thereby leaving a record of scouring depth that could not be obtained by periodic sediment surface height measurements. Twelve cores were divided among 3 blocks located between the 4 blocks of germination plots described above, and were precisely located by offset markers to allow accurate re-sampling. Cores within blocks were separated by 10 m, and blocks were separated by 50 m, the width of 1 block of germination plots. Indicator cores were installed by first extracting a sediment core of the same depth from inside a slightly larger concentric tube, preventing the collapse of sediment into the evacuated space. The indicator core, with its bottom surface held in the core tube by a layer of weak tape, was installed with its surface sediments level with the surrounding bottom. The outermost tube was removed, then the indicator core sediments were held in place by an 8 cm diameter plunger while the core's tube was withdrawn, breaking the tape seal at the bottom and leaving the tracer particle layers buried at 1 cm intervals below the surface. In addition to the

indicator cores, 3 poles with inscribed height marks were deployed to assess net deposition or erosion. The poles were driven as far as possible into the substrate to ensure that they remained stationary, and were located within the 3 blocks of indicator cores.

On each sampling date, 1 indicator core was removed from each block. The original core tube was inserted into the sediment, the top of the tube sealed with a rubber stopper, and the core removed and returned to the lab for analysis. A plunger was used to slowly extrude the core in 0.5 cm intervals, and tracer particles in each interval were identified and counted. The depth of each tracer particle layer from the sediment surface in the recovered core was used to calculate 2 metrics of sediment change, since the depth of each layer at installation was known. The difference between the known installation depth and the recovery depth of intact tracer layers (i.e. those with abundant particles maintaining well-defined horizontal bands) was interpreted in 0.5 cm intervals as the net change in sediment height. For example, square tracer particles were deployed in the 2.0 cm deep layer, so if a layer of square particles was recovered in the 3.0–3.5 cm deep layer, we scored the net change in sediment height as +1.0 cm; this reflects our choice to score 0 cm change for tracer layers found in the 0.5 cm interval below their depth at installation, rather than the 0.5 cm interval above, to compensate for any slight compaction of cores that might have occurred after installation. All undisturbed layers from 1 core reflected the same net change in sediment height, so multiple layers were used in scoring. The second metric, disturbance depth, was defined as the installation depth of the deepest tracer layer from which almost all deployed particles were missing at retrieval. For the previous example, if the layer of circular particles installed at 1.0 cm was missing, disturbance depth would be 1.0 cm. This example illustrates the ability of our indicator core method to detect multiple events that first scour sediments deeply and later leave accumulated sediments that might otherwise be interpreted as only sediment deposition. Our estimate of disturbance depth is conservative, as disturbance could have extended almost to the next lower tracer particle layer.

Net change in sediment surface height was also measured against the permanent height reference poles on each sampling date. To avoid effects of erosion at the base of each pole, a 1 m bar placed on the sediment surface was used to estimate the integrated local surface height.

## RESULTS

### Seed germination test plots

Seeds retrieved from independent germination test plots at monthly intervals showed little evidence of germination failure for either surface seeds or buried seeds (Fig. 1). Of all seeds retrieved in the January, March, and April samplings, 90% had germinated

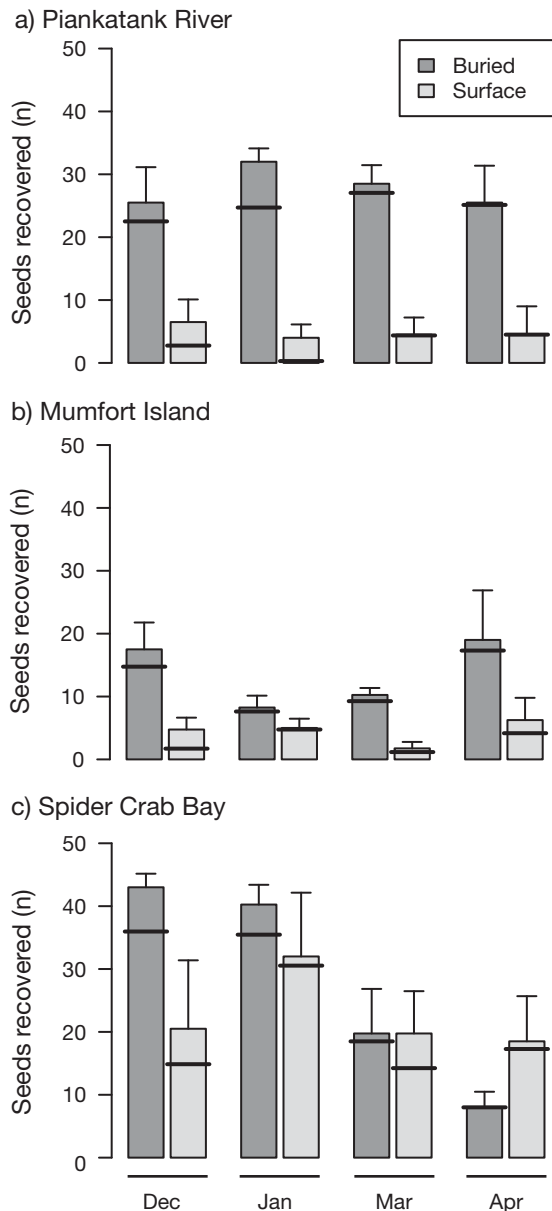


Fig. 1. *Zostera marina*. Mean number of seeds (+SE) recovered out of 50 deployed in 0.03 m<sup>2</sup> germination test plots (n = 4). Buried seeds were injected to a depth of 2 to 3 cm, while surface seeds were pressed 1 to 2 mm into the sediment surface by a 1 mm sieve. Horizontal lines through bars indicate the mean number of recovered seeds that had germinated

(December was excluded because germination was likely not complete by that point), and the proportion was similar for both buried and surface seeds. However, most surface seeds were lost from plots before retrieval (90, 91, and 55% of all deployed seeds at the Piankatank River, Mumfort Island, and Spider Crab Bay sites, respectively). Significantly fewer buried seeds (44%) were lost at the Piankatank River (2-way ANOVA,  $df = 1, 24$ ;  $p < 0.0001$ ) and at Mumfort Island (73%;  $df = 1, 24$ ;  $p < 0.001$ ). At Spider Crab Bay, 45% of buried seeds were lost, not significantly fewer than for surface seeds ( $p = 0.31$ ). Most seeds were lost between plot establishment in October and the first sampling in December, and there were no significant effects of month on seed loss except for buried seeds at Spider Crab Bay (1-way ANOVA,  $df = 3, 12$ ;  $p < 0.001$ ), where the seed germination plots may have been scoured by filamentous algae accumulating on seedlings or on nearby PVC stakes in March (see description in 'Video-monitored seedling plots' below). Variation in seed loss among the 4 replicate plots was high, especially at Spider Crab Bay, with individual plots showing excellent seed retention and others complete loss. Over 90% of retrieved seeds were found within the central 0.09 m<sup>2</sup> scoop at all 3 sites, with the remainder found in the adjacent peripheral scoops.

### Video-monitored seedling plots

In plots with seeds deposited at the sediment surface, monthly video monitoring through the winter showed moderate initial seedling abundance (approximately 15% of seeds deposited) followed by substantial seedling loss at the Piankatank River and Spider Crab Bay sites (Fig. 2a,c). At the Mumfort Island site, few seedlings (approximately 2% of seeds deposited) were observed in most surface-seeded plots, except for 1 plot positioned in a region of sediment accretion (see sediment analysis in 'Sediment disturbance depth' below) that showed 19% seedling emergence (Fig. 2b). Very few seedlings were observed in the surveyed region surrounding each video plot. Repeated-measures ANOVAs showed significantly greater seedling abundance in plots with seeds buried at 2 to 3 cm than in surface-seed plots at the Piankatank River ( $df = 1, 10$ ;  $p < 0.0001$ ) and Mumfort Island sites ( $df = 1, 10$ ;  $p = 0.016$ ). No significant effects of month or interactions were detected at those sites. At Spider Crab Bay, the effect of seed depth was non-significant ( $df = 1, 10$ ;  $p = 0.095$ ), but seedling abundance declined significantly over time for both depths ( $df =$

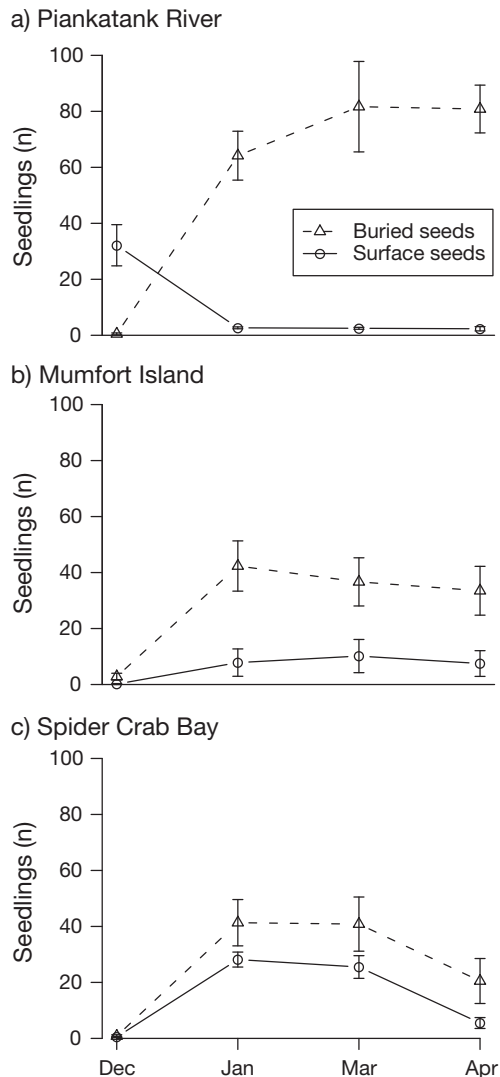


Fig. 2. *Zostera marina*. Mean number of seedlings ( $\pm$  SE) observed in repeated observations of 0.25 m<sup>2</sup> video-monitored plots (n = 6) receiving 200 seeds each. Buried seeds were injected to a depth of 2 to 3 cm, while surface seeds were pressed 1 to 2 mm into the sediment surface by a 1 mm sieve

2, 20;  $p < 0.0001$ ). Seedling loss after the peak month was less severe for buried seeds (1, 21, and 50% of mean peak seedling abundance lost at the Piankatank River, Mumfort Island, and Spider Crab Bay sites, respectively) than for surface seeds (93, 26, and 80%). Overall 6 mo seedling establishment rates at the same sites were 1.2, 3.8, and 2.8% for surface seeds, and 40.4, 16.8, and 10.3% for buried seeds.

Spider Crab Bay was influenced in March and April by an overgrowth of the filamentous brown alga *Ectocarpus*, which coated much of the bottom and most of the emerging seedlings. In April, many algal-covered seedlings had exposed rhizomes and were retained in the sediment only by root hairs.

Divers observed algal-covered, barely-attached seedlings being nearly dislodged by wave motion. Masses of macroalgae had also accumulated on nearby PVC stakes used to delineate plots, resulting in some scouring of the bottom and loss of seedlings. Macroalgae were not observed at the other sites.

### Sediment disturbance depth

Indicator core results showed widespread disturbance of sediments to depths that could have dislodged early seedlings developing from surface seeds, and to a lesser degree seedlings from buried seeds (Fig. 3). Substantial variability in disturbance depth and net sediment height change was observed within sites and across sampling periods. In many cases, disturbance of the 1 cm deep tracer layer was evident despite net sediment accretion during the time period. Half of all Mumfort Island and Piankatank River cores showed sediment disturbance to at least 1 cm (Fig. 3b), and the depth of disturbance was deeper than net sediment loss in all cores (Fig. 3a). Different cores at the Mumfort Island and Piankatank River sites showed deposition and erosion within the same time period, suggesting sediment movement within sites rather than deposition or erosion across a whole site during a given time period. At Spider Crab Bay, no cores recorded any net sediment loss or sediment disturbance. Where net sediment accumulation was documented, it ranged from 0.5 to 3 cm.

Net sediment height change measured against permanent reference poles showed high variability within sites (Fig. 4) and was not well correlated with height change recorded in nearby indicator cores (Fig. 3a). Of 33 possible pairs of core versus pole observations within the same block for the same time period (3 cores did not provide reliable sediment height change data), 20 differed by less than 2 cm, but 5 differed by 5 cm or more. These pairs were due to individual poles at Mumfort Island and Piankatank River with substantial sediment accumulation, and 2 poles at Spider Crab Bay with sediment loss that was not reflected in nearby indicator cores.

### DISCUSSION

Our experiments tracking germination, emergence, and loss of *Zostera marina* seedlings through the winter months revealed that seed loss and post-germination seedling loss, rather than germination



failure, were responsible for a low rate of seedling establishment observed 6 mo after seeding. Seed loss generally occurred within the first month of the experiment, and was most severe for seeds at the sediment surface. Seed burial did not have a detectable effect on germination rates, as germination rates were similarly high for seeds at the surface and for buried seeds, but burial clearly enhanced the subsequent survival of emerging seedlings. Our findings help clarify the mechanism and general timing of a substantial *Z. marina* seedling establishment bottleneck for seeds that escape summertime seed predation in our region, demonstrating that the processes pivotal to *Z. marina* recruitment dynamics in unvegetated sediments involve physical sediment–seedling interactions rather than germination failure. They

also demonstrate the potential utility of seed burial for maximizing seedling yield from limited seed supplies in *Z. marina* restoration efforts.

### Role of seed burial

Seed burial has generally been viewed as important mostly for minimizing seed predation in terrestrial and submersed angiosperms (Fishman & Orth 1996, Hulme & Borelli 1999, Christian & Stanton 2004). In the Chesapeake Bay region, seeds distributed in October have shown higher establishment rates than seeds present in the summer (Marion & Orth 2010a) when seed predators are abundant and active (Fishman & Orth 1996), and seed burial

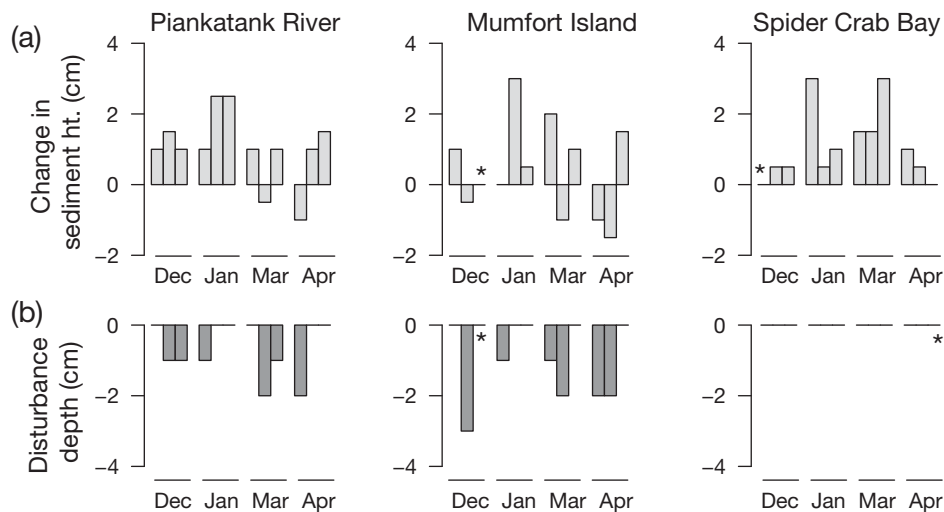


Fig. 3. Sediment changes recorded in cores with tracer particles layered at 1 cm intervals. Each bar shows 1 core, with cores from the left, center, and right sections of the experimental region shown in that order within each month. Asterisks indicate no data for an individual core. (a) Net sediment height change between core establishment in October and the indicated month. (b) Depth of sediment disturbance inferred from missing tracer particles in the same cores as in (a)

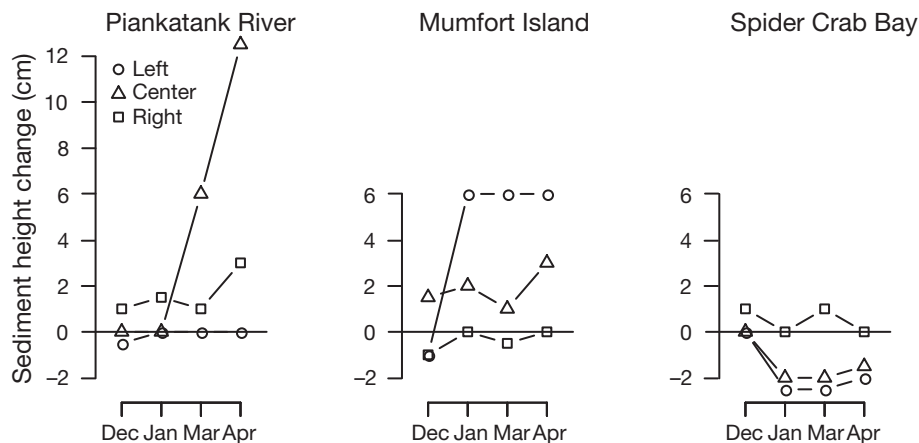


Fig. 4. Sediment height changes recorded against permanent poles deployed in the left, center, and right sections of the experimental region

Table 1. Sediment grain size distribution and organic content at the experimental sites in the Chesapeake Bay area (USA). Mean (SD), n = 3

Site	% Sand	% Silt+clay	% Organic content
Mumfort Island	96.6 (0.6)	3.4 (0.6)	0.62 (0.00)
Piankatank River	98.6 (0.5)	1.4 (0.5)	0.73 (0.07)
Spider Crab Bay	86.2 (4.6)	13.8 (4.1)	1.41 (0.36)

increased success for seeds present in summer (Marion & Orth 2010a). In addition to reducing the potential for seed predation, our results show that seed burial benefits *Zostera marina* by reducing seedling loss after germination. While seed predators may have removed some surface seeds in our plots, their activity was likely reduced during our winter study compared to previous work on seed predation conducted during the warmer months, and our finding of substantial survival for surface seeds even into January at the site with potentially the most seed predators, Spider Crab Bay (Fig. 1c), suggests limited influence of predation on our results. More importantly, the observed pattern of disturbance depths of tracer particles suggests that physical forces (waves and currents) were responsible for removal of shallow seeds and seedlings. These physical processes are potentially also important for seed dispersal and seedling retention in other submerged macrophytes (Koch et al. 2010).

Some previous data exist suggesting a direct seed burial effect on germination (Granger et al. 2000, Tanner & Parham 2010), and our results from seed-buried plots in December showing a high proportion of germinated seeds are consistent with a seed burial effect on germination (Fig. 1), although variability in the number of seeds recovered and the proportion germinated was high, especially for Spider Crab Bay. However, our results for surface seeds are also consistent with the findings of Moore et al. (1993) that seeds held in oxygenated conditions in the field germinated later than seeds in anoxic conditions, but eventually reached similar, high germination rates. We think that the initial sampling in December reflected delayed germination of viable surface seeds, and suggest that the subtle pattern of higher proportions of germinated seeds found in later months resulted from the eventual germination of most seeds, rather than progressive loss of ungerminated seeds over time.

Seedling establishment rates for buried seeds at the Mumfort Island and Piankatank River sites were substantially higher than for surface seeds, and were

the highest we have ever recorded for any seeds at these sites. Burial conferred relatively less of an advantage to seeds at the Spider Crab Bay site, where surface seeds performed much better than surface seeds at other sites. This is consistent with previous observations of higher broadcast seed success in the coastal bays and attenuated seed burial effects relative to Chesapeake Bay sites (Orth et al. 2003, Marion & Orth 2010a). The higher initial retention of seeds and seedlings at Spider Crab Bay is likely a result of that site's finer sediment grain size and higher organic content (Table 1), which may provide a more consolidated foundation for anchoring developing seedlings and reduce erosion around developing shoots (Titus & Hoover 1991). Spider Crab Bay was the only site with potential sources of extraneous seeds; these potential sources were 0.4 ha plots seeded 2 yr prior, with the closest plots either 480 m in the direction of tidal current flow, or 250 m perpendicular to the current. Our interspersed treatments would have been robust to any bias due to extraneous seed input, which we consider unlikely based on diver observations of minimal seedling abundance in the meter surrounding each plot. The few seedlings that were found outside plots were likely from seeds that moved out of the plots, not seeds arriving from external sources.

### Seed loss and germination

The germination status of the seeds lost from germination test plots is unknown. Missing surface seeds in the 0.03 m<sup>2</sup> germination test plots could have been uncovered and transported out of the 0.81 m<sup>2</sup> sampling area, or they could have germinated *in situ* and subsequently been scoured out by waves. Since 90% of the retrieved seeds were found within the central 0.09 m<sup>2</sup> sampling area, and divers examining the area surrounding the plots found very few seedlings, local seed redistribution does not seem to explain the patterns observed. Previous experiments have repeatedly found very few seedlings farther than 2 m from the point of seed deposition on the surface (Orth et al. 2003), and in the present experiment, the 'surface' seeds were covered by a few millimeters of sediment, reducing the likely importance of pre-germination seed redistribution.

Instead, we hypothesize that many surface seeds germinated and were later scoured out. Results from the Piankatank River video-monitored plots show earlier leaf emergence for surface seeds than for buried seeds, and provide a clear example of sub-

stantial, early shallow seedling loss. Earlier emergence is consistent with shallower seedling position in the sediment, and with exposure to a longer period of potentially erosive storm waves. Despite the early loss of many seeds, the data from recovered seeds show that relatively few seeds remain ungerminated *in situ* through the winter.

Although wave data at the sites are unavailable, we examined wind records from the nearest available weather station to assess storm events that might have differentially affected our sites during each sampling period (Table 2). We restricted data to winds blowing onshore for the Mumfort Island and Piankatank River sites, but used all data for the Spider Crab Bay site, which has no nearby protective shoreline. Differences in shoal width and fetch make direct comparisons among sites difficult, but the site with the greatest October to December seed retention in surface plots, Spider Crab Bay, had very high exposure to winds over 25 knots during that period. Comparison among sampling periods within sites shows the greatest duration and intensity of strong onshore winds at Mumfort Island in January to March, and in December to January at the Piankatank River. The early loss of surface seeds at these sites is therefore not parsimoniously explained by the timing of storm events; instead, we think the vulnerability of early-stage, shallow seedlings resulted in high losses in the first sampling period.

### Video-monitored seedling plots

The video technique used to follow seedling development and loss through the winter was effective at documenting net seedling loss rates. Our estimates of

seedling emergence are likely conservative because there may have been some undetected loss of seedlings offset by newly emerged seedlings between observation time points, especially early in the study. For seeds placed at the sediment surface, seedling establishment rates at 6 mo were generally consistent with our low historical expectations (overall mean of 2.6%), and also consistent with patterns of low establishment at the Mumfort Island and Piankatank River sites and higher establishment in the coastal bays (Marion & Orth 2010a). While surface seeds at Mumfort Island produced very few seedlings except in 1 anomalous plot, the low April seedling counts at the Piankatank and Spider Crab sites clearly reflect loss of previously germinated seedlings, rather than germination failure.

### Sediment disturbance depth

The indicator core technique used to estimate sediment disturbance depth and net sediment height change is a novel approach for documenting the physical processes expected to influence *Zostera marina* seeds and seedlings. Bioturbation can profoundly influence sediment biogeochemistry and particle movement, especially seeds (Dumbauld & Wyllie-Echeverria 2003, Meysman et al. 2006). However, we think that disturbance by bioturbation was substantially lower than would be expected during the warmer months, as we found very few organisms in recovered cores and saw little evidence of burrowers and other sediment disruption in and around experimental plots.

Sediment height change recorded in indicator cores is expected to be more reliable than that recorded against protruding stakes, because stakes can cause local deposition or provide attachment sites for macroalgae that can subsequently scour sediments. Any bias in the core-derived measure is expected to be positive (more apparent sediment accumulation) due to the potential for compaction of the layered core. There is some evidence for this process, as intact tracer particle layers recovered near the bottom of cores were in some cases separated by slightly less than 1 cm upon retrieval, suggesting compaction during transport. The mean sediment height changes measured by cores were accumulations of 0.5, 1.0, and 1.2 cm at the Mumfort Island, Piankatank River, and Spider Crab Bay sites, respectively. Any positive bias in sediment height affecting our disturbance depth inferences would result in conservative (shallow) estimates of disturbance depth,

Table 2. Number of hours with mean wind speed exceeding specified thresholds (20, 25, and 30 knots) for each sampling interval between *Zostera marina* seeding in October and final seedling assessment in April. Wind data from the nearest available NOAA CO-OPS stations (<http://tidesandcurrents.noaa.gov>) were used, and only onshore winds were included (160–310° for Mumfort Island, 310–140° for Piankatank River, all directions for Spider Crab Bay)

Interval	Mumfort Island			Piankatank River			Spider Crab Bay		
	>20	>25	>30	>20	>25	>30	>20	>25	>30
Oct–Dec	14	3	0	10	0	0	180	65	8
Dec–Jan	10	2	0	21	2	0	149	49	18
Jan–Mar	22	7	1	6	1	0	149	44	5
Mar–Apr	17	7	2	12	1	0	202	45	10

although the magnitude of any potential bias seems small relative to the 1 cm resolution of our tracer particle layers.

Our measurements of sediment disturbance, deposition, and erosion are generally consistent with literature values within and outside vegetated areas (Koch et al. 2006). These changes can be rapid, influencing decimeters of sediment in a few hours (Paling et al. 2003), and are capable of removing deeply rooted plants and causing large-scale landscape changes (Koch et al. 2006). Our data show that sediment re-suspension and erosion clearly have the potential to remove developing seedlings, especially at early stages before their rhizomes and root hairs are able to locally stabilize sediments (Titus & Hoover 1991). Variability in these processes was seen over spatial scales of tens of meters. Within experimental plots, we observed accretion of up to 3 cm and erosion to 1.5 cm. However, our permanent depth poles suggested that accretion could be as high as 12.5 cm over 6 mo, and observations of rhizome layer depth from previous experiments have also shown high accretion levels (R. Orth unpubl. data).

## CONCLUSIONS

This wintertime experiment showed that seed germination is not the primary factor limiting *Zostera marina* seedling establishment in unvegetated, sandy sediments in our region. Instead, we showed that germination rates are generally high, but many developing seedlings at or near the sediment surface are subsequently lost due to sediment disturbance, while seedlings developing from buried seeds have a higher retention rate. Winter conditions and seed burial depth may provide an important piece of the puzzle regarding the low seedling establishment rates in previous seed experiments. Investigating the factors that affect seed burial depth such as bioturbation (Dumbauld & Wyllie-Echeverria 2003, Meysman et al. 2006) and seed morphology (Benvenuti 2007), as well as the sedimentary characteristics of sites that enhance seedling retention, may be key to understanding the colonization potential of different seagrass species, as well as to the development of optimal restoration strategies.

Physical disturbance of developing seedlings may also be an important process influencing the natural dynamics of *Zostera marina* meadows due to the potential feedback of an existing seagrass canopy on seedling success. Seagrass canopies decrease wave energy reaching the sediment–water interface, and

consequently reduce sediment resuspension (Koch et al. 2006, Hansen & Reidenbach 2012, this TS). Higher initial success of seedlings might therefore be expected within established canopies, although experiments in *Ruppia maritima* canopies found no enhancement for surface seeds (Marion & Orth 2010b), and seedlings establishing within *Z. marina* canopies subsequently face light competition from adult plants (Olesen 1999). We suggest that *Z. marina* is more likely than *R. maritima* to reduce wave and current energy within the meadow, enhancing seedling establishment within sand gaps and creating a positive feedback cycle for meadow persistence. Conversely, the increased potential for seedling loss due to sediment disturbance in regions where the *Z. marina* canopy has been lost may create a negative feedback cycle, maintaining an unvegetated state by limiting recolonization after canopy loss. Further investigation of seedling success relative to existing canopies is necessary to understand the importance of these potential feedbacks for meadow persistence and recovery.

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# Recovery trajectories during state change from bare sediment to eelgrass dominance

Karen J. McGlathery<sup>1,\*</sup>, Laura K. Reynolds<sup>1</sup>, Luke W. Cole<sup>1</sup>, Robert J. Orth<sup>2</sup>,  
Scott R. Marion<sup>2</sup>, Arthur Schwarzschild<sup>1</sup>

<sup>1</sup>Department of Environmental Sciences, University of Virginia, PO Box 400123, Charlottesville, Virginia 22903, USA

<sup>2</sup>Virginia Institute of Marine Science, School of Marine Science, 1208 Greate Road, College of William and Mary, Gloucester Point, Virginia 23062, USA

**ABSTRACT:** Seagrasses are important foundation species in shallow coastal ecosystems that provide critical ecosystem services including stabilizing sediment, sequestering carbon and nutrients, and providing habitat and an energy source for a diverse fauna. We followed the recovery of functional (primary productivity, carbon and nitrogen sequestration, sediment deposition) and structural (shoot density, biomass, plant morphometrics) attributes of *Zostera marina* (eelgrass) meadows in replicate large plots (0.2 to 0.4 ha) restored by seeding in successive years, resulting in a chronosequence of sites from 0 (unvegetated) to 9 yr since seeding. Shoot density was the structural metric that changed most significantly, with an initial 4 yr lag, and a rapid, linear increase in plots 6 to 9 yr after seeding. Changes in *Z. marina* aerial productivity, sediment organic content, and exchangeable ammonium showed a similar trend with an initial 4 yr lag period before differences were observed from initial bare sediment conditions. After 9 yr, *Z. marina* meadows had 20× higher rates of areal productivity than 1 to 3 yr old meadows, double the organic matter and exchangeable ammonium concentrations, 3× more carbon and 4× more nitrogen, and had accumulated and retained finer particles than bare, unvegetated sediments. These results demonstrate the reinstatement of key ecosystem services with successful large-scale restoration, although none of the parameters reached an asymptote after 9 yr, indicating that at least a decade is required for these attributes to be fully restored, even in an area with high habitat suitability. Survivorship along a depth gradient showed that ~1.6 m (mean sea level) is the maximum depth limit for *Z. marina*, which matches the 'tipping point' for survival predicted for this system from a non-linear hydrodynamic/seagrass growth model.

**KEY WORDS:** Eelgrass · *Zostera marina* · Restoration · Seagrass · Coastal bays · Ecosystem services

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## INTRODUCTION

Seagrasses are recognized as important foundation species in shallow coastal ecosystems that both modify the physical habitat and modulate ecosystem processes. The complex structure of seagrasses compared to unvegetated sediments enhances biodiversity (Duffy 2006) and alters local hydrodynamics by attenuating wave energy and currents, which influences sediment suspension and deposition (Ward et al. 1984, Fonseca & Fisher 1986, Gacia et al. 2002,

Folkard 2005, Gruber & Kemp 2010, Hansen & Reidenbach 2012, this Theme Section). Seagrass habitats also sequester carbon (C) and nutrients, support higher trophic levels, and provide energy and biomass subsidies to other marine ecosystems (Duarte et al. 2005, 2010, Heck et al. 2008, Kennedy et al. 2010). Long-term trends in seagrass abundance and distribution indicate that the rate of habitat loss due to degraded water quality, disturbance, and disease is accelerating worldwide and is accompanied by a loss of the services that these ecosystems provide (Orth et

\*Email: kjm4k@virginia.edu

al. 2006a, Waycott et al. 2009, Short et al. 2011). Modeling of future climate scenarios suggests that seagrass habitats are also vulnerable to an increase in storm frequency and temperature, and that synergistic effects between water quality and climate change could further accelerate seagrass decline (Carr et al. 2010, Carr et al. 2012, this Theme Section). Small changes in environmental factors (light, temperature, nutrients, disturbance) may lead to a rapid and catastrophic loss of seagrass, and a shift to a bare, unvegetated state (van der Heide et al. 2007, Carr et al. 2010). Although there have been some successes in large-scale restoration efforts to mitigate these losses, these have not been enough to reverse the declining trends globally (Orth et al. 2006a).

Restoration efforts have the greatest probability of success if habitat suitability is high (i.e. the habitat has not been degraded or degradation has been reversed), and if donor material is from comparable conditions and has high genetic diversity (Short et al. 2002, van der Heide et al. 2007, van Katwijk et al. 2009, Fonseca 2011, Reynolds et al. 2012, this Theme Section). The most important habitat characteristics influencing restoration success are water column light attenuation, sediment characteristics (e.g. porewater redox, sulfide, organic content, grain size), and local hydrodynamics (Koch 2001, Short et al. 2002, van der Heide et al. 2007, van Katwijk et al. 2009). The existence of alternative states of clear water/seagrass conditions and turbid water/no seagrass in shallow ecosystems occurs primarily because of the positive feedback of seagrasses on dampening sediment suspension and improving water clarity (van der Heide et al. 2007, Carr et al. 2010). Recent studies have suggested that this feedback also should be incorporated into guidelines for restoration (van Katwijk et al. 2009).

Which metrics are best used to judge restoration success is a matter of some debate (Palmer et al. 1997, Fonseca et al. 1998, Short et al. 2002), but include structural and/or functional attributes of the system. Generally, structural aspects for seagrass ecosystems include shoot density, plant morphometrics, and biomass as proxies for habitat structure (Fonseca et al. 1996a, 1998, Evans & Short 2005, Leschen et al. 2010, Li et al. 2010). Functional aspects are typically measures of primary productivity, faunal abundance and community composition, and sediment trapping (Fonseca et al. 1996b, 1998, Evans & Short 2005, Leschen et al. 2010). Metrics in restored habitats are generally compared with natural, reference meadows, although the selection of suitable reference sites by which to judge appropriate restoration endpoints can be problematic, especially

in degraded habitats or in areas where seagrass loss is widespread. Assessment of functional trajectories describing the development of ecological functions over time can be used to assess when functional equivalency is reached in seagrass restoration projects (Craft et al. 2003, Evans & Short 2005, Bell et al. 2008). Our study is the first to monitor long-term (9 yr) trajectories of recovery in seagrass meadows following restoration by seeding.

The local extinction of *Zostera marina* in the Virginia, USA, coastal bay region in the early 1930s due to disease and storm disturbance is a striking example of the loss of ecosystem services with seagrass habitat loss (Orth et al. 2006b, Orth & McGlathery 2012, this Theme Section). The extirpation of *Z. marina* led to an immediate decline of commercially and recreationally important species, including bay scallops *Argopecten irradians* and brant *Branta bernicla* (Milne & Milne 1951, Orth et al. 2006b). Following the loss of the sediment-stabilizing service of *Z. marina*, it was debated whether the continued absence of *Z. marina* was the result of light or seed limitation. Recent studies have indicated that much of the seafloor in the coastal bays is suitable habitat for *Z. marina* recolonization based on light attenuation (Lawson et al. 2007) and that the lack of recovery was likely due to seed limitation (Orth et al. 2012, this Theme Section). The discovery of a small patch of *Z. marina* in the late 1990s spurred a large-scale effort to restore *Z. marina* that has resulted in approximately 1700 ha of *Z. marina* habitat from an original 125 ha seeded throughout the Virginia coastal bays as of 2012 (Orth et al. 2006b, 2012). Here we describe the recovery trajectories of both functional and structural attributes of *Z. marina* meadows restored by seeding in successive years, resulting in a chronosequence of sites from 0 (unvegetated) to 9 yr since seeding. We follow the terminology of Elliot et al. (2007) where 'restoration' refers to 're-creating habitat that was present within historical records.' We measured plant and sediment parameters that quantify the reinstatement of key ecosystem services, i.e. primary productivity, C and nutrient sequestration, and sediment deposition, with successful large-scale restoration initiated by seeding.

## MATERIALS AND METHODS

### Site description

The seed additions were done within the Virginia Coast Reserve Long Term Ecological Research (VCR



LTER) site on the eastern shore of Virginia. The coastal bay system is bound to the west by the Delmarva Peninsula and to the east by barrier islands; exchange with Atlantic Ocean waters is through narrow inlets between the islands. Water residence times are spatially variable, averaging 16 d, and ranging from 30 d near the mainland margin to 2 tidal cycles near the inlets (Fugate et al. 2006). The coastal bays are shallow, with 50% <1 m at mean low water (Oertel 2001), and have a tidal range of 1.2 to 1.3 m (Table 1). Watershed nitrogen (N) loading to the coastal bays averages  $2.1 \text{ g N m}^{-2} \text{ yr}^{-1}$  (Cole 2011) and is extremely low compared to similar shallow coastal bays in the US and other countries (McGlathery et al. 2007). As a result, water quality (based on dissolved nutrients, chlorophyll) is high and has shown no negative trends for the last 2 decades (VCR LTER data base, [www1.vcrlter.virginia.edu/home1/?q=data\\_wq](http://www1.vcrlter.virginia.edu/home1/?q=data_wq)). The high water quality and availability of long-term data on primary productivity and nutrient cycling (e.g. McGlathery et al. 2001, Tyler et al. 2001, 2003, Anderson et al. 2003, 2010) make this an ideal location to understand the consequences of state change from an algal- to a seagrass-dominated system. The seed plots were located at (37° 24' 47" N, 75° 43' 36" W, 'Hog Island Bay') and (37° 15' 54" N, 75° 48' 50" W, 'South Bay') within the coastal bay system and were separated by mudflats and a tidal

channel. The sites are comparable with respect to bathymetry and water depth, sediment and water column characteristics in unvegetated regions (representing initial conditions), and current speeds (Table 1). Both have light penetration capable of supporting *Zostera marina* production (Lawson et al. 2007, Orth et al. 2012).

### Experimental design

Plots were seeded in 2001, 2006, 2007, and 2008, and *Zostera marina* and sediment parameters (see below) were measured annually mid-summer in 2007 to 2010, which resulted in a gradient in *Z. marina* colonization from 1 to 9 yr. Meadows seeded in South Bay in 2001 were sampled 6 to 9 yr after seeding; meadows seeded in Hog Island Bay in 2006 to 2008 were sampled 1 to 4 yr after seeding. Vegetated sites were compared with nearby 'bare' unvegetated plots ( $n = 12$ , 6 at each site), which represented the initial condition (0 yr time point; Table 1). Seeds were harvested by hand from stable *Z. marina* populations in nearby Chesapeake Bay and subsequently from the expanding meadow in South Bay in the VCR; full details of the collection and seeding methodology can be found in Orth et al. (2012). Briefly, reproductive shoots with mature seeds were collected in late spring and were held in flowing outdoor seawater tanks; released seeds were separated from detritus and were kept in seawater until they were distributed by hand broadcasting in the fall just prior to the normal period of seed germination in this region (Moore et al. 1993). In total, 4.4 million seeds were added to the plots included in the present study, and 37.9 million seeds throughout all of the coastal bays (Orth et al. 2012). Establishment rates for seeds broadcast in the fall and assessed as seedlings the following spring in these coastal bays were 2 to 7% (Orth et al. 2012).

Seeds were broadcast in 2001 and 2008 into 0.4 ha plots at densities of 100 000 seeds  $0.4 \text{ ha}^{-1}$ ; we followed 6 plots seeded in 2001 and 6 plots seeded in 2008. The plots seeded in 2006 to 2008 were located along a depth gradient from 0.9 to 1.6 m at mean sea level (MSL). In 2006 and 2007, seeds were broadcast in a  $2 \times 2$  factorial design to compare plot size (0.2 and 0.4 ha) and seed density (50 000 and

Table 1. Comparison of hydrodynamic, sediment, and water column characteristics of eelgrass restoration sites in Hog Island Bay (HIB) and South Bay (SB) in the Virginia coastal bay system, USA. MSL: mean sea level

	HIB	SB
Depth (MSL, m)	0.9–1.6	0.9–1.6
Water column <sup>a</sup>		
Median annual turbidity (NTU)	5.2–11.4	7.8–16.9
Median annual chlorophyll ( $\mu\text{g l}^{-1}$ )	4.8–7.0	3.8–6.9
Hydrodynamics <sup>b</sup>		
Tidal range (m)	1.24	1.32
Current		
Tidally-averaged mean ( $\text{cm s}^{-1}$ )	1.3–3.5	3.5–4.4
Maximum ( $\text{cm s}^{-1}$ )	7.1–18.0	13.2–18.0
Sediment		
% organic matter	$1.68 \pm 0.23$	$1.17 \pm 0.19$
% N	$0.01 \pm 0.005$	$0.008 \pm 0.001$
% C	$0.35 \pm 0.06$	$0.33 \pm 0.05$
% sand (63–250 $\mu\text{m}$ )	$80.19 \pm 4.39$	$84.36 \pm 5.47$
% silt/clay (<63 $\mu\text{m}$ )	$19.81 \pm 4.29$	$15.64 \pm 5.47$
Exchangeable $\text{NH}_4^+$ ( $\mu\text{mol N g}^{-1}$ dry wt of sediment)	$0.0239 \pm 0.003$	$0.0236 \pm 0.002$
<sup>a</sup> Data from Orth et al. (2012)		
<sup>b</sup> Data calculated from coastal ocean circulation model, FVCOM (Chen et al. 2006), run for 5 tidal cycles to reach steady-state conditions		

100 000 seeds  $0.4 \text{ ha}^{-1}$ ), with 7 plots treatment<sup>-1</sup> in 2006 and 6 plots treatment<sup>-1</sup> in 2007. The uneven plot replication between years was due to variation in the number of seeds available in a given year. Analyses of sediment and plant parameters showed no significant effects of plot size or seed density in either 2006 or 2007, and so all plots were pooled for each of these years for comparisons of the different aged plots. Here we refer to 'age' as time since seeding. This resulted in sample sizes from 6 to 28 plots for a given age. Within each plot, 3 to 10 replicate samples were analyzed for specific plant and sediment parameters (detailed below), so individual plot replication incorporated the variation within a plot. Replicate samples were collected along transects extending 50 m in 0.4 ha plots and 25 m in 0.2 ha plots through the center of each plot. Samples were collected equidistant along the transect line depending on the number of replicates.

### Eelgrass metrics

In each plot, *Zostera marina* shoot density was determined by counting individual shoots *in situ* in 10 quadrats ( $0.25 \text{ m}^2$  each). Quadrats were placed at a random distance perpendicular to the transect line at each sampling point. For biomass estimates, 3 cores (15.24 cm inner diameter) containing shoots were collected, including sediment and eelgrass biomass to 20 cm depth, in each plot. The cores were sieved *in situ* to remove sediments. In the laboratory, shoots were separated into aboveground and belowground biomass for each core, and the number of shoots was counted to obtain estimates of total, above-, and belowground biomass per shoot. To separate biomass into above- and belowground portions, we cut the shoots at the root primordia, where the presence of 2 small bumps at the base of the shoot indicated the formation of a new rhizome internode. Canopy height was determined in the laboratory as the average height of the 3 tallest leaves. Pooled samples of leaf tissue and rhizome/root tissue for each core were dried at  $60^\circ\text{C}$  to a constant weight and ground to a fine powder using a Digital Wig-L-Bug<sup>®</sup> Mixer/Amalgamator. C and N contents were determined on 3 to 7 mg samples for plant tissue and 20 to 30 mg samples for sediments using a Carlo Erba Elemental Analyzer with a  $1020^\circ\text{C}$  combustion tube and  $650^\circ\text{C}$  reduction tube, and helium as a carrier gas.

In 4 plots for each age class, plant productivity was measured on all shoots within a  $20 \times 10 \text{ cm}$  anchored grid using the leaf-marking method, where the blades

were punctured with a 22-gauge needle using the top of the sheath bundle as the reference point. Shoots were collected 10 to 15 d after marking, and leaf area and dry weights of the older growth (above the scar) and the new growth (below the scar, but excluding the sheath bundle and including new shoots) were measured.

### Sediment metrics

Sediments were collected in  $60 \text{ cm}^3$  syringe corers in each vegetated and bare plot for determination of sediment organic content, C and N contents, exchangeable ammonium, and grain size distribution. We standardized our sampling to collect the top 5 cm where we expected to see meadow effects on these sediment parameters independent of the accumulation of belowground biomass. Five cores were collected in each plot for each analysis; the reported values represent the integrated measure for the 0 to 5 cm depth interval. Sediment organic content was calculated as loss on ignition from sediment dry weight after combustion in a  $500^\circ\text{C}$  muffle furnace for 8 h. C and N contents were measured on dried sediment ( $60^\circ\text{C}$  for at least 48 h) using a Carlo Erba Elemental Analyzer as described above. Exchangeable ammonium was determined by KCl extraction of sediments (Keeney & Nelson 1982); ammonium concentrations were measured on a Lachat 8500 autoanalyzer.

Sediment grain size distributions were determined on samples collected in 2010 and were compared between *Zostera marina*-vegetated sediments and bare sediments. Two sediment cores were taken in each replicate plot down to a depth of 5 cm using a  $60 \text{ cm}^3$  syringe corer. Sediments were homogenized and a 10 ml subsample was used for the grain size analysis. Organic matter (OM) was removed from the sediments by addition of a 50:50 bleach:water mixture; large organic material was removed by hand. After the sediment had completely settled and there was no evidence of OM, the excess water was decanted; the samples were then rinsed with deionized water and decanted 5 times to remove all bleach. A 5% sodium hexametaphosphate solution was then added to each sample as a dispersant, and the grain size analysis was then done on an LS 13 320 Laser Diffraction Particle Size Analyzer (PSA; Beckman Coulter). Approximately 0.5 to 1.0 ml of solution was added to the PSA. The PSA reports grain size distribution as % volume for particle diameters of 0 to 500  $\mu\text{m}$ .

### Benthic chlorophyll

Five surface sediment samples were collected in each plot to a depth of 1 cm with a 10 cm<sup>3</sup> syringe corer for analysis of benthic chlorophyll. Collected samples were kept in the dark on ice until they were frozen at -80°C immediately after returning to the laboratory. Samples were later thawed and extracted in a 45:45 methanol:acetone solution overnight in the freezer after a 1 min sonication. Benthic chlorophyll was determined spectrophotometrically using the equations of Lorenzen (1967).

### Statistics

To test for differences between different-aged plots all replicates were averaged within a plot, and then an analysis of variance (ANOVA) was used, followed by a Tukey test to determine which specific age categories were different. Individual tests of means between bare and vegetated sediments were done using the Student's *t*-test, as noted. Differences were considered significant at  $p < 0.05$ .

## RESULTS

### Eelgrass metrics

The density of shoots was the metric that changed most significantly across the 9 yr age gradient. There was an initial 4 yr lag in shoot density, with densities ranging from 14.2 to 105.4 shoots m<sup>-2</sup>. Shoot densities increased linearly in plots 6 to 9 yr after seeding, from 249.3 to 616.7 shoots m<sup>-2</sup>, and were significantly different from plots 1 to 4 yr after seeding (Fig. 1). The average canopy height was lower for the 1 yr plants than for all other ages, although there were no significant differences in the canopy height for different-aged meadows due to the high variability in the 1 yr meadows seeded and sampled in different years (averages 22.01 to 29.10 cm for 1 yr meadows vs. 32.24 to 44.90 cm for 2 to 9 yr meadows).

The average total biomass shoot<sup>-1</sup> varied both within a given year and between meadows seeded in different years (0.260 to 0.781 g dry weight shoot<sup>-1</sup>). However, there were no significant trends in biomass per shoot related to meadow age. The ratio of above- to belowground biomass was also variable, and there were no significant differences between meadows of different ages. Variability within an age class was higher for the 1 to 4 yr meadows than for the 8 to 9 yr meadows.

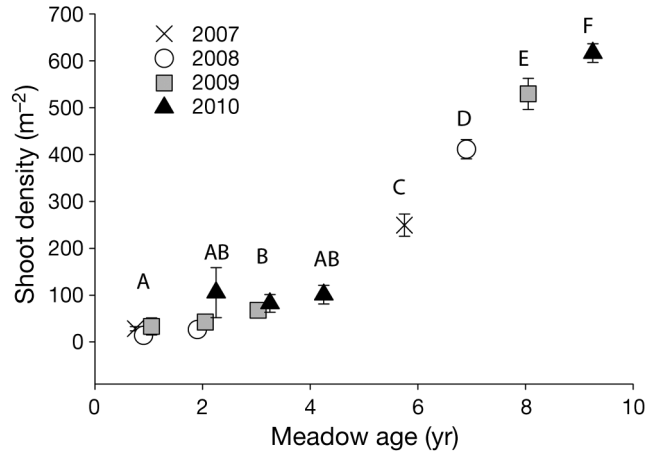


Fig. 1. *Zostera marina*. Shoot density in replicate 0.2 to 0.4 ha plots of eelgrass meadows restored by seeding in successive years, resulting in a chronosequence of sites from 1 to 9 yr since seeding. Symbols denote the sampling year, and meadow age represents the time since seeding. Each data point represents 60 to 280 shoot counts; error bars are  $\pm$ SE. Different letters indicate significant differences between years. Shoot density increased slowly 1 to 4 yr after seeding and increased significantly each year in meadows 6 to 9 yr after seeding

Aboveground productivity, calculated as cm<sup>2</sup> shoot<sup>-1</sup> d<sup>-1</sup>, during the peak summer periods varied considerably between meadows of different ages and between years when measurements were done, with no trends in individual shoot productivity over time since seeding (Fig. 2a). The higher productivity in the 7 yr old meadow compared to the 8 yr old meadow was likely related to lower temperatures in 2009 that caused lower shoot-specific productivity that year. Aerial rates of aboveground productivity (cm<sup>2</sup> m<sup>-2</sup> d<sup>-1</sup>) increased with meadow age (Fig. 2b,  $p < 0.0001$ ) and showed a trend similar to the eelgrass density, with an initial lag in productivity in the first few years after seeding. Productivity was not significantly higher until the meadows were 7 to 8 yr old.

### Sediment metrics

Sediment OM varied significantly with time since seeding (Fig. 3a,  $p = 0.001$ ). Initially, for meadows 1 to 2 yr after seeding, there were no differences between the sediment OM in the meadows and the nearby bare sediments. There was an increasing trend in %OM in meadows 4 and 7 yr after seeding, and the OM was significantly higher in meadows 9 yr after seeding. The accumulation of OM in eelgrass-vegetated sediments resulted in a doubling of OM in eelgrass meadows compared to

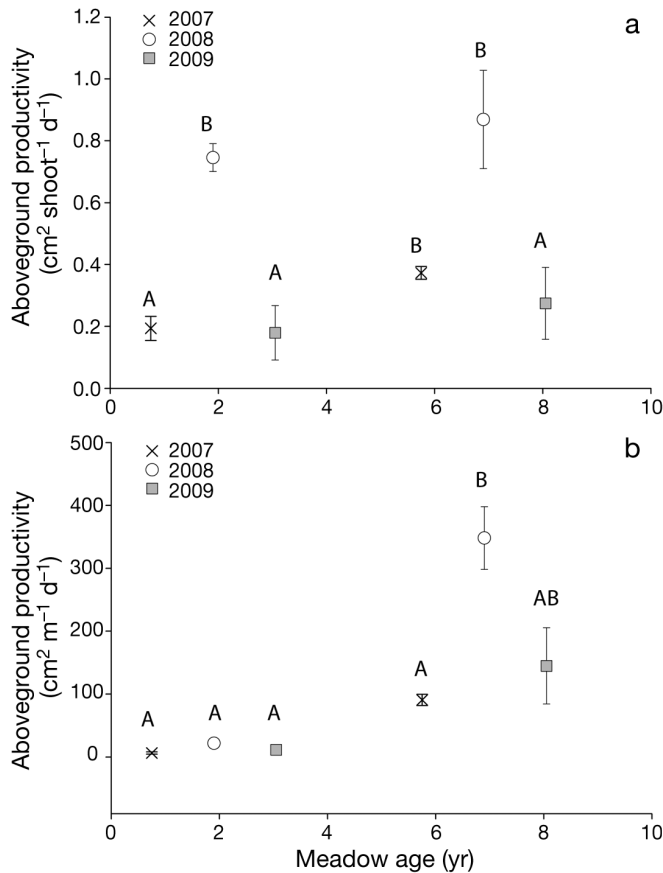


Fig. 2. *Zostera marina*. Rates of aboveground productivity in restored meadows as a function of time since seeding. (a) Productivity rates per shoot were variable from year to year and did not change significantly over time, whereas (b) areal productivity tracked patterns of shoot density. After 9 yr, meadows had 20 times higher rates of aerial productivity than 1 to 3 yr meadows. Error bars are  $\pm$ SE; different letters indicate significant differences between years

nearby bare sediments after 9 yr (mean  $\pm$  SE:  $2.35 \pm 0.13$  vs.  $1.39 \pm 0.22$ ).

Exchangeable ammonium concentrations in sediments showed a similar significant trend of increasing concentrations with time since seeding (Fig. 3b,  $p < 0.0001$ ). Meadows 1 to 2 yr since seeding had exchangeable  $\text{NH}_4^+$  concentrations similar to those of nearby bare sediments, and there was a trend of increasing concentrations in 4 yr meadows. Nine years after seeding, exchangeable  $\text{NH}_4^+$  concentrations had doubled in *Zostera marina* meadows compared to bare sediments.

There were no significant trends evident in either %C or %N in sediments as *Zostera marina* meadows developed over time due to high variability both within and between age classes. C concentrations ranged from 0.23 to 0.47% C. N concentrations were always low, near the detection limit, and averages

never exceeded 0.05% N. However, both the average %C and % N contents in the sediments of the 9 yr meadows were higher than nearby bare sediments (C:  $0.467 \pm 0.021$  [mean %  $\pm$  1 SE] vs.  $0.227 \pm 0.040$ ,  $p < 0.004$ , N:  $0.027 \pm 0.002$  vs.  $0.008 \pm 0.003$ ,  $p < 0.0001$ , Student's *t*-test).

Sediment grain size distribution changed significantly over time since seeding, with fining of the sediment as meadows developed. This is evident in the PSA results, which showed a consistent decrease in the % volume of sediment between 175 and 100  $\mu\text{m}$  diameter comparing bare sediments with sediments from meadows 2, 3, 4, and 9 yr since seeding, and an increase in the % volume of sediment between 0 and 60  $\mu\text{m}$  diameter (Fig. 4a). Most of the sediment below 63  $\mu\text{m}$  was coarse silt; only  $<0.5\%$  of the total sedi-

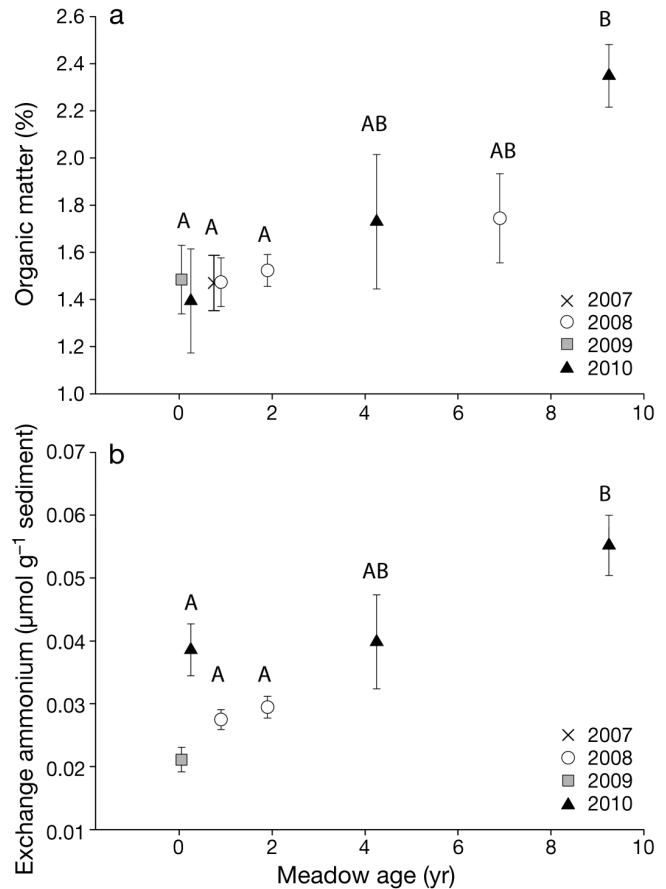


Fig. 3. (a) Sediment organic matter and (b) exchangeable ammonium concentrations in vegetated sediments in restored *Zostera marina* meadows as a function of time since seeding. Nearby bare, unvegetated sediments are represented as '0' age. Error bars are  $\pm$ SE; different letters indicate significant differences between years. Nine years after seeding, sediment organic matter and exchangeable ammonium concentrations in *Z. marina*-vegetated sediments had doubled compared to bare sediments

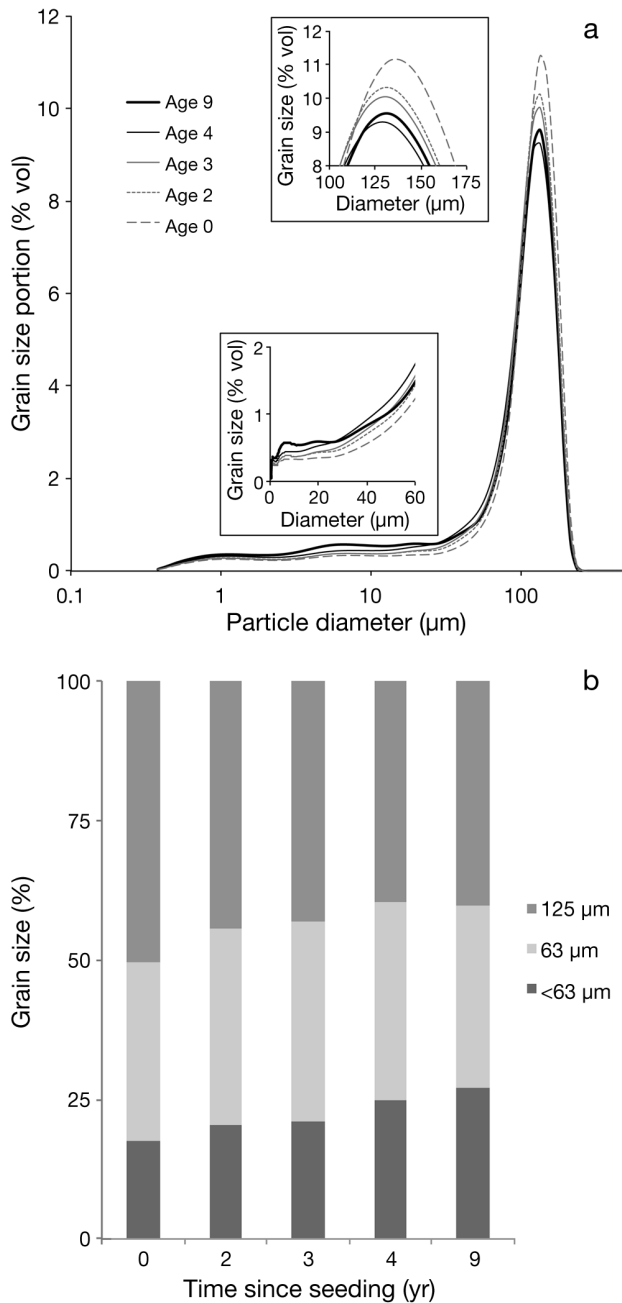


Fig. 4. (a) Grain size distributions in *Zostera marina* sediments. Small graphs show decrease in the % volume of sediment between 175 and 100  $\mu\text{m}$  diameter comparing bare sediments with sediments from meadows 2, 3, 4, and 9 yr since seeding, and an increase in the % volume of sediment between 0 and 60  $\mu\text{m}$  diameter. (b) Pooled grain size data for the grain size categories: sand/clay (<63  $\mu\text{m}$ ), very fine sand (63 to 125  $\mu\text{m}$ ), fine sand (>125  $\mu\text{m}$ ). There was no sediment coarser than 250  $\mu\text{m}$

ment volume was <31  $\mu\text{m}$  (clay to medium silt). The data from the PSA can also be binned to show the relative percent of the sediment volume in different size categories (Fig. 4b). Sediments from all sites were

in the fine sand or smaller size classes (<250  $\mu\text{m}$ ). Fig. 4b shows the consistent increase in the proportion of the sediment of grain size below <63  $\mu\text{m}$  (very fine sand and silt) during meadow development and a related decline in the proportion of the sediment of grain size >125  $\mu\text{m}$  (fine sand). There was no sediment in the medium and coarse sand size classes (>250  $\mu\text{m}$ ).

### Benthic chlorophyll

There was no significant trend in benthic chlorophyll concentrations with time during meadow development, and concentrations in the *Zostera marina* meadows were similar to those in adjacent bare sediments. Concentrations ranged from 11.9 to 16.9  $\text{mg m}^{-2}$  in the bare sediments and 12.0 to 26.3  $\text{mg m}^{-2}$  in the eelgrass-vegetated sediments.

### Depth distribution

Seeds germinated in all plots along the depth gradient (0.9 to 1.6 m MSL); survival rates of *Zostera marina* shoots over time give an indication of the maximum depth limit for *Z. marina* at this site. After 4 yr, for plots seeded in 2006, no shoots survived at depths of 1.6 m MSL or greater (Fig. 5a). In addition, plots between 1.5 and 1.58 m MSL all showed a decrease in density, while density increased from 0 to 1750 % in plots located at 0.9 to 1.5 m MSL depth (Fig. 5b). Taken together, these results suggest a threshold for survival related to light availability at 1.5 to 1.6 m MSL.

## DISCUSSION

### Habitat suitability

Habitat degradation is often a challenge for successful restoration, since in most regions the decline in seagrass has been attributed to multiple stressors, including eutrophication, sedimentation, and toxicity (Orth et al. 2006a, van der Heide et al. 2007). This is not the case in the Virginia coastal bays; recent work points to seed limitation rather than poor habitat quality as the limiting factor for recolonization and expansion of *Zostera marina* in the bays (Orth et al. 2012). The impressive areal expansion of the eelgrass meadows in these coastal bays (Orth et al. 2012) and the significant increase in eelgrass density described

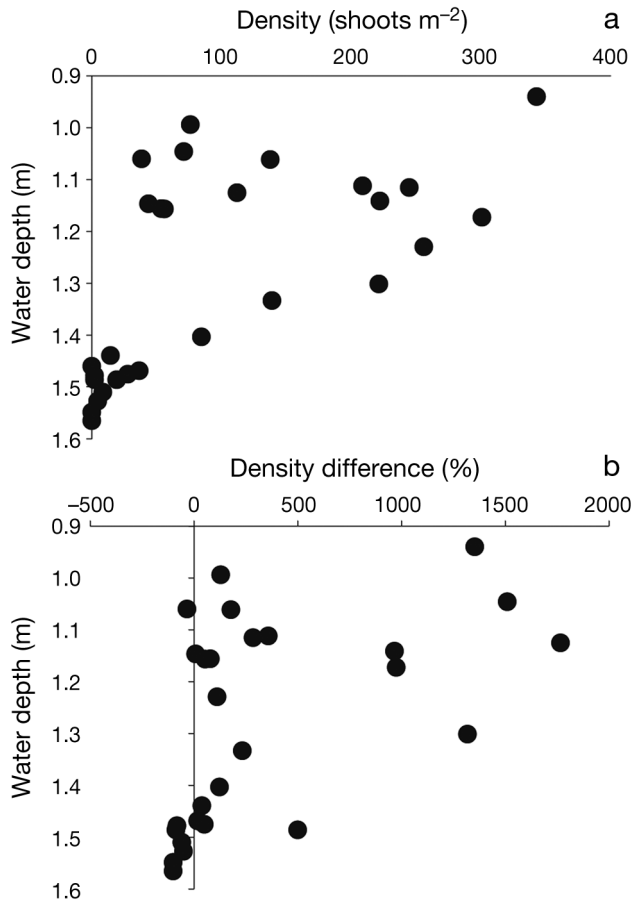


Fig. 5. *Zostera marina*. (a) Shoot density as a function of water depth at mean sea level, and (b) changes in shoot density for plots seeded in 2006 after 4 yr. Negative values represent a loss of shoots between 2007 and 2010

in the present study (up to 1750% in 4 yr) indicate that conditions are conducive to rapid meadow development. Nutrient loading to the coastal bays is low ( $2.1 \text{ g N m}^{-2} \text{ yr}^{-1}$ ; Cole 2011), and is considerably lower than most other coastal bay systems (McGlathery et al. 2007). This is due to the low population density ( $<30 \text{ people km}^{-2}$ ) in the rural watersheds, the small ratio of watershed area to bay area, and the regional land use. The watersheds are dominated by forest and crop agriculture (corn, soybean, tomato); poultry farms do not contribute significantly to N loading as they do to other coastal bay systems in the mid-Atlantic (Stanhope et al. 2009, Cole 2011, Giordano et al. 2011).

Long-term data from the VCR LTER water quality monitoring program from 1992 to the present indicate that water quality parameters typically used to assess habitat suitability for *Zostera marina* have remained high and show no trends of decline over a nearly 20 yr period ([www1.vcrlter.virginia.edu/](http://www1.vcrlter.virginia.edu/)

home1/?q=data\_wq). Average annual concentrations of chlorophyll *a* ( $1 \text{ to } 6 \mu\text{g l}^{-1}$ ), dissolved inorganic N ( $1 \text{ to } 7 \mu\text{M}$ ), and dissolved inorganic phosphorus ( $0.3 \text{ to } 1.3 \mu\text{M}$ ) from monthly mainland-inlet transects across the bays are well within the range that can support growth of *Z. marina* in shallow shoals ( $<2 \text{ m}$  at mean low water [MLW]; Dennison et al. 1993, Moore et al. 1996, Greve & Krause-Jensen 2005). Previous work has shown that wind-driven sediment suspension is the dominant factor influencing light availability in these coastal bays, and accounts for periodic spikes in total suspended solid concentrations (Lawson et al. 2007). Despite this variability, Lawson et al. (2007) showed that  $>60\%$  of 1 of the coastal bays (Hog Island Bay) was suitable for seagrass growth based on light availability.

Sediment characteristics and local hydrodynamics in the Virginia coastal bays are also conducive to *Zostera marina* growth. The sediments are low in OM ( $<2.5\%$ ) and are well below the average organic content suggested to generally limit seagrass growth (5%, Barko & Smart 1983, Koch 2001), although some *Z. marina* sediments have organic content as high as 16% (Koch 2001). Highly organic sediments typically have high concentrations of sulfides and ammonium, and low oxygen levels associated with decomposition, that may limit plant growth unless plants oxygenate the rhizosphere sufficiently to counteract the negative effects (Goodman et al. 1995, Lee & Dunton 2000). Grain size also influences the accumulation of sulfides and ammonium and the depletion of oxygen in sediments (Holmer & Nielsen 1997, Franke et al. 2006) because permeable sediments facilitate the exchange of overlying water column with porewater, which oxygenates the sediments (Huettel et al. 2003, Precht & Huettel 2003). The bare sediments we studied had  $\sim 80\%$  sand, and after 9 yr the eelgrass-vegetated sediments had  $\sim 75\%$  sand, both well within the range of sand content found in healthy seagrass meadows (Koch 2001). Current and wave conditions were also sufficient to induce porewater exchange (Table 1; Hansen & Reidenbach 2012).

### Recovery trajectories

We expected to observe an initial lag phase after seeding before rapid meadow development occurred based on the reproductive phenology of *Zostera marina* in this region. Seedlings typically flower and produce seeds in their second year and thereafter, and those seeds that germinate and survive then again produce seeds after 2 yr. Our data clearly show

this initial 4 yr lag in newly seeded meadows and a linear increase in shoot density in meadows 6 to 9 yr since seeding. This pattern is analogous to the trends in recovering *Z. marina* meadows observed on larger spatial scales in Chincoteague Bay and Chesapeake Bay, where there was an initial lag of several years before meadow expansion increased rapidly, and is likely a general pattern for this region (Orth et al. 2010). The other plant and sediment parameters that changed over the 9 yr period, including areal *Z. marina* productivity and sediment organic and  $\text{NH}_4^+$  concentrations, also showed the same lag response in recovery, as these changes were strongly influenced by shoot density.

The recovery trajectory in the Virginia coastal bays is slower than that observed for *Zostera marina* meadows restored by transplanting in other regions. Evans & Short (2005) showed that functional equivalence (with reference to natural meadows) of habitat structure based on shoot density, canopy height, and leaf area index was achieved after 3 yr, which is the typical time frame for monitoring programs (Evans & Short 2005). Other studies of restoration by transplanting have also shown similarly fast recovery of habitat structure and specific faunal communities (Fonseca et al. 1996a,b, Leschen et al. 2010), but Bell et al. (2008) found that percent cover of transplanted *Halodule wrightii* took more than 3 yr to reach 100% coverage equivalent to reference areas. Though perhaps slower to recover, an obvious advantage of the seeding technique we used is the large area that can be restored (>1700 ha in this study after 9 yr; Orth et al. 2012). Specific recovery rates for seed-based restoration will likely depend both on the seed density used and the landscape context (e.g. proximity to adjacent meadows). The high rates of areal expansion of the eelgrass meadows in the Virginia coastal bays suggest that disturbance was not a significant factor limiting the success of restoration, as has been shown in other systems (Fonseca 2011, Valdemarsen et al. 2011).

Assessing the time to achieve functional equivalence of restored areas ideally involves reference sites that are of similar scale, located in similar conditions, and within close proximity (Addy 1947, Palmer et al. 1997, Craft et al. 2003, Simenstad et al. 2006, Bell et al. 2008, Fonseca 2011). These requirements were not possible to find for the restored *Zostera marina* meadows in the Virginia coastal bays; we initially followed *Z. marina* meadows in Chesapeake Bay and in southern-most coastal bay near the mouth of Chesapeake Bay. We eventually abandoned these as reference sites, as they were exposed to different

environmental conditions than our restored sites (e.g. nutrients, hydrodynamics). Instead we use the trajectories to show the development of ecological function (or structural proxies for function) over time with the expectation that these characteristics will reach an asymptote, reflecting some long-term persistence, with natural fluctuations related to environmental variability or disturbance. These kinds of long-term studies are not common in seagrass meadows, and have been done in salt marshes on a 5 to 25 yr time frame (Zedler 1996, Tyler & Zieman 1999, Craft et al. 2002, 2003, Evans & Short 2005). After 9 yr in the Virginia coastal bays, none of the eelgrass or sediment parameters we monitored have reached an asymptote, indicating that a decade or more is required to restore key functions in restored *Z. marina* meadows by seeding, even in a region with high habitat suitability. It is possible that low N concentrations in the Virginia coastal bays limit the rate of seagrass meadow development, as has been shown for restored marshes (Zedler 1996).

### Ecosystem services

In this study we focused on 3 ecosystem services (functions) that eelgrass habitats provide: primary productivity, C and N sequestration, and sediment deposition. The conceptual diagram in Fig. 6 summarizes the changes we observed. We found that shoot density was the primary driver of areal rates of primary productivity, as there were no differences in productivity rates per shoot. As the meadows developed over time, productivity increased nearly 20-fold, from an average of  $13.2 \text{ cm}^2 \text{ m}^{-2} \text{ d}^{-1}$  in the first 3 yr after seeding to  $246.5 \text{ cm}^2 \text{ m}^{-2} \text{ d}^{-1}$  in 7 to 8 yr old meadows. This latter rate accounts for significant year-to-year variation related to temperature, and is comparable to rates reported for mature *Z. marina* meadows (Dennison & Alberte 1982). There were no differences in biomass per shoot, and areal biomass tracked changes in density, which agrees with the literature review of Olesen & Sand-Jensen (1994), who showed that the maximum summertime leaf biomass of different populations of *Z. marina* did not vary with density. They suggested that self-thinning was not an important phenomenon in natural *Z. marina* meadows, presumably because the period of high leaf biomass was too short for negative feedbacks to occur. The densities in our meadows may not have approached the point where shoots would be expected to undergo density-dependent growth due to reduced light availability.

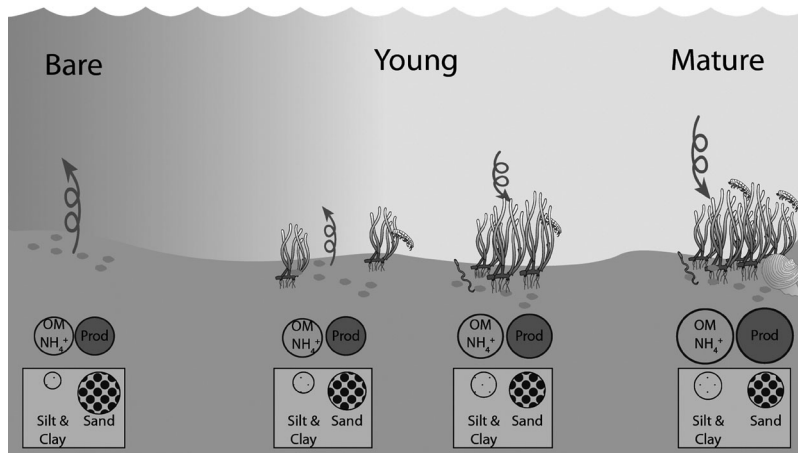


Fig. 6. *Zostera marina*. Conceptual diagram of the measured changes in structural and functional characteristics over time of restored meadows in the Virginia, USA, coastal bays. Arrows represent sediment suspension or deposition and show that sediment stability increases with meadow development. The relative proportions of sand and silt fractions change with meadow development (indicated by size of circles), with a fining of the sediments over time. The size of the circles for organic matter (OM) and  $\text{NH}_4^+$  concentrations indicates that both pools increase as meadows develop over time. The same is true for rates of productivity (Prod)

C and nutrient sequestration is an important, and relatively understudied, ecosystem service of seagrass meadows and other productive coastal habitats (Keith et al. 2009, Duarte et al. 2010, Kennedy et al. 2010, Donato et al. 2011). We calculated differences in the C and N concentrations in unvegetated sediments and 9 yr *Zostera marina* meadows as a first-order estimate of the accumulation of C and N standing stocks in *Z. marina* meadows, assuming that the bare sediment represented the initial condition (Fig. 7). Sediment standing stocks were determined from measures of %C, %N, and bulk density in surface sediments to a depth of 5 cm. Vegetated sediments stored more than twice as much C (278.9 versus 138.7  $\text{g m}^{-2}$ ) and 3 times more N (16.2 vs. 5.1  $\text{g m}^{-2}$ ) than unvegetated sediments. Sediments comprised a larger pool of stored C and N than vegetation, similar to what is observed for salt marsh ecosystems (Craft et al. 1988, Morris et al. 2002, Chmura et al. 2003). Including the C temporarily stored in *Z. marina* biomass during this period of maximum production, eelgrass habitats stored 3 times the C and over 4 times the N compared to unvegetated sediments. We are continuing this work to determine annual accumulation rates of C in sediments, but these initial estimates clearly demonstrate the potential role of seagrass restoration in increasing C and N sequestration in shallow coastal ecosystems (Irving et al. 2011).

The presence of dense seagrass meadows can improve local water quality by attenuating wave energy and near-bed currents, increasing particle deposition, and reducing sediment suspension (Ward et al. 1984, Fonseca & Fisher 1986, Gacia et al. 2002, Hendriks et al. 2008, Gruber & Kemp 2010). We show significant fining of the sediments in eelgrass meadows as shoot densities increased over time, indicating that finer (clay/silt) particles were being deposited and retained within the meadows. These results are consistent with a study conducted concurrently showing that the restored *Zostera marina* meadows reduced near-bottom current velocities by 70 to 90 % and wave heights by 45 to 70 % compared to nearby unvegetated sediments and that expansion of the meadows has changed the

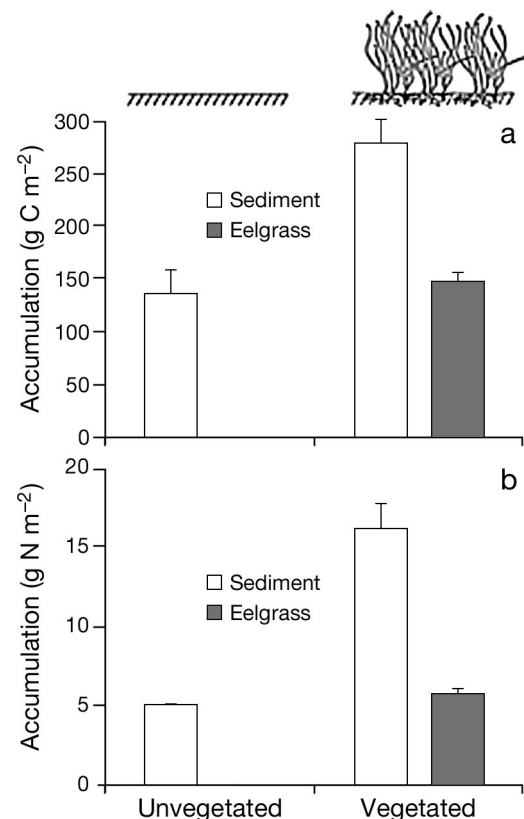


Fig. 7. Calculated accumulation of (a) C and (b) N in restored *Zostera marina* habitats determined by comparing vegetated sediments of meadows 9 yr after seeding with bare, unvegetated sediments. C and N temporarily bound in eelgrass aboveground biomass is also shown. Error bars are +SE



seafloor from being an erosional to a depositional environment (Hansen & Reidenbach 2012). In addition, water clarity measurably improved in the South Bay meadows as *Z. marina* became denser from 2002 to 2010 (Orth et al. 2012). Sediment OM and exchangeable  $\text{NH}_4^+$  also were twice as high in 9 yr meadows compared to 1 to 3 yr meadows, indicating that the restored eelgrass meadows are important repositories of OM, some of which accumulates as discussed above, and some of which is decomposed *in situ*. Given the consistently low water column chlorophyll concentrations, it is likely that either re-suspended benthic microalgae from nearby unvegetated areas or eelgrass detritus are the largest contributors to the OM accumulation in these eelgrass meadows. This agrees with what Gacia et al. (2002) found for a *Posidonia oceanica* meadow where benthic diatoms and filamentous algae dominated the organic particle flux to seagrass sediments, except during times of phytoplankton blooms.

### Depth limits and future trajectories

The positive feedback of *Zostera marina* on sediment stability and light availability is strong enough to induce depth-dependent bistable dynamics in these shallow coastal bays, with the 2 states being a *Z. marina* meadow and bare, unvegetated sediments (Carr et al. 2012). Our experimental data show that restored eelgrass did not survive at 1.6 m MSL, and that densities declined at water depths below 1.5 m MSL. These field results match remarkably well with the model results of Carr et al. (2012) that identify 1.6 m MSL as the 'tipping point,' or maximum depth that can support expanding seagrass meadows under current conditions in this system. The model indicates the bistable range to be 1.6 to 1.8 m MSL, where either *Z. marina* meadows or the bare sediment state could exist, depending on initial densities and environmental conditions (e.g. temperature). Modeling of future climate change scenarios suggests that meadows in the bistable range have limited resilience and that increases in water column temperatures would push a meadow past a critical bifurcation point to a bare sediment state from which recovery would not be possible (Carr et al. 2012). In effect, this would decrease the depth limit of *Z. marina* and reduce the suitable area for eelgrass expansion in the coastal bays. Changes in temperature and/or wave exposure associated with future climate change may also influence the minimum depth limit of *Z. marina* in this system, pushing the mead-

ows to deeper waters and 'squeezing' the suitable habitat to a narrower depth range. By continuing field monitoring of restored meadows, and incorporating mechanistic studies of habitat effects on productivity and minimum light requirements of *Z. marina* and modeling, we can achieve a better understanding of the potential for continued recovery in the region.

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# Eelgrass restoration by seed maintains genetic diversity: case study from a coastal bay system

Laura K. Reynolds<sup>1,\*</sup>, Michelle Waycott<sup>2</sup>, Karen J. McGlathery<sup>1</sup>, Robert J. Orth<sup>3</sup>,  
Joseph C. Zieman<sup>1</sup>

<sup>1</sup>Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia 22904, USA

<sup>2</sup>School of Marine and Tropical Biology, James Cook University Townsville, Queensland 4811, Australia

<sup>3</sup>Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, Virginia 23062, USA

**ABSTRACT:** Genetic diversity is positively associated with plant fitness, stability, and the provision of ecosystem services. Preserving genetic diversity is therefore considered an important component of ecosystem restoration as well as a measure of its success. We examined the genetic diversity of restored *Zostera marina* meadows in a coastal bay system along the USA mid-Atlantic coast using microsatellite markers to compare donor and recipient meadows. We show that donor meadows in Chesapeake Bay have high genetic diversity and that this diversity is maintained in meadows restored with seeds in the Virginia coastal bays. No evidence of inbreeding depression was detected ( $F_{IS}$  –0.2 to 0) in either donor or recipient meadows, which is surprising because high levels of inbreeding were expected following the population contractions that occurred in Chesapeake Bay populations due to disease and heat stress. Additionally, there was no evidence for selection of genotypes at the restoration sites, suggesting that as long as donor sites are chosen carefully, issues that diminish fitness and survival such as heterosis or out-breeding depression can be avoided. A cluster analysis showed that, in addition to the Chesapeake Bay populations that acted as donors, the Virginia coastal bay populations shared a genetic signal with Chincoteague Bay populations, their closest neighbor to the north, suggesting that natural recruitment into the area may be occurring and augmenting restored populations. We hypothesize that the high genetic diversity in seagrasses restored using seeds rather than adult plants confers a greater level of ecosystem resilience to the restored meadows.

**KEY WORDS:** Seagrass · *Zostera marina* · Restoration · Genetic diversity · Microsatellite DNA

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## INTRODUCTION

A high level of genetic diversity in plant populations is associated with increased benefits for plant survival and ecosystem services (Booy et al. 2000). The loss of genetic diversity may cause reduced adaptability to environmental change through loss of fitness (Reed & Frankham 2003). In both marine and terrestrial systems, experimental studies have demonstrated the benefits of genetic diversity to the capacity of populations to resist stressors such as disease, predation, and physical disturbance (Zhu et al.

2000, Hughes & Stachowicz 2004, Reusch et al. 2005, Johnson et al. 2006, Hughes & Stachowicz 2011). In marine systems, lower genetic diversity in the seagrass *Zostera marina* (eelgrass) has been shown to reduce survivorship following disturbance (Hughes & Stachowicz 2004, 2011, Reusch et al. 2005). In terrestrial systems, genetically diverse assemblages of primrose plants *Oenothera biennis* were found to serve as a better habitat and support more species of arthropods than less diverse assemblages (Johnson et al. 2006). Also, genetically diverse rice *Oryza sativa* fields have been found to be less susceptible to

\*Email: lkreynolds@virginia.edu

disease (Zhu et al. 2000). Given the positive benefits associated with higher levels of genetic diversity, it should be considered an essential component of ecosystem restoration.

Strategies to enhance the likelihood of increased genetic diversity through restoration focus on 2 alternatives. The first is to maximize the use of genetic resources incorporated into captive breeding programs. The second is to use the diversity present in natural populations. Both strategies have been adopted widely, such as when salmon hatcheries that have captive animals sourced from a variety of locations have been used to mitigate population declines in the wild (Waples 1991, 1994). Captive breeding and reintroduction of young into wild populations also have been employed as tactics to increase the genetic diversity of the endangered Hawaiian thrush and big horn sheep (Kuehler et al. 2000, Ostermann et al. 2001). However, this strategy, where material is sourced from a variety of origins, has also been criticized, because new genotypes are introduced into remnant populations and this potentially results in less fit progeny (Knapp & Dyer 1998).

Many estuarine and coastal areas are experiencing increasing levels of disturbance and/or stress related to human activities, such as eutrophication, low dissolved oxygen, increasing temperatures, and invasive species (Jackson et al. 2001, Lotze et al. 2006, Halpern et al. 2008, Waycott et al. 2009). Knowledge of the value of the plant and animal species that occupy these habitats has resulted in significant efforts to reduce anthropogenic stressors and to emphasize restoration of species and habitats.

Seagrasses (marine angiosperms), of which there are approximately 72 species, are often the dominant macrophytes in estuaries, shallow coastal bays, and lagoons worldwide (Green & Short 2003, Short et al. 2011). Globally, seagrasses are declining (Orth et al. 2006a, Waycott et al. 2009), most often as a result of increasing nutrients and sediments from watersheds being altered by human activities (Waycott et al. 2009). In many degraded systems efforts are being made to mitigate seagrass decline and to improve habitat for seagrass restoration (Greening & Janicki 2006, Orth et al. 2010). There is a growing body of evidence that indicates that genetically diverse assemblages of seagrasses are fitter (Williams 2001) and more resistant to a variety of disturbances (Hughes & Stachowicz 2004, 2011, Reusch et al. 2005). In the seagrass ecosystems dominated by a single species that are typical of northern hemisphere seagrass communities, adopting appropriate restoration strategies to capture adequate levels of

genetic diversity is an important and realistic goal. Monospecific seagrass meadows can act as case studies for evaluating the relative success of implementing different restoration strategies based on maintaining genetic diversity.

*Zostera marina* is a seagrass found in temperate and sub-temperate regions of the North Atlantic and North Pacific Oceans and in the Mediterranean Sea (Green & Short 2003). This species of seagrass has been observed to undergo periods of extreme population fluctuations, especially in the North Atlantic (Cottam 1934, 1935). The most notable broad-scale population decline was associated with the spread of *Labyrinthula zosterae*, a fungal parasite, in the 1930s (Rasmussen 1977). While many populations eventually recovered from the impact of this disease (Cottam & Munro 1954), populations in a number of coastal bays in the mid-Atlantic region of the United States did not (Orth et al. 2006b). Most recovery in the Virginia coastal bays is the result of large-scale restoration (Orth et al. 2006b, 2012, this Theme Section). While the scale and success of the restoration in the Virginia coastal bays is somewhat unique, mitigation to compensate for seagrass loss through restoration is becoming more globally widespread (Paling et al. 2009, van Katwijk et al. 2009)

One concern surrounding seagrass restoration is the possible loss of genetic diversity when adult plants are used for re-establishing populations (Williams & Davis 1996, Williams 2001). Depending upon the size of the clone, it is entirely possible that adult plants for a small-scale restoration effort could be drawn from a single clone with low genetic diversity. The use of seeds harvested from multiple parents, rather than adult plants, could offset this genetic bottleneck. The successful re-establishment of *Zostera marina* into unvegetated coastal bays in the mid-Atlantic region of the United States using seeds from a number of source beds (Orth et al. 2012) offered a unique opportunity to test the hypothesis that genetic diversity is not eroded when seeds are used as in restoration. Here we present results from our analysis of genetic diversity from both natural *Z. marina* beds in Chesapeake Bay, several of which have served as source beds for restoration, and the restored beds in the Virginia coastal bays.

## MATERIALS AND METHODS

A total of 9 *Zostera marina* meadows were sampled in 3 distinct regions. These included both natural beds in Chesapeake Bay (mouth of the York River,

YR; Mobjack Bay, MB; Hungar's Creek, HC; and Fisherman Island, FI), 1 bay to the immediate north of the restoration sites (Chincoteague Bay, CB), and restored beds in 3 Virginia coastal bays (South Bay, SB; Spider Crab Bay, SC; and 2 sites in Hog Island Bay, HR6, HR7) (Fig. 1). These Virginia coastal bays are part of the Virginia Coast Reserve Long Term Ecological research site. Several of the Chesapeake Bay sites were sources of seeds (YR, MB, and HC) used in the coastal bay restoration (Table 1). Restored beds sampled in South Bay were seeded from a variety of western Chesapeake Bay sources, including MB and YR. Restored beds sampled in Spider Crab Bay were seeded from SB seeds in 2008. Restored beds sampled in Hog Island Bay were seeded either from Hungar's Creek in 2006 (labeled HR6) or from South Bay (SB) in 2007 (labeled HR7).

Methods for collection, storage, and disbursing of seeds can be found in Marion & Orth (2010). Because we were interested in whether genetic diversity would be maintained in restored beds developed with seeds, we compared donor sites and restored beds for genetic diversity, resulting in 7 comparisons of donor meadows and recipients. Natural populations at Fisherman Island (FI) and Chincoteague Bay (CB) were also sampled as they represent populations immediately south and north of the restored sites.

At each sampling site, whole eelgrass shoots were haphazardly collected by hand from areas approximately 5 m apart, to avoid collecting shoots from the same clones. Leaf tissue was dried and stored at room

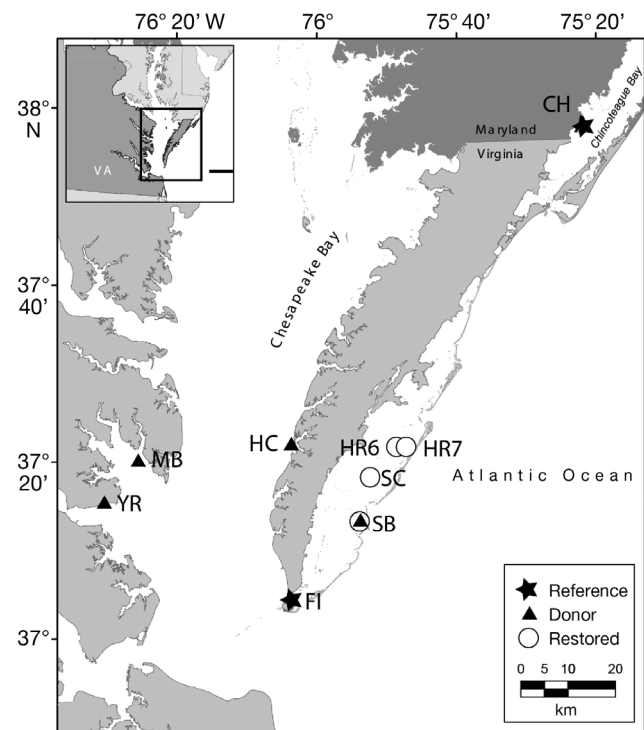


Fig. 1. Site map. Chincoteague Bay (CH), Fisherman Island (FI), Hungar's Creek (HC), Mobjack Bay (MB), and York River (YR) are natural *Zostera marina* meadows. Seeds from various Chesapeake Bay sites including MB and YR were used to restore South Bay (SB). HC was used as a donor for HR6 in 2006, and seeds from the restored meadow in SB were used in a restoration in Hog Island Bay in 2007 (HR7) and in a restoration in Spider Crab Bay (SC) in 2008

Table 1. *Zostera marina*. Summary of multilocus genetic diversity estimates for all 9 populations based on 8 microsatellite loci. Sites refer to locations shown on Fig. 1.  $N$ : sample size;  $N_A$ : total number of alleles per population;  $A_R$ : allelic richness;  $A$ : average number of alleles per locus;  $A_{<25\%}$ : uncommon alleles;  $H_o$ : observed heterozygosity;  $H_e$ : expected heterozygosity;  $F$ : Wright's inbreeding coefficient; VA: Virginia, USA

Site	Site description	$N$	$N_A$	$A_R$	$A$	$A_{<25\%}$	$H_o$	$H_e$	$F$
Fisherman Island (FI)	Southern natural meadow at the mouth of Chesapeake Bay	46	47	4.4	6.7	0.6	0.7	0.6	-0.2
Hungar's Creek (HC)	Natural Chesapeake Bay meadow	48	71	5.5	10.1	0.6	0.8	0.7	-0.1
Mobjack Bay (MB)	Natural Chesapeake Bay meadow	30	63	5.4	9.0	0.6	0.6	0.6	0.0
York River (YR)	Natural Chesapeake Bay meadow	30	66	5.7	9.4	0.7	0.7	0.7	0.0
South Bay (SB)	VA coastal bay meadow restored in 2002 using seed from MB and YR	95	79	5.6	11.3	0.7	0.7	0.7	0.0
Hog Island Bay (HR6)	VA coastal bay meadow restored in 2006 using seed from HC	167	94	5.6	13.4	0.7	0.7	0.7	-0.1
Hog Island Bay (HR7)	VA coastal bay meadow restored in 2007 using seed from SB	46	66	5.3	9.4	0.7	0.8	0.7	0.0
Spider Crab Bay (SC)	VA coastal bay meadow restored in 2008 using seed from SB	48	70	5.3	10.0	0.7	0.8	0.7	-0.2
Chincoteague Bay (CH)	Northern natural VA coastal bay meadow	48	60	4.6	8.6	0.6	0.7	0.6	-0.1

temperature using silica gel desiccant (AGM, mixture of white and indicating beads) until DNA extraction. All plant samples were collected during the summer months (June to August) of 2008 and 2009.

Total genomic DNA was extracted using DNeasy plant extraction kits (Qiagen) following manufacturer's instructions. A total of 8 microsatellite loci previously used for this species (Reusch et al. 1999) were amplified using fluorescently tagged primers (CT17H, CT3, CT35, GA2, CT19, CT20, GA3, and GA6). Amplification of PCR products followed the procedures recommended by Reusch et al. (1999). PCR products were analyzed by capillary electrophoresis on a MegaBACE 1000 (GE Biosciences) with ET 400-Rox (GE Biosciences) internal size standard in each sample, as per manufacturer's instructions. Fragment lengths for each allele, at each locus, were determined using Fragment Profiler V1.2 (GE Biosciences).

Standard measures of genetic diversity were calculated for each population sampled. Allelic richness ( $A_R$ ), standardized to the smallest population size by rarefaction, was computed using FSTAT 2.9.3.2 (Goudet 2001). Total number of alleles per population ( $N_A$ ), the average number of alleles per locus ( $A$ ), the average number of low-frequency alleles ( $A_{<25\%}$ ) at each locus, the mean observed ( $H_o$ ) and mean expected heterozygosity ( $H_e$ ), and Wright's inbreeding coefficient ( $F$ ) were calculated using GenAlEx 6.3 (Peakall & Smouse 2006). Differences in genetic diversity between donor and recipient populations were analyzed using both a paired  $t$ -test and a chi-squared goodness-of-fit test, where paired donor meadows were treated as expected values.

The population structure or relatedness of geographically separated meadows was compared using the standard measure of population differentiation,  $F_{st}$ , calculated in GenAlEx 6.3 (Peakall & Smouse 2006). Within-population inbreeding was estimated using  $F_{is}$ , calculated in GenAlEx 6.3 (Peakall & Smouse 2006). A test of population assignment using Bayesian modeling of all samples was conducted using the software STRUCTURE for assigned numbers of populations of  $K = 1$  to 10 and with 10 replicates with a random start for each value of  $K$  (Pritchard et al. 2000). The number of distinct population clusters was determined using the delta  $K$  method (Evanno et al. 2005). The relationship between the cluster to which a sample was assigned and geographic origin were analyzed with a Kruskal-Wallis 1-way analysis of variance. Pairwise differences were analyzed with individual Mann-Whitney  $U$ -tests, with Bonferroni-corrected alpha values.

## RESULTS

Moderate to high levels of allelic diversity were detected across the 9 *Zostera marina* meadows sampled from the Chesapeake Bay, Virginia coastal bays, and Chincoteague Bay. All loci conformed to Hardy-Weinberg equilibrium in at least some of the populations. All populations sampled showed relatively high allelic richness (mean  $A_R = 5.3$ ), with York River having the highest value at 5.7 and Fisherman Island having the lowest value at 4.4. Across all sites both the observed (mean  $H_o = 0.72$ ) and expected (mean  $H_e = 0.67$ ) heterozygosities were high, which is typical of *Z. marina*. Although in many population samples  $H_o$  was greater than  $H_e$ , which resulted in slightly negative inbreeding coefficients ( $F = -0.2$  to 0), the  $F$ -values were not significantly different (Table 1).

Restored meadows did not show a significant reduction in allelic richness, mean number of rare alleles, or expected heterozygosity relative to their donor meadows (Fig. 2). A paired  $t$ -test between donor and recipient meadows resulted in  $p$ -values of 0.39 or greater, and a chi-squared goodness-of-fit using donor values as expected values resulted in  $p$ -values equal to or greater than 0.98. The inbreeding coefficient within populations ( $F_{is}$ ) approached zero, and there was no significant deviation between donor and recipient pairs (Fig. 2;  $t = 0.62$ ,  $p = 0.58$  and  $\chi^2 = 1.08$ ,  $p = 0.77$ ).

All Chesapeake Bay sites and restored Virginia coastal bay sites were closely related when analyzed with permuted  $F_{st}$  values ( $<0.1$ ). Higher pairwise  $F_{st}$  values were observed in comparisons with Fisherman Island and Chincoteague Bay. This was expected as these sites acted as external non-donor recipient reference sites for the present study. Fisherman Island had pairwise  $F_{st}$  values  $>0.1$  with all other meadows. Chincoteague Bay showed a similar deviation from Chesapeake Bay and South Bay sites; however, it had lower pairwise  $F_{st}$  values when compared with Hog Island (2006 and 2007) and Spider Crab Bay, the more northerly restored Virginia coastal bay sites (Table 2).

The relative distinctiveness of sampled meadows was assessed by assigning individuals based on genetically homogenous groups, rather than on sampled locations, using a Bayesian cluster approach with the software STRUCTURE (Pritchard et al. 2000) and by implementing the ad hoc statistic ( $\Delta K$ ) (Evanno et al. 2005). The highest values for the  $\Delta K$  statistic identify 4 groups, or genetic populations, that were present among the 9 sampled locations



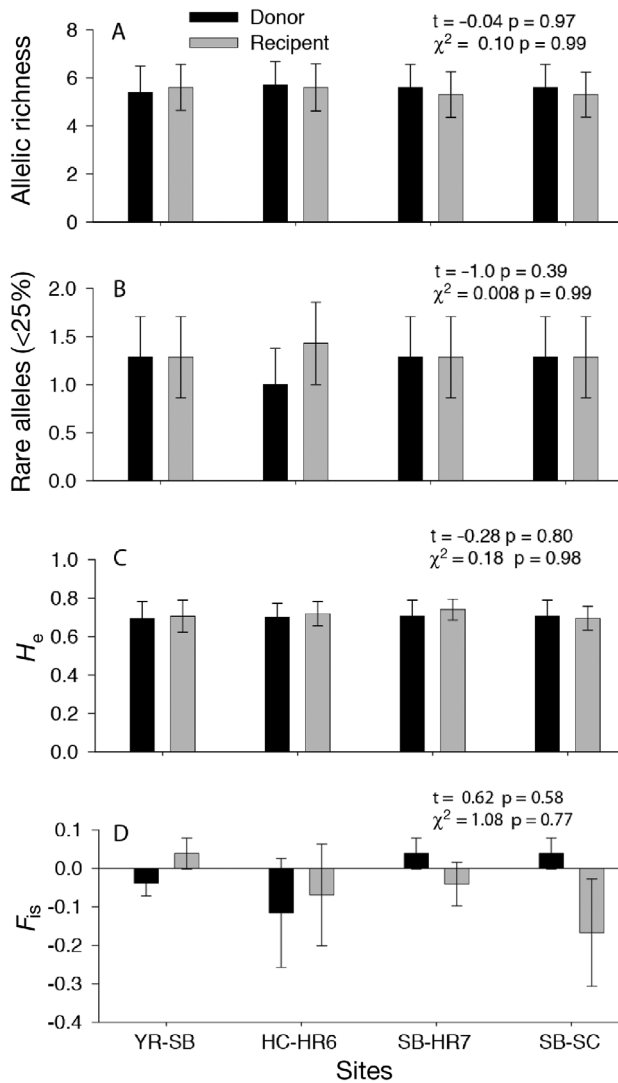


Fig. 2. *Zostera marina*. Four measures of genetic diversity were used to compare donor (dark) and recipient (light) eelgrass meadows ( $\pm$  SE): (A) allelic richness, (B) frequency of rare alleles (<25%) per population, (C) expected heterozygosity ( $H_e$ ), and (D) Wright's inbreeding coefficient ( $F$ ). Site abbreviations, see Fig. 1

(Fig. 3). Samples from different geographic locations were assigned to each of these 4 different clusters with high probability (Kruskal-Wallis:  $\chi^2 = 72$ ,  $p < 0.0001$ ). Significant differences in pairwise comparisons, made with individual Mann-Whitney  $U$ -tests using a Bonferroni corrected alpha of 0.001, were observed for many of the comparisons, particularly between reference sites and the donor-recipient locations (Table 3). The southern-most site near the mouth of the Chesapeake Bay, Fisherman Island (FI), was assigned to Cluster 4 and was significantly distinct from each of the other locations. All Chesapeake Bay locations (HC, YR, MB) and the restored meadow at South Bay (SB) were assigned to both Clusters 1 and 2; however, they were not significantly different from one another. The Hog Island Bay beds restored in 2006 (HR6) were not different from the donor meadow of Hungar's Creek (HC). HR6 differed from the York River site and the South Bay site. The Hog Island Bay beds restored in 2007 (HR7) were not different from the South Bay donor meadow, but like HR6 differed from the York site. The Spider Crab Bay site (SC) was similar to the South Bay donor site, as well as the restorations in Hog Island Bay. The northern natural Virginia coastal bay site, Chincoteague Bay, differed from Fisherman Island, all Chesapeake Bay sites, and the older restoration sites of South Bay and HR6. Spider Crab Bay and HR7 were not statistically different from Chincoteague Bay (Table 3).

The proportional assignment of individual samples to each of the 4 modeled genetic clusters supports the observation that the diversity in the restored meadows was equivalent to that in the donor meadows (Fig. 4). Fisherman Island was distinctive, with few individuals from other sites having a high likelihood of sharing this group; these were assigned to Cluster 4 (Fig. 4). Chesapeake Bay samples (HC, MB, YR), as well as the restored meadows in the Virginia coastal

Table 2. *Zostera marina*. Pairwise  $F_{st}$  estimates for all 9 *Z. marina* populations based on 8 microsatellite loci. \*Values not significantly different from zero

	FI	HC	MB	YR	SB	HR6	HR7	tSC	CH
Fisherman Island (FI)	0.000								
Hungar's Creek (HC)	0.124	0.000							
Mobjack Bay (MB)	0.187	0.048	0.000						
York River (YR)	0.159	0.031	0.012	0.000					
South Bay (SB)	0.143	0.019	0.014	0.005*	0.000				
Hog Island Bay (HR6)	0.117	0.006	0.056	0.031	0.023	0.000			
Hog Island Bay (HR7)	0.114	0.030	0.095	0.062	0.044	0.018	0.000		
Spider Crab Bay (SC)	0.109	0.026	0.082	0.051	0.046	0.014	0.037	0.000	
Chincoteague Bay (CH)	0.136	0.092	0.148	0.111	0.095	0.064	0.067	0.023	0.000

bays, were similar and assigned across Clusters 1 and 2. Neither Cluster 1 nor 2 was specific to 1 location (Fig. 4). Chincoteague Bay samples were distinct and assigned to Cluster 3, along with numerous samples in the Virginia coastal bays, especially Hog Island Bay, which is closest in proximity (Fig. 4). The same patterns persisted when data were grouped as averages of plants collected from 1 location (Fig. 4).

## DISCUSSION

Our results demonstrate that the restoration of *Zostera marina* with seeds in Virginia coastal bays has maintained overall population genetic structure and diversity compared to the donor populations. This finding is in contrast to the significant reduction in genetic diversity observed in a restored *Z. marina* meadow in Southern California, USA, where adult plants were used in the restoration effort and were collected from a very small area (200 to 12 000 m<sup>2</sup>) (Williams & Davis 1996, Williams 2001). A genetically diverse donor population is required to achieve a genetically diverse restored population, and this was the case with Chesapeake Bay and the Virginia

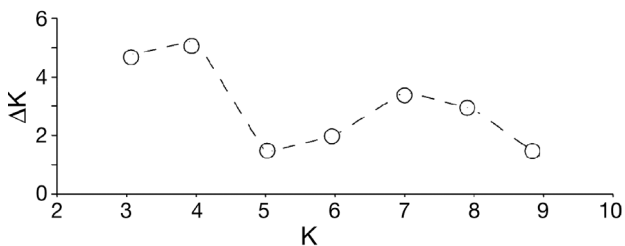


Fig. 3. The 9 geographically separated *Zostera marina* meadows sampled were grouped into 4 genetically distinct clusters based on the ad hoc statistic  $\Delta K$ .  $\Delta K$  was calculated based on 10 runs of the model following Evanno et al. (2005)

coastal bays. Despite population fluctuations in Chesapeake Bay's *Z. marina* populations in the last 80 yr, since the 1930s decline (Orth & Moore 1983, 1984, Orth et al. 2010), current populations exhibit relatively high genetic diversity (Tables 1 & 4).

In the restoration efforts evaluated here, measures of genetic diversity and levels of inbreeding did not differ between the paired donor meadows and recipient meadows (Fig. 1). There is no evidence currently that genotypes are being selected for in the restoration sites. Donor and recipient pairs appear as highly connected, undifferentiated population pairs through high gene flow and low  $F_{st}$  values ( $N_m$  ranges between 4.95 and 8.61, while  $F_{st}$  ranges between 0.005 and 0.05), and this supports the conclusion that the donor and recipient populations are genetically comparable. We propose that the success in maintaining genetic diversity in restored populations of the Virginia coastal bays is due to a combination of high levels of genetic diversity present in the donor meadows, collection of seeds from a broad area that does not result in oversampling of closely related individuals, and the introduction of adequate numbers of seeds into donor sites in a manner reflecting relatively 'natural' recruitment processes. While this is an improvement over previous analyses of restorations using adult plants (Williams & Davis 1996, Williams 2001), those studies incorporated plants that were collected from a small area and restorations were relatively small in numbers of transplant units. If adult plants were collected from a large area within a genetically diverse region, reductions in genetic diversity could be improved; however, logistically, it is easier to collect and transplant large numbers of seeds than to transplant large numbers of adult plants. This is underscored by the small scale (<0.5 ha) of most adult transplant restoration efforts (Paling et al. 2009).

Table 3. The *Zostera marina* samples from each of 9 geographically separated meadows were assigned to different genetically distinct clusters using STRUCTURE. Mann-Whitney  $U$ -tests were used to determine differences in populations, and p-values are reported

	FI	HC	PC	PR	SB	HR6	HR7	SC	CH
Fisherman Island (FI)									
Hungar's Creek (HC)	<0.0001								
Mobjack Bay (MB)	<0.0001	0.4							
York River (YR)	<0.0001	0.004	0.2						
South Bay (SB)	<0.0001	0.2	0.9	0.202					
Hog Island Bay (HR6)	<0.0001	0.06	0.004	<0.0001	<0.0001				
Hog Island Bay (HR7)	<0.0001	0.03	0.002	<0.0001	0.0003	0.05			
Spider Crab Bay (SC)	<0.0001	<0.0001	<0.0001	<0.0001	0.002	0.0004	0.9		
Chincoteague Bay (CH)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.12	0.004	

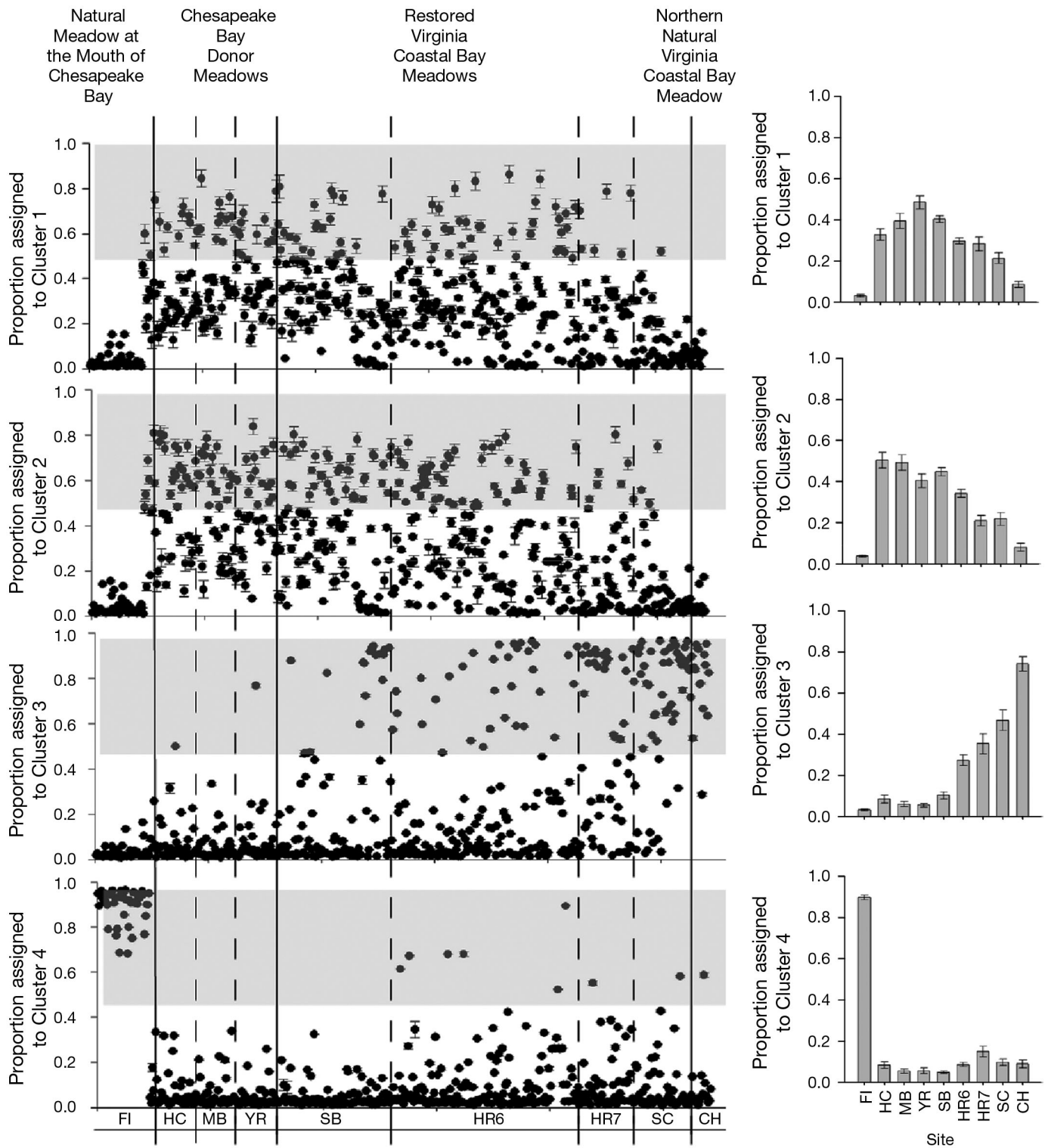


Fig. 4. *Zostera marina*. Each sample was assigned to 1 of 4 genetic clusters using the Bayesian cluster model STRUCTURE (Pritchard et al. 2000), and samples tended to cluster regionally. Plots of outcomes for proportional assignments of individual samples to each of the 4 genetic clusters based on 10 independent runs are presented. For each panel, the left plot depicts the mean proportional assignment ( $\pm$ SE) for each individually sampled seagrass shoot; the grey shaded area highlights where assignment to the cluster was  $>0.5$ . The right plot depicts the mean proportion assigned to that genetic cluster for all samples collected within that location ( $\pm$ SE). Samples are arranged by geographical location from south to north. Site abbreviations, see Fig. 1

Table 4. *Zostera marina*. Summary of multilocus microsatellite-based genetic diversity around the world.  $A_R$ : allelic richness;  $A$ : average number of alleles per locus;  $H_e$ : expected heterozygosity;  $F_{is}$ : inbreeding coefficient

Location	$A_R$	$A$	$H_e$	$F_{is}$	Source
<b>Eastern Pacific</b>					
San Juan Archipelago, Washington, USA	2.3–3.1	2.5–10.4	0.29–0.51	–0.11 to 0.05	Wyllie-Echeverria et al. (2010)
San Francisco Bay, California, USA	2.7–5.4	3.1–6	0.24–0.60	–0.2 to 0.19	Talbot et al. (2004) Ort et al. (2010)
Eastern Pacific, USA and Canada	4.9–6.4		0.17–0.51	–0.14 to 0.16	Olsen et al. (2004)
Baja California, Mexico		1.5–4.0	0.13–0.54	–0.04 to 0.36	Coyer et al. (2007)
<b>Western Pacific</b>					
Mikawa Bay, Japan		10.5–12	0.70–0.85	–0.04 to 0.03	Yoshida et al. (2009)
<b>Eastern Atlantic</b>					
Baltic Sea, Germany		5–5.9	0.40–0.44	–0.02 to 0.09	Reusch et al. (2000)
Baltic Sea, Finland	1.17		0.17–0.44	–0.67 to 0.01	Olsen et al. (2004)
Margot, France	3.33		0.32	0.01	Reusch et al. (2000)
Eastern Atlantic, Europe	1.5–2.7		0.33–0.52	–0.26 to 0.038	Olsen et al. (2004)
<b>Western Atlantic</b>					
Nova Scotia, Canada	5.67		0.46	–0.022	Reusch et al. (2000)
Western Atlantic Ocean, USA and Canada	2.9–4.9		0.41–0.56	–0.09 to 0.19	Olsen et al. (2004)
New Jersey coastal bays, New Jersey USA		6.8–10.5	0.69–0.83	0.55 to 0.71	Campanella et al. (2009)
Chesapeake Bay, Virginia, USA	5.4–5.7	9–10.1	0.6–0.7	–0.1 to 0	Present study
Virginia Coastal Bays, Virginia, USA	4.6–5.6	6.7–9.1	0.6–0.7	–0.2 to 0	Present study

Using seeds from local or regional provenances that are likely locally adapted to appropriate environmental conditions would enhance restoration success. In addition to immediate restoration outcomes, the presence of high levels of genetic diversity in restored populations suggests that the populations will be less likely to show signs of genetic erosion.

Overall, our estimates of genetic diversity are high, but are consistent with the range of values observed in previous studies (Table 4). Olsen et al. (2004) found an insignificant trend of increased diversity with decreased latitude along the western Atlantic coast. Our study adds additional data from closer to the geographic margin of the species, and further supports the observation of a trend of increased diversity with decreased latitude. Compared to the western Atlantic and eastern Pacific populations studied (Reusch et al. 2000, Olsen et al. 2004, Talbot et al. 2004, Coyer et al. 2007, Ort et al. 2010, Wyllie-Echeverria et al. 2010), the Chesapeake and Virginia coastal bay populations described here are more diverse. The only meadows found to have higher values of heterozygosity and numbers of alleles per locus were in Mikawa Bay, Japan (Yoshida et al. 2009; Table 4, present study). The high levels of diversity found in Virginia were unexpected due to the

population history of *Zostera marina* in the region. Over the last century, the *Z. marina* meadows in Virginia have experienced many disturbances including disease, reduced water quality and clarity, bioturbation by rays, and high temperature stress (Orth 1975, 1976, Orth & Moore 1984, Moore & Jarvis 2008). The large-scale decline of *Z. marina* populations in the 1930s, which was attributed to disease (Orth & Moore 1984), would be expected to have created a population bottleneck, with subsequent high levels of inbreeding and reduced genetic diversity in remnant populations in the Chesapeake Bay and Chincoteague Bay. While a recently published study found that *Z. marina* populations from both New Jersey and 1 site in the Chesapeake Bay showed significant signs of inbreeding ( $F_{is} > 0.6$ ; Campanella et al. 2009) (Table 4), our data from Chesapeake and Chincoteague *Z. marina* meadows do not, despite finding similar levels of allelic diversity.

The mechanism by which natural seagrass meadows in Virginia maintain such a high diversity may be quite similar to the mechanism by which restoration by seed maintains high genetic diversity: large numbers of seeds added to open space. The disturbances in Chesapeake Bay (i.e. Orth 1975, 1976, Orth & Moore 1984, Moore & Jarvis 2008) remove seagrass,

Table 5. *Zostera marina*. Pairwise estimates of gene flow for all 9 populations based on 8 microsatellite loci. Values above the diagonal are  $N_m$  values calculated based on  $F_{st}$  in GenALEX 6.3 (Peakall & Smouse 2006), and values below the diagonal are calculated based on rare alleles using GenePop (Raymond & Rousset, <http://wbiomed.curtin.edu.au/genepop/index.html>)

	FI	HC	MB	YR	SB	HR6	HR7	SC	CH
Fisherman Island (FI)		1.762	1.084	1.325	1.494	1.883	1.948	2.037	1.588
Hungar's Creek (HC)	1.168		5.006	7.763	13.249	42.906	7.988	9.348	2.469
Mobjack Bay (MB)	0.516	5.608		20.989	18.067	4.193	2.387	2.792	1.435
York River (YR)	0.639	4.678	3.731		54.986	7.734	3.764	4.641	1.994
South Bay (SB)	0.682	6.234	5.663	4.945		10.448	5.402	5.243	2.380
Hog Island Bay (HR6)	1.362	8.608	6.058	8.245	9.235		13.327	17.192	3.632
Hog Island Bay (HR7)	1.307	4.366	2.194	2.603	4.974	10.833		6.449	3.485
Spider Crab Bay (SC)	1.280	7.123	4.049	4.089	6.800	13.257	6.953		10.536
Chincoteague Bay (CH)	1.362	3.262	3.311	2.689	3.680	4.233	4.089	4.705	

which reduces competition and thus seedling survival. Phillips et al. (1983) showed that *Zostera marina* flowering increased due to environmental stress and disturbance, which suggests an increase in the source of seeds in disturbed areas. Modeling of clonal terrestrial plants has shown that frequent disturbance and high seedling recruitment can increase overall genotypic diversity (Watkinson & Powell 1993).

Seeds used in the coastal bay restoration sites were collected from as far as 80 km away, as no local source populations were available from the Virginia coastal bays. We detected no direct evidence of genetic erosion through outbreeding depression. Outbreeding depression occurs when locally adapted genotypes interbreed with non-adapted genotypes, resulting in reduced fitness of the progeny (Hufford & Mazer 2003), and usually occurs when different populations mix. Another potential genetic impact of population mixing is heterosis, often referred to as hybrid vigor, that occurs when deleterious alleles are masked or when an increase in heterozygosity results in progeny which are fitter relative to their parents (Hufford & Mazer 2003). Although heterosis is a positive effect of genetic mixing among the first-generation population hybrids, the next generation may experience reduced fitness as deleterious genetic traits are expressed in future generations. Since genetic structure was maintained by restoration with seeds, as long as donor sites are chosen carefully, these problems are more likely to be minimized.

The use of donor material for restoration from the closest populations, the coastal bay meadows in Chincoteague Bay (CH) or the very small population at the mouth of Chesapeake Bay (FI), may result in problems not encountered when Chesapeake Bay populations were used as donors. These 2 natural meadows in the Virginia coastal bay region have little gene flow and relatively high  $F_{st}$  values among

them and with Chesapeake Bay to the west ( $N_m$  ranges between 1.362 and 1.588,  $F_{st} = 0.136$ ) (Tables 2 & 5). Because they are geographically separated and differ genetically, it is possible they may have acquired distinct adaptations through either genetic drift or due to selection for fitness to local conditions. Using seeds from these locations could result in outbreeding depression if environmental conditions differ from those in the restoration sites.

We observed the genetic signature of Chincoteague Bay in the restored Virginia coastal bays, principally Hog Island Bay (HB6). When all samples were analyzed using a Bayesian cluster model, 4 distinct genetic clusters emerged, with Chincoteague Bay being relatively unique, except for a few samples in the more northern coastal bays (Fig. 4). This genetic signature could have been the result of 2 alternative mechanisms. First, small-scale (4 m<sup>2</sup>) test plots in South Bay seeded in 1999 used plants from Chincoteague Bay. These plots spread rapidly, and it is possible that flowering shoots with seeds could have drifted to Hog Island Bay and released seeds. Alternatively, flowering shoots with seeds could have drifted out of Chincoteague Bay south along the Atlantic coast and entered the coastal inlet near Hog Island Bay, releasing seeds as they floated over the bay. Flowering shoots with mature seeds can disperse long (150 km) distances (Harwell & Orth 2002, Källström et al. 2008), and it was suggested that natural recruits observed in 1997 in South Bay may indeed have developed from Chincoteague populations (Harwell & Orth 2002). Based on these previous studies, the Virginia coastal bays are within the colonization envelope of Chincoteague Bay *Zostera marina* populations. The detection of Virginia coastal bay *Z. marina* populations that share a specific genetic signal with Chincoteague Bay (Fig. 4) suggests recruitment via such long-distance dispersal events is likely occurring, although a more

targeted analysis would be needed to confirm the most likely source. Natural recruitment into the area suggests that a slow recovery may have already begun before restoration intervention was initiated.

The present study demonstrates that large-scale *Zostera marina* restoration with seed as the source of propagules maintains comparable levels of genetic diversity in donor populations. The donor meadows used in our study had a high genetic diversity, and the subsequent high diversity in the restored areas likely contributed to the success of the restoration by increasing resistance to ecosystem disturbances (for discussion see Hughes & Stachowicz 2004, 2011, Reusch et al. 2005). The positive effect of high genetic diversity is not limited to marine systems, and the use of seeds in the restoration of clonal terrestrial plants might also be advantageous. It should be noted that the Virginia coastal bays experience good water quality ([www1.vcrllter.virginia.edu/home1/?q=data\\_wq](http://www1.vcrllter.virginia.edu/home1/?q=data_wq)), and this has undoubtedly been important to the restoration success in this area given that eutrophication is the most common cause of seagrass loss (Orth et al. 2006a). Where restoration attempts are made with marginal water quality, stresses and disturbances are likely to reduce plant growth and survival. Previous studies suggest that genetically diverse assemblages of seagrass will be better at surviving disturbances, such as intense grazing events, temperature stress, and algal blooms (Hughes & Stachowicz 2004, 2011, Reusch et al. 2005). The present study also suggests that source material for *Z. marina* restoration can be collected from a relatively great distance away from the recipient site without a concern for genetic problems such as outbreeding depression.

The maintenance of genetic diversity can be used as one measure of restoration success, since high genetic diversity is associated with increased benefits for plant survival and ecosystem services (Booy et al. 2000). In our system, we demonstrate a method of restoration that maintains genetic diversity, and the results of that restoration are positive in terms of increased seagrass coverage and feedbacks on sediment and water-quality characteristics (Hanson & Reidenbach 2012 this Theme Section, McGlathery et al. 2012 this Theme Section, Orth et al. 2012 this Theme Section).

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# Nitrogen fixation in restored eelgrass meadows

Luke W. Cole\*, Karen J. McGlathery

Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia 22904, USA

**ABSTRACT:** Biological nitrogen ( $N_2$ ) fixation is the primary input of new nitrogen (N) to marine systems, and is important in meeting the N demands of primary producers. In this study, we determined whether restoration of the eelgrass *Zostera marina* L. in a shallow coastal bay facilitated increasing rates of  $N_2$  fixation as the meadows aged. Rates of  $N_2$  fixation were measured in a system that had been devoid of eelgrass following local extinction in the 1930s until restoration by seeding began in 2001. Restored meadows of different ages were compared to nearby bare sediment sites during summer peak metabolism over 2 yr. Nutrient addition by  $N_2$  fixation was enhanced as the meadows aged. Rates of  $N_2$  fixation in the older (7 to 8 yr old) meadows were 2.7 times more than the younger (2 to 3 yr old) meadows (average 390 and 146  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ , respectively), and 28 times more than bare sediments (average 14  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ). Heterotrophic epiphyte bacteria fixed approximately 90% of the total  $N_2$  in *Z. marina* meadows of both age classes. Both sediment and epiphyte  $N_2$  fixation were strongly related to *Z. marina* density and sediment organic content, suggesting that shoot density increases the positive feedback of plant presence on  $N_2$  fixation through the release of organic carbon exudates into the rhizosphere and phyllosphere, and the build up of sediment organic matter also increases. The N provided through fixation represented a large fraction (20.5 to 30%) of the total N demand to support eelgrass aboveground growth during this period of peak summertime production.

**KEY WORDS:** Nitrogen fixation · Seagrass · *Zostera marina* · Restoration · State change · Epiphytes · Sediments

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## INTRODUCTION

In many temperate coastal marine systems, nitrogen (N) is often the limiting nutrient for benthic and pelagic primary producers (Howarth 1988, Taylor et al. 1995, Tyler et al. 2003). Although dinitrogen ( $N_2$ ) gas constitutes 62.6% of the gases dissolved in seawater when at equilibrium with air (Pilson 1998),  $N_2$  is biologically unavailable except to specialized prokaryotic  $N_2$ -fixing bacteria (Howarth et al. 1988, Welsh et al. 2000) and some archaea (Dekas et al. 2009). To meet the N demand required for growth, primary producers rely on external N sources as well as internal recycling of N. In the open ocean, pelagic  $N_2$ -fixing bacteria provide a large source of N on an areal basis compared to sediments, although deep-sea benthic  $N_2$  fixation has only been minimally

assessed (Carpenter & Capone 2008, Dekas et al. 2009). In estuarine and lagoonal systems, pelagic  $N_2$  fixation is rarely considered to be an important process, and N inputs from benthic  $N_2$  fixation can vary, with higher rates typically measured in tropical systems (Howarth & Marino 2006). Although rates of benthic  $N_2$  fixation have often been assumed to be low in temperate coastal systems (Howarth & Marino 2006), studies have shown high rates of benthic  $N_2$  fixation, both in bare sediments (Gardner et al. 2006, Fulweiler et al. 2007) and in macrophyte-dominated sediments (Capone & Budin 1982, Howarth et al. 1988, McGlathery et al. 1998, McGlathery 2008). Thus  $N_2$  fixation can be a major source of N to benthic macrophytes (e.g. McRoy et al. 1973, O'Donohue et al. 1991, Moriarty & O'Donohue 1993, Welsh et al. 1996a), particularly in systems where pools of inor-

\*Email: lwc8j@virginia.edu

ganic N in the porewater are depleted (O'Donohue et al. 1991). N fixation is among the many ecosystem services provided by organisms associated with seagrass habitats in shallow coastal systems. Given the significant loss of seagrass habitats globally (Waycott et al. 2009), and concurrent large-scale restoration efforts (Orth et al. 2010), it is important to understand the influence of seagrass habitats on N inputs via fixation.

Seagrass-vegetated sediments often have higher rates of N<sub>2</sub> fixation than bare sediments, although there can be considerable overlap in rates. Studies in temperate estuarine and shallow bay systems report rates from 7 to 520  $\mu\text{mol N m}^{-2} \text{d}^{-1}$  (McGlathery 2008 and sources therein), while in bare sediments free of macrophytes or cyanobacterial mats, reported rates range from 0 to 300  $\mu\text{mol N m}^{-2} \text{d}^{-1}$  (Howarth et al. 1988 and sources therein, Nixon et al. 1996). In shallow, vegetated (sub)tropical systems, much higher N<sub>2</sub> fixation rates have been measured in the rhizosphere of *Zostera capricorni* (1785 to 2800  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ; O'Donohue et al. 1991) and in *Thalassia testudinum* (360 to 10 000  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ; McGlathery 2008 and sources therein). In vegetated sediments of some temperate systems, N<sub>2</sub>-fixing bacteria have been shown to provide 5 to 12% of the annual N requirement to the plant (Welsh et al. 1996a, McGlathery et al. 1998).

Heterotrophic N<sub>2</sub> fixers are typically the dominant functional group in seagrass-vegetated sediments (McGlathery et al. 1998), although autotrophic N<sub>2</sub> fixers are common on illuminated surface sediments (Stal et al. 1985, Paerl et al. 1996). Heterotrophic N<sub>2</sub>-fixing bacteria require organic carbon to fuel N<sub>2</sub> fixation (Penhale & Smith 1977, Kirchman et al. 1984, Welsh et al. 1996a), and the supply of organic carbon is enhanced by the presence of seagrasses through biological and physical mechanisms. Seagrass photosynthesis results in passive diffusion of photosynthate (dissolved organic carbon) into the rhizosphere, contributing a carbon source for heterotrophic N<sub>2</sub>-fixing bacteria (McRoy & Goering 1974, Penhale & Smith 1977, Oremland & Taylor 1977, Moriarty & Pollard 1982). Seagrasses also apply drag to the overlying water column, slowing water velocities and causing organic particles to fall out of solution and accumulate in seagrass sediments (Fonseca et al. 1982, Marbá et al. 2006). This increase in sediment organic matter potentially provides a carbon source for sediment N<sub>2</sub> fixers.

The relative importance of epiphyte N<sub>2</sub> fixation in seagrass meadows has been less studied than sediment N<sub>2</sub> fixation, and most studies have focused on

tropical systems (Goering & Parker 1972, Patriquin & Knowles 1972, McRoy et al. 1973, Capone & Taylor 1977, Capone et al. 1979, O'Donohue et al. 1991). Epiphytic N<sub>2</sub> fixers can be either autotrophic (Capone & Taylor 1977, Welsh et al. 2000) or heterotrophic (Goering & Parker 1972, Capone & Taylor 1977). Reported ratios of autotrophic to heterotrophic epiphyte N<sub>2</sub> fixation have ranged from one-third to two-thirds (Goering & Parker 1972, Capone & Taylor 1977), although most studies have been performed in the light and thus have been limited to gross autotrophic N<sub>2</sub> fixation. Heterotrophic epiphytes require an external carbon source, primarily photosynthate excreted from leaves (McRoy et al. 1973, McRoy & Goering 1974, Capone & Taylor 1977, Wetzel & Penhale 1979). It has been shown that the seagrass *Zostera marina* excretes photosynthate in proportion to the rate of primary production (Penhale & Smith 1977), suggesting that plants with high rates of primary productivity may in turn support high rates of epiphyte N<sub>2</sub> fixation.

The chronic, global loss of seagrass has resulted in a reduction in the capacity of shallow coastal systems to act as carbon and nutrient sinks (Duarte et al. 2010). Given the positive feedback between seagrass and N<sub>2</sub> fixation, restoring seagrass in a system devoid of rooted macrophytes can potentially increase the quantity of N introduced by fixation in these ecosystems. The Virginia (USA) coastal bays became a benthic algae-dominated system following the loss of *Zostera marina* due to disease and massive coastal storms in 1933. Following a nearly 70 yr absence of *Z. marina*, a large-scale restoration program by seeding, begun in 2001, has resulted in approximately 4500 acres (~1821 ha) of eelgrass habitat from 200 acres (~81 ha) initially seeded (Orth et al. 2006, 2010). A restoration program of this scale provides the opportunity to quantify the effects of a state change from an algae-dominated to eelgrass-dominated system on N cycling processes by comparing replicate plots of different ages (i.e. time since seeding). Here we report the effects of this state change on N<sub>2</sub> fixation, including those occurring in the sediments and by epiphytes in the eelgrass canopy.

## MATERIALS AND METHODS

### Site description

Over 200 km of shallow coastal bays extend from Delaware to Virginia, USA, bounded to the east by barrier islands and to the west by the Delmarva

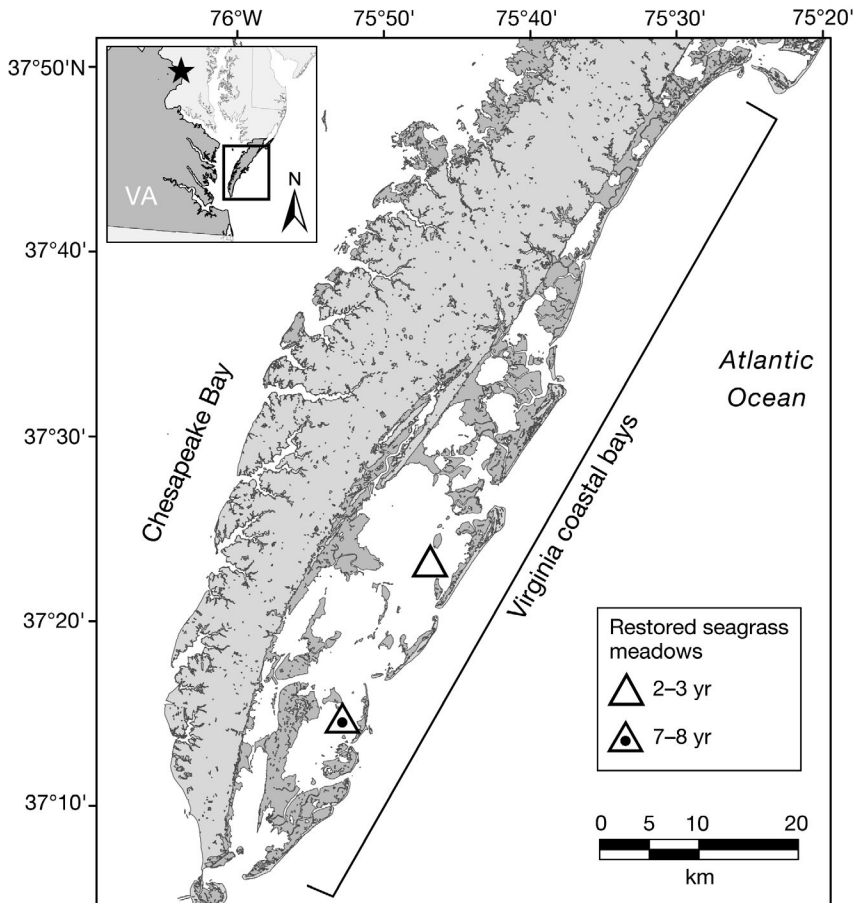


Fig. 1. Virginia (VA) coastal bays, USA. The 2 to 3 and 7 to 8 yr old eelgrass sites are within Hog Island Bay and South Bay, respectively. Land: light grey; marsh: dark grey; ★: Washington, DC

Peninsula (Fig. 1). Our study sites are within the Virginia coastal bay system, which is part of the Virginia Coast Reserve Long Term Ecological Research (VCR LTER) site. A large-scale experiment to mimic a state change from an algae- to an eelgrass-dominated system was initiated in 2001 by broadcasting seeds into 1 acre (~0.4 ha) plots at  $10^5$  seeds acre<sup>-1</sup> (Orth et al. 2006, 2010), 4 of which were monitored in the present study. In 2006, a similar area was seeded in 1 acre plots with  $10^5$  seeds acre<sup>-1</sup>, 4 of which were monitored in the present study. Four bare sites were identified adjacent to each eelgrass restoration site and were sampled concurrently. Here we compare the bare sites to the 2 to 3 yr old (seeded 2006; hereafter 2/3 yr old) and 7 to 8 yr old (seeded 2001, hereafter 7/8 yr old) eelgrass vegetated sites.

The study sites were located within 20 km of each other (37°24'47" N, 75°43'36" W and 37°15'54" N, 75°48'50" W) and were separated only by mudflats and a tidal channel (Fig. 1). The sites are similar with respect to bathymetry, hydrodynamics, and water

column and sediment characteristics. Both sites were shallow (<1 m at low tide) and had light penetration capable of supporting the seagrass *Zostera marina* to -1.6 m mean sea level (MSL) (Lawson et al. 2007, Carr et al. 2010). The vegetated sites were located between 0.9 and 1.4 m MSL while the bare sites ranged from 1.1 to 1.6 m MSL. The tidal amplitude was similar at both sites (1.3 versus 1.2 m; McGlathery et al. 2012, this Theme Section [TS]). Bare sediment characteristics at the sites, including organic matter, N and carbon content, exchangeable NH<sub>4</sub><sup>+</sup>, and grain size, were also not significantly different (McGlathery et al. 2012). The largest input of N to the Virginia coastal bay system is direct atmospheric deposition, and the total N load from all sources was calculated to be approximately ~2.1 g N m<sup>-2</sup> yr<sup>-1</sup> (authors' unpubl. data), which is extremely low compared to similar shallow coastal bay systems (McGlathery et al. 2007).

### N<sub>2</sub> fixation

In June and July of 2008 and 2009, sediment and epiphyte N<sub>2</sub> fixation rates were quantified using the acetylene-reduction technique (methods modified from Capone & Taylor 1977, Capone 1982). Data from June and July were selected to represent peak rates of N<sub>2</sub> fixation based on seasonal and monthly data from 2008 and 2009, respectively. Two sediment cores were collected in each of the 4 replicate plots of each eelgrass age class and the 8 bare sites. The 2 sediment cores were taken by hand in both vegetated and bare sediment using a 1 inch (~2.5 cm) inner diameter clear acrylic corer at random locations within each replicate eelgrass plot, making sure the aboveground eelgrass biomass was not included. Cores were closed on either end with rubber stoppers, held at ambient temperature in site water, and taken into the laboratory where the top stoppers were removed and the cores were bubbled with an aquarium motor equipped with a diffuser until processing (not more than 2 h) in the dark. For the incubation, a sub-core was taken from each large core using a 5 cm<sup>3</sup> syringe corer down to 5 cm. The sub-core was split lengthwise to provide adequate exposure to the acetylene and to give a depth-integrated estimate of N<sub>2</sub> fixation

rates. Each half was placed in a 40 cm<sup>3</sup> incubation vial with a glass bead and 0.7 ml of ammonium-free artificial seawater, and capped with a screw-top collar holding a septum in place. Samples were sparged with argon gas for 2 min, after which time 10 ml of acetylene (C<sub>2</sub>H<sub>2</sub>) gas was injected into the vials. The vials were then shaken for 10 s and vented to atmospheric pressure. Vials were kept in the dark at field temperature and incubated for 6 to 8 h. Transferring the gas samples into a pre-evacuated vial ended the incubation. Gas samples were run on a Varian Star 3400 Cx gas chromatograph equipped with a flame ionization detector and a 1.86 × 0.32 × 0.25 cm Teflon-coated column filled with Porapak N 80/100. For scaling purposes, the halved sub-core samples were summed and extrapolated to area units using the aperture of the core. Rates of sediment acetylene reduction were scaled to fixed N<sub>2</sub> (as NH<sub>4</sub><sup>+</sup>) using a 3:1 molar conversion (Seitzinger & Garber 1987). An initial analysis to determine the potential for autotrophic sediment N<sub>2</sub> fixation in vegetated sediments showed no differences within a 2 × 2 design for oxic versus anoxic and light versus dark incubation ( $F = 1.76$ ,  $p = 0.2$ ), with all rates comparable to N<sub>2</sub> fixation rates in dark anoxic sediment. Autotrophic N<sub>2</sub> fixation was calculated as the difference between incubations in the light (reflecting activity of both autotrophs and heterotrophs) and dark (heterotrophic activity only). An additional analysis to measure the percentage of N<sub>2</sub> fixation by sulfate-reducing bacteria was performed on replicate cores using the presence/absence of molybdate (40 mM Na<sub>2</sub>MoO<sub>4</sub>) to suppress sulfate reduction (Howarth et al. 1988), and the treatment resulted in no significant differences between replicates. Based on these findings, and to increase the number of replicates, we used anoxic and dark conditions without molybdate in subsequent incubations.

A profile of sediment N<sub>2</sub> fixation with depth was done at 1 cm intervals down to 5 cm in both bare and vegetated plots in May 2009. Samples were inoculated with C<sub>2</sub>H<sub>2</sub> and incubated in the same manner as described above.

Shoots of *Zostera marina* were collected haphazardly in each of the 4 replicate plots in the 2/3 and 7/8 yr old meadows, placed into clear, zip-top bags filled with site water, and kept at field temperature. Shoots were taken directly to the laboratory (within 2 h of collection) for analysis. Eight shoots per bay were analyzed for N<sub>2</sub> fixation, chosen randomly from the 5 shoots collected in each replicate plot (2 per replicate plot were analyzed). For each shoot, the length and width of each leaf was recorded to calcu-

late leaf area, and the top 15 cm of leaves 2 and 4 were placed into 120 ml serum vials filled with 90 ml of filtered site water, and then 10 ml of acetylene-saturated, filtered site water was added. The vials were corked with a septum, crimped with an aluminum collar, shaken for 30 s, and incubated at field temperatures. For the epiphytes, an initial test comparing light and dark incubations similar to that done for the sediments indicated that 99% of the epiphyte N<sub>2</sub> fixation was heterotrophic (dark incubations), and so subsequent incubations were done in the dark. Incubations lasted for 4 to 6 h, and were ended by shaking the bottle vigorously for 30 s, waiting 10 s to allow bubbles trapping gases to dissolve, and then the headspace was drawn into a pre-evacuated vial using a double-ended needle for storage until it was run on the gas chromatograph. As for the sediments, rates of epiphyte acetylene reduction were scaled to fixed N<sub>2</sub> (as NH<sub>4</sub><sup>+</sup>) using a 3:1 molar conversion (Seitzinger & Garber 1987). The fixed N<sub>2</sub> was representative of the leaf area of the top 15 cm of the leaves incubated. To scale to an areal rate of N<sub>2</sub> fixation, the average of the 2 leaves incubated was scaled up per shoot, using the area of the top 15 cm of the leaves on the shoot. We incubated the top 15 cm of the leaf knowing that N<sub>2</sub> fixation is less at the base of the leaf since epiphyte colonization is a function of leaf age, and we observed little epiphyte biomass below the top 15 cm of the leaves. However, since our calculated values do not include the entire leaf surface of the plant, our method may give a conservative estimate of epiphyte N<sub>2</sub> fixation. This value was multiplied by shoot density to calculate an areal N<sub>2</sub> fixation rate in  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ .

### Site characterization

Eelgrass density and plant productivity were measured concurrently with the N<sub>2</sub> fixation measurements in both 2008 and 2009. At each of the 4 replicate plots for each age category, 10 PVC quadrats (0.25 m<sup>2</sup>) were thrown and the shoots were counted. Plant productivity was measured on all shoots ( $n = 5$  to 20) within 2 anchored grids (20 × 10 cm) using the leaf marking method with the top of the sheath bundle as the reference point, puncturing the blades with a 22-gauge needle (Zieman 1974). Shoots were collected 10 to 15 d after marking. Leaf area and dry weight of the older growth (above the scar) and the new growth (below the scar, but excluding the sheath bundle and including new shoots) were measured separately.

Sediment organic and C:N contents were determined in July in both 2008 and 2009 at the 4 replicate plots in both the 2/3 yr old and 7/8 yr old eelgrass meadows and the 8 adjacent bare sites. Five cores were collected equidistant along a 50 m transect in the middle of each plot using a 60 ml syringe, bagged, and placed immediately on ice. Sediment organic content was calculated as loss on ignition from sediment dry weight after combustion in a 500°C muffle furnace for 8 h. Carbon and N contents were measured on dried sediment (60°C for at least 48 h) using a Carlo Erba Elemental Analyzer with a 1020°C combustion tube and 650°C reduction tube and helium as a carrier gas.

### Statistics

For sediment characteristics, replicate samples from each replicate plot were averaged to provide values for each of the bare sites and for the vegetated sites ( $n = 8$  for bare,  $n = 4$  for each eelgrass age class). An initial test showed that the sediment characteristics of the bare sites at the 2 locations were not significantly different, so all bare sites were pooled for further comparisons with the eelgrass sites in each age class. For the eelgrass characteristics, replicate measurements of densities and productivities within each plot were also averaged, and all replicate plots for each age class were then averaged. Differences in

pooled sediment and seagrass parameters were tested using analysis of variance (ANOVA); all parameters conformed to ANOVA assumptions (heteroscedasticity and normality). Epiphyte N<sub>2</sub> fixation rates were first scaled to the whole shoot, and then rates were averaged within each replicate plot based on average shoot densities for that plot; these values were then averaged to obtain mean and variance for the 2 eelgrass age classes. Analyses of epiphyte and sediment N<sub>2</sub> fixation, eelgrass characteristics, and sediment profiles as a function of *Zostera marina* meadow age (time since seeding) were done using a 2-way ANOVA, and pairwise differences were tested using post hoc Tukey's tests (SAS version 9.2). Linear regressions were used to analyze relationships between N<sub>2</sub> fixation rates and plant characteristics or chemical variables using statistical software (Minitab). For all comparisons,  $p$ -values  $\leq 0.05$  were considered significant.

## RESULTS

### Sediment N<sub>2</sub> fixation

Heterotrophs were the dominant functional group fixing N<sub>2</sub> in the sediments, as indicated by the similar rates between light and dark incubations. Sulfate-reducing N<sub>2</sub>-fixing bacteria were responsible for a relatively small percentage of the sediment N fixation, contributing 16% of the sediment N<sub>2</sub> fixation in both bare and vegetated sites. Sediment N<sub>2</sub> fixation rates were significantly higher in 2008 than in 2009, possibly due to low temperatures in 2009 (Fig. 2). In 2008, peak sediment N<sub>2</sub> fixation rates (June to July) in the 7/8 yr old eelgrass meadows (mean  $\pm$  SE =  $32.5 \pm 1.2 \mu\text{mol N m}^{-2} \text{d}^{-1}$ ) were significantly higher than in the 2/3 yr old meadows ( $21 \pm 2.0 \mu\text{mol N m}^{-2} \text{d}^{-1}$ ) and bare sediments ( $18 \pm 1.5 \mu\text{mol N m}^{-2} \text{d}^{-1}$ ; Fig. 2). In 2009, however, the only significant differences were between the 7/8 yr old meadows ( $18 \pm 1.0 \mu\text{mol N m}^{-2} \text{d}^{-1}$ ) and bare sediments ( $9.7 \pm 3.0 \mu\text{mol N m}^{-2} \text{d}^{-1}$ ; Fig. 2). There were no significant differences between the bare sediments and 2/3 yr old meadows in either year. Depth profiles of sediment N<sub>2</sub> fixation showed that rates increased with depth up to 5 cm in the eelgrass-vegetated sediments and decreased with depth in the bare sediments (Fig. 3).

Shoot density, areal rates of primary productivity, and sediment organic matter were identified as environmental variables that could influence sediment N<sub>2</sub> fixation rates in eelgrass-vegetated sediments of different ages (time since seeding; Table 1). The in-

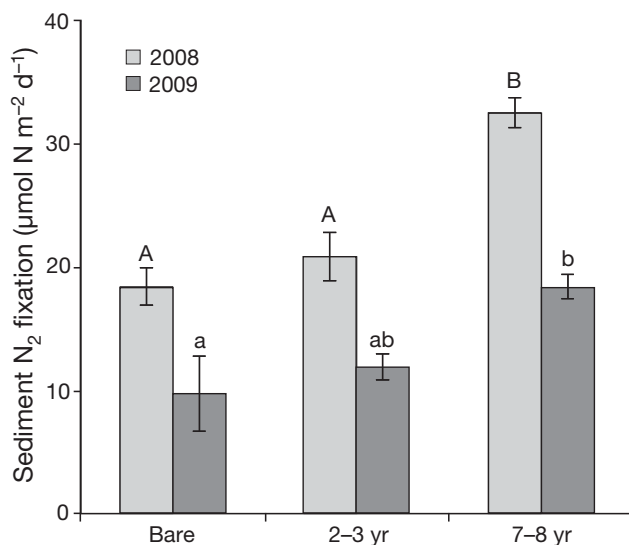


Fig. 2. Sediment N<sub>2</sub> fixation in bare sediment (bare) and in the rhizosphere of restored *Zostera marina* meadows (2 to 3 and 7 to 8 yr old). Different letters indicate statistically significant differences (ANOVA, post hoc Tukey's test;  $p < 0.05$ ); case of letter is relative to year. Means  $\pm$  1 SE

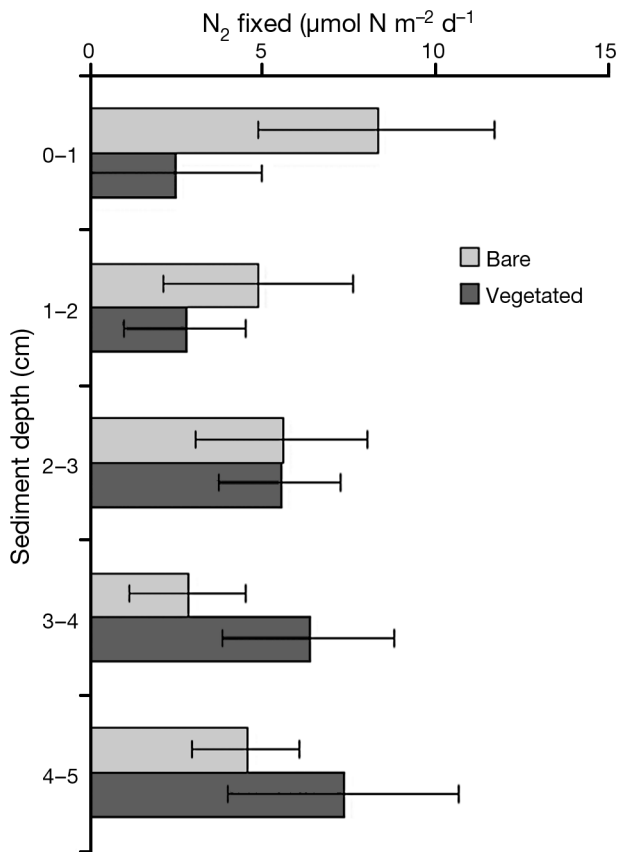


Fig. 3. Depth profile of sediment  $N_2$  fixation in bare and *Zostera marina* vegetated sediment. No significant differences across depth or sediment type are apparent, although peak rates and depth relationships are different in the presence versus absence of eelgrass. Means  $\pm$  1 SE

crease in *Zostera marina* shoot density was positively correlated with sediment  $N_2$  fixation in both 2008 and 2009 (Fig. 4). Additionally, the higher rates of areal productivity were positively correlated with higher rates of sediment  $N_2$  fixation, though only significant in 2008 (Fig. 4). Lastly, higher sediment organic mat-

ter was positively correlated with sediment  $N_2$  fixation, though again only significant in 2008 (Fig. 4). Despite having an  $r^2 = 0.93$ , the low number of samples in 2009 yielded a linear regression that was not significant.

### Epiphyte $N_2$ fixation

Rates of epiphyte  $N_2$  fixation were higher in the 7/8 yr old *Zostera marina* meadows than in the 2/3 yr old meadows during both years (Fig. 5). Heterotrophs were the dominant functional group of the epiphytic  $N_2$  fixers in both the 7/8 and 2/3 yr old meadows; our initial test indicated that autotrophs fixed <1% of the total  $N_2$  fixed. Rates of epiphyte  $N_2$  fixation in 2009 were significantly higher than in 2008 (Fig. 5), an opposite trend than what was measured for sediment  $N_2$  fixation (Fig. 2).

Areal rates of epiphyte  $N_2$  fixation were positively correlated with *Zostera marina* shoot density, though not significantly in either year (Fig. 6). Furthermore, shoot-specific productivity did not vary between sites of different ages, but did vary by year (Table 1). Because epiphyte  $N_2$  fixation rates were not significantly correlated with shoot density, but were positively correlated with areal productivity rates in both 2008 and 2009, we show that the areal productivity effect occurred independently of shoot density (Fig. 6). The slopes of the regressions for epiphyte  $N_2$  fixation versus density and productivity were considerably steeper in 2008 than in 2009 (Fig. 6).

To determine what variable was affecting the difference in rates of  $N_2$  fixation and plant productivity, we analyzed temperature records during the sampling periods. We found a significant difference in air temperature for June and July in 2008 and 2009, with a higher mean temperature in June and July 2008 of 0.81 °C (VCR LTER, [www.vcr.lter.virginia.edu/data/metdata/index.html](http://www.vcr.lter.virginia.edu/data/metdata/index.html)).

Table 1. *Zostera marina*. Eelgrass and sediment parameters of restored *Z. marina* meadows and associated bare sediment. Means  $\pm$  SE

	Density (shoots $m^{-2}$ )	Shoot productivity ( $cm^2 d^{-1}$ )	C:N (sediment)	Organic matter (sediment) (%)
Bare	–	–	9.0 $\pm$ 1.8	1.48 $\pm$ 0.1
2–3 yr old				
2008	33 $\pm$ 5	0.75 $\pm$ 0.05	18.7 $\pm$ 6.7	1.3 $\pm$ 0.3
2009	88 $\pm$ 51	0.18 $\pm$ 0.09	5.9 $\pm$ 2.2	–
7–8 yr old				
2008	412 $\pm$ 21	0.87 $\pm$ 0.16	5.2 $\pm$ 0.7	1.74 $\pm$ 0.6
2009	515 $\pm$ 22	0.27 $\pm$ 0.12	8.1 $\pm$ 1.0	–

### Eelgrass and sediment parameters

Eelgrass and sediment parameters for the bare sediments and different-aged eelgrass meadows showed significant differences by both meadow age and sampling year (Table 1). The 7/8 yr old *Zostera marina* meadows had significantly higher shoot densities than the 2/3 yr old meadows in

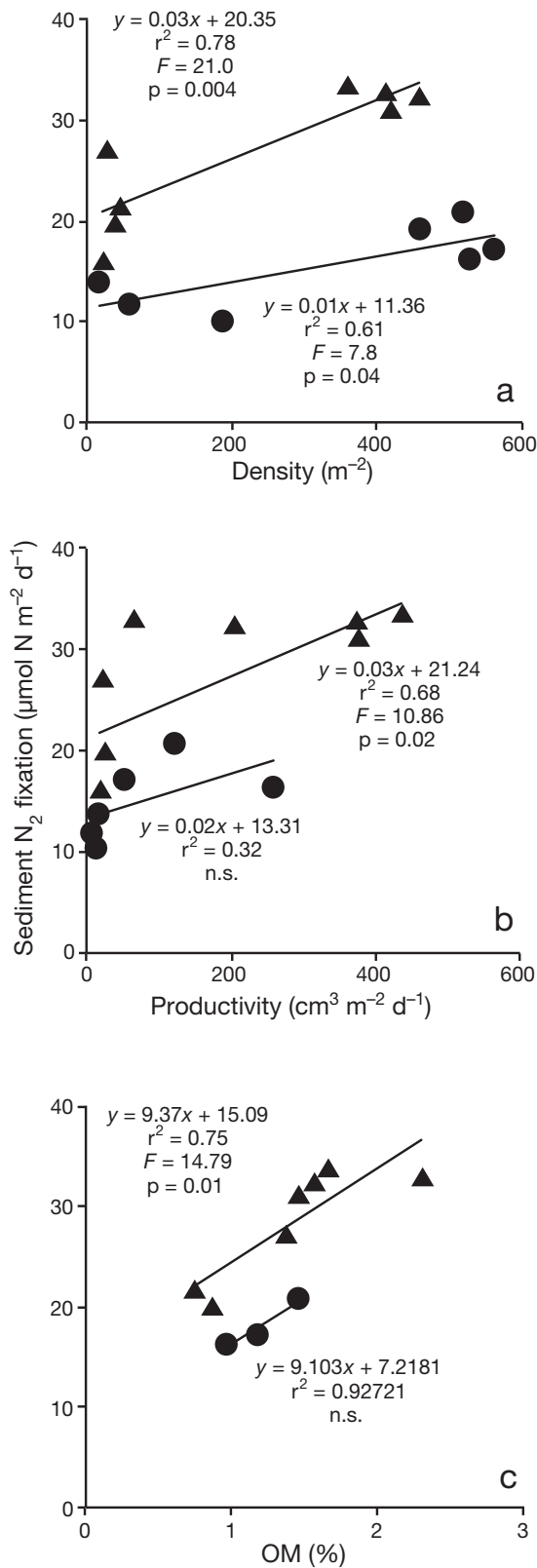


Fig. 4. Sediment N<sub>2</sub> fixation regressed with (a) eelgrass *Zostera marina* shoot density and (b) areal productivity, and (c) sediment organic matter (OM) for the years 2008 (▲) and 2009 (●)

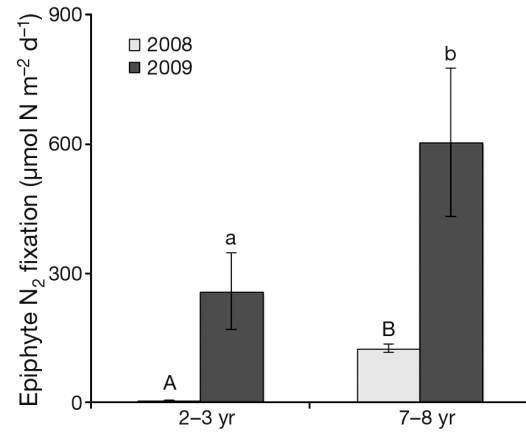


Fig. 5. *Zostera marina*. Epiphyte N<sub>2</sub> fixation in the eelgrass meadows of different ages (2 to 3 and 7 to 8 yr old). Significant differences represented by difference in within-case letter coding (ANOVA, post hoc Tukey's test;  $p < 0.05$ ); case is relative to year. Means ± 1 SE

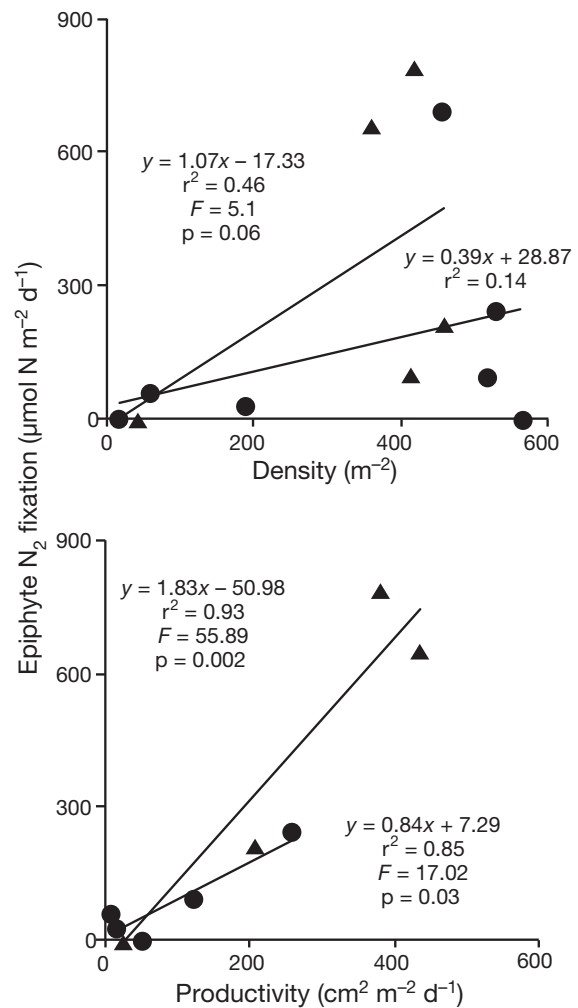


Fig. 6. *Zostera marina*. Regressions of epiphyte N<sub>2</sub> fixation with eelgrass shoot density and areal productivity for the years 2008 (▲) and 2009 (●)

Table 2. *Zostera marina*. ANOVA table of p-values ( $\alpha = 0.05$ ) for biological and physical eelgrass characteristics between meadow ages (2 to 3 and 7 to 8 yr old) and over the course of this study

Block	Comparison	Density	Productivity	Foliar N	Foliar C	C:N ratio
2–3 yr	2008 vs. 2009	0.26	0.005	–	–	–
7–8 yr	2008 vs. 2009	0.01	0.04	–	–	–
2008	2–3 vs. 7–8 yr	<0.001	0.54	–	–	–
2009	2–3 vs. 7–8 yr	<0.001	0.56	–	–	–
All	2–3 vs. 7–8 yr	<0.001	<0.001	<0.001	0.33	<0.01

both 2008 and 2009 (Table 2). The rates of productivity per shoot were not different between the two meadow types in 2008 or 2009. However, productivity rates per shoot were higher at both eelgrass sites in 2008 than in 2009, presumably due to the higher mean water temperature in 2009 (Table 2). The average N content of *Z. marina* tissue was significantly higher in the 2/3 yr old meadows (2.3% versus 1.2% in the older meadows). Average C:N indicated that the younger meadows were significantly less N-limited than the older meadows (C:N 20.6 versus 37.3). The 7/8 yr old meadows had a significantly higher sediment organic matter than the younger meadows and the bare sediments; however, there were no differences in sediment organic matter between the 2/3 yr old meadows and bare sediments ( $p = 0.32$ ).

## DISCUSSION

### Total rates of N<sub>2</sub> fixation

Our data show that N<sub>2</sub> fixation in restored eelgrass meadows was enhanced as the meadows aged. The markedly higher rates of N<sub>2</sub> fixation in the 7/8 yr old *Zostera marina*-vegetated system compared to the unvegetated sediments illustrate the effects of the state change from a bare to a macrophyte-dominated benthos. N<sub>2</sub>-fixing bacteria in the older meadows fixed on average 390  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ , 2.7 times more than the younger meadows (146  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ), and 28 times more than bare sediment sites (14  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ), and the majority of the N<sub>2</sub> fixed was by heterotrophs (Fig. 7). The higher variance at the vegetated sites was a result of the high and variable rates of epiphyte N<sub>2</sub> fixation. Our findings are in agreement with previous studies showing a positive feedback between seagrass presence and rates of N<sub>2</sub> fixation (Capone 1988, Welsh et al. 2000, Carpenter & Capone 2008), but show for the first time that this

feedback is based on seagrass density. N<sub>2</sub> fixation rates in the younger meadows where shoot densities were on average <100 shoots  $\text{m}^{-2}$  were not different than nearby unvegetated sediments, indicating there this is a lag time after initial seeding before this feedback is apparent at the  $\text{m}^{-2}$  scale. Although we do not know the exact threshold density where vegetated sediments had higher N<sub>2</sub> fixation rates than bare sediments, our data show that by 7 to 8 yr since seeding when densities were >400 shoots  $\text{m}^{-2}$ , rates were significantly greater.

The rates of N<sub>2</sub> fixation in the older meadows of the Virginia coastal bays are within the range reported for seagrass meadows in other temperate regions, indicating that by 7 to 8 yr since seeding, this function had been restored relative to established meadows. In a study of *Zostera marina* in the Chesapeake Bay, Capone (1982) found rates of N<sub>2</sub> fixation (279 to 464  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ) comparable to our 7/8 yr old site. The results of 2 European studies of *Z. marina* and *Z. noltii* are also consistent with our findings, with rates of N<sub>2</sub> fixation ranging from 300 to 429  $\mu\text{mol N m}^{-2} \text{d}^{-1}$  (McGlathery et al. 1998) and 143 to 521  $\mu\text{mol N m}^{-2} \text{d}^{-1}$  (Welsh et al. 1996a), respectively. It is important to note that the total N<sub>2</sub> fixation rates were equivalent primarily because our epiphyte N<sub>2</sub> fixation rates were very high. Our rates of total N<sub>2</sub> fixation only include heterotrophic N<sub>2</sub> fixation based on our find-

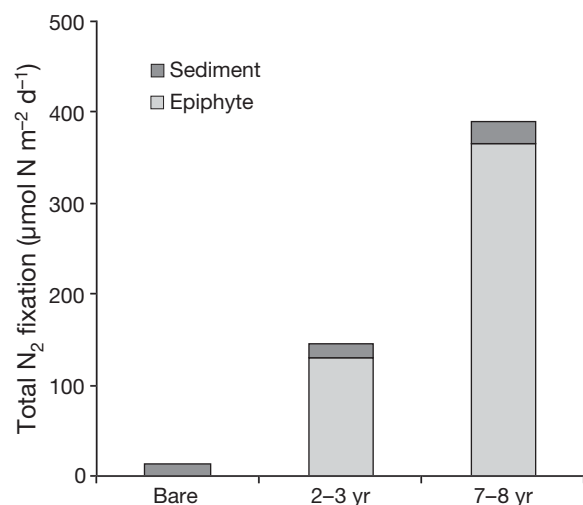


Fig. 7. *Zostera marina*. Total rates of N<sub>2</sub> fixation. Bare: sediment N<sub>2</sub> fixation in bare sediment only. Epiphyte: *Zostera marina* vegetated sediments; sum of sediment N<sub>2</sub> fixation and epiphyte N<sub>2</sub> fixation. Variance of rates and statistics are reported in 'Results' and 'Discussion'



ings that autotrophic N<sub>2</sub> fixation contributed a minor amount of N<sub>2</sub> fixed, and thus may be a slightly conservative estimate of total N<sub>2</sub> fixation rates.

### Contributions of epiphyte and sediment N<sub>2</sub> fixation

The majority of total N<sub>2</sub> fixation (epiphyte + sediment N<sub>2</sub> fixation) in the eelgrass meadows of both age classes was attributed to epiphytes on the eelgrass blades, which accounted for 89 and 93% of the total N<sub>2</sub> fixed in the younger and older meadows, respectively (Fig. 7). On an areal basis, epiphyte N<sub>2</sub> fixation was higher in the older *Zostera marina* meadows in both years. Because shoot-specific rates of N<sub>2</sub> fixation and primary productivity were not different between meadows in the different age classes, we attribute the higher epiphyte N<sub>2</sub> fixation in the older meadows to differences in shoot densities that were a function of eelgrass meadow age (Table 1, Fig. 6).

The dominance of N<sub>2</sub> fixation by epiphytes compared to sediments has not been shown previously in other temperate seagrass meadows. We know of only 1 study in a tropical system, the Gulf of Carpentaria, Australia, where N<sub>2</sub> fixation was dominated by epiphytes for the seagrass *Enhalus acoroides* (300  $\mu\text{mol N m}^{-2} \text{d}^{-1}$  compared to rinsed roots at 21  $\mu\text{mol N m}^{-2} \text{d}^{-1}$  and rhizomes at 7  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ) during summer months (Moriarty & O'Donohue 1993). In our study, summertime epiphyte N<sub>2</sub> fixation rates ranged from 2 to 256  $\mu\text{mol N m}^{-2} \text{d}^{-1}$  (mean 129  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ) in the younger meadows, and rates were significantly higher (123 to 604  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ; mean 364  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ) in the older seagrass meadows (Fig. 5). For temperate seagrass meadows, previous studies have shown that *Zostera marina* leaves collected in North Carolina, USA, had low to undetectable rates of epiphyte N<sub>2</sub> fixation (McRoy et al. 1973), and *Ruppia maritima* collected from brackish water in Chesapeake Bay had rates of 5  $\mu\text{mol N m}^{-2} \text{d}^{-1}$  (Lipschultz et al. 1979). A comparison with tropical systems shows some similar rates to those we measured, where N<sub>2</sub> fixation associated with *Thalassia testudinum* leaves ranged from 82 to 228  $\mu\text{mol N m}^{-2} \text{d}^{-1}$  (Capone et al. 1979) to 285 to 357  $\mu\text{mol N m}^{-2} \text{d}^{-1}$  (Capone & Taylor 1977). The high rates of epiphyte N<sub>2</sub> fixation in the Virginia coastal bays may be in part related to the low nutrient status and high water clarity of these coastal bays. External nutrient loading rates to the Virginia coastal

bays are very low compared to other shallow coastal bay systems (McGlathery et al. 2007, Giordano et al. 2011, authors' unpubl. data), and the 16 yr database from the VCR LTER shows that water column chlorophyll was very low (chl *a*: 1 to 6.5  $\mu\text{g l}^{-1}$ ) and has shown no trends of decreasing water quality during this period ([www1.vcr.lter.virginia.edu/home1/?q=data\\_wq](http://www1.vcr.lter.virginia.edu/home1/?q=data_wq)). Additionally, Orth et al. (2012, this TS) report median turbidity values to be very low, typically <10 NTU from 2003 to 2009.

Sediment N<sub>2</sub> fixation measured in the rhizosphere of *Zostera marina* only comprised on average 11% of the total N<sub>2</sub> fixed in the younger meadows and 7% in the older meadows. Rates of N<sub>2</sub> fixation in the 7/8 yr old *Z. marina* meadows were significantly higher than in the younger meadows in 2008, but not in 2009, although the trend of increasing sediment N<sub>2</sub> fixation with eelgrass meadow age was evident that year. This difference may be due to the lower temperatures in 2009 compared to 2008, which could have translated into lower eelgrass productivity rates. We found that rates of sediment N<sub>2</sub> fixation in the rhizosphere of the restored *Z. marina* meadows during the summer months were lower than previously reported values for vegetated sediments in coastal Virginia (16 to 25  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ , present study; 279 to 464  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ , Capone 1982). Peak sediment N<sub>2</sub> fixation rates reported from temperate European seagrass meadows were also consistently higher than those found in the present study (143 to 521  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ , Welsh et al. 1996a; 300 to 429  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ , McGlathery et al. 1998). We suspect that the rates of sediment N<sub>2</sub> fixation in the present study were much lower than the older, more established seagrass meadows reported in the literature because the shoot densities in our study location are comparatively low (our Table 1; Neckles et al. 1993, Moore & Wetzel 2000), and this influences the positive feedback between seagrass presence and N<sub>2</sub> fixation.

### Drivers of N<sub>2</sub> fixation

The mechanisms behind the positive relationship between *Zostera marina* shoot density and areal sediment and epiphyte N<sub>2</sub> fixation rates that were apparent in the meadows of different ages during both years are likely the stimulation of bacterial activity in the rhizosphere by the excretion of organic carbon from eelgrass roots and also by accumulation of organic matter in the sediments

(Figs. 2 & 6). The majority of the  $N_2$  fixation (both sediment and epiphyte) in the Virginia coastal bays was heterotrophic, and it is well known that heterotrophs in seagrass-vegetated sediments can be stimulated by carbon inputs from both plant metabolism (McRoy & Goering 1974, Penhale & Smith 1977, Kirchman et al. 1984, Moriarty et al. 1986) and by accumulation of organic carbon (Moriarty et al. 1986, Welsh et al. 1996b, McGlathery et al. 1998). The depth profiles of  $N_2$  fixation in the sediment highlighted the influence of eelgrass on  $N_2$  fixation, showing peak rates in the rhizosphere of vegetated sediments where maximum root biomass occurred (4 to 5 cm below surface), while the peak in bare sediment cores was at the surface (0 to 1 cm; Fig. 3). This is consistent with other studies showing depth-related differences in bacterial processes in seagrass-vegetated sediments (O'Donohue et al. 1991, Moriarty & O'Donohue 1993, McGlathery et al. 1998). The sediments in the older, more dense eelgrass meadows, where the sediment organic content was significantly higher, also had higher sediment  $N_2$  fixation than bare sediments (Fig. 2). But unlike many other temperate systems with more mature seagrass meadows, the role of  $N_2$ -fixing sulfate-reducing bacteria was relatively low; sulfate reducers fixed only 12 to 20% of the total  $N_2$  compared to >80% (Capone 1982, Welsh et al. 1996b), although similar rates of  $N_2$  fixation by sulfate-reducing bacteria have been observed in some meadows (25% of total  $N_2$  fixed, McGlathery et al. 1998). The low concentrations of organic matter in the rhizosphere (1 to 1.8%) may limit sulfate reduction in these sandy sediments.

The low levels of sediment organic matter likely contributed to the overall low levels of sediment  $N_2$  fixation in the Virginia coastal bays compared to other sites. We suspect that the consistently low anthropogenic N input rates, water column N, and chlorophyll concentrations in the Virginia coastal bays (Giordano et al. 2011, authors' unpubl. data, VCR LTER database, [www1.vcr.lter.virginia.edu/home1/?q=data\\_wq](http://www1.vcr.lter.virginia.edu/home1/?q=data_wq)) limit the input of organic carbon to the benthos (Nixon et al. 2009). With low inputs of organic carbon, heterotrophic  $N_2$  fixers cannot produce the ATP required for  $N_2$  fixation (e.g. Herbert 1975, Welsh et al. 2000). As seagrass meadows develop over time, both in size and shoot density, the seagrass community will exert more drag on the overlying water column and will increase the deposition of organic particles from the overlying water column and reduce sediment suspension (Fonseca et al. 1982, Moore 2004, Marbá et al. 2006, Chen et al.

2007, Gruber & Kemp 2010, Hansen & Reidenbach 2012, this TS). Our findings suggest the mechanism of drag-induced particle deposition can be affected by meadow age as seagrass density and sediment organic matter increase because both of these variables correlated positively to an increase in sediment  $N_2$  fixation (Fig. 4).

### Contribution to *Zostera marina* N demand

N demand was calculated from the N content of the eelgrass leaves, the shoot-specific primary production rate, and shoot density. During the study period, the N demand for the 2/3 yr old eelgrass meadow was  $488 \mu\text{mol N m}^{-2} \text{d}^{-1}$  and  $1903 \mu\text{mol N m}^{-2} \text{d}^{-1}$  for the 7/8 yr old meadow. The 16.4 and  $25.8 \mu\text{mol N m}^{-2} \text{d}^{-1}$  supplied by sediment  $N_2$  fixation in the younger and older meadows, respectively, had the potential to supply 3.4 and 1.4% of the total N demand of each eelgrass meadow. The larger amount of  $N_2$  fixed by epiphytes had the potential to provide 19 to 26.5% to the total N demand in the older and younger meadows, respectively. Combining the sediment and epiphyte  $N_2$  fixation rates, we see that  $N_2$  fixation could supply 29.8% of the total *Z. marina* N demand in the younger meadows and 20.5% in the older meadows, respectively, during this period of high summer production. These contributions are higher than those reported previously for temperate seagrass meadows on an annual basis (5 to 12% of the N requirement, Welsh et al. 1996a, McGlathery et al. 1998), largely due to the significant contribution of epiphyte  $N_2$  fixation. The C:N ratios for the eelgrass tissues in the restored meadows (younger: 21, older: 37) and the low N content of the leaves (younger: 2.3%, older: 1.2%) suggest that the plants were N limited (Duarte 1990), and internal recycling of N before tissue senescence may reduce the demand for external sources (Pedersen & Borum 1993).

We expect that as the restored eelgrass beds continue to age, remineralized N will provide a greater proportion of the N required to support growth as sediment organic matter and standing stock nutrient concentrations increase (Bronk & Steinberg 2008, McGlathery 2008). Tyler et al. (2003) found this pattern in a *Spartina alterniflora*-dominated salt marsh chronosequence of sites in the VCR ranging in age from 7 to 150 yr, where sediment  $N_2$  fixation decreased with stand age presumably as a result of increased porewater  $\text{NH}_4^+$  concentrations.

### System-wide scaling

After over a 70 yr period when the coastal bays of the VCR were devoid of seagrasses, restored *Zostera marina* meadows (7 to 8 yr old in this study) fixed N<sub>2</sub> at rates comparable to established mature meadows primarily because of high rates of heterotrophic epiphyte N<sub>2</sub> fixation, and these rates were nearly 30-fold higher than bare sediments. If the rates of N<sub>2</sub> fixation for the older eelgrass meadows are scaled to *Z. marina* potential habitat in the VCR coastal bays (depths at MSL between 0.6 and 1.6 m; Carr et al. 2010, 2012, this TS), we see the potential for nearly  $3.3 \times 10^5$  kg N to be fixed each year, which equates to 77% of the allochthonous N from the watersheds of the VCR (authors' unpubl. data). In comparison, if the same potential habitat was to remain unvegetated, the N<sub>2</sub> fixed would be only 3% of the total allochthonous N delivered to the coastal bays. Our study indicates that continued spread of *Z. marina* would facilitate a positive feedback of N<sub>2</sub> fixation that supplements the plant and bacterial communities, potentially supporting continued expansion of the eelgrass meadow into uncolonized, bare sediment.

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# Eelgrass survival in two contrasting systems: role of turbidity and summer water temperatures

Kenneth A. Moore\*, Erin C. Shields, David B. Parrish, Robert J. Orth

Virginia Institute of Marine Science, School of Marine Science, 1208 Greate Road, College of William and Mary, Gloucester Point, Virginia 23062, USA

**ABSTRACT:** Eelgrass *Zostera marina* L. distribution patterns in the mid-Atlantic region of the USA have shown complex changes, with recovery from losses in the 1930s varying between the coastal lagoons and Chesapeake Bay. Restoration efforts in the coastal bays of Virginia introduced *Z. marina* back to this system, and expansion since 2005 has averaged 66% yr<sup>-1</sup>. In contrast, Chesapeake Bay has experienced 2% expansion and has undergone 2 significant die-off events, in 2005 and 2010. We used a temperature-dependent light model to show that from 2005 to 2010 during daylight periods in the summer, coastal bay beds received at least 100% of their light requirements 24% of the time, while beds in the lower Chesapeake Bay only met this 6% of the time. Summer light attenuation ( $K_d$ ) and temperatures from continuous monitoring at 2 additional Chesapeake Bay sites in 2010 suggest that the greater tidal range and proximity of the coastal bays to cooler ocean waters may ameliorate influences of exposure to stressful high water temperature conditions compared to Chesapeake Bay. A temperature difference of 1°C combined with a  $K_d$  difference of 0.5 m<sup>-1</sup> at 1 m depth results in a 30% difference in available light as a proportion of community light requirements. These differences are critical between survival and decline in these perennial populations growing near the southern limits of their range. Without an increase in available light, Chesapeake Bay populations may be severely reduced or eliminated, while coastal bay populations, because of their proximity to cooler Atlantic waters, may become the refuge populations for this region.

**KEY WORDS:** Chesapeake Bay · Coastal ecosystem · Water quality · Turbidity · Seagrass ecosystems · *Zostera marina* · Restoration · Climate variation · Western mid-Atlantic

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## INTRODUCTION

Seagrass populations in estuarine and coastal bay systems have been declining globally (Green & Short 2003, Orth et al. 2006a, Waycott et al. 2009), with nearly 25% of all seagrass species considered threatened or near threatened (Short et al. 2011). While numerous factors have been cited for these losses, reduced water quality and more specifically, reduced light availability related directly or indirectly to anthropogenic inputs of nutrients and sediments, have been documented as principal factors (Orth et al. 2006a, Waycott et al. 2009). Recently, warming sea surface temperatures have been sug-

gested as another major factor that could have serious deleterious effects on seagrasses, especially at their range limits (Short & Neckles 1999, Short et al. 2011). In the western mid-Atlantic coastal region, eelgrass *Zostera marina* grows near the southern limits of its range (Green & Short 2003, Moore & Short 2006), so the potential for increased water temperatures to become a major stressor is high, not only as regards the direct effects of high temperature on the eelgrass, but also as regards the potential interactions of high temperatures with other stressors (Koch & Erskine 2001).

Light requirements for *Zostera marina* have been shown to increase with temperature (Wetzel & Pen-

\*Email: moore@vims.edu

hale 1983, Murray & Wetzel 1987, Moore et al. 1997, Moore 2004), and photosynthetic capacities decrease at high summertime temperatures (Evans et al. 1986, Zimmerman et al. 1989), thereby decreasing growth and production (Zharova et al. 2001, Pastres et al. 2004). Increases in water temperatures can also increase sediment and water column community respiration, potentially resulting in increased periods of hypoxia or anoxia (Moore 2004, Greve et al. 2005). Temperature can have a substantial influence on meristematic oxygen balance (Borum et al. 2006), with plant respiration increasing more with increasing temperatures than photosynthesis (Evans et al. 1986). Greater diffusion and loss of oxygen from eelgrass roots into the sediments due to increasing temperature-dependent, microbial respiration rates can further stress the plants (Holmer & Bondgaard 2001). Staehr & Borum (2011) found that while there can be seasonal acclimation of *Z. marina* to temperature, in their laboratory-based experiments, respiration increased significantly more than photosynthesis especially at temperatures above 20°C, and they concluded that:

high temperatures may have more severe negative effects on seagrass survival than just from reducing the carbon balance of the plants. (p. 145)

Periods of sediment hypoxia have been associated with declines of seagrasses in several areas (Robblee et al. 1991, Carlson et al. 1994, Koch et al. 2007). Greve et al. (2003) found that oxygen supply to *Zostera marina* tissues is highly variable and is dependent on plant photosynthesis, water column and sediment oxygen concentrations, and *Z. marina* community oxygen consumption rates. Toxic metabolites such as ethanol and lactate can accumulate within plant tissues under anoxic conditions (Crawford & Braendle 1996, Vartapetian & Jackson 1997). Additionally, sulfide, which is strongly phytotoxic (Koch & Erskine 2001), can diffuse from the sediments into plant tissues under low oxygen conditions (Frederiksen et al. 2006), reducing photosynthesis and growth (Goodman et al. 1995, Erskine & Koch 2000, Holmer & Bondgaard 2001, Borum et al. 2005, 2006), which can result in significant losses of vegetation (Carlson et al. 1994). Laboratory studies of *Z. marina* found it very sensitive to short-term periods of exposure to anoxic seawater, with significantly reduced photosynthesis and growth at increased temperature (Pulido & Borum 2010).

The effects of short-term stress have been shown to have long-term effects on eelgrass persistence. Moore et al. (1997) found that short-term periods of

high turbidity for as little as 20 d resulted in a die-off of *Zostera marina* transplants in Chesapeake Bay (USA), in contrast to transplant growth and survival in a comparable area which did not receive the turbidity stress. Periods of high summertime water temperatures were found to be related to diebacks of *Z. marina* in Danish waters (Greve et al. 2005). In the summer of 2005, a die-off of *Z. marina* in Chesapeake Bay was associated with low water column dissolved oxygen concentrations of 1 to 3 mg l<sup>-1</sup> which occurred only 3 to 5% of the time in July and August of that year, as well as a higher proportion of time when water temperatures exceeded 29°C during the same period (Moore & Jarvis 2008).

The lower Chesapeake Bay and nearby eastern shore coastal lagoons have historically supported extensive *Zostera marina* populations (Orth & Moore 1984, Orth et al. 2006b, 2010a, Koch & Orth 2003). However, in the 1930s, impacts from the 'wasting disease' caused by a virulent strain of the pathogenic slime mold *Labyrinthula zosterae* devastated populations in these regions (Rasmussen 1977). Recovery from this event was not quantified, but *Z. marina* did eventually return to the higher-salinity regions of Chesapeake Bay (Orth & Moore 1984), while recovery in the more isolated eastern shore lagoons was limited, due possibly to limited propagule availability (Orth et al. 2012, this Theme Section).

More recently, *Zostera marina* populations in Chesapeake Bay have shown a pattern of decline, reaching current levels which are 65% of those observed only several decades ago (Orth et al. 2010a; Fig. 1, inset). A wide range of restoration efforts attempted during that period have had only limited success (Orth et al. 2010a). In contrast, *Z. marina* populations in the Virginia coastal lagoons, which were started by seeds initially collected from Chesapeake Bay populations (Orth et al. 2012), have shown dramatic increases since populations were first established from seeds in 1997. While reduced light availability has been the primary factor affecting *Z. marina* success throughout Chesapeake Bay (Moore et al. 1996, 1997, Kemp et al. 2004, Orth et al. 2010b), it has not been a factor limiting *Z. marina* growth and abundance in the coastal bays (Lawson et al. 2007, McGlathery et al. 2012, this Theme Section, Orth et al. 2012). While summertime die-offs associated with high temperatures were occurring in Chesapeake Bay in 2005 (Moore & Jarvis 2008), *Z. marina* in the Virginia coastal bays expanded (Orth et al. 2012), apparently unaffected by the stressful conditions occurring in the neighboring Chesapeake system.

The persistence and spread of *Zostera marina* in these coastal bays, unlike Chesapeake Bay populations, suggests some ameliorating effects of the environment in the coastal bays during summertime periods associated with diebacks in Chesapeake Bay. The objectives of this paper were therefore to assess both the levels and variability in the water quality conditions associated with the successful growth of *Z. marina* in the coastal bays system, and compare these to conditions in declining *Z. marina* areas in the lower Chesapeake Bay, to determine what factors and levels of these factors may be influencing these divergent population responses.

## MATERIALS AND METHODS

### Study sites

Four study sites with existing *Zostera marina* beds were studied for water quality conditions and *Z. marina* persistence (Fig. 1). One site, South Bay, is located within a large *Z. marina* restoration site in 1 sub-basin along the Virginia and Maryland (USA) Delmarva peninsula's coastal bay system (see Orth et al. 2012). South Bay is part of the Virginia Coast Reserve Long-Term Ecological Research site. Three other sites (Goodwin Island, Ware River, and Horn Harbor) are located along the western littoral zone of the lower Chesapeake Bay in or near areas vegetated by *Z. marina*. All 4 study sites are of comparable depths (0.5 to 1.0 m mean sea level).

### Water quality

Water quality measurements were made at each of the study sites using YSI 6600 EDS V2 multi-parameter sondes which were fixed to pilings and located between 0.25 and 0.51 m above the bottom at each site. The South Bay station was deployed from 2005 through 2010. Four 2 to 3 wk deployments were conducted each year between 2006 and 2009, while in 2005, only three 2 wk deployments were conducted during the study period. In 2009, a sonde was deployed continuously from 28 July through 18 December, and in 2010 continuously from 8 March through 31 December. A sonde at the Goodwin Island, Chesapeake Bay station, which is part of the Chesapeake Bay National Estuarine Research Reserve in Virginia (CBNERRVA) monitoring network, was deployed continuously from 2005 to 2010. The 2 other Chesapeake Bay stations, Horn Harbor and Ware River, had sondes deployed from 1 April to 30 November 2010. Water quality was measured at 15 min intervals throughout each deployment. Measurements included turbidity (in NTU), chlorophyll fluorescence, temperature, salinity, pH,

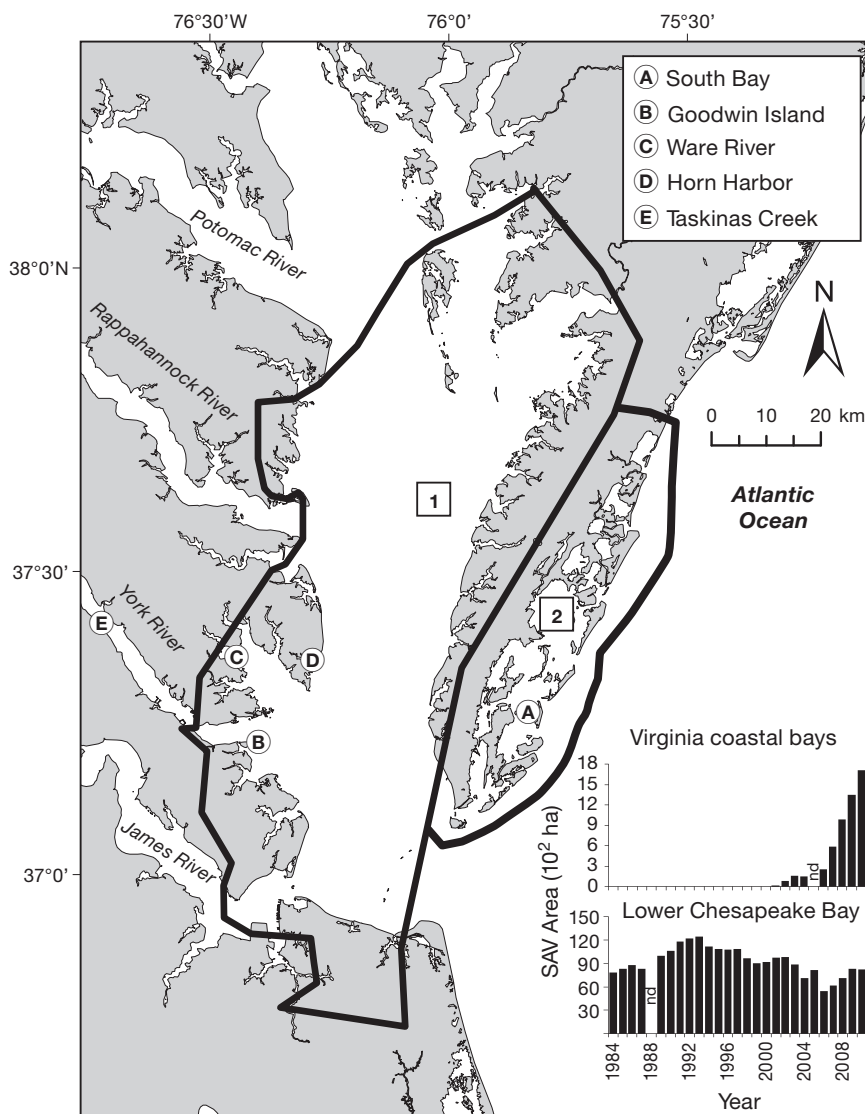


Fig. 1. *Zostera marina*. Occurrence region in the lower Chesapeake Bay (1) and the Virginia coastal bays region (2), USA. A–D are water quality monitoring sites; E is the meteorological monitoring site. Inset histograms show bed areas (in 10<sup>2</sup> ha) of submerged aquatic vegetation (SAV) monitored annually over time. nd: not determined. Figure modified from Orth et al. (2010a)

dissolved oxygen, and depth. Sondes were replaced at 1 to 3 wk intervals, depending on fouling rates, with newly calibrated instruments. All sensors on the YSI 6600 EDS sondes were both calibrated pre-deployment and checked post-deployment according to YSI standard procedures. Diffuse downwelling attenuation of photosynthetically available radiation (PAR) was determined using triplicate water column measurements of downwelling photosynthetic photon flux density measured with a LI-192SA underwater quantum sensor (LI-COR) taken every 25 cm from 10 cm below the surface to 25 cm above the bottom. YSI turbidity measurements were modeled to light attenuation by least squares regression relating turbidity to downwelling light attenuation coefficients ( $K_d$ ) using the simultaneously measured light profiles and turbidities taken throughout the course of the studies. Bio-optical models of light attenuation within similar seagrass habitats in coastal estuarine and bay systems (Gallegos 1994, 2001, Biber et al. 2008) have shown that turbidity, chlorophyll, and colored dissolved organic matter (CDOM) can form variable optical components of the water. In the coastal bay and lower estuarine areas studied here, both chlorophyll and CDOM have little effect on light attenuation coefficients, while total suspended solids dominated (Cercio & Moore 2001, Lawson et al. 2007). Downwelling insolation measurements of PAR were continuously recorded at 15 min intervals at Taskinas Creek (Fig. 1) using a LI-190SA terrestrial quantum sensor (LI-COR) from 2005 to 2010.

### Aerial imagery

*Zostera marina* bed areas for both the Chesapeake Bay and South Bay regions were derived from 1:24 000 scale aerial photography acquired annually during the May to July period of highest abundance of *Z. marina* in this mid-Atlantic region (Moore et al. 2000). Details for the acquisition, photo-interpretation and processing of the seagrass signatures, and *Z. marina* abundance data are provided by Moore et al. (2009) and Orth et al. (2010b, 2012). Ground surveys were conducted each year to confirm the occurrence of *Z. marina* in these areas.

### Compensating light ( $I_c$ ) and light to the bottom ( $I_z$ ) calculations

*Zostera marina* community compensating light requirement ( $I_c$ ) at each of the study sites was deter-

mined using a linear least squares regression model developed for this mid-Atlantic area (Wetzel & Penhale 1983, Moore et al. 1997) that relates *in situ* measures of *Z. marina* community light compensation to water temperature ( $\log(I_c) = 0.057 \times \text{temp} + 1.01$ ;  $r^2 = 0.99$ ). Water temperatures were recorded every 15 min using the YSI sondes at each site.

To better understand the water quality trends over the tidal cycles at each station relative to tidal influences, the water depth values that were determined every 15 min by the non-vented YSI sonde at each location were corrected from the barometric pressure set at calibration prior to deployment to the actual barometric pressures at sampling times. This was done using 15 min interval barometric measurements from the Taskinas Creek meteorological station (Fig. 1). The fixed distance of each moored sonde above the bottom was then added to each of the 15 min corrected depth values to get the actual water depths at each site. The long-term average water level relative to the bottom of these water depths was determined using all available depth data from each site, and the differences between the long-term mean for South Bay and the long-term means for each of the other stations were calculated. To normalize the 3 Chesapeake Bay sites relative to the South Bay site for light to the bottom ( $I_z$ ) calculations, each depth value at each station was then adjusted to this consistent average water level reference.

The percent light through water to the bottom (PLW) was calculated using Eq. (1), where  $K_d$  is modeled from the 15 min turbidity values and  $z$  is the 15 min depth values taken at each site and normalized to a common sea level datum:

$$\text{PLW} = 100 \times e^{(K_d \times z)} \quad (1)$$

PLW was then used to calculate the light to the bottom ( $I_z$ ) using Eq. (2), where PAR was the 15 min reading taken from the Taskinas Creek meteorological station:

$$I_z = (\text{PAR} \times \text{PLW})/100 \quad (2)$$

### Statistical analyses

Continuous water quality measurements made at the fixed monitoring stations at South Bay, Goodwin Island, Ware River, and Horn Harbor during the June to August 2010 deployment periods were compared among sites using Kruskal-Wallis rank sum tests. Paired post hoc comparisons followed procedures originally published in Siegel & Castellan (1988). Regression analysis was used to develop sep-



arate conversions between fixed station turbidity measurements and underwater light attenuation coefficients for the South Bay station and the combined Chesapeake Bay stations, as the optical properties of the water can differ between the coastal and estuarine regions due to varying dissolved and particulate components in the water column (Gallegos et al. 1990, Gallegos 2005, Biber et al. 2008). Comparison of long-term mean frequency distributions of water temperatures, dissolved oxygen, light attenuation coefficients, and proportion of light requirements met by light availability were accomplished using Kolmogorov-Smirnov tests for significance (StatView).

## RESULTS

*Zostera marina* abundance in the Virginia coastal bays increased gradually during the early phases of the restoration efforts (Orth et al. 2012) through 2006, when 253 ha were recorded, and then more rapidly through 2010 when 1715 ha were reported (Fig. 1). *Z. marina* abundance in Chesapeake Bay declined from 1993 through 2006. There was a significant dieback observed in Chesapeake Bay between the aerial mapping surveys in 2005 and 2006 (Fig. 1) which occurred at the end of the summer of 2005 (Moore & Jarvis 2008, Jarvis & Moore 2010, Orth et al. 2010b). Since then there has been only a modest increase with little change between the 2009 and 2010 surveys, which for 2010 was 34 % less than the eelgrass abundance reported in 1993. Field observations at the end of August 2010 (R. J. Orth pers. obs.) revealed widespread diebacks of the *Z. marina* beds throughout the lower Chesapeake Bay, in contrast to little dieback evident at the South Bay restoration area.

Goodwin Island had a proportionally higher duration of water temperatures  $27^{\circ}\text{C}$  and above, with the cumulative distribution of higher temperatures at Goodwin Island exceeding that of South Bay from June through August for the period of 2005 to 2010 ( $p < 0.01$ , Fig. 2). Light-turbidity models developed separately for the coastal bay and Chesapeake Bay stations resulted in a good fit between the 2 different measurements of water clarity: South Bay  $K_d = 0.0885$  (Turbidity) + 0.5529,  $r^2 = 0.716$ ; Chesapeake Bay  $K_d = 0.0869$ (Turbidity) + 1.1976,  $r^2 = 0.771$ . Higher ( $p < 0.01$ ) levels of light attenuation were observed at Goodwin Island, with the distribution shifted approximately  $0.5 \text{ m}^{-1}$  higher at that site compared to the South Bay site. The resultant distributions of percentage light available to light required ( $I_z/I_c$ ) for Goodwin Island

and South Bay (Fig. 3) showed that it was significantly lower ( $p < 0.01$ ) at the Chesapeake Bay site. This was related to both the warmer water temperatures, which increased light demand ( $I_c$ ), and the more turbid conditions, which reduced the light availability ( $I_z$ ).

Water-column dissolved oxygen concentrations measured at or just above the *Zostera marina* canopy rarely fell below  $4 \text{ mg l}^{-1}$  during the summer periods of 2005 to 2010 at either Goodwin Island or South Bay. For example, during the summer of 2010, dissolved oxygen levels of 2 to  $4 \text{ mg l}^{-1}$  occurred only 6 times at South Bay and 6 times at Goodwin Island for an average duration of 7.5 and 7.8 h, respectively, during these events. Overall, the measured dissolved oxygen levels were slightly, but significantly ( $p < 0.01$ ), higher at Goodwin Island than at South Bay (Fig. 2).

Comparisons of 1 d means of water temperatures and light attenuation for the South Bay site and the 3 Chesapeake Bay sites for the period of May through September 2010 (Fig. 4) revealed that in spite of considerable variability in daily temperatures and turbidities, the South Bay site was consistently cooler and less turbid (lower light attenuation) than the Chesapeake Bay areas (Fig. 5). Overall, water temperature differences between South Bay and the Chesapeake Bay sites were larger during the periods of June through August when water temperatures were at their seasonally highest levels. Additionally, short periods of a few days to a week could be observed when water temperature differences were much greater between the coastal lagoon *Zostera marina* bed and the Chesapeake Bay beds. Light attenuation also showed considerable temporal variability, with very high levels usually associated with storms or other high wind events. For example, short-term spikes in light attenuation observed in May and September 2010 (Fig. 4) corresponded to average winds from the north of  $11.7 \text{ m s}^{-1}$ , with average gusts of  $14.1 \text{ m s}^{-1}$  on 8 to 9 May, and north winds of  $11.9 \text{ m s}^{-1}$ , with average gusts of  $15.0 \text{ m s}^{-1}$  on 3 September (NOAA National Ocean Service Station KPTV2 – Kiptopeke, VA,  $37.167^{\circ} \text{ N}$ ,  $75.988^{\circ} \text{ W}$ ). In comparison, average wind speeds from May through September 2010 were  $4.4 \text{ m s}^{-1}$  with variable direction.

Median water quality conditions for the 2010 June through August period (Fig. 5) showed significantly ( $p < 0.05$ ) higher water temperatures comparing the Chesapeake Bay sites and the South Bay coastal bay restoration area, with the Goodwin Island *Zostera marina* bed lying intermediate between the other 2 Chesapeake Bay sites and South Bay. Similarly, median light attenuation was significantly lower at

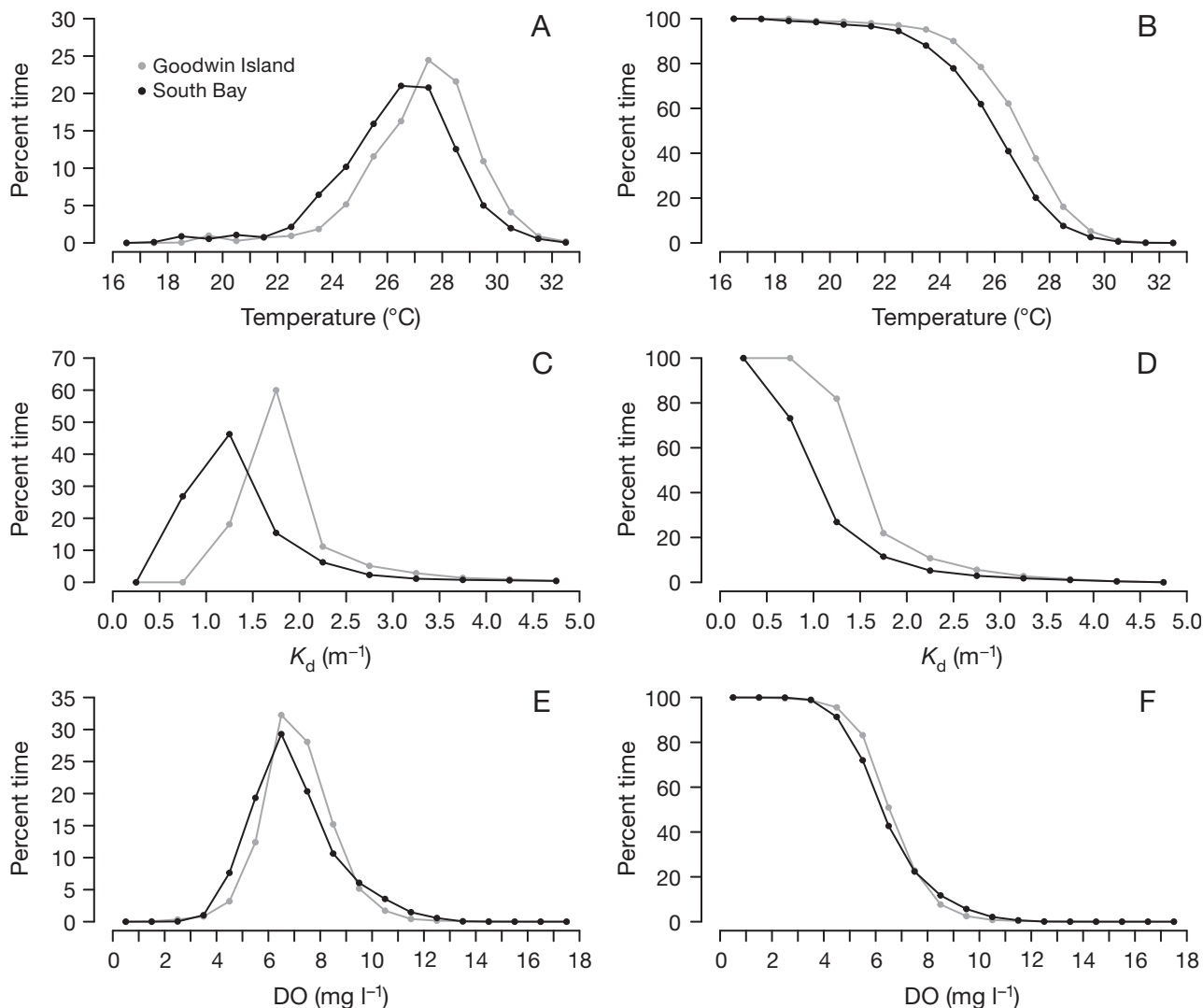


Fig. 2. (A,C,E) Frequency distributions and (B,D,F) cumulative frequency distributions for June through August 2005 to 2010 water temperatures, light attenuation ( $K_d$ ), and dissolved oxygen (DO) at Goodwin Island and South Bay. Graphs present mean binned frequencies of all data for each parameter and site. In (A,C,E), note differences in y-axis scales

South Bay than the other areas. The resultant determinations of percentage light available relative to compensating light requirements ( $I_z/I_c$ ) revealed a significantly higher proportion of light requirements met at South Bay compared to the Chesapeake Bay sites, with the lowest level of light requirements met at Horn Harbor due to its highest levels of water temperatures and light attenuation.

Analysis of temperature and turbidity records for the period of June through August 2010 relative to tidal periods revealed marked differences among the sites (Fig. 6). First, the overall tidal range was greater at South Bay compared to the Chesapeake Bay sites where the average tidal range of South

Bay was 1.18 m, as compared to 0.64 m at Goodwin Island, 0.50 m at Horn Harbor, and 0.71 m at Ware River. At low tidal levels, water temperatures were more similar at South Bay to those at the 3 Chesapeake Bay sites. At high tides, the water temperatures were reduced by nearly 2°C compared to low tide, while high tides at the Chesapeake Bay sites resulted, on average, in only 0.5°C or less reduction in water temperatures. Light attenuation showed evidence of higher attenuation levels at lower tidal periods at the 3 Chesapeake Bay sites, while at South Bay there was evidence that higher turbidities were associated with the initial flooding periods after low tides.

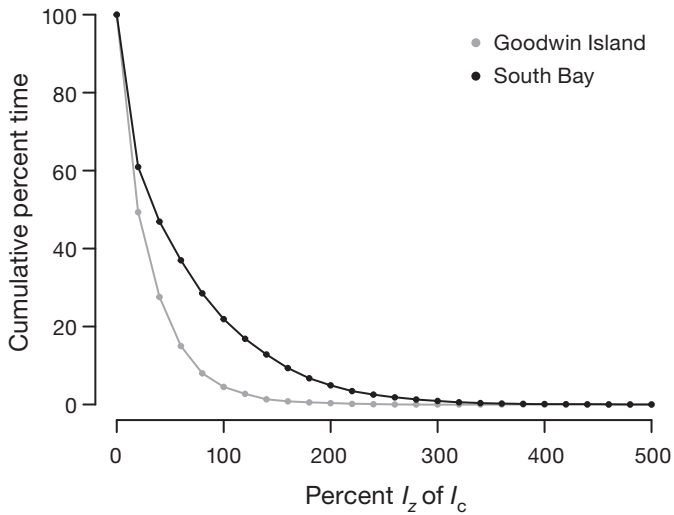


Fig. 3. Cumulative frequency distribution of percent light available (light to the bottom,  $I_z$ ) of compensating light requirement ( $I_c$ ) at Goodwin Island and South Bay for June through August 2005 to 2010. Graphs present mean binned frequencies of all data for each site

**DISCUSSION**

Results of this study indicate that a combination of factors, including lower summertime water temperatures and lower light attenuation, interact to both increase the proportion of light available for *Zostera marina* photosynthesis and decrease the *Z. marina* community light requirements at the South Bay restoration site compared to sites in the lower Chesapeake Bay. This results in markedly improved conditions for *Z. marina* growth and meadow expansion in South Bay compared to the lower Chesapeake Bay. Since 2005, seed-based *Z. marina* restoration efforts in the Virginia Coastal Bay region have resulted in bed expansion at a rate of 66% yr<sup>-1</sup>. In contrast, the lower Chesapeake Bay *Z. marina* beds have only shown an expansion rate of 2% yr<sup>-1</sup> following a significant die-off event by the end of the summer of 2005 (Moore & Jarvis 2008). The application of the temperature-dependent compensating light model demonstrates that during the summers from 2005 to

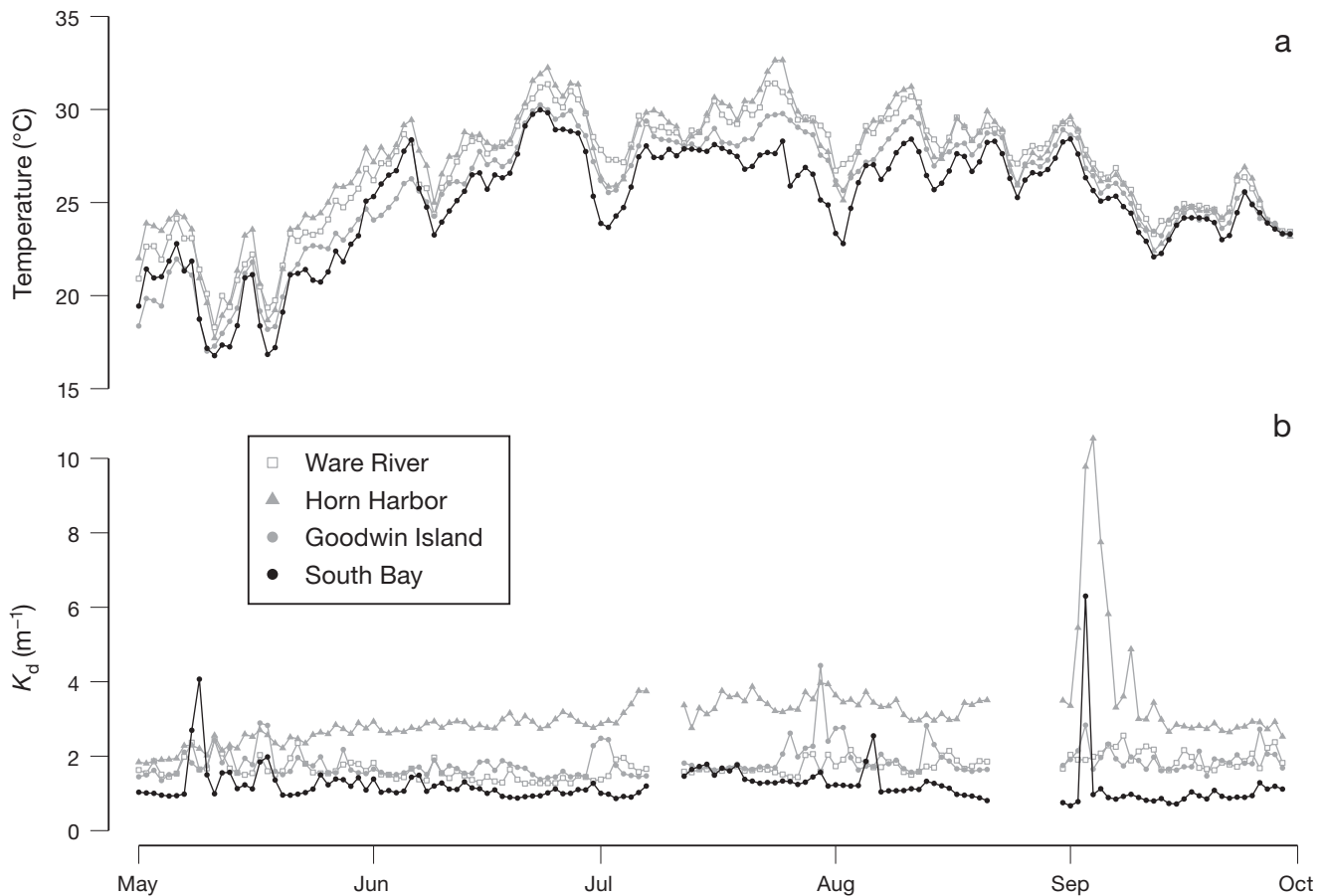


Fig. 4. May to October 2010 (a) daily mean water temperatures and (b) light attenuation ( $K_d$ ) for Ware River, Horn Harbor, Goodwin Island, and South Bay

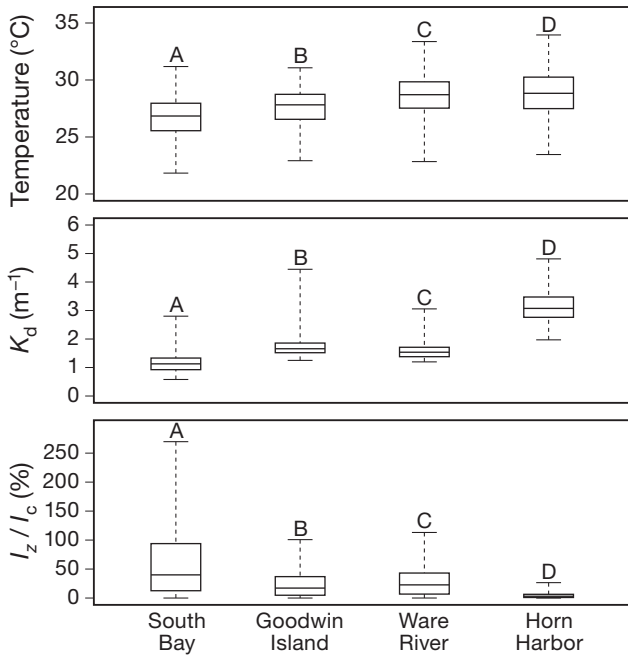


Fig. 5. Water temperature, light attenuation ( $K_d$ ), and percent light available (light to the bottom,  $I_z$ ) of compensating light requirement ( $I_c$ ) for South Bay, Goodwin Island, Ware River, and Horn Harbor for June through August 2010, showing median, 25th and 75th percentiles, and the minimum and maximum of the lower 99% of the data. Data are 15 min records. Identical letters indicate no significant differences ( $p > 0.05$ ) in median values between the sites

2010, the South Bay beds received at least 100% of their community-compensating light requirements 24 % of the time during the day, while populations in the Goodwin Island region of the lower Chesapeake Bay received compensating light only 6 % of the time. In the summer of 2010, the addition of 2 other *Z. marina* monitoring sites in the lower Chesapeake Bay confirmed this trend, with median light levels as a proportion of light requirements significantly higher at South Bay (40%) compared to Goodwin Island (16%), Ware River (21%), and Horn Harbor (3%) in Chesapeake Bay.

Reductions in attainment of compensating light levels are related to reductions in the distribution and abundance of *Zostera marina*, as well as other seagrass species in coastal areas (Dennison & Alberte 1982, 1985, Zimmerman et al. 1994, Lee et al. 2007). Our work adds to the results of these studies and suggests that relatively modest changes near the upper tolerance limits in water temperature and clarity can result in pronounced differences in the trajectories of *Z. marina* population expansion or contraction. Analyses here suggest that a summertime temperature difference of 1°C, combined with a  $K_d$  difference of 0.5  $m^{-1}$ , would result in a 30% difference in available light at 1 m depths as a proportion of the *Z. marina* community light requirements. This difference may represent a threshold between expansion and die-

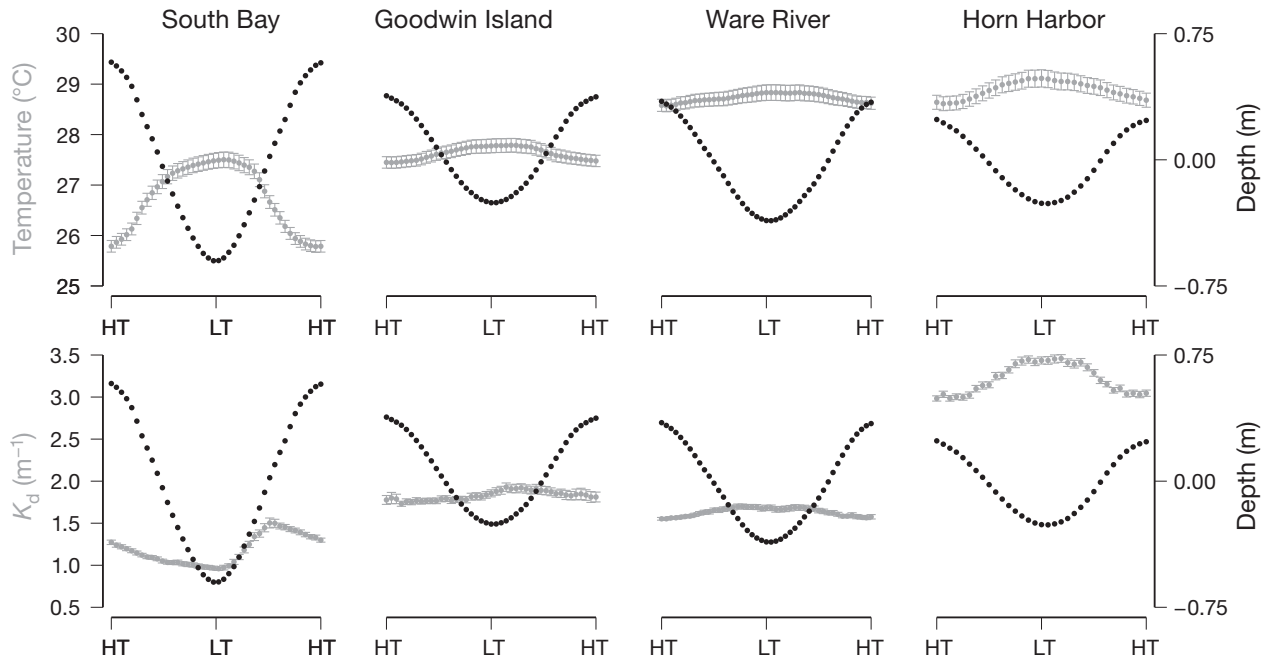


Fig. 6. Aggregated tidal periodicity in water temperatures (mean  $\pm$  SE), light attenuation ( $K_d$ ; mean  $\pm$  SE), and water depths (mean, relative to mean sea level) for June through August 2010. HT: high tide, LT: low tide

back of *Z. marina* beds growing in this region of the mid-Atlantic, which is near the southern limit of its range along this coast (Koch & Orth 2003).

In addition to climate and latitudinal effects on temperature, site-specific factors such as tides can play an important role in the balance between *Zostera marina* habitat suitability and sustainability. Analyzing summer light attenuation and temperatures from continuously recording datasondes at 4 stations in 2010 suggests that the greater tidal range and proximity of the coastal bays to cooler ocean waters may be an ameliorating influence on the duration of exposure to stressful conditions. The significant decreases in water temperatures at high tide, on average, compared to low tide is likely to be very important in reducing the overall light requirements of the *Z. marina* community. In comparing South Bay to Goodwin Island (Fig. 6), while maximum summertime water temperatures during low tidal periods were very similar, water temperatures at South Bay were below those of Goodwin Island for approximately 50% of each day. During 2 to 3 h at high tide, the difference was on average nearly 2°C. Monitoring data at the other Chesapeake Bay sites show similar patterns of very limited change in water temperatures with tidal phase, suggesting that the surface water masses in the vicinity of each station were relatively consistent in temperature. In contrast, the warming and cooling cycles observed at the South Bay site suggest that water masses of differing temperatures influenced the *Z. marina* beds there. The summer water temperatures at the Ware River and Horn Harbor sites were significantly higher than at the Goodwin Island site (Fig. 5). As these sites are located in more embayed areas than Goodwin Island, it suggests that the physical location of the *Z. marina* beds along the shore can also be a factor affecting temperature stress.

Water clarity levels were also observed to vary markedly with tidal phase (Fig. 6). At South Bay, the rapid increase in turbidity with flooding tide suggests that in addition to wind- and storm-generated re-suspension (Koch et al. 2006, Chen et al. 2007, Lawson et al. 2007), tidal energies were sufficient at the site to re-suspend sediments and reduce water clarity. Much less effect was seen at all 3 Chesapeake Bay sites. Although tidal currents were not measured here, the much larger tidal range and broader shallow flats at South Bay compared to the Chesapeake Bay sites may result in higher tidal currents during the early flood. All 3 Chesapeake Bay sites showed, on average, slightly to markedly lower water clarity, around low tide versus high tide compared to the

South Bay site. This may reflect the relatively greater effect of wind waves on the shallow water bottoms at low tidal levels in these areas. Moore (2004) reported that the effect of *Zostera marina* bed structure was not evident until seagrass cover reaches 25 to 50%. The relatively dense *Z. marina* beds at South Bay (Orth et al. 2012), compared to the much more sparse and less expansive beds at the Chesapeake Bay sites (Orth et al. 2010b), may influence the beds' capacities to attenuate re-suspension, especially during lower tides (Hansen & Reidenbach 2012, this Theme Section).

Orth et al. (2012) observed that the water clarity in South Bay has improved significantly since 2003 as the *Zostera marina* vegetated areas have increased. This suggests that water clarity conditions at South Bay were better in 2010 than in the recent past. However, in comparison to the Goodwin Island site monitored here since 2005, water clarity in South Bay was at least as good or better over this entire time period. The additional positive effects of cooler water temperatures on *Z. marina* persistence at South Bay support the results of transplant studies in both Chesapeake and South Bay sites over this time period (Orth et al. 2010a), which conclude that conditions have been more suitable for *Z. marina* restoration in South Bay compared to Chesapeake Bay.

Water temperatures in 2010 at the Chesapeake Bay and South Bay sites all demonstrated similar seasonal trends, as well as short-term variability over days to weeks (Fig. 4). These are likely reflective of variation in air temperatures over the summer. However, differences in water temperatures between the coastal South Bay site and the Chesapeake Bay sites were not consistent, and differences ranged from <1°C to >5°C (Fig. 4). This suggests varying influence of offshore waters on the coastal bay, which might be related to short-duration upwelling events or other factors affecting nearshore water conditions in this region (Clemente-Colon & Yan 1999, Austin 2002). The effect of this variability could be significant. For example, very hot periods during the summer, especially during weeks when low tides are occurring around mid-day, can result in very elevated water temperatures in the nearshore, shallow vegetated areas. This has been observed to cause significant diebacks of *Zostera marina* in Chesapeake Bay, even if the events only occur for a few days to a week (Orth & Moore 1986). However, alongshore southern winds, which are relatively normal during these periods (Clemente-Colon & Yan 1999), can produce offshore surface Ekman flows that regularly bring colder water to the surface (Glenn et al. 1996) and

potentially into the coastal bays, thus buffering the *Z. marina* there from the pulses of high temperatures.

The effect of high temperatures of short duration on *Zostera marina* in Chesapeake Bay was observed in 2005 when there was a nearly complete dieback of aboveground vegetation by the end of August following a period of unusually high water temperatures (Moore & Jarvis 2008). This dieback phenomenon was not observed in the coastal bays (Orth et al. 2010a,c). This difference was recorded in 2006 when overall abundance of aerial mapping of *Z. marina* in Chesapeake Bay declined, while populations in the coastal bays continued to increase (Fig. 1). Similarly, in June 2010, air temperatures were observed to be unusually high for several weeks (data not shown), with concurrent increases in shallow water temperatures (Fig. 4). As in 2005, a general dieback in *Z. marina* in Chesapeake Bay was observed subsequent to this but again, not in the coastal bays (R. J. Orth & E. C. Shields pers. obs.). *Z. marina* can become stressed at 25°C (Rasmussen 1977), and an upper limit range from 28 to 30°C has been proposed as a critical threshold for growth (Evans et al. 1986, Orth & Moore 1986, Abe et al. 2008). Temperatures exceeding 30°C for prolonged periods have been associated with large-scale declines and transplant failures in the Chesapeake Bay (Moore & Jarvis 2008, Golden et al. 2010, Jarvis & Moore 2010, Tanner et al. 2010) as well as in laboratory studies (Pulido & Borum 2010, Staehr & Borum 2011). While daily mean temperatures were not consistently above 30°C over the entire 2010 summer, there were varying periods in late June and July in which plants were exposed to these stressful temperatures. The apparent connection between high temperatures and dieback, at different periods during the summer, suggests that temperature stress, even at varied periods during the summer, can have long-term effects on plant persistence (Staehr & Borum 2011). Burke et al. (1996) showed that having adequate carbon reserves is very important for *Z. marina* persistence through the summer. Various stresses can reduce carbon reserves such that summertime survival can be compromised even if conditions return to more normal patterns. Given the persistently higher summertime temperatures for Chesapeake Bay populations combined with generally higher turbidities, pulsed stresses, even for short durations, can have long-lasting effects.

Low water-column oxygen concentrations can have a compounding effect on temperature and light stresses (Greve et al. 2003, Borum et al. 2005, 2006). Pulido & Borum (2010) demonstrated that short-term exposure (<24 h) to water significantly reduce

*Zostera marina* photosynthetic performance and growth and can increase mortality, and that these effects increase with temperature. At the sites studied here, measured water-column dissolved oxygen rarely dropped below 4 mg l<sup>-1</sup> and never became anoxic, even during the highest temperature periods in the summer. These observations are similar to those observed previously by Moore (2004) for the Goodwin Island *Z. marina* site. This suggests that while low water-column dissolved oxygen may be a continuing summertime stress to the *Z. marina* here, specific low dissolved oxygen events were likely not the precipitating factor causing the widespread die-off in 2010. While water-column dissolved oxygen levels remained relatively high at the monitoring stations, it is certainly possible that in some other areas with low water movement and very high bacterial respiration due to high organic matter accumulations, water column anoxia, especially as it may affect meristematic tissues near the sediment surface, may result in plant mortality (Pulido & Borum 2010). In fact, isolated diebacks which may be examples of this have been observed in other lower Chesapeake Bay *Z. marina* areas in the past (Orth & Moore 1986).

The results of our study of these estuarine and coastal bay systems are important for 2 reasons. First, they provide critical data on key processes of growth and survival that have allowed *Zostera marina* to survive and spread in the coastal bays compared to Chesapeake Bay populations. Second, they are relevant in the debate surrounding the implications of climate change for plant and animal populations as this influences the overall temperature regime. These temperature scenarios suggest that without an increase in available light, Chesapeake Bay populations may be severely reduced or possibly eliminated, while coastal bay populations, because of their proximity to cooler Atlantic waters, may become the refuge populations for this region.

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# Enhancement of sediment suspension and nutrient flux by benthic macrophytes at low biomass

S. E. Lawson\*, K. J. McGlathery, P. L. Wiberg

Department of Environmental Sciences, University of Virginia, 291 McCormick Rd., Charlottesville, Virginia 22904, USA

**ABSTRACT:** In shallow coastal ecosystems where most of the seafloor typically lies within the photic zone, benthic autotrophs dominate primary production and mediate nutrient cycling and sediment stability. Because of their different structure and metabolic rates, the 2 functional groups of benthic macrophytes (seagrasses, macroalgae) have distinct influences on benthic–pelagic coupling. Most research to date in these soft-bottomed systems has focused on mature seagrass meadows where shoot densities are high and on dense macroalgal mats that accumulate in response to eutrophication. Relatively little is known about the influence of low-biomass stands of seagrass and macroalgae on nutrient fluxes and sediment suspension. Using an erosion microcosm with controlled forcing conditions, we tested the effects of the eelgrass *Zostera marina* L. and the invasive macroalga *Gracilaria vermiculophylla* on sediment suspension and nutrient fluxes under high-flow conditions. At low densities, *G. vermiculophylla* increased sediment suspension and increased the nutrient flux from the sediment to the water column. For macroalgae, increased sediment suspension is likely due to dislodgement of sediment particles by bedload transport of the algae. In this case, the increase in sediment transport was reflected in an increase in nutrient flux from the sediment, showing that modification of physical forcing by benthic primary producers can also affect nutrient flux. The presence or absence of *Z. marina* did not have a significant effect on nutrient flux. However, the results suggest that there may be a range of low shoot densities for which storm-like flows increase sediment suspension to values higher than those expected for a bare sediment bed.

**KEY WORDS:** Benthic macrophytes · Sediment suspension · Nutrient flux · Seagrass · Benthic–pelagic coupling · Coastal bay · Lagoon

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## INTRODUCTION

In shallow coastal ecosystems where the seafloor typically lies within the photic zone, benthic autotrophs dominate primary production and mediate nutrient cycling and sediment stability (Sand-Jensen & Borum 1991, McGlathery et al. 2007). The strong benthic–pelagic coupling in these systems is influenced by both the distinct structure and the metabolic rates of the 2 functional groups of benthic macrophytes, viz. seagrasses and macroalgae (McGlathery et al. 2007). Almost all studies on the effects of seagrasses and macroalgae have been conducted on mature or high-density beds (e.g. Gambi et al.

1990, Fonseca & Cahalan 1992, Escartín & Aubrey 1995, Sfriso et al. 2005). Compared to these high-density populations, we know little about the effects of lower densities of benthic macrophytes on sediment and nutrient fluxes, even though it is common for seagrasses and macroalgae to occur at low densities. For example, multiple stressors, including sediment and nutrient runoff, algal blooms, and physical disturbances from storms, boat traffic, and some fishing practices, can cause thinning and/or patchiness in seagrass populations in addition to wholesale losses of seagrass habitat (Duarte 2002, Orth et al. 2006). Restoration of seagrass meadows, especially by seeding, also results initially in low-density populations

\*Email: sel7b@virginia.edu

that increase in density as the meadows develop over time (McGlathery et al. 2012, Orth et al. 2012, both in this Theme Section). Macroalgae can also be present at low densities in shallow coastal systems depending on availability of attachment structures or advection of ephemeral populations by wind-driven currents (Thomsen & McGlathery 2005).

The effect of benthic macrophytes on near-bed hydrodynamics and the resulting sediment/nutrient fluxes may be different at low density than at high density. Extensive research has shown that dense seagrass canopies reduce current velocity (Peterson et al. 2004) and dampen wave energy (Fonseca & Cahalan 1992), leading to decreased sediment resuspension and increased particle deposition (Heiss et al. 2000, Peterson et al. 2004). Observations of sediment stabilization in seagrass beds and increased turbidity following seagrass decline (e.g. Cottam & Munro 1954, Christiansen et al. 1981, Stumpf et al. 1999) have supported a conceptual model that sediment suspension increases when seagrass is replaced by macroalgae (e.g. Sand-Jensen & Borum 1991, Boynton et al. 1996). However, dense macroalgal mats can have similar stabilizing effects. Thick mats decrease sediment suspension (Sfriso & Marcomini 1997, Romano et al. 2003) and shear flow at the sediment surface (Escartín & Aubrey 1995). Both dense seagrass meadows (Gambi et al. 1990) and dense macroalgal mats (Escartín & Aubrey 1995) deflect flow around the bed, creating an area of low shear stress at the sediment surface within the bed. However, research on other types of emergent features in coastal systems, such as polychaete worm tubes, has shown that low-density stands typically displace velocity around individual features, rather than over or around the larger-scale bed/patch, leading to increased erosion around the features (Eckman et al. 1981, Friedrichs et al. 2000, Widdows et al. 2002). When flow is diverted around isolated low-density features, turbulent wake structures can cause areas of high bed shear stress to develop around the features, with the potential to cause scour (e.g. Sadeque et al. 2008), similar to that commonly observed around an isolated bridge pier. Macrophyte density, patch size, and flow velocity interact to determine whether flow, or some portion of the flow, is diverted around the entire bed/patch or individual features. Isolated areas of scour and decreased sediment accumulation have been documented in field, laboratory, and modeling studies of low-density stands of emergent features (Bouma et al. 2007).

Most of the experimental studies to date on the effects of macrophytes on nutrient fluxes across the sediment–water interface have been conducted in

artificially-calm hydrodynamic conditions such as stirred laboratory cores or *in situ* chambers. It is well known that local hydrodynamics influence nutrient fluxes through porewater advection (Huettel & Webster 2001), desorption from suspended sediments (Morin & Morse 1999), and thinning of the diffusive boundary layer (Kelly-Gerrey et al. 2005). Benthic macrophytes can have significant effects on these fluxes physically, by deflecting flow and changing the hydrodynamic conditions at the sediment surface, and biologically, by uptake and indirect effects on bacterial nutrient transformations in the sediment (McGlathery et al. 2007).

Here we report results from a study using controlled microcosm experiments to measure the effects of low-density seagrass and macroalgal populations on sediment suspension and nutrient fluxes. The motivation for this study was the local extinction and subsequent large-scale restoration of the seagrass *Zostera marina* (eelgrass) in the Virginia coastal bays that are part of the Virginia Coast Reserve Long Term Ecological Research (VCR LTER) site (McGlathery et al. 2012, Orth et al. 2012). Extensive seagrass populations were decimated in this region in the 1930s as a result of 2 natural events: a slime mold wasting disease and a severe hurricane. The loss of eelgrass in the mid-Atlantic region of the USA produced a state change in which the perennial, rooted eelgrass was replaced by ephemeral, attached and drift macroalgae, including the invasive species *Gracilaria vermiculophylla*. Fishery species, including scallops, declined and turbidity increased (Cottam & Munro 1954), similar to changes seen in other systems (Stumpf et al. 1999, Yamamuro et al. 2006).

Our experiments were carried out using a small-scale microcosm with controlled hydrodynamic conditions, allowing us to isolate the effects of the primary producers on sediment stability and nutrient fluxes. The use of microcosms with a controlled flow field permits both a mechanistic look at how flow conditions and primary producers interact and direct comparisons between 2 types of primary producers at varying densities. While this approach does not provide ecosystem-level results, microcosm-scale experiments are valuable to direct ecosystem-level studies and parameterize models.

## MATERIALS AND METHODS

To determine the effects of *Zostera marina* and *Gracilaria vermiculophylla* on sediment suspension and nutrient flux, sediment cores with varying bio-

mass were exposed to flow conditions that generate a known bed shear stress in bare sediment cores. Five sampling sites with patchy distributions of eelgrass and macroalgae (attached and detached) were selected randomly within a region of restored eelgrass meadows in South Bay at the VCR LTER site (Fig. 1). At each site, 5 sediment cores (4 bare sediment and 1 with eelgrass) were taken. Each site was sampled on 1 day (18 to 22 June 2007), and bare sediment cores were randomly assigned to a control, low macroalgal biomass, medium macroalgal biomass, or high macroalgal biomass treatment (no medium algal biomass treatment was done on Day 3 due to the loss of 1 sediment core). The study was designed as a randomized complete block design, blocked by day and plot, with 5 cores planned per treatment (1 core was lost and 1 was re-classified resulting in 5 cores for all treatments except low algae). Sediment and water column samples were also taken at each site to characterize the sites for any differences in initial conditions.

### Shear stress experiments

#### Sample collection, storage, and preparation

Sediment samples for the experiments were taken by hand on a falling tide (water depth approximately 0.8 to 1 m), within 1 h of predicted low tide, using

polycarbonate core tubes with an internal diameter of 10.8 cm and a height of 22.5 cm, with a 10 cm water column preserved above the sediment surface. Core tubes were sealed using rubber plumbing end caps and kept in the dark at 4°C during transport back to the lab. Ambient water was collected in 20 l carboys to use as replacement water during the experiments and for core storage until analysis. Upon arrival at the lab, within 1 h of core collection, the cores were submerged in a cooler of ambient water oxygenated by an aquarium bubbler and maintained in low light at ambient temperatures. Macroalgae were collected in South Bay and maintained in a container of ambient water at 4°C for transport to the lab, then kept in oxygenated ambient water until use. Immediately prior to the experiment, the overlying water in each sediment core was gently replaced with the same water that would be used for replacement water during the experiment. No sediment suspension or disturbance was noted during this step.

Bare sediment cores were assigned randomly to 1 of 4 treatments (control, low macroalgae, medium macroalgae, and high macroalgae), and analysis order of the treatments was assigned randomly. Macroalgae treatments were defined as 2 (low), 4 (medium), or 6 (high) g wet weight (WW) of macroalgae, standardized by blotting excess water with a paper towel. Macroalgae were added to a bare sediment core immediately before the start of the experiment.

At the end of the experiment, the macroalgae were collected and dried at 60°C to a constant weight to determine dry weight (DW) biomass. The macroalgae treatments, based on the average field biomass of 54 g DW m<sup>-2</sup> found in an adjacent bay (Thomsen et al. 2006), resulted in a range of dry biomass with averages ± SE of 18 ± 0.83 g DW m<sup>-2</sup> (low), 37 ± 2.1 g DW m<sup>-2</sup> (medium), and 66 ± 4.4 g DW m<sup>-2</sup> (high).

Height limitations of the microcosm required eelgrass to be trimmed to a maximum height of 8 cm prior to running the experiments. Trimming to this height allowed the blades to remain submerged without interfering with the functioning of the microcosm. While the trimming created shoots that were shorter than found in the field during June (the time of this experiment), the shorter length is representative of early spring and late fall shoots found

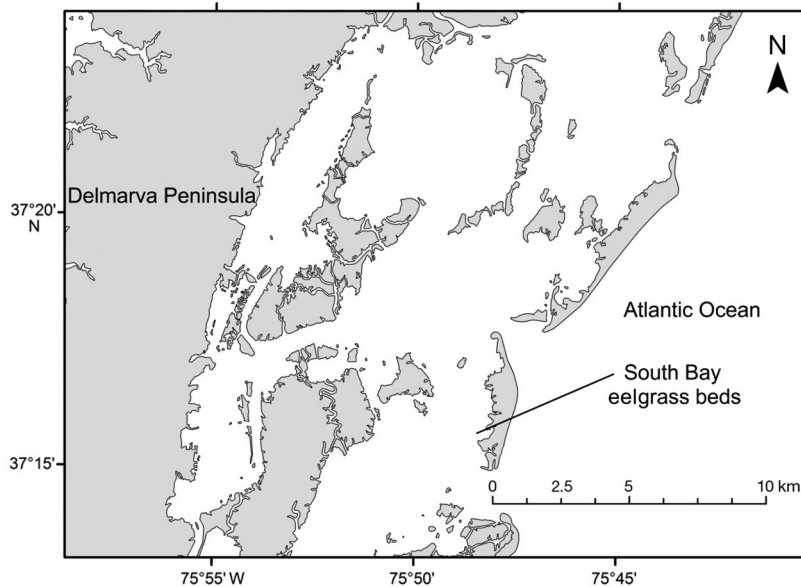


Fig. 1. Study site. South Bay is a shallow coastal lagoon on the east side of the Delmarva Peninsula, USA. Eelgrass transplant efforts and natural recolonization have led to the development of patchy eelgrass beds in the area. Cores with eelgrass or bare sediment were taken in South Bay and exposed to stress under controlled forcing conditions using the Gust erosion microcosm; macroalgae were added to some of the cores

in the nearby Chesapeake Bay (Orth & Moore 1986). The eelgrass cut at the beginning of the experiment was dried at 60°C and weighed. At the end of the experiment, all remaining eelgrass aboveground biomass was cut and also dried and weighed so that the biomass of the eelgrass cut before the experiment, the aboveground biomass in the cores during the experiment, and the total aboveground biomass could be calculated for each core. Total aboveground biomass of the eelgrass (before trimming) in the cores ranged from 64 to 470 g DW m<sup>-2</sup>. This is comparable to 76 to 558 shoots m<sup>-2</sup>, based on field measurements of densities and biomass per shoot from the study site (McGlathery et al. 2012). The aboveground biomass of the eelgrass in the cores during the experiments (i.e. the 8 cm tall blades that remained after trimming) ranged from 41 to 184 g DW m<sup>-2</sup>.

### Shear stress manipulation

The Gust microcosm used in these experiments consisted of an erosion head with a rotating plate and a push-through water system fitted over a polycarbonate core tube (Fig. 2; Thomsen & Gust 2000). With a level, bare sediment bed, the combination of the rotation of the plate and the suction of the water system in the center of the core generates a near-uniform bed shear stress and diffusive boundary

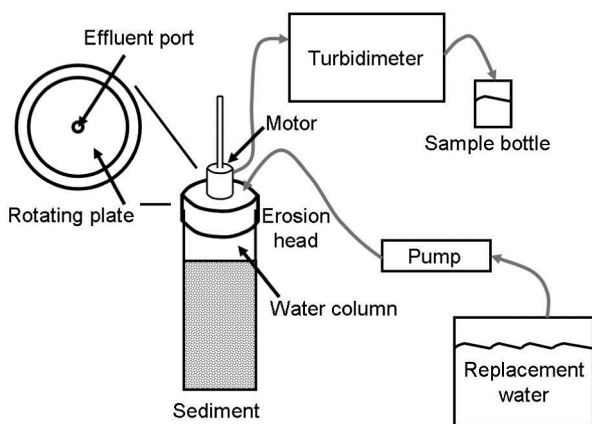


Fig. 2. Gust erosion microcosm. The erosion head contains inflow and outflow ports and a rotating plate that generates a flow producing a relatively uniform shear stress on the sediment surface of a core sample. The rotation and pumping rates are controlled by a datalogger. The turbidity from the effluent is continuously recorded on the turbidimeter, and the outflow is collected in a sample bottle for filtering. The measured sample volumes and sediment and nutrient concentrations were used to calculate fluxes for bare sediment, 3 densities of macroalgae, and seagrass cores

layer thickness, though not a uniform pressure distribution (Tengberg et al. 2004). Because sediment conditions were similar between treatments and the hydrodynamic forcing was controlled, changes in sediment suspension should be a direct result of alterations to the near-bed hydrodynamics. Across treatments, any increase in the mass of sediment eroded from the bed should reflect localized increases in bed shear stress caused by the treatment. The use of Gust microcosms for studying benthic fluxes is further examined in the 'Discussion'.

Immediately prior to the experiment, a water sample was pumped from the replacement water to provide background concentration values. The cores were then exposed to low forcing conditions (forcing that produces a low shear stress of 0.01 N m<sup>-2</sup> in bare sediment cores) for 20 min as a flushing step, followed by high forcing conditions (forcing that produces a shear stress of 0.32 N m<sup>-2</sup> in bare sediment cores) for 40 min. This shear stress is in the range of storm-generated bed shear stresses in the VCR (Lawson et al. 2007, Mariotti et al. 2010) and roughly corresponds to an average velocity of 0.25 m s<sup>-1</sup> in the microcosm (without obstructions), similar to the flow rates used in other studies of sediment suspension/stabilization in *Zostera marina* beds (e.g. Fonseca & Fisher 1986). Effluent water was collected in 1 l Nalgene bottles exchanged every 5 min for the higher shear stresses. All effluent water from the erosion tests was collected, subsampled, and filtered for total suspended solids (TSS), NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3-</sup>, and NO<sub>3</sub><sup>-</sup>. No fragmentation of macroalgae or eelgrass was noted during the experiments.

### Site characterization

Bottom sediment samples were taken at each plot to characterize porewater nutrients, sediment grain size, exchangeable ammonium (NH<sub>4</sub><sup>+</sup>), and benthic chlorophyll. Five replicate samples for grain size and NH<sub>4</sub><sup>+</sup> were taken to a depth of 2 cm with a modified 60 ml syringe, and 5 replicate samples for benthic chlorophyll were taken to 1 cm depth with a modified 5 ml syringe. Three replicate sediment cores were collected in polycarbonate tubes (8 cm internal diameter, 23 cm height) for laboratory sampling of porewater. All samples were kept at 4°C during transport to the lab. Samples for benthic chlorophyll and extractable NH<sub>4</sub><sup>+</sup> were immediately frozen; grain size samples were maintained at 4°C until analysis. Porewater samples were extracted from the sediment cores at 2 cm depth intervals to 10 cm depth using a

stainless steel probe (Berg & McGlathery 2001) and were immediately filtered (0.45  $\mu\text{m}$ ) and frozen. Exchangeable  $\text{NH}_4^+$  was extracted for 1 h with an equal volume of 1 N KCl solution. Porewater samples (for  $\text{NH}_4^+$ , phosphate  $\text{PO}_4^{3-}$ , and nitrate+nitrite  $\text{NO}_3^-$ ), and exchangeable  $\text{NH}_4^+$  samples were analyzed on a Lachat QuickChem 8500 using standard methods (Hach). Benthic chlorophyll was determined spectrophotometrically following extraction with a 45% acetone, 45% methanol, 10% deionized water solution using the equations of Lorenzen (1967). Sediment grain size was analyzed as 1 bulk sample per plot, from 5 pooled samples (0 to 2 cm depth), using a combination of wet sieving for sands and analysis on a Sedigraph 5100 particle size analyzer for silt and clay.

### Laboratory analyses

All sediment and water samples were analyzed using standard methods. TSS (sample volumes 150 to 550 ml) was analyzed by weight difference after filtration onto pre-combusted, pre-weighed Whatmann GF/F filters (nominal particle retention 0.7  $\mu\text{m}$ ). Sub-samples for nutrients (20 ml) were filtered (0.45  $\mu\text{m}$ ) and frozen until analysis.  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ , and  $\text{NO}_3^-$  were analyzed using standard methods on a Lachat QuikChem 8500 (as described above).  $\text{NO}_3^-$  and nitrite ( $\text{NO}_2^-$ ) were not separated because  $\text{NO}_2^-$  values were frequently below the detection level. The combined value is presented as  $\text{NO}_3^-$  throughout this study.

To correct for the mass of measured components carried in the replacement water, all fluxes were corrected for background concentration. For the nutrient fluxes, the average concentration of all background samples for the day was used as the background concentration. For the mass of sediment eroded, the lowest measured concentration of TSS during the analysis of the core was used. Data from

the flushing step were discarded, and only data from the higher shear stress step were analyzed. One macroalgal treatment was reclassified (from low to medium) at the end of the experiment based on the measured DW of the macroalgae (33 g DW  $\text{m}^{-2}$ ).

## RESULTS

### Site characterization

We found no significant differences in the initial conditions at the 5 plots in South Bay (Table 1). Sediment extractable  $\text{NH}_4^+$  ranged from 0.065 to 0.087  $\mu\text{mol g DW}^{-1}$  with an average  $\pm$  SE of  $0.08 \pm 0.00 \mu\text{mol g DW}^{-1}$ . Porewater concentrations of  $\text{NH}_4^+$  ranged from 0 to 152  $\mu\text{M}$ ,  $\text{PO}_4^{3-}$  from 0 to 16  $\mu\text{M}$ , and  $\text{NO}_3^-$  from 1.2 to 6  $\mu\text{M}$ . The sediment in all plots was very fine sand, with between 5 and 11% mud (average grain size = 71  $\mu\text{m}$ ). Water column nutrient concentrations, as measured from the replacement water, were  $0.5 \pm 0.1 \mu\text{M NH}_4^+$ ,  $0.4 \pm 0.1 \mu\text{M PO}_4^{3-}$ , and  $0.3 \pm 0.1 \mu\text{M NO}_3^-$ , similar to values measured in neighboring Hog Island Bay (McGlathery et al. 2001).

### Sediment suspension

During the 40 min high-forcing experiments, an average of  $0.26 \pm 0.03 \text{ g}$  of sediment was eroded for all treatments, which is equivalent to an average erosion depth of  $2.8 \times 10^{-3} \text{ m}$ , assuming a bulk density (DW of sediment per unit volume of bed) of  $1.0 \text{ g cm}^{-3}$ , or  $28.7 \text{ g m}^{-2}$  (see Figs. 3 & 4). There was no significant effect of eelgrass presence on sediment suspension compared to bare sediment for the flow conditions and biomass used in these experiments (paired *t*-test,  $p = 0.4$ , Fig. 3). However, closer analysis of the variation in eelgrass biomass within the replicate cores indicates that at low biomass (corresponding to shoot densities of approximately 76 to

Table 1. Summary of site characteristics for each plot in South Bay. All plots showed similar sediment and nutrient conditions. Values are averages (SE) of samples as described in 'Materials and methods'

	Average grain size ( $\mu\text{m}$ )	Benthic chlorophyll ( $\text{mg m}^{-2}$ )	$\text{NH}_4^+$ ( $\mu\text{M}$ )	$\text{PO}_4^{3-}$ ( $\mu\text{M}$ )	$\text{NO}_3^-$ ( $\mu\text{M}$ )	Extractable $\text{NH}_4^+$	Porewater		
							$\text{NH}_4^+$ ( $\mu\text{M}$ )	$\text{PO}_4^{3-}$ ( $\mu\text{M}$ )	$\text{NO}_3^-$ ( $\mu\text{M}$ )
Day 1	76	4.7 (0.8)	0.4 (0.2)	0.3 (0.1)	0.5 (0.1)	0.06 (0.00)	62 (5)	4.3 (0.5)	2.2 (0.2)
Day 2	70	5.2 (0.2)	0.2 (0.1)	0.2 (0.0)	–	0.09 (0.00)	43 (5)	3.5 (0.6)	1.7 (0.3)
Day 3	76	5.6 (0.9)	0.6 (0.2)	0.4 (0.2)	0.2 (0.1)	0.08 (0.00)	82 (7)	5.7 (0.4)	2.0 (0.1)
Day 4	67	4.5 (0.6)	0.6 (0.1)	0.4 (0.0)	0.3 (0.1)	0.07 (0.00)	34 (9)	4.7 (0.2)	2.6 (0.3)
Day 5	66	6.1 (0.8)	0.7 (0.1)	0.5 (0.2)	0.3 (0.2)	0.08 (0.02)	42 (3)	3.4 (0.5)	2.6 (0.3)

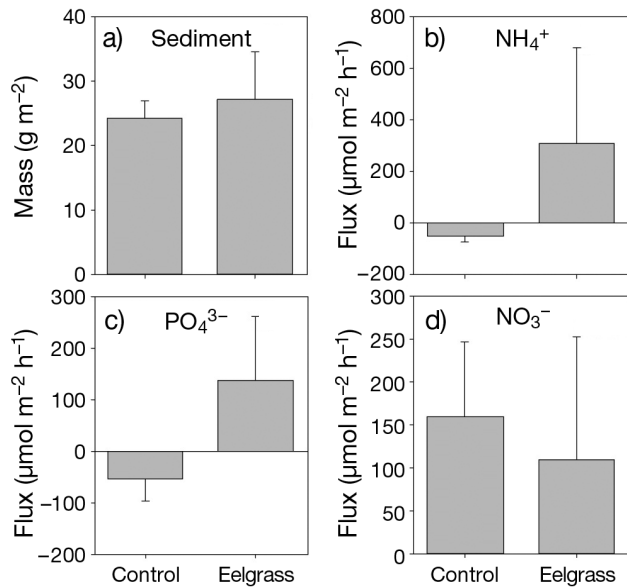


Fig. 3. Summary of sediment eroded and nutrient flux for eelgrass (error bars = SE;  $n = 5$ ). (a) Mass of sediment eroded, (b)  $\text{NH}_4^+$  flux, (c)  $\text{PO}_4^{3-}$  flux, and (d)  $\text{NO}_3^-$  flux. Nutrient fluxes generally mirrored the mass of sediment eroded, although SEs were large. The variability of densities and lack of replicates at different eelgrass densities make comparisons between the control and eelgrass cores difficult and lead to no significant differences between sediment suspension in eelgrass and control cores (paired  $t$ -test,  $p = 0.4$ )

239 shoots  $\text{m}^{-2}$ ), sediment suspension appeared to increase with increasing biomass, whereas at higher biomass, sediment suspension dropped to almost 0. Moore (2004) found a lack of sediment trapping in seagrass beds of similar low biomass, but a mechanistic look at internal resuspension has not previously been conducted. A more complete understanding of the structure of this relationship and the biomass or density corresponding to peak suspension would require considerably more data than are available in these experiments and is beyond the scope of this study. However, the existence of conditions in which seagrass may de-stabilize, rather than stabilize, sediment beds is considered in the 'Discussion'.

Macroalgal biomass had a significant effect on sediment suspension, with an initial increase above control values for the low biomass treatment, followed by a decrease for the medium and high biomass treatments (ANOVA,  $p = 0.04$ ,  $n = 14$ , Fig. 4). If we take into account the variability in macroalgal biomass for each replicate and consider the macroalgal biomass, excluding the control, as a continuous variable, the mass of sediment eroded was strongly negatively dependent on biomass ( $R^2 = 0.55$ ,  $p = 0.002$ ,  $n = 14$ , Fig. 5). The control treatment was

excluded from this regression analysis because of the initial increase in sediment erosion from the control to low biomass treatments.

### Nutrient flux

For the macroalgae treatments, the  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  fluxes showed a similar pattern to the sediment suspension, with an increase in flux between the control and low biomass treatment, followed by a decrease for the medium and high biomass treatments (Fig. 4). Although high variability in nutrient fluxes within each treatment resulted in no significant differences between treatments for macroalgae (ANOVA,  $\text{NH}_4^+$   $p = 0.9$ ,  $\text{PO}_4^{3-}$   $p = 0.4$ ,  $\text{NO}_3^-$   $p = 0.2$ ) or eelgrass ( $t$ -test,  $\text{NH}_4^+$   $p = 0.2$ ,  $\text{PO}_4^{3-}$   $p = 0.08$ ,  $\text{NO}_3^-$   $p = 0.4$ ), some trends in the data are apparent. The sediment flux was related to the  $\text{NH}_4^+$  flux ( $R^2 = 0.98$ ,  $p = 0.007$ , Fig. 6) for the macroalgal treatments and the control. The close relationship between macroalgal biomass and mass of sediment eroded (Fig. 5) makes it difficult to distinguish effects of nutrient uptake by macroalgae from effects of hydrodynamic forcing.

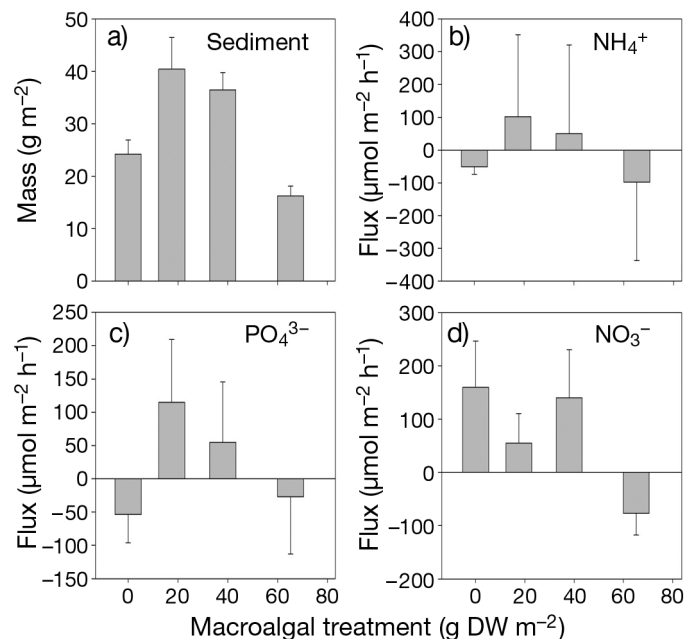


Fig. 4. Summary of sediment eroded and nutrient fluxes for the macroalgae treatments (error bars = SE;  $n = 5$  for control, medium, and high biomass treatments,  $n = 4$  for low biomass). (a) Mass of sediment eroded, (b)  $\text{NH}_4^+$  flux, (c)  $\text{PO}_4^{3-}$  flux, and (d)  $\text{NO}_3^-$  flux. The mass of sediment eroded varied with macroalgal biomass (ANOVA,  $p = 0.04$ ,  $n = 14$ ), with an increase at low biomass (18 g dry weight, DW,  $\text{m}^{-2}$ ) followed by a decrease at higher biomasses (37 and 54 g DW  $\text{m}^{-2}$ ). Nutrient fluxes did not vary significantly but generally mirrored the mass of sediment eroded

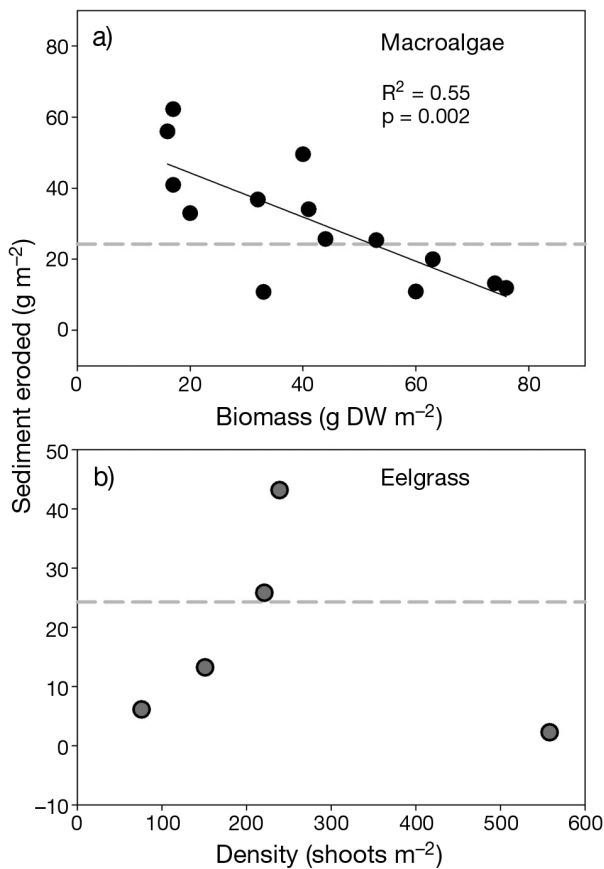


Fig. 5. Relationship between suspended sediment and primary producer biomass. (a) Macroalgae showed decreased suspension with increasing biomass ( $R^2 = 0.55$ ,  $p = 0.002$ ), but sediment suspension at low biomass was higher than control levels (dashed gray line). (b) Eelgrass showed increasing suspension with increasing biomass, a novel finding compared to characterization of seagrass as a sediment stabilizer. The range of eelgrass biomass that showed increasing suspension is roughly equivalent to 76 to 239 shoots  $m^{-2}$ . Sediment suspension is presented as the total mass eroded scaled to 1  $m^2$  area for the duration of the high flow conditions (40 min). Sediment suspension is not scaled to the hour because the sediment flux would likely decrease over time, consistent with Type I erosion (Sanford & Maa 2001)

However, if nutrient fluxes were dependent on biomass, the control point would not fit the regression of mass of sediment eroded and nutrient flux, because the control point is not part of the linear relationship between mass of sediment eroded and biomass (Fig. 4).

## DISCUSSION

Our data provide evidence that low-biomass macroalgal populations can increase both sediment suspension and nutrient efflux under high-flow con-

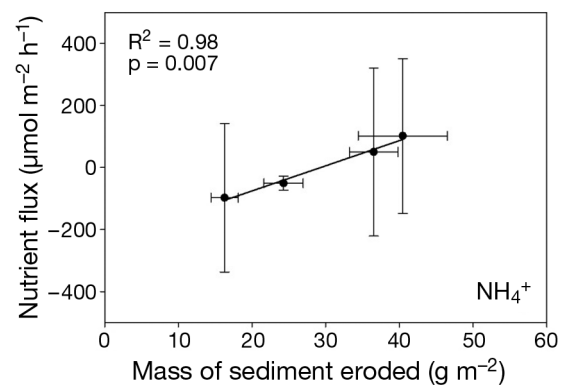


Fig. 6. Relationships between nutrient flux and mass of sediment eroded (error bars = SE). Flux of  $\text{NH}_4^+$  ( $R^2 = 0.98$ ,  $p = 0.007$ ) is positively correlated with mass of sediment eroded for the macroalgal treatments and the control (second point from the left). This relationship indicates that when shear stresses are high and macroalgal biomass is low macroalgae may increase the nutrient efflux from the sediment. In coastal bays in which macroalgae typically reduce the flux of nutrients from the sediment to the water column, this may be an important process for supplying nutrients to pelagic producers

ditions. The data also suggest that at low densities, seagrass may not be effective at stabilizing sediment and may in fact enhance sediment suspension. Most previous research on high-biomass populations has shown that seagrass and macroalgal mats reduce sediment suspension (Sfriso & Marcomini 1997, Heiss et al. 2000, Romano et al. 2003, Peterson et al. 2004) and nutrient efflux (Thybo-Christesen et al. 1993, Sundback et al. 2000, McGlathery et al. 2004, 2007), although recent experiments on macroalgae by Canal-Vergés et al. (2010) are an exception. Because our results differ from those of most previous studies, these experiments give insight into conditions that may favor sediment destabilization by benthic primary producers.

## Applicability of microcosm studies to field conditions

While microcosms can never exactly mimic field conditions, the Gust microcosm has been widely used to represent near-bed flows for the purpose of measuring benthic fluxes (Ziervogel & Bohling 2003, Porter et al. 2004a, Stevens et al. 2007, Dickhudt et al. 2009, Wiberg et al. in press). The Gust microcosm creates a relatively uniform bed shear stress through the action of a spinning plate just below the water surface and a push-through water system. With this forcing, flow paths in the Gust microcosm occur in a logarithmic spiral with an upward vortex in the mid-

dle of the core (Thomsen & Gust 2000). Because the flow path includes a significant vertical component, it more closely resembles shallow wave action or a combined wave-current flow than a simple uniform current, which is appropriate for shallow lagoons where wave forcing is dominant (Lawson et al. 2007, Mariotti et al. 2010). Gust microcosms have been used to measure benthic fluxes on sediment cores from a wide range of environments, including the Baltic Sea (Ziervogel & Bohling 2003), Adriatic shelf (Stevens et al. 2007), Chesapeake Bay tributaries (Porter et al. 2004a, Dickhudt et al. 2009), the northern Atlantic off the coast of the UK (Black et al. 2003), and tidal flats in southwest Washington State, USA (Wiberg et al. in revision). Sediment erodibility measured using Gust microcosms has been shown to compare well with measurements made using other techniques (e.g. *in situ* inverted flume; Dickhudt et al. 2009) and to yield calculated suspended sediment concentrations in good agreement with field measurements (Traykovski et al. 2007).

An extensive comparison of the Gust microcosm and other benthic chambers showed that the Gust microcosm produced the most uniform bed shear stress and diffusive boundary layer thickness of the chambers studied (Tengberg et al. 2004). This uniformity is important when introducing obstructions to the chamber, making the Gust microcosm an appropriate choice for this experiment. To confirm that the forcing conditions chosen for the experiments reasonably reflect the forcing conditions in benthic environments, the ratio of mean velocity ( $0.25 \text{ m s}^{-1}$ ) to the shear velocity ( $0.018 \text{ m s}^{-1}$ , assuming a saltwater density of  $1030 \text{ kg m}^{-3}$ ) in the chamber was compared with the ratios found in natural benthic environments (Porter et al. 2004b). The calculated value for the flow conditions without obstructions (14.1) is within the range seen in natural environments (13.9 to 22.8, Porter et al. 2004b). The presence of macrophytes is expected to decrease average bed shear stress because of drag on the macrophytes.

### Stabilizing versus destabilizing effects of seagrass

Sediment stabilization from seagrass beds typically occurs due to the high flow resistance offered by a dense seagrass bed (Gambi et al. 1990, Bouma et al. 2007). However, at low densities and high-flow rates, water predominantly moves through, rather than over or around a seagrass bed. In sparse seagrass beds, suspended sediment concentrations may be equal to those in unvegetated areas because the tur-

bulent stresses are similar (Luhar et al. 2008). The interaction of flow with individual shoots causes wakes to form behind the shoots. At Reynolds numbers,  $Re = Ud/\nu > \approx 200$ , where  $U$  is velocity,  $d$  is shoot diameter, and  $\nu$  is kinematic viscosity, the wakes are turbulent (Nepf 1999). At relatively low shoot densities, increases in wake-generated turbulence production can be large enough relative to frictional decreases in velocity to cause an increase in turbulent kinetic energy (Nepf 1999) and local bed shear stresses (Sadeque et al. 2008) when vegetation is present compared to unvegetated conditions. If the wake-generated shear stresses at the bed exceed the critical shear stress of the bed sediment, scour and increased sediment suspension can result, as seen with other isolated emergent features (e.g. Eckman et al. 1981, Friedrichs et al. 2000, Widdows et al. 2002). Assuming a representative blade width of 3 mm (Hansen & Reidenbach 2012, this Theme Section),  $Re \approx 600$  for our experiments.

In addition to density and flow velocity, flow characteristics (i.e. wave or current dominated) may affect the ability of the seagrass to stabilize sediment. When seagrass is exposed to wave forcing, a mean current is generated within the canopy rather than a skimming flow developing over the top of the canopy (Luhar et al. 2010). Also, while seagrass blades in unidirectional flow bend and physically shield the sediment bed, seagrass blades in oscillatory flows move with the flow and can enhance the exchange between the sediment bed and the water column (Koch & Gust 1999). Taller seagrass in unidirectional flow may shield the bed (as noted by Fonseca & Fisher 1986); however, short seagrass shoots that are more representative of early spring and late fall conditions in the field do not provide bed shielding in wave-like flow conditions. Previous research has shown that these low-density and low-biomass beds are ineffective at trapping suspended sediment under riverine conditions (Moore 2004), but their influence on internal resuspension and wave-dominated flows has not been studied.

### Stabilizing versus destabilizing effects of macroalgae

Density and flow conditions may have similar effects on the ability of macroalgae to stabilize sediments. While dense macroalgal mats deflect flow around the mat (Gambi et al. 1990, Escartín & Aubrey 1995) similar to dense seagrass beds, isolated individual or individuals in patchy distributions are directly exposed to the undisturbed flow conditions.



Macroalgae such as *Gracilaria vermiculophylla*, which are frequently found detached, are often transported as bedload. Bedload transport of macroalgae is an important component of macroalgal transport (Flindt et al. 1997, 2004), and has been shown to significantly increase sediment suspension at densities of 17 to 33 g DW m<sup>-2</sup> (Canal-Vergés et al. 2010). Combining that study with the results of our experiment and the results seen in high-density mats (Escartín & Aubrey 1995, Sfriso & Marcomini 1997, Romano et al. 2003, Sfriso et al. 2005) shows that high-biomass populations of macroalgae stabilize sediment while low-biomass populations destabilize sediment. Given the high seasonal variability in macroalgal biomass (e.g. Thomsen et al. 2006), the stabilizing/destabilizing effect of macroalgae is also likely seasonal. A vertical component of flow, found in the microcosm and in wave-dominated flows, may make the macroalgae more likely to be transported because fronds are lifted away from the bed and into the faster-moving flow in the water column, whereas in unidirectional flows, the macroalgae likely lie closer to the sediment surface. The movement of these fronds in current-versus wave-dominated flows may be analogous to the differences seen in seagrass movement in unidirectional and oscillatory flow conditions (Koch & Gust 1999).

Bedload transport of macroalgae can destabilize sediments by dislodging particles in a process similar to the widely documented increase in sediment suspension due to saltating or abrading particles (e.g. Houser & Nickling 2001, Thompson & Amos 2002, 2004). In fine-grained beds, the critical shear stress required to initiate erosion is often greater than the stress required to maintain the sediment in suspension. Under these conditions, an object (e.g. macroalgae) that impacts or scrapes the bed while moving across it can dislodge particles and significantly increase sediment suspension/erosion. We observed higher sediment erosion in experiments in which the macroalgae were in motion compared to cases in which the macroalgae were stationary.

### Factors affecting nutrient fluxes

The results of this study show that the effects of macrophytes on benthic nutrient fluxes are not limited to biotic effects. Modification of near-bed flow by benthic macrophytes can influence sediment–water column nutrient fluxes. Field and modeling studies have shown that increased hydrodynamic activity and sediment suspension influenced nutrient flux in

areas such as Lake Okeechobee, Florida, USA (Chen & Sheng 2003, 2005), the Satilla River Estuary, Georgia, USA (Zheng et al. 2004), and the Mediterranean shelf (Gremare et al. 2003). Modification of near-bed flow by benthic primary producers can produce similar effects. Benthic primary producers are well known to indirectly affect nutrient cycling, including effects on nitrogen fixation, nitrification, and nitrification/denitrification (e.g. Viaroli et al. 1996, Hansen et al. 2000, McGlathery et al. 2004). Nutrient uptake by benthic primary producers can also significantly reduce the flux of remineralized nutrients to the water column (Thybo-Christesen et al. 1993, Sundbäck et al. 2000, McGlathery et al. 2004, 2007). The total impact of benthic primary producers on the flux of nutrients between the sediment and water column is thus the result of both physical modification and biotic influences, yet these have rarely been studied in tandem.

Our data suggest that the effect of primary producers on nutrient flux during a forcing event depends on the balance between uptake and physical modification of the flow near the sediment surface. In low-flow cores or microcosm incubations, it has been shown that benthic algae can prevent the efflux of nutrients from the sediment to the water column (e.g. Sundbäck et al. 2000, Tyler et al. 2001), with the balance between benthic autotrophy and heterotrophy having a significant influence on nutrient efflux (Eyre & Ferguson 2002). This uptake effectively decouples nutrient cycling in the sediment and the water column (Kristensen et al. 2000, Anderson et al. 2003). Our results show that when macroalgal biomass is low and flow conditions are relatively high, physical dynamics result in an enhancement of nutrient flux to the water column in addition to increased sediment suspension. This can be seen in the relationship between sediment suspension and NH<sub>4</sub><sup>+</sup> fluxes (Fig. 6). Under high-flow conditions, processes such as porewater advection and desorption of nutrients from suspended particles increase nutrient fluxes above the levels supported by diffusion alone (Ward et al. 1984, Huettel et al. 1998, Morin & Morse 1999). The movement of algae caused by the increased shear stress likely enhances this effect. In a related study, we investigated the mechanisms accounting for the increased nutrient efflux from sediments in our microcosm experiments and showed that hydrodynamically-forced fluxes (advection, suspension/desorption) were similar in magnitude to diffusive fluxes, indicating that low-flow incubations may underestimate nutrient fluxes by 50% if higher flows are present (Lawson 2008).

In summary, our data show that seagrass and macroalgae can have both stabilizing and destabilizing effects on the sediment when exposed to forcing conditions that reflect the high flows representative of storm conditions in shallow bays. Low biomass, or densities, of macroalgae can destabilize sediments, leading to increased sediment suspension and nutrient efflux; the same may be true of low-density seagrass beds. The mechanisms responsible for the destabilization are different for the 2 macrophyte functional groups: increases in near-bed turbulence as flow moves through seagrass shoots versus dislodgement of sediment particles by bedload transport of macroalgae. The threshold between stabilizing and destabilizing effects is biomass-dependent, and suggests a 'window of vulnerability' to negative feedbacks when biomass or densities are low and flows are high. This is relevant to restored seagrass populations where seeding results in initially low-density populations that eventually increase in biomass and density as the meadow develops over time. It is also relevant to shallow coastal systems that have been impacted by nutrient over-enrichment where multiple stresses create patchiness and thinning of seagrass populations, and macroalgal biomass may accumulate on seasonal time scales.

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# Wave and tidally driven flows in eelgrass beds and their effect on sediment suspension

Jennifer C. R. Hansen, Matthew A. Reidenbach\*

Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia 22904, USA

**ABSTRACT:** Seagrass beds alter their hydrodynamic environment by inducing drag on the flow, thereby attenuating wave energy and near-bottom currents. This alters the turbulent structure and shear stresses within and around the seagrass bed that are responsible for the suspension and deposition of sediment. To quantify these interactions, velocity, pressure, and sediment measurements were obtained across a density gradient of an eelgrass *Zostera marina* bed within a shallow coastal bay (1 to 2 m depth). Eelgrass beds were found to reduce near-bottom mean velocities by 70 to 90%, while wave heights were reduced 45 to 70% compared to an adjacent unvegetated region. Wave orbital velocities within the eelgrass bed were reduced by 20% compared to flow above the bed, primarily acting as a low-pass filter by removing high-frequency wave motion. However, relatively little reduction in wave energy occurred at lower wave frequencies, suggesting that longer period waves were able to effectively penetrate the seagrass meadow. Average bottom shear stresses ( $\tau_b$ ) at the unvegetated region were  $\tau_b = 0.17 \pm 0.08 \text{ N m}^{-2}$ , significantly larger than the critical stress threshold necessary for sediment entrainment of  $0.04 \text{ N m}^{-2}$ . Within the eelgrass bed,  $\tau_b = 0.03 \pm 0.02 \text{ N m}^{-2}$  and stresses were below the critical stress threshold during 80% of the time period of measurement. Expansion of eelgrass within the coastal bay has thus altered the dynamics of the seafloor from an erosional environment to one that promotes deposition of suspended sediment, enhancing light penetration throughout the water column and creating a positive feedback for eelgrass growth.

**KEY WORDS:** *Zostera marina* · Seagrass · Waves · Turbulence · Boundary layer · Sediment resuspension

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## INTRODUCTION

Seagrass ecosystems have been viewed as depositional environments for sediment because the structure of the meadow serves to reduce flow and attenuate bottom shear stresses (Ward et al. 1984, Koch et al. 2006, Gruber & Kemp 2010). Studies of direct particle trapping by seagrasses and seagrass epiphytes confirm that seagrass meadows buffer against sediment resuspension and increase sediment retention, therefore reducing erosion in the coastal zone (Gacia & Duarte 2001, Agawin & Duarte 2002). When fluid flow encounters individual seagrass blades or a seagrass bed, drag is imparted on the flow and a velocity

gradient develops, creating a boundary layer (Denny 1988). Momentum loss due to drag by the canopy leads to the reduction in resuspension; however, reduced resuspension and increased sediment accumulation may not occur at the same rate everywhere within the meadow, and are dependent upon local flow dynamics and sediment supply from within and outside the meadow (Chen et al. 2007). Whether bulk flow is able to move through the seagrass canopy or is diverted above or around it is dependent on shoot density and bed spatial heterogeneity (Fonseca & Koehl 2006, Nepf et al. 2007). Reduction in velocities within seagrass beds is often accompanied by skimming flow, which increases velocities above the sea-

\*Corresponding author. Email: reidenbach@virginia.edu

grass canopy relative to ambient flow conditions (Fonseca et al. 1982). This creates an inflection point of instability in the velocity profile, and shear layers are developed near the top of the canopy (Widdows et al. 2008, Bouma et al. 2009), which can locally enhance turbulent mixing into the canopy (Nepf & Vivoni 2000). Canopy friction exhibits a strong positive relationship to the percent of the water column occupied by the seagrass (Fonseca & Fisher 1986), and greater flow reductions are found inside canopies with increasing shoot density (Peterson et al. 2004). However, the impact of seagrass on within-canopy turbulence is less clear, and suggests that mixing rates and turbulent kinetic energy (TKE) are highly dependent upon seagrass density and morphology (Worcester 1995, Granata et al. 2001, Widdows et al. 2008).

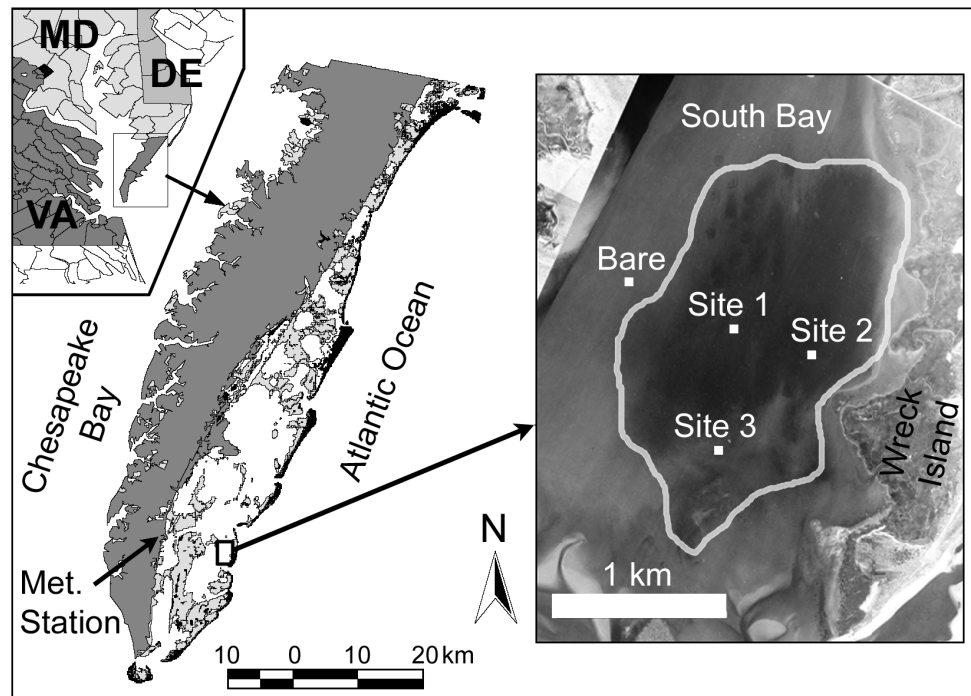
Within shallow-water environments where seagrasses are abundant, local flow dynamics can be drastically altered depending on whether currents are dominated by tides, wind-generated waves, storm surge, or a combination of these factors (Fonseca & Cahalan 1992, Koch & Gust 1999, Koch et al. 2006). The increase in oscillatory flows caused by waves has been linked to enhanced turbidity (Granata et al. 2001). The nonlinear interaction between waves and currents leads to changes in the hydrodynamics and shear stresses imposed on the seafloor from those expected under either condition independently (Jing & Ridd 1996). Typically when waves are present in unvegetated seafloor regions, an oscillatory wave boundary layer develops that is more strongly sheared than the boundary layer formed under steady conditions alone (Grant & Madsen 1979). This wave boundary layer results in greater drag on the mean flow and increased bottom shear stresses. Sediment resuspension has been found to be predominately controlled by waves in shallow coastal bays, occurring periodically and corresponding to high wind events (Lawson et al. 2007), suggesting sediment suspension is episodic in nature. Many studies have described how seagrasses attenuate waves (Fonseca & Cahalan 1992, Koch & Gust 1999, Bradley & Houser 2009); however, how this attenuation impacts bottom shear stresses and within-canopy turbulence, which are ultimately responsible for the suspension of sediments from the seafloor, is largely unknown.

Most studies that have addressed the impacts of seagrass density and morphology on flow and sediment dynamics have been based on laboratory flume experiments or numerical modeling (Heller 1987, Chen et al. 2007, Bouma et al. 2009, Carr et al. 2010).

These studies have determined that the thickness of the shear layer at the top of the canopy is directly related to the vegetation density; as density is increased under a constant velocity, the exchange is reduced, as is the penetration depth of the mixed layer into the canopy (Ghisalberti & Nepf 2002). For waves that are typically generated in fetch-limited shallow coastal bays, wave energy decreases with depth, and the magnitude of wave energy that reaches the seafloor depends heavily on the wave period, wave height, and overall water depth (de Boer 2007). According to modeling efforts, wave energy attenuation increases with increasing shoot density (Chen et al. 2007), but the degree of flow reduction by the canopy can also be a function of distance from the edge of the canopy and the mean depth at which the seagrass resides below the surface (Fonseca & Fisher 1986, Verduin & Backhaus 2000). However, the lack of field studies on these interactions makes it difficult to understand the role of seagrass structure on near-bottom turbulence, bottom shear, and sediment dynamics (de Boer 2007). Consequently, it is important to explore the dynamic interactions between seagrass meadows and fluid motion on multiple spatial and temporal scales.

Within the coastal bays of Virginia, USA, the once-dominant seagrass species *Zostera marina* (eelgrass) virtually disappeared during the 1930s due to a pandemic infestation of a parasitic fungus, called wasting disease, combined with a destructive hurricane (Short et al. 1987, Orth et al. 2006). The discovery of several small, natural patches of eelgrass in the mid-1990s suggested that certain locations within the coastal bays were adequate for plant growth (Orth et al. 2006). Survival and expansion of these plots initiated an intensive, large-scale eelgrass seeding program to re-establish the Virginia coastal bays (Fig. 1) as a self-sustaining seagrass ecosystem (Orth et al. 2012, this Theme Section). The shallow depths of Virginia coastal bays, typically <2 m depth, make the bottom sediments susceptible to current- and wave-induced sediment suspension. These coastal bays also lack any significant riverine discharge, and therefore turbidity is primarily controlled by local resuspension (Lawson et al. 2007). In addition, low pelagic primary productivity in the coastal bays suggests that light attenuation is primarily controlled by non-algal particulate matter (Sand-Jensen & Borum 1991, McGlathery et al. 2001). High suspended sediment concentrations (SSC) can attenuate light penetration through the water column that can limit benthic primary production (Zimmerman et al. 1995). As a result, subsequent changes in the fluid environ-

Fig. 1. Study sites, located off the eastern shore of the Delmarva peninsula, Virginia, USA. The 4 study sites within and outside the eelgrass bed are labeled on the aerial image of South Bay. Location of the eelgrass bed within South Bay is outlined in white. Bare: a predominantly un-vegetated site



ment due to the eelgrass expansion now being observed in these coastal bays (Orth et al. 2012, this Theme Section) may improve water quality and reduce turbidity, thus increasing light penetration to the seafloor, which has been shown to have a positive feedback effect on seagrass growth (Madsen et al. 2001, Carr et al. 2010).

In coordination with the seagrass seeding program, the focus of the present study was on the relationships between physical and biological forces controlling water circulation, flow structure, and suspended sediment within the seagrass beds and the Virginia coastal bay system. The goals were to quantify: (1) the spatial variation of *Zostera marina* morphology across the meadow, (2) the velocity and turbulence levels above and within the *Z. marina* canopy, (3) the bottom shear stresses due to combined currents and waves, (4) and the response of the SSC to local turbulence and shear stress magnitudes. These parameters were compared to flow and sediment dynamics occurring in an adjacent unvegetated region.

## MATERIALS AND METHODS

Seagrass studies were performed in South Bay, one of the coastal bays within the Virginia Coast Reserve (VCR), where ongoing seagrass restoration efforts are being performed. The VCR is characterized by contiguous marsh, shallow bay, and barrier island

systems and is a National Science Foundation–Long Term Ecological Research (NSF-LTER) program site (Fig. 1). Reseeding of eelgrass *Zostera marina* in South Bay began in 2001 (Orth et al. 2012, this Theme Section). Field studies were performed in May and June of 2010, with instrument deployments and field data collection occurring over a 72 h period at each site. Three seagrass sites were chosen of varying density, each surrounded by a *Z. marina*-dominated area of at least 700 m<sup>2</sup>. During summer 2010, the total eelgrass cover in South Bay was estimated to be 1020 ha (Orth et al. 2012, this Theme Section). A predominantly unvegetated site, containing only very few, small patches of eelgrass within the region, was also monitored as a reference for flow characteristics in the absence of any considerable benthic vegetation ('Bare' in Fig. 1). Eelgrass density was measured in the field via 0.25 m<sup>2</sup> quadrat shoot counts, while blade length and width were measured in the laboratory from eelgrass collected at each site (Table 1). The 3 sites, labeled Sites 1, 2, and 3 in Fig. 1, contained *Z. marina* of varying densities of  $560 \pm 70$ ,  $390 \pm 80$ , and  $150 \pm 80$  shoots m<sup>-2</sup>, respectively. Mean blade lengths ranged from 16 to 28 cm. Densities between each of the 3 sites, as well as mean blade lengths, were significantly different from one another (ANOVA,  $p < 0.05$ ). Measurements were taken throughout 3 consecutive weeks from June to July 2010, a period in the growing season when the eelgrass is near peak biomass (Orth et al. 2006).

Table 1. *Zostera marina*. Morphometrics during the summer of 2010 at 3 neighboring sites in South Bay, Virginia, USA. Eelgrass density was measured using *in situ* 0.25 m<sup>2</sup> quadrat shoot counts. Blade length is the mean length of all eelgrass blades measured, maximum length is the average of the longest 10% of all blades, and blade width was measured at the midpoint along the length of the blade. Values are  $\pm$  SD.  $n_{\text{blades}}$ : number of blades measured for length and width;  $n_{\text{density}}$ : number of 0.25 m<sup>2</sup> quadrats measured

	Blade length (cm)	Max. length (cm)	Blade width (cm)	$n_{\text{blades}}$	Density (shoots m <sup>-2</sup> )	$n_{\text{density}}$
Site 1	21 $\pm$ 8	33 $\pm$ 3	0.29 $\pm$ 0.08	158	560 $\pm$ 70	9
Site 2	28 $\pm$ 13	51 $\pm$ 3	0.41 $\pm$ 0.12	176	390 $\pm$ 80	8
Site 3	16 $\pm$ 9	34 $\pm$ 4	0.26 $\pm$ 0.07	73	150 $\pm$ 80	21

Mean water depth at all study sites ranged between 1.4 and 1.8 m. Bathymetry for the coastal bays throughout the VCR is provided in Fagherazzi & Wiberg (2009).

### Instrumentation

A coordinated package of instrumentation was deployed at each site for a minimum of 72 h. Two Nortek Vector acoustic Doppler velocimeters (ADV) were deployed at each site, and velocity was quantified at a single point within a 1 cm<sup>3</sup> sampling volume located 15 cm below each sensor. One ADV was located within the meadow with its sampling volume at  $z = 0.1$  m above the seafloor, and the other ADV was located above the meadow with its sampling volume at  $z = 0.5$  m. To measure velocities within the meadow, a small eelgrass patch of 15 cm diameter (the size of the instrument probe) was removed to prevent blades from blocking the sampling volume. In addition, values with poor correlation factors, often due to the probes being exposed at low tide, were removed. Velocities were measured at each site in 10 min bursts every 20 min at a sampling rate of 32 Hz. Each ADV was equipped with a pressure sensor, which was used to determine the water depth and characterize the wave climate. Wind data were obtained from a meteorological station in Oyster, Virginia, located approximately 4 km from South Bay (Fig. 1).

Sediment grain size distribution was characterized at each site using a laser diffraction particle size analyzer (Beckman Coulter LS I3 320). Sediment samples were sieved at 1 mm to remove large organic matter, such as eelgrass blades and roots, and then bleached to remove particulate organic matter. The size class distribution was found, and  $D_{84}$ , the grain size diame-

ter for which 84% of the sample grain diameters are smaller, was computed from the resulting size distribution curve. At least 2 samples were collected and analyzed from each site. Sediment  $D_{84}$  at the bare site was  $157 \pm 7$   $\mu\text{m}$ , while, at the eelgrass sites, sediments were finer at  $130 \pm 17$   $\mu\text{m}$ . SSCs were measured using 2 optical backscatter sensors (OBSs; Campbell Scientific OBS3+). One OBS was placed within the meadow to quantify SSC at  $z = 0.1$  m, and another, above the canopy at  $z = 0.5$  m, where  $z$  is the vertical distance above the seafloor. To perform laboratory calibrations of the OBS, sediment samples were collected, suspended, and known volumes of suspended sediment were mixed into 60 l of filtered seawater. Suspended sediment sample volumes were then dried and weighed, and a linear regression was formed between the backscatter intensity from the OBS and the SSC. Each calibration had an  $R^2 > 0.99$ .

### Wave-turbulence decomposition

Instantaneous velocity measurements were collected in the east-north-up (ENU) reference frame using the internal compass and tilt sensors of the velocimeters and were time-averaged independently for every 10 min burst interval. Velocities were then rotated for each burst interval into the dominant direction of horizontal flow,  $u$ . Mean velocity and turbulence statistics were computed along the dominant flow direction ( $u$ ), transverse direction ( $v$ ), and vertical direction ( $w$ ). The 10 min time interval was chosen because in statistical tests, 10 min often emerges as the best balance between obtaining convergence of the mean statistics while minimizing velocity drift due to changes in flow conditions (Gross & Nowell 1983).

In flows with both waves and currents, the variance in velocity associated with waves is often much larger than that associated with turbulence and some form of wave-turbulence decomposition must be performed (Trowbridge 1998). When waves and currents are present, the instantaneous horizontal and vertical velocities can be written as:

$$\begin{aligned} u &= u' + \tilde{u} + \bar{u} \\ w &= w' + \tilde{w} + \bar{w} \end{aligned} \quad (1)$$

where  $\bar{u}$  and  $\bar{w}$  are the horizontal and vertical components of the mean velocity,  $\tilde{u}$  and  $\tilde{w}$  are the wave-induced orbital velocities, and  $u'$  and  $w'$  are the turbulent velocities. To determine motions that contribute to the turbulent Reynolds stress,  $\overline{u'w'}$ , turbulent motions must be separated from those of waves. The method of wave-turbulence decomposition that



was employed uses spectral decomposition, known as the phase method (Bricker & Monismith 2007), where the phase lag between the  $u$  and  $w$  components of the surface waves are used to interpolate the magnitude of turbulence under the wave peak. The wave stress is calculated through the spectral sum:

$$\overline{\tilde{u}\tilde{w}} = \int_{-f_{Nyquist}}^{f_{Nyquist}} S_{\tilde{u}\tilde{w}}(f) df \quad (2)$$

where  $S_{\tilde{u}\tilde{w}}(f)$  is the 2-sided cross-spectral density (CSD) of the wave-induced orbital velocities,  $f$  is frequency and  $f_{Nyquist}$  is the Nyquist sampling frequency, which is half the sampling frequency of the discrete signal. The turbulence spectrum can be expressed as the difference between the spectrum of raw velocities and that of wave-induced orbital velocities such that:

$$S_{u'w'}(f) = S_{uw}(f) - S_{\tilde{u}\tilde{w}}(f) \quad (3)$$

The CSD of the spectra in Eq. (3) are then integrated to obtain the turbulent Reynolds stress:

$$\overline{u'w'} = \overline{uw} - \overline{\tilde{u}\tilde{w}} \quad (4)$$

$U_j = U(f_j)$  and  $W_j = W(f_j)$  are the Fourier transforms of  $u(t)$  and  $w(t)$  at the frequency  $f_j$  in the Fourier transform. For a finite data series, the integral of the wave stress becomes:

$$\overline{\tilde{u}\tilde{w}} = \sum_{j=-N/2}^{j=N/2} \tilde{U}_j \times \tilde{W}_j \quad (5)$$

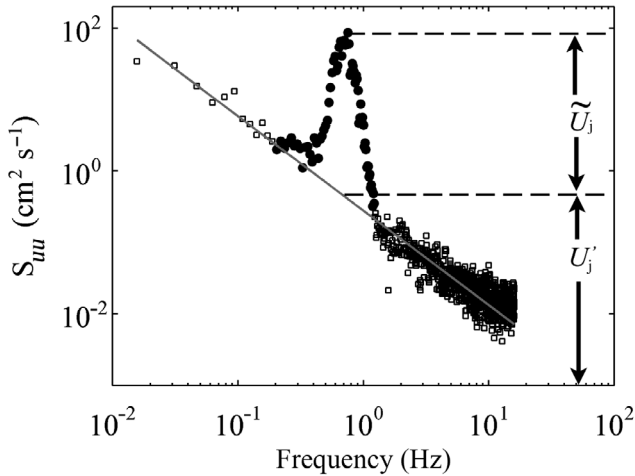


Fig. 2. Power spectral density (PSD) of the horizontal velocity,  $S_{uu}$ , for a 10 min representative data series computed at  $z = 0.1$  m at the bare site, where  $z$  is the vertical distance above the seafloor. Solid circles: region encompassing wave peak; squares: region of the spectra outside the wave domain. Grey solid line: least squares fit to the data outside the wave domain. The wave component of the stress is removed by subtracting the PSD formed above the grey line that encompasses the region of the wave peak

where  $N$  is the number of data points used in the Fourier transform and the magnitude of  $\tilde{W}_j$  is the difference between the raw  $W_j$  and the turbulence  $W'_j$  interpolated below the wave peak, via a least-squared fit straight line, as shown in Fig. 2. The magnitude of  $\tilde{W}_j$  is the difference between the raw  $W_j$  and the turbulence  $W'_j$  interpolated below the wave peak, via a least-squared fit straight line, as shown in Fig. 2.  $\tilde{W}_j$  is solved for by expressing the wave stress in terms of power spectral density (PSD):

$$S_{ww_j} = \frac{1}{df} |W_j|^2 \quad (6)$$

The same method is used to solve for  $\tilde{U}_j$ . The Fourier coefficients can be written in phasor notation as:

$$\overline{\tilde{u}\tilde{w}} = \sum_{j=\text{wave\_peak}} \tilde{U}_j \times \tilde{W}_j = \sum_{j=\text{wave\_peak}} |\tilde{U}_j| |\tilde{W}_j| \cos(\angle W_j - \angle U_j) \quad (7)$$

where  $\angle W_j$  and  $\angle U_j$  are the phases of the Fourier coefficients. The wave stress is then found by integrating the wave component of the CSD,  $S_{\tilde{u}\tilde{w}}(f)$ , over the width of the wave peak and subtracting it from the integral of the total stress,  $S_{uw}(f)$ , over the full frequency spectrum in order to obtain Reynolds stress, as in Eq. (4).

### Wave orbital velocities

The spectral density of surface elevation,  $S_{\eta\eta p}$ , was computed using the pressure signal from the ADV located at  $z = 0.5$  m as:

$$S_{\eta\eta p} = \left[ \frac{\cosh(kh)}{\cosh(kz)} \right]^2 \frac{S_{pp}}{\rho^2 g^2} \quad (8)$$

where  $S_{pp}$  is the spectral density of the pressure,  $k$  is the wave number ( $\text{m}^{-1}$ ,  $=2\pi/L$  where  $L$  is wavelength),  $h$  is the mean water depth,  $z$  is the vertical distance above the seafloor,  $g$  is gravitational acceleration and  $\rho$  is density (Dean & Dalrymple 1991). Significant wave height ( $H_s$ ) and average period ( $T$ ) were then computed using the first ( $m0$ ) and second ( $m2$ ) moments from the  $S_{\eta\eta p}$  power spectrum:

$$H_s = 4\sqrt{m0} \quad T = \sqrt{m0/m2} \quad (9)$$

where  $m0 = \int S_{\eta\eta p}(f) df$  and  $m2 = \int f^2 S_{\eta\eta p}(f) df$ .

To determine the interaction between eelgrass structure and wave-induced flows, wave orbital velocities were calculated and compared above and within the eelgrass meadow. Adjacent to the sea

floor, vertical orbital velocity approaches zero and thus the horizontal component of the orbital velocity accounts for the majority of particle motion. The horizontal orbital velocity can be calculated from spectra of the horizontal velocity components,  $u$  and  $v$ , by summing the contributions from wave spectra across each frequency component (Wiberg & Sherwood 2008):

$$u_{os}^2 = 2 \left[ \sum_j S_{\bar{u}\bar{u}_j} \Delta f_j + \sum_j S_{\bar{v}\bar{v}_j} \Delta f_j \right] \quad (10)$$

where  $u_{os}$  is equivalent to the root-mean-squared (rms) orbital velocity. Since only the wave spectra are used and not the full spectrum, this method removes water motion due to turbulence and currents driven by the tides. Eq. (10) can then be applied to velocities above and within the canopy to determine seagrass impacts on orbital wave motion.

## RESULTS

Physical characteristics of wind speed, water temperature and depth, as well as depth-averaged water currents, significant wave height, and SSCs measured at each *Zostera marina* site are shown in Fig. 3. The bay had a mean depth of approximately 1.6 m, and the average wind speed between May and June 2010 was  $2.3 \pm 1.2 \text{ m s}^{-1}$ . Over the course of the sampling period, water temperatures in the bay steadily increased from 21 to 30°C. The flow regime in South Bay was tidally dominated; therefore, water veloci-

ties were low at high and low tide and intensified during ebbing and flooding tides. In addition, flows were typically not symmetric, with ebbing tides characterized by slightly higher velocity magnitudes than flooding tides. Mean tidal amplitude and significant wave height at each site are shown in Fig. 4A. Tidal amplitudes ranged from 0.58 to 0.73 m, while  $H_s$  ranged from  $0.18 \pm 0.08 \text{ m}$  at the bare site to  $0.05 \pm 0.02 \text{ m}$  at eelgrass Site 3. Time-averaged velocities at  $z = 0.5$  and  $0.1 \text{ m}$  at each site are shown in Fig. 4B. The bare site showed a 40% reduction in velocity between  $z = 0.5$  and  $0.1 \text{ m}$  due to frictional interaction with the seafloor. In comparison, velocities within the seagrass canopy ( $z = 0.1 \text{ m}$ ) showed a 70% reduction in velocity compared to  $z = 0.5 \text{ m}$  due to the combined influence of friction by the seafloor and drag induced by the seagrass canopy.

Waves within these coastal bays were predominantly formed by winds, the  $H_s$  of which is controlled by the fetch and bottom topography over which they propagate (Lawson et al. 2007). At the bare site, strong winds were mostly to the north. During these periods, wind-generated waves traveled over stretches of South Bay devoid of eelgrass, with average wind speeds ( $\pm 1 \text{ SD}$ ) of  $2.6 \pm 1.4 \text{ m s}^{-1}$ . At the eelgrass sites, there were no consistent patterns in the wind direction, with winds oscillating from northward to southward (Fig. 3A). Average wind conditions at Sites 1, 2, and 3 were  $2.3 \pm 1.2$ ,  $2.4 \pm 1.1$ , and  $1.9 \pm 1.0 \text{ m s}^{-1}$ , respectively. For all sites, there was a minimum of 5 km of fetch to both the north and

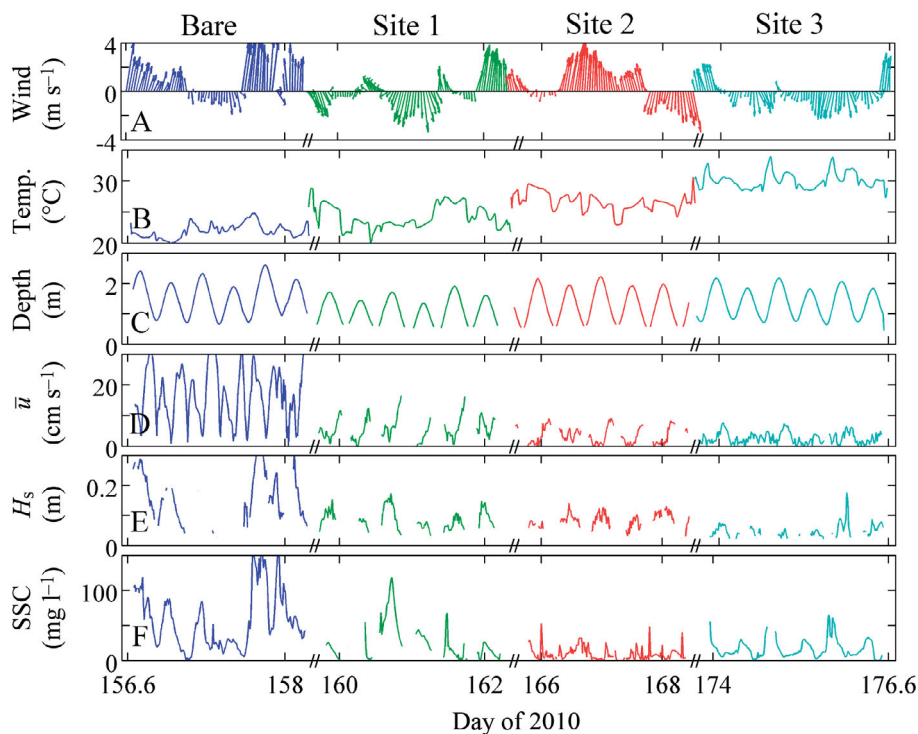


Fig. 3. (A) Wind magnitude and direction, (B) water temperature, (C) water depth, (D) magnitude of burst-averaged water speed ( $\bar{u}$ ) at  $z$  (vertical distance above the seafloor) = 0.5 m, (E) significant wave height ( $H_s$ ), and (F) suspended sediment concentration (SSC) at  $z = 0.1 \text{ m}$ . Locations of missing or no data correspond to time periods when velocimeters or sediment sensors had poor signal quality. Note the non-continuous time record, where days between successive monitoring of sites have been removed

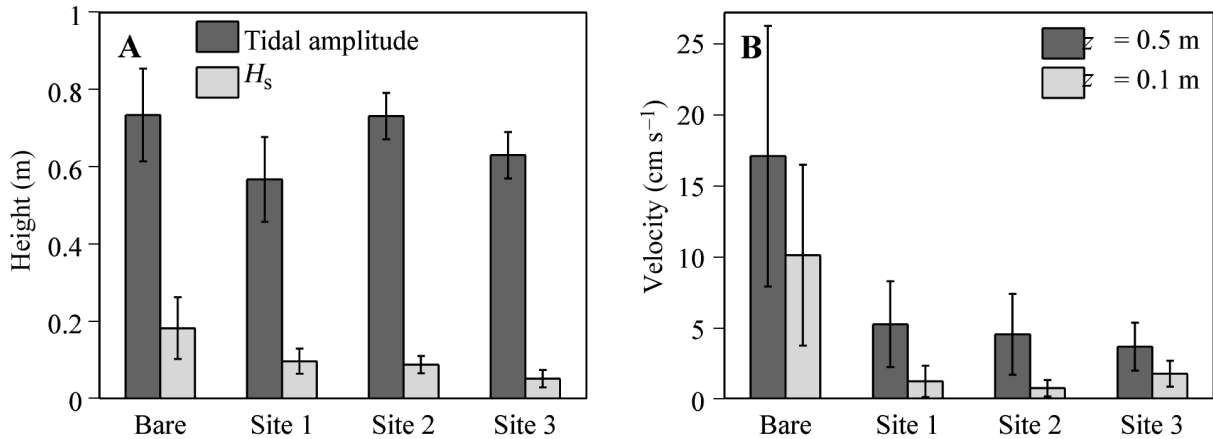


Fig. 4. (A) Average tidal amplitudes and significant wave heights ( $H_s$ ) with error bars representing  $\pm 1$  SD. The average across all sites was  $0.65 \pm 0.11$  m. (B) Time-averaged velocity magnitude of the study sites during tidally dominant flow conditions ( $\pm 1$  SD).  $z$ : vertical distance above the seafloor

south.  $H_s$  increased at all sites during periods surrounding high tide and was reduced during low tide (Fig. 3E). Overall, there was a 45 to 70% reduction in measured  $H_s$  at the eelgrass sites compared to the bare site, although changes in wind magnitude and direction during sampling played a factor in wave development and the overall amount of wave reduction (Fig. 5). In general, the rate of increase in  $H_s$  with increasing wind speed was considerably smaller at the eelgrass sites than at the bare site.

### Turbulent kinetic energy and Reynolds stress

At each site, flows were separated into time periods where wave action was a significant contributor to the overall flow and those with minimal wave action where flows were dominated by tidally driven currents. Tidally dominated flows were those where the PSD of the wave component of the frequency spectrum,  $S_{\bar{u}\bar{w}}$ , constituted  $< 10\%$  of the overall power density,  $S_{u\bar{w}}$  measured at  $z = 0.5$  m. Overall, at the bare site, tidally dominated flows occurred 15% of the time, while, at the eelgrass sites, due to wave attenuation across the canopy, tidally dominated flows occurred approximately 25% of the time. There was no statistical difference in time-averaged mean velocity during periods in which tidally dominated or wave-dominated flows occurred. Both Reynolds stress ( $\overline{u'w'}$ ) and turbulent kinetic energy,  $\text{TKE} = 0.5(u'^2 + v'^2 + w'^2)$ , were computed at each site (Fig. 6), where  $u'$ ,  $v'$ , and  $w'$  are the turbulent velocity fluctuations in the dominant horizontal, transverse, and vertical directions, respectively. At the bare site during tidally dominated time periods, Reynolds stresses,  $\overline{u'w'}$ , were

similar at the  $z = 0.5$  and  $0.1$  m elevations, whereas there was up to a 60% reduction in Reynolds stress magnitude at the eelgrass sites. Although standard deviations for TKE and Reynolds stress were large at all locations, this was primarily due to variations in velocity magnitude caused by waves and tides. Confidence intervals for mean estimates were typically quite small, and locations where significant differences were found between mean estimates of TKE and  $\overline{u'w'}$  at  $z = 0.1$  and  $0.5$  m are denoted with an asterisk in Fig. 6. Comparisons of  $\overline{u'w'}$  within the canopy versus that above the canopy at each site indicated that the largest reduction in  $\overline{u'w'}$  coincided with the

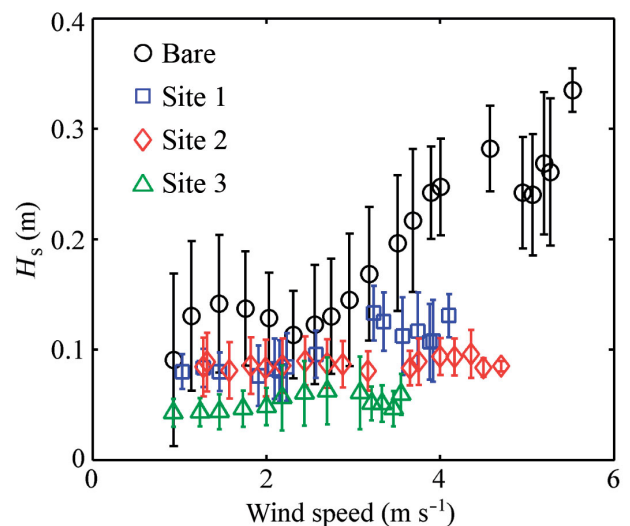


Fig. 5. Significant wave height ( $H_s$ ) as a function of mean wind speed. Bars represent  $\pm 1$  SD. Estimates of  $H_s$  were computed as running means over a  $\pm 0.5$  m s<sup>-1</sup> averaging window of wind speed

most dense eelgrass canopy, Site 1, followed by the mid-density, Site 2, and then the most sparse canopy, Site 3. TKE also showed reductions within the canopy versus above the canopy, but the magnitude of the reduction was significantly less than that of the Reynolds stress. This may be due to the added stem-generated TKE formed by flow interaction with the seagrass blades (Verduin & Backhaus 2000).

To compute Reynolds stress and TKE for wave-dominated flow conditions, the wave components of the PSD were first removed, as in Eq. (3), and then the remaining turbulent component of the PSD was integrated. Overall, the addition of waves did not statistically alter the magnitude of Reynolds stress or TKE at either the bare site or eelgrass sites (Fig. 6C,D). Reynolds stress was typically reduced within the seagrass canopy compared to above the canopy, although trends of greater reductions of within-canopy  $\overline{u'w'}$  with higher seagrass densities were not as evident under wave-dominated conditions as they were in tidally dominated flows.

### Quadrant analysis of turbulence

Quadrant analysis is a useful technique to describe how turbulent fluctuations contribute to the transport of momentum, sediment, and gases throughout the bottom boundary layer (Lu & Willmarth 1973). Depending upon the flow field, high-momentum fluid overlaying the seagrass canopy can be advected downwards into the canopy, or low-momentum fluid residing within the canopy can be advected upwards out of the canopy. Velocity fluctuations,  $u'$  and  $w'$ , were normalized by their respective standard deviations and were divided into 4 quadrants based on the sign of their instantaneous values. Contours of the turbulent probability distribution function (pdf) are shown in Fig. 7 for turbulent motions within and above the eelgrass bed at Site 1 during periods with no wave action. Quadrants are listed from Q1 to Q4. The 2 dominant quadrants responsible for momentum transfer are Q2 ( $u' < 0, w' > 0$ ), where turbulent

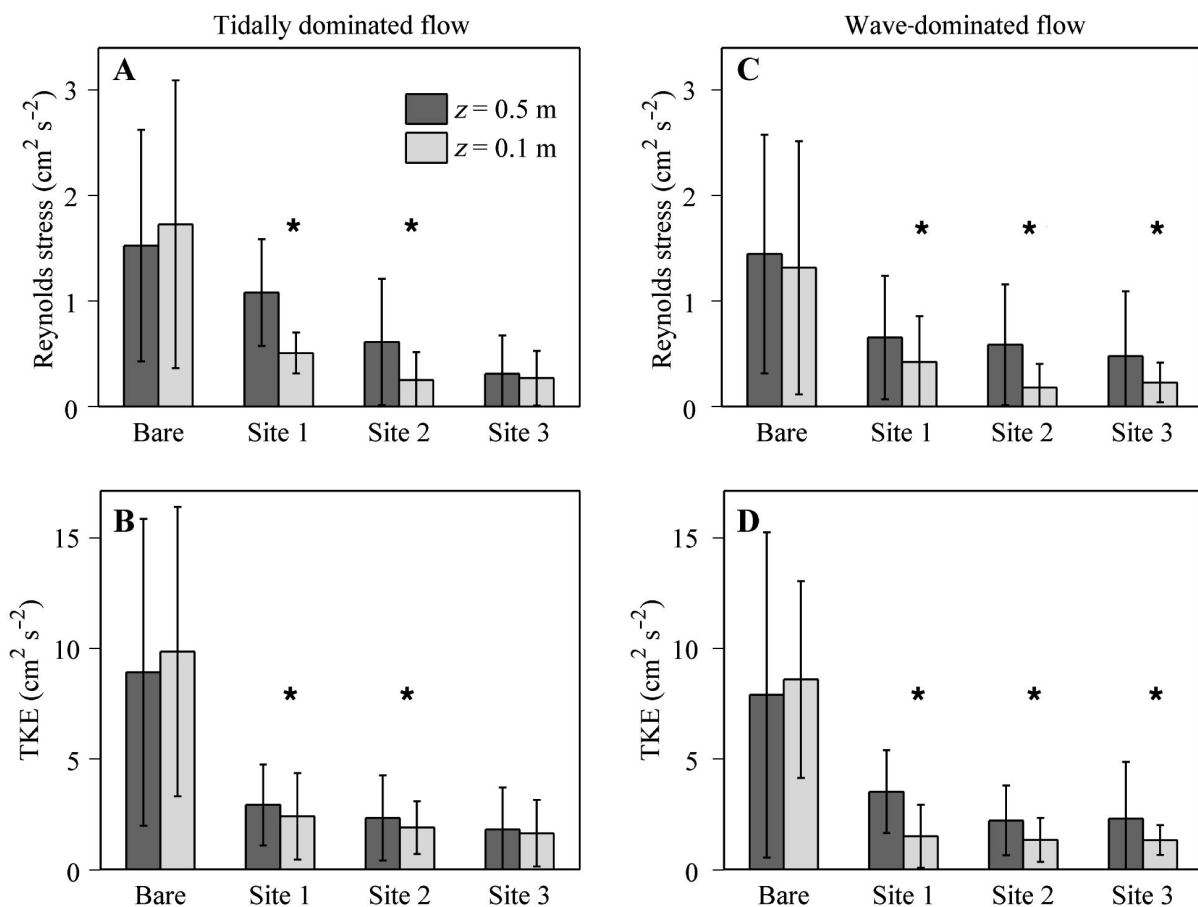


Fig. 6. (A) Reynolds stress ( $-\overline{u'w'}$ ,  $\pm 1$  SD), (B) turbulent kinetic energy (TKE,  $\pm 1$  SD) for tidally dominated flow conditions, (C) Reynolds stress and (D) TKE for wave-dominated flow conditions. \* Locations where confidence intervals of mean estimates were significantly different between  $z$  (vertical distance above the seafloor) = 0.1 and 0.5 m

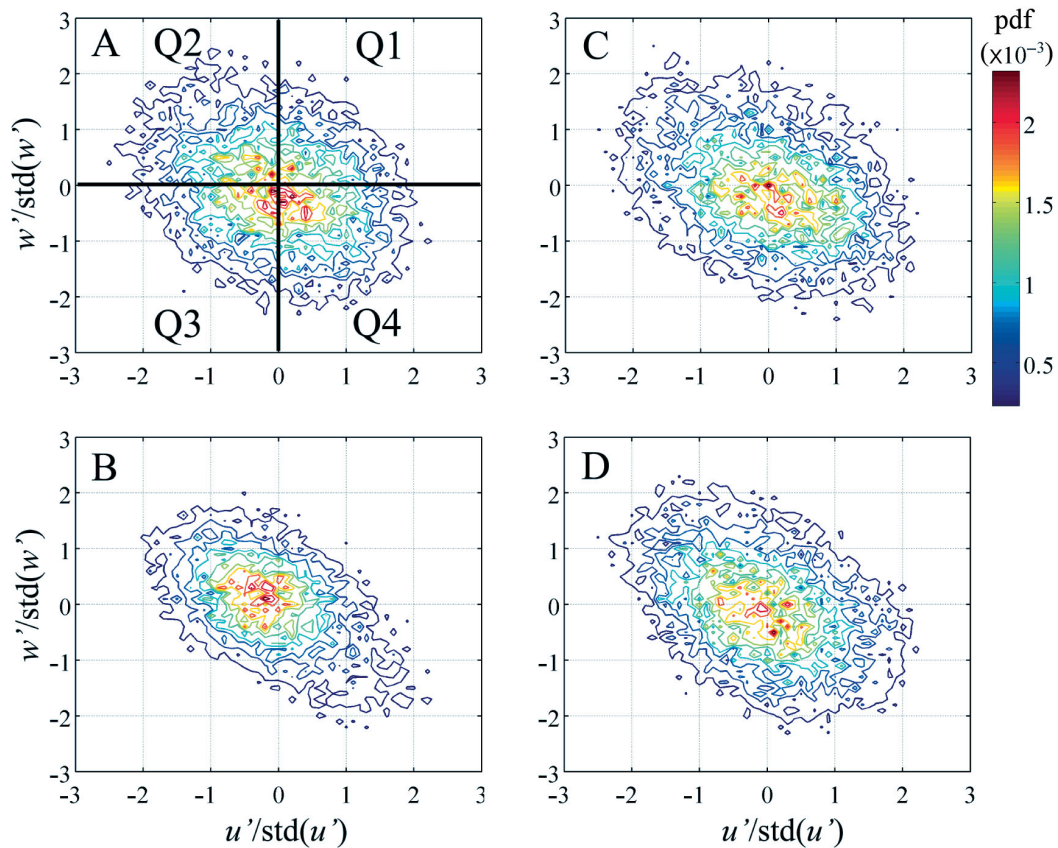


Fig. 7. Quadrant analysis of the probability density functions (pdf) of  $u'$  and  $w'$  distributions normalized by their standard deviation (std) at (A,B) *Zostera marina* Site 1 for (A)  $z$  (vertical distance above the seafloor) = 0.5 m and (B)  $z$  = 0.1 m, showing the dominance of turbulent sweeping events in Quadrant 4 (Q4), and at (C,D) the bare site for (C)  $z$  = 0.5 m and (D)  $z$  = 0.1 m

ejections of low-momentum fluid are transported vertically upwards, and Q4, where sweeping events transport high-momentum fluid downward towards the seagrass meadow. These ejection/sweep phenomena result in intermittent flushing of water masses from within the canopy (Grass 1971). Typically, momentum transport is dominated by ejection and sweeping events and shows a predominance of values in Q2 and Q4, which held true for flows above the eelgrass canopy (Fig. 7A). The total contribution to Reynolds stress within each quadrant was found by summing the absolute value of the  $u'w'$  contributions within each quadrant and dividing by the total contribution from all quadrants: Q1 (15%), Q2 (36%), Q3 (15%), and Q4 (34%). This indicated that the combined Q2 and Q4 contributions accounted for approximately 70% of the total Reynolds stress, which is similar to results determined in other studies of flow over high-roughness topographies (Bennet & Best 1996, Lacey & Roy 2008). There was a fairly even distribution of stresses between Q2 and Q4, indicating

that the turbulent ejection of low-momentum fluid from near the top of the seagrass canopy was similar to that of sweeps of high-momentum fluid towards the canopy.

Within the seagrass canopy, at  $z$  = 0.1 m, distinct changes in the contribution to the stress from various components occurred (Fig. 7B), where motions within Q4 were dominant. The total contribution to Reynolds stress within each quadrant was: Q1 (7%), Q2 (32%), Q3 (6%), and Q4 (55%). This signifies that the principal exchange of water masses within the canopy was driven by sweeps of high-momentum fluid from above the canopy downwards into the canopy. For the bare site (Fig. 7C,D), there was still dominance in Q2 and Q4, with 75 and 79% of the total Reynolds stresses found at  $z$  = 0.5 and 0.1 m, respectively. However, there was a fairly even distribution of stresses between Q2 and Q4 at both elevations, similar to the flow structure formed above the seagrass canopy. For instance at  $z$  = 0.1 m, contributions were: Q1 (11%), Q2 (40%), Q3 (10%), and Q4 (39%).

### Velocity spectra and wave orbitals

PSDs of the horizontal velocities during tidally dominated flow conditions at the bare site showed a distinct  $-5/3$  slope, indicative of an inertial subrange at both  $z = 0.1$  and  $0.5$  m (Fig. 8A). There was general agreement between the 2 elevations, with slightly higher energy at low frequencies ( $<1$  Hz) for  $z = 0.5$  m, indicative of higher mean flows, but lower energy at higher frequencies ( $>1$  Hz), indicating lower turbulent energy within the inertial subrange. Within the eelgrass meadow, there was a reduction in the magnitude of the PSD across all frequencies within the canopy compared to flow above the canopy (Fig. 8B).

PSD of wave-dominated flow conditions showed similar trends outside the wave domain of the fre-

quency spectrum for both the bare and eelgrass sites. Within the wave domain at the bare site (Fig. 8C), there was little wave energy attenuation, indicating that oscillatory motion due to waves was effective at generating wave-induced orbital motions near the seafloor. Within the wave domain at the eelgrass site (Fig. 8D), wave energy was still able to penetrate the eelgrass canopy, with little reduction of wave energy at  $z = 0.1$  m at frequencies smaller than the wave peak. However, at both the bare and eelgrass sites, but especially within the *Zostera marina* canopy (Fig. 8D), the bulk of energy loss occurred at wave frequencies within the wave domain at approximately  $f \geq 1$  Hz, where short period wave oscillations ( $T = 1/f$ ) attenuated before reaching the bottom. This agrees with wave theory that suggests waves will attenuate before

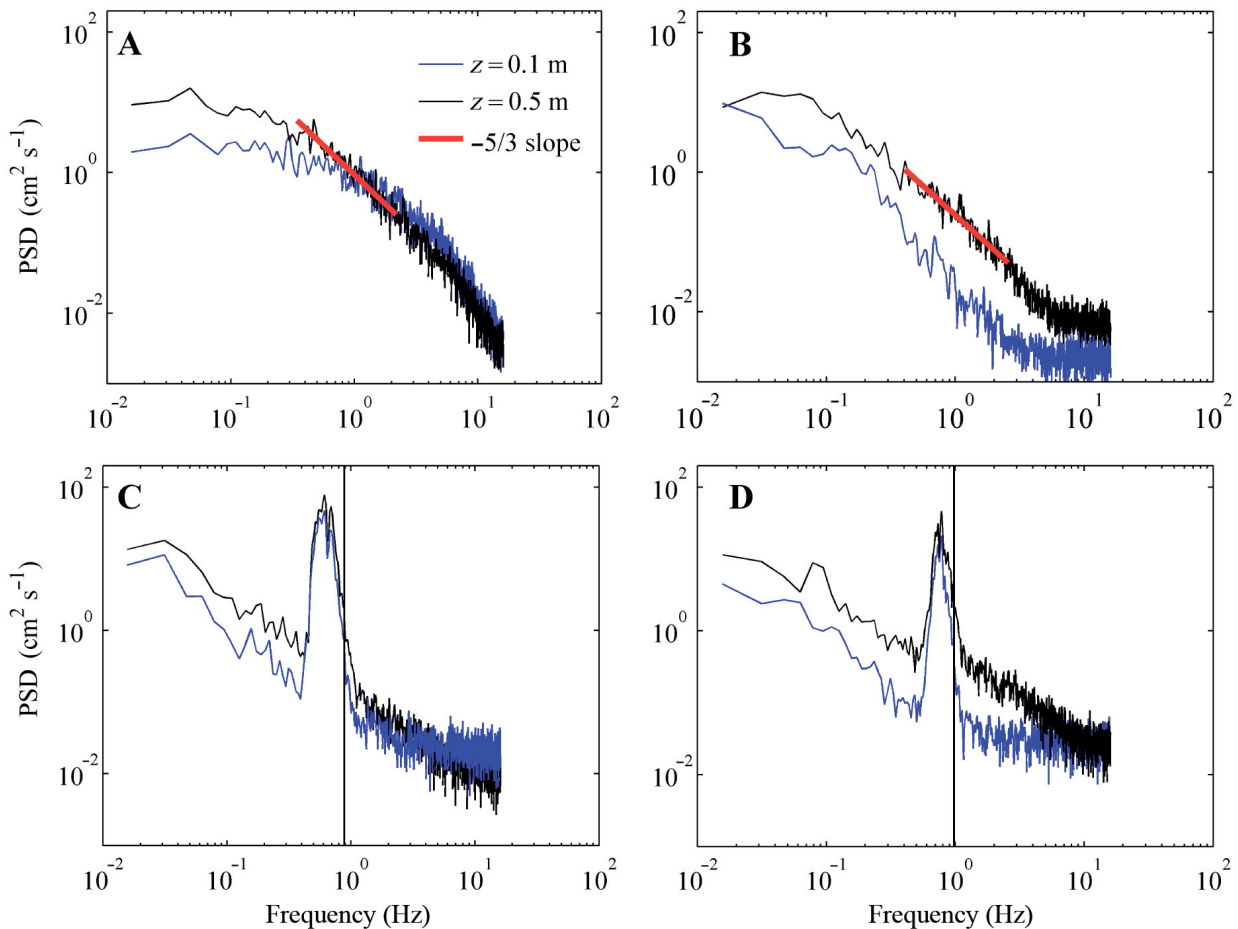


Fig. 8. Power spectral density (PSD) of horizontal velocity at the (A) bare site (horizontal component of the mean velocity  $[\bar{u}] = 9.7 \text{ cm s}^{-1}$ ) and (B) Site 2 ( $\bar{u} = 8.4 \text{ cm s}^{-1}$ ) during tidally dominated flow conditions. Spectra are formed by averaging 9 independent spectra of  $n = 2048$  velocity records that occur within a 10 min time period. PSD for the (C) bare site ( $\bar{u} = 10.8 \text{ cm s}^{-1}$ , significant wave height [ $H_s$ ] = 19 cm) and (D) mid-density eelgrass Site 2 ( $\bar{u} = 6.2 \text{ cm s}^{-1}$ ,  $H_s = 14$  cm) during wave-dominated flow conditions. Vertical line represents the frequency at and above which wave motion is expected to be attenuated at  $z$  (vertical distance above the seafloor) = 0.1 m according to linear wave theory. Flattening of the power density at high frequencies ( $>0.4$  Hz in Panels B, C, and D) indicates that the noise floor of the instrument has been reached and the velocity signal is indistinguishable from noise

reaching the seafloor for  $f > \sqrt{g/(4\pi h)}$  (Wiberg & Sherwood 2008). At a given elevation above the seafloor,  $z$ , waves with  $f > \sqrt{g/[4\pi(h-z)]}$  will be attenuated.

PSD for frequencies between 0 and 2 Hz for both horizontal and vertical velocities are shown for the bare site in Fig. 9. These plots include the tidally dominated contributions to the spectra at small frequencies (i.e.  $f < 0.3$  Hz), as well as the wave component of the PSD, which typically spans the range  $0.3 < f < 1$  Hz. Frequency analysis within the wave domain of the frequency spectra for the bare site indicate that there was a general trend of a reduction of wave energy near the seafloor for the vertical velocity,  $S_{ww}$  PSD, but a fairly uniform distribution of wave energy throughout the water column for the horizontal,  $S_{uu}$  PSD. Peak wave frequency at high tide was approximately  $f = 0.5$  Hz ( $T = 2$  s), but as the water depth decreased, peak wave frequency increased to approximately  $f = 1$  Hz, indicating a reduction in the peak wave period to  $T = 1$  s.

For Site 2, similar trends emerged in a reduction of vertical wave energy ( $S_{ww}$ ) within the seagrass bed compared to flow above the canopy (Fig. 10). There were also reductions in wave energy within the horizontal component of the velocity,  $S_{uu}$ , due to the

wave-dampening effect that the eelgrass had on the flow. However, as is also shown in Fig. 8 for an individual spectrum, much of the reduction in wave energy occurred at the high frequencies within the wave band ( $f > 1$  Hz), which can be attributed both to attenuation due to the interaction with the seagrass and due to the natural attenuation of the waves with depth. The shorter period waves that formed over the seagrass bed (average  $T = 1.4 \pm 0.3$  s) tended to attenuate with depth to a greater extent than the longer period waves that formed over the bare site (average  $T = 1.7 \pm 0.4$  s), as predicted from linear wave theory. Also of note was the reduction in power density at low frequencies,  $f < 0.3$  Hz, indicating a reduction in the energy of the mean flow,  $u$ , within the canopy.

The wave-orbital velocity,  $u_{os}$ , at  $z = 0.5$  and  $0.1$  m was computed following Eq. (10), and estimates for each site are shown in Fig. 11. At the bare site, there was only a 3% difference in measured  $u_{os}$  between the  $z = 0.5$  and  $0.1$  m elevations. At all 3 eelgrass sites there was an overall reduction in wave orbital motion by  $20 \pm 5\%$  within the canopy compared to above, and orbital velocities at the eelgrass sites were less than half that produced over the bare site. To determine the extent to which this orbital wave reduction within the eelgrass canopy was due to natural atten-

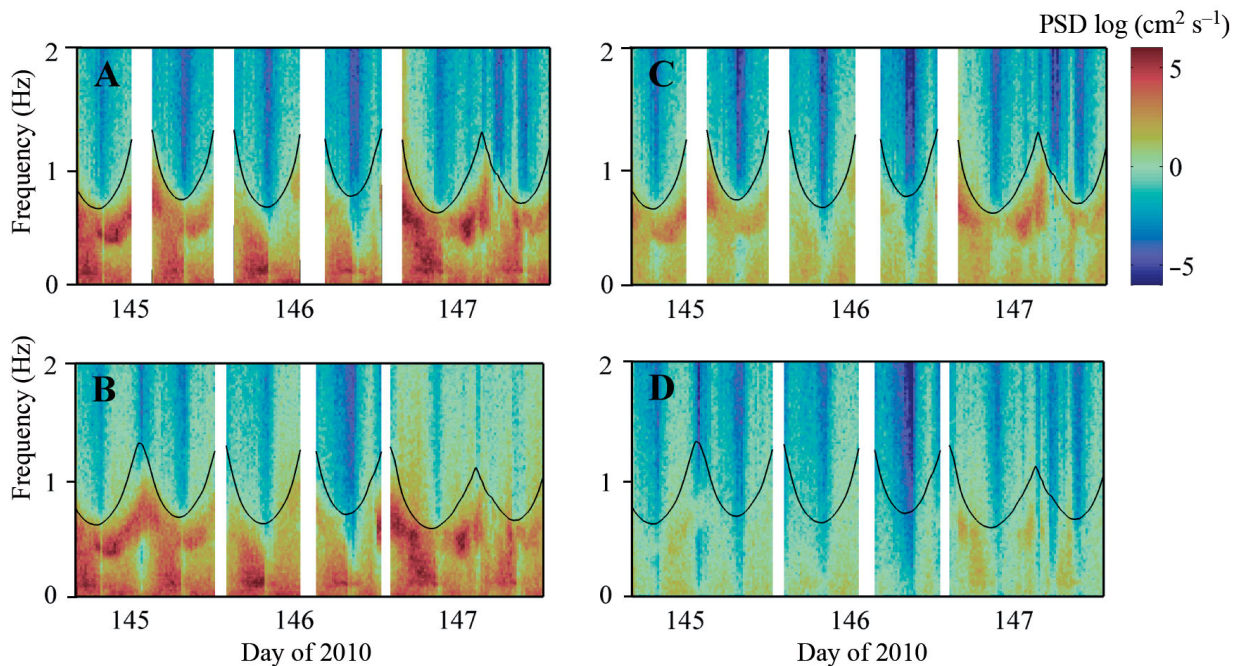


Fig. 9. Two-dimensional power spectral density (PSD) at the bare site of horizontal velocities (A)  $S_{uu}$  at  $z = 0.5$  m and (B)  $S_{uu}$  at  $z = 0.1$  m, showing only minor attenuation of the wave frequency band. Two-dimensional PSD of vertical velocities (C)  $S_{ww}$  at  $z = 0.5$  m and (D)  $S_{ww}$  at  $z = 0.1$  m, showing substantial attenuation of the wave frequency band, as predicted by wave theory. Black lines indicate  $f = \sqrt{g/[4\pi(h-z)]}$ , where  $g$  is gravitational acceleration,  $h$  is water depth,  $z$  is the vertical distance above the seafloor, and  $f$  is the frequency at and above which linear wave theory predicts wave motion is attenuated. Regions with no data are during low tide when velocimeters had poor signal quality

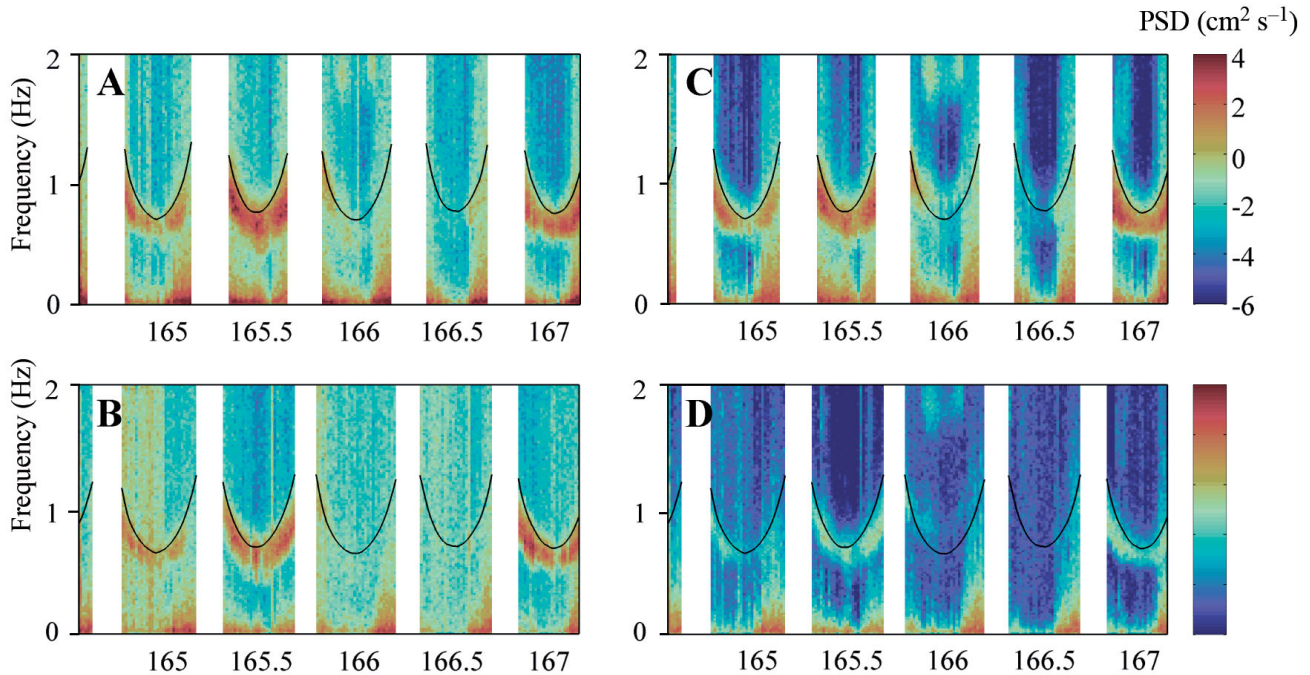


Fig. 10. Two-dimensional power spectral density (PSD) at Site 2 of horizontal velocities (A)  $S_{uu}$  at  $z = 0.5$  m and (B)  $S_{uu}$  at  $z = 0.1$  m. Attenuation of the wave energy within the canopy primarily occurs at high frequency ( $f$ ) within the wave band of the spectrum. Two-dimensional PSD of vertical velocities (C)  $S_{ww}$  at  $z = 0.5$  m and (D)  $S_{ww}$  at  $z = 0.1$  m, showing substantial attenuation of wave energy at all  $f$ -values within the wave band. Black lines indicate  $f = \sqrt{g/[4\pi(h-z)]}$ , where  $g$  is gravitational acceleration,  $h$  is water depth,  $z$  is the vertical distance above the seafloor, and  $f$  is the frequency at and above which linear wave theory predicts wave motion is attenuated

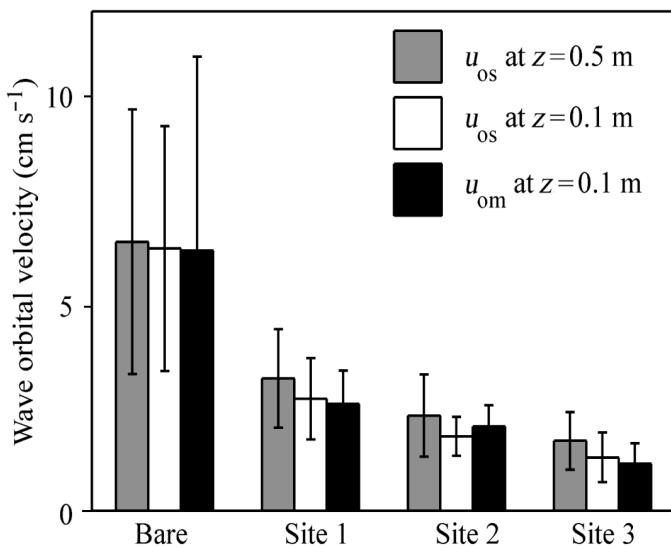


Fig. 11. Wave orbital velocities,  $u_{os}$  (Eq. 10), computed utilizing spectra of horizontal wave velocities,  $S_{\bar{u}\bar{u}}$  and  $S_{\bar{v}\bar{v}}$ , at  $z$  (vertical distance above the seafloor) = 0.5 m and  $z = 0.1$  m and wave orbital velocities,  $u_{om}$  (Eq. 12), at  $z = 0.1$  m, computed utilizing linear wave theory and estimates of free-surface displacements. Agreement between  $u_{os}$  and  $u_{om}$  at  $z = 0.1$  m suggests wave velocity decay is primarily due to wave attenuation of high-frequency wave motion with depth, and not from interaction with the eelgrass

uation with depth or due to interaction with the eelgrass bed, orbital velocities computed through local velocity spectra (Eq. 10) were compared to estimates of bottom wave-orbital velocities using pressure sensor measurements of displacement of the free surface. Using linear wave theory for small-amplitude, monochromatic waves, the horizontal component of orbital velocity,  $u_o$ , can be computed as:

$$u_o = \frac{\pi H \cosh(kz)}{T \sinh(kh)} \cos(kx - \omega t) \quad (11)$$

where  $H$  is the rms of wave height (m),  $\omega$  is the radian of wave frequency ( $\text{rad s}^{-1}$ ),  $x$  is the position in the wave orbital (m), and  $t$  is time (s). As the velocities vary sinusoidally with the  $x$ - and  $t$ -values throughout the wave period, the above equation can be simplified to the rms of the maximum orbital velocity when  $|\cos(kx - \omega t)| = 1$ :

$$u_{om} = \frac{\pi H \cosh(kz)}{T \sinh(kh)} \quad (12)$$

Linear wave theory assumes the bed is frictionless; therefore, estimating wave velocity decay with depth using linear wave theory (Eq. 12) and comparing it to computed orbital velocities using local velocity mea-



surements within the canopy (Eq. 10) should indicate the relative dampening of wave velocity due to frictional interaction with the seagrass canopy. Fig. 11 indicates there is good agreement between measured within-canopy orbital velocities and those estimated from linear wave theory, suggesting that within-canopy dampening of wave orbitals was primarily due to natural attenuation of high-frequency wave motion with depth and not from flow interaction with the eelgrass.

### Bottom shear stresses and suspended sediment

The total stress imparted to the seafloor was quantified using a combined bottom shear stress,  $\tau_b$ , calculated as the square root of the sum of the squares of the shear stress due to currents,  $\tau_{\text{current}}$ , and due to waves,  $\tau_{\text{wave}}$  (Wiberg & Smith 1983) such that:

$$\tau_b = \sqrt{\tau_{\text{wave}}^2 + \tau_{\text{current}}^2} \quad (13)$$

Wave shear stress was determined by:

$$\tau_{\text{wave}} = \frac{1}{2} f_w \rho u_b^2 \quad f_w = 0.04 \left[ \frac{u_b T}{2\pi k_b} \right]^{-0.25} \quad (14)$$

such that  $f_w$  is the wave friction factor,  $u_b$  is the bottom orbital velocity, which can be approximated as  $u_{os}$  measured near the seafloor (Wiberg & Sherwood 2008), and  $k_b$  is the characteristic roughness length of the bottom, which is defined as  $3D_{84}$  (Lawson et al. 2007).  $D_{84}$  is the sediment grain diameter such that 84% of grain diameters are smaller, and was measured to be 157  $\mu\text{m}$  at the bare site and 130  $\mu\text{m}$  within the seagrass canopy. Computation of the current shear stress within vegetation is not well described, especially in the presence of wave activity, but under unidirectional currents, a useful parameterization is through estimates of the near-bottom TKE (Stapleton & Huntley 1995, Widdows et al. 2008):

$$\tau_{\text{current}} = \rho u_{\text{current}}^2 = 0.19\rho(\text{TKE}) \quad (15)$$

Although waves can alter Reynolds stresses and TKE in the presence of a mean current (Grant & Madsen 1979), our findings within South Bay suggest only minor alteration in the magnitude of TKE in the presence of small-amplitude wind waves; therefore, Eq. (15) can be reasonably applied.

Fig. 12 shows the magnitude of the total bottom shear stress at each site. At the bare site, median bottom shear stress was  $\tau_b = 0.17 \text{ N m}^{-2}$ , but within the eelgrass bed, median  $\tau_b \leq 0.035 \text{ N m}^{-2}$  at all sites.

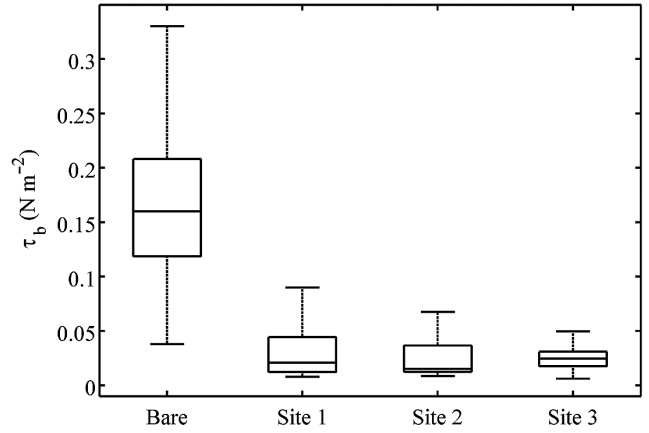


Fig. 12. Bottom shear stress ( $\tau_b$ ) at each site calculated as the square of the sums of the wave and current bottom stresses. Horizontal line within the box indicates median  $\tau_b$ , while the lower and upper edges of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box indicate the minimum and maximum measured  $\tau_b$ .

Although the critical shear stress that can initiate sediment resuspension was not directly quantified in South Bay, estimates within Hog Island Bay, which is directly adjacent to South Bay in the VCR, were performed by Lawson et al. (2007) and found to be  $\tau_{\text{cr}} = 0.04 \text{ N m}^{-2}$  over unvegetated sites adjacent to seagrass beds. This value is in relative agreement with  $\tau_{\text{cr}} = 0.05 \text{ N m}^{-2}$  found by Widdows et al. (2008) for sediments in the North Sea, where adjacent *Zostera marina* beds had a  $\tau_{\text{cr}} = 0.07 \text{ N m}^{-2}$ . The increase in sediment stabilization within the *Z. marina* bed was found to be due to increased abundance of the microphytobenthos and lower densities of grazers. This suggests that  $\tau_{\text{cr}} = 0.04 \text{ N m}^{-2}$  is likely a reasonable estimate for unvegetated regions, and a conservative estimate for the eelgrass bed in South Bay. At the 3 eelgrass sites, measured  $\tau_b$  was below the critical threshold for sediment entrainment during 80% of the sampling time period. However,  $\tau_b$  at the unvegetated site was significantly larger than  $\tau_{\text{cr}}$ , suggesting that sediment resuspension was occurring during a significant fraction of the sampling period.

OBSs were deployed at each site, measuring SSCs at  $z = 0.1$  and  $0.5$  m. SSC estimates at these elevations were averaged to create a mean SSC within the water column. Since flow and suspended sediment measurements were conducted at different time periods at each site, direct cross-site comparisons of SSC for the same time periods could not be conducted. Therefore, to determine if events of elevated magnitudes of bottom shear stress correlated to increases in SSC, a Pearson linear correlation was performed,

which is defined as the covariance of the 2 variables divided by the product of their standard deviations. Elevated SSC events were determined by taking the temporal mean of the 72 h data set from each site and locating periods of time when  $\tau_b$  was greater than its mean. At the bare site (mean SSC = 56 mg l<sup>-1</sup>), there was a Pearson correlation coefficient of 0.72 between elevated levels of  $\tau_b$  and increased SSC. At Site 1 (mean SSC = 43 mg l<sup>-1</sup>), which is closest to the bare site, there was a correlation of 0.58, while at Site 2 (mean SSC = 23 mg l<sup>-1</sup>) and Site 3 (mean SSC = 27 mg l<sup>-1</sup>) there were correlation coefficients of 0.26 and 0.23, respectively. Therefore, within the eelgrass meadow there was a decreased tendency for periods of high SSCs to correspond with periods of high bottom shear stress, suggesting that elevated levels of SSC within the seagrass bed were derived, in part, from non-local resuspension.

## DISCUSSION

*Zostera marina* beds in this Virginia coastal bay were found to substantially lower overall mean currents compared to adjacent bare site flow conditions. Average velocities were 2 to 3 times higher at the bare site than at the eelgrass sites, without a substantial change in tidal forcing. In addition, near-bed flows were dramatically reduced, with mean velocity at  $z = 0.1$  m at the bare site of 10.0 cm s<sup>-1</sup>, while average flows at the 3 eelgrass sites were 1.2 cm s<sup>-1</sup>, a 70% reduction compared to flow above the eelgrass canopy. Along the density gradient, ranging from 150 to 560 shoots m<sup>-2</sup>, the greatest flow reduction within the canopy compared to above the canopy occurred at Site 2, which was the mid-density site with 390 ± 80 shoots m<sup>-2</sup>. The eelgrass at this site, however, also had a significantly higher mean (28 cm) and maximum blade length (51 cm) compared to the other two *Z. marina* sites. Although seagrass density has been found to play an important role in flow reduction (Ackerman & Okubo 1993), the blade length is also important in modifying the canopy friction (Fonseca & Fisher 1986), leading to a proportional reduction in fluid velocity (Gacia et al. 1999, Thompson et al. 2004). The smallest within-canopy flow reduction occurred at Site 3, which had the lowest eelgrass density and smallest average blade length. This suggests that both eelgrass density and blade length can have an impact on flow processes. Overall, the presence of seagrass structure serves to substantially reduce velocity magnitude as compared to the unvegetated seafloor (Hasegawa et al. 2008).

Tidally and wave-dominated flows were separated using the magnitude of the power density within the wave band of the frequency spectra. For tidally dominated flow conditions, the magnitude of the turbulent Reynolds stress typically decreased within the canopy compared to flow above, and within-canopy  $\overline{u'w'}$  reductions increased with increasing seagrass density. The highest relative turbulence levels within the eelgrass bed were found at the lowest eelgrass density, Site 3, and were of similar magnitude within and above the canopy. This transition from low turbulence in high-density beds to elevated turbulence in low-density beds agrees with findings within other seagrass systems and laboratory measurements, which indicate enhanced turbulence due to stem-generated wake turbulence (Nepf et al. 1997, Widows et al. 2008). This enhanced turbulence can be intensified at the canopy–water interface (Abdelrhman 2003), where strong shear layers develop. Under wave-dominated flows, this relationship between turbulence intensity and seagrass density became less apparent, but both Reynolds stresses and TKE values were of similar magnitude with and without the presence of wave action. Quadrant analysis indicated that much of the turbulent motions within the canopy were dominated by sweeping events, where high-momentum fluid was transported downward into the canopy. These sweeps constituted 55% of the turbulent motion, compared to 32% for ejections of low-momentum fluid transported upwards out of the canopy. Strong sweeping motions into the canopy were also observed by Ghisalberti & Nepf (2006), who found that sweeps were followed by weak ejection events ( $u' < 0$ ,  $w' > 0$ ) which occurred at frequencies twice that of the dominant frequency of the coherent vortex formed at the top of the canopy.

### Wave attenuation due to interaction with the eelgrass canopy

Significant wave heights and wave periods were computed from pressure records obtained from the ADVs at a sampling rate of 32 Hz. Wave periods at all sites ranged between 1 and 2 s, representing wind-generated gravity waves. There was a 45 to 70% reduction in average  $H_s$  at the eelgrass sites compared to the bare site. For both the bare and eelgrass sites, the majority of energy lost within the wave band occurred at high wave frequencies,  $\geq 1$  Hz. This is in general agreement with wave theory, which predicts that waves with  $f > \sqrt{g/(4\pi h)}$  will be attenu-

ated before reaching the bottom (Wiberg & Sherwood 2008). Mean wave periods were  $T = 1.7 \pm 0.4$  s and  $T = 1.4 \pm 0.3$  s at the bare and eelgrass sites, respectively. As evident from PSD graphs, waves were not monochromatic and typically spanned a range of frequencies between  $f = 0.3$  and  $1 \text{ s}^{-1}$ . At the bare site, measured mean wave orbital velocities were within 3% of each other at both  $z = 0.5$  and  $0.1$  m. With the presence of eelgrass structure, the horizontal orbital velocities were found to decrease by approximately 20% within the canopy versus above. That shorter period waves formed over the eelgrass canopy suggests that natural wave attenuation with depth was a major contributing factor in the measured reduction in orbital velocities within the canopy. However, little reduction in low-frequency wave motion within the canopy indicates that oscillatory flows were also effective at penetrating the eelgrass canopy for longer period waves. This suggests that storm events, which tend to increase  $H_s$  and  $T$  in combination with storm surge (Chen et al. 2005), may be effective in generating oscillatory flows through the seagrass canopy, increasing bottom shear stresses and ultimately leading to sediment resuspension. This agrees with the results of Bradley & Houser (2009), who found that the ability for seagrasses to attenuate wave energy decreases as wave heights increase, but that seagrasses also serve as a low-pass filter where higher frequencies in the spectra tend to be more attenuated. Their results also suggest that the rate of energy dissipation is not uniform over a range of wave frequencies, and waves at higher frequencies are attenuated, but waves at lower frequencies are less affected by the seagrass.

### Bottom shear stress and sediment dynamics

Total bottom shear stress was estimated by summing contributions from orbital wave motion due to waves and turbulence from the mean current. Overall, bottom shear stress at the bare site was found to be  $\tau_b = 0.17 \pm 0.08 \text{ N m}^{-2}$ , which was 5 times greater than at any of the eelgrass sites. Within the eelgrass canopy the mean  $\tau_b$  at the 3 sites ranged between  $0.025 \pm 0.02$  and  $0.032 \pm 0.03 \text{ N m}^{-2}$ . A conservative estimate of the critical shear stress for sediment erosion was  $\tau_{cr} = 0.04 \text{ N m}^{-2}$ , which was measured in an adjacent coastal bay with similar water depth and sediment characteristics (Lawson et al. 2007). Bottom shear stress was found to be below the critical bottom stress threshold for erosion 80% of the time within the eelgrass meadow. In the absence of eelgrass,

depth-averaged SSC =  $56 \text{ mg l}^{-1}$ , and elevated levels of SSCs were well correlated throughout the water column with periods of high bottom shear stress (Pearson linear correlation coefficient = 0.72). However, with the addition of eelgrass structure, increases in depth-averaged suspended sediment were no longer well correlated with enhanced bottom shear stress. Correlation between  $\tau_b$  and elevated SSC occurred most closely at Site 1 (mean SSC =  $43 \text{ mg l}^{-1}$ , correlation coefficient = 0.58), which is located closest to unvegetated sites within the bay and the edge of the eelgrass meadow (Fig. 1), where sediment resuspension is expected to be most pronounced. Further from regions devoid of eelgrass, correlations between elevated  $\tau_b$  and SSC drop considerably, where at Site 2 (mean SSC =  $23 \text{ mg l}^{-1}$ ) and Site 3 (mean SSC =  $27 \text{ mg l}^{-1}$ ) correlation coefficients were 0.26 and 0.23, respectively. This suggests that suspended sediment measured within the canopy was, to some magnitude, advected into the canopy from non-vegetated regions of the bay. Although wave activity has been shown to be a dominant driver initiating the suspension of sediment within these shallow coastal bays, previous studies have shown that wave activity alone does not necessarily induce the transport of sediment (Heller 1987). Rather, the combination of both waves and currents acts to distribute sediment throughout the seagrass bed. The stress exerted by wave motion acts to suspend sediment above the seafloor, but, ultimately, unidirectional currents will cause a net transport of sediment, even if such a unidirectional current alone produces conditions below the threshold to initiate sediment suspension.

Overall, our results indicate that turbulence, wave heights, and wave orbital velocities were reduced in magnitude by the eelgrass canopy. This resulted in lowered suspended sediment within the eelgrass meadow as compared to adjacent unvegetated areas. In addition, between 2003 through 2009, monthly turbidity levels within the vegetated areas were found to be significantly lower than levels outside of the vegetated areas under summer conditions (Orth et al. 2012). Within the eelgrass canopy, SSC showed significant decreases over time, with median SSC decreasing approximately 75% between 2003 and 2009, and coincided with increased retention of fine sediments. These findings relate well with trends found in previous studies where flow reduction by seagrass meadows ranged from 25 to 80% (Koch & Gust 1999, Nepf 1999, Lacy & Wyllie-Echeverria 2011) and turbulence was reduced 30 to 50% (Granata et al. 2001). In a study by Gruber & Kemp (2010) with a

similar current and wave regime as that in our study, reductions in SSC of up to 60% were found, which varied directly with seagrass biomass via seasonal senescence of a *Stuckenia pectinata* meadow. Our findings using different density sites within the same eelgrass bed suggest the opposite trend of lower SSC values at less dense seagrass sites. These opposing trends are likely due to site location within the meadow and local exposure to wind and wave activity. Locations that were further from the canopy edge and locations where waves propagated further distances across the eelgrass canopy had greater reductions in flow and suspended sediment. These findings suggest that seagrass density and meadow size both play roles in sediment suspension.

There is evidence to suggest that seagrass patches below a minimum size, and possibly locations close to the canopy edge, act to enhance near-bed turbulence and cause scouring (Heller 1987, Fonseca & Koehl 2006). Once the bed reaches a minimum density and patch width, turbulence reduction occurs, switching the local flow environment from erosional to depositional. Although it is still unclear what minimum size causes this transition, our findings suggest that the expansion of the eelgrass canopy within South Bay has altered the hydrodynamics from a net erosional environment to one that promotes deposition of suspended sediment. This has enhanced light penetration throughout the water column and created a positive feedback for eelgrass growth.

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# Modeling the effects of climate change on eelgrass stability and resilience: future scenarios and leading indicators of collapse

Joel A. Carr\*, Paolo D'Odorico, Karen J. McGlathery, Patricia L. Wiberg

University of Virginia Dept. of Environmental Sciences, 291 McCormick Rd, Clark Hall, Charlottesville, Virginia 22904, USA

**ABSTRACT:** Seagrass meadows influence local hydrodynamics in coastal bays, resulting in a decrease in the shear stress acting on the underlying bed sediment. The reduced sediment suspension and water column turbidity creates a more favorable light environment for further seagrass growth. This positive feedback is strong enough to induce depth-dependent bistable dynamics with 2 possible stable states, an extant meadow and a bare sediment surface. A coupled vegetation-growth hydrodynamic model was used to investigate eelgrass stability and leading indicators of ecosystem shift under the effects of sea-level rise and increases in water temperature associated with climate change. The model was applied to Hog Island Bay, a shallow coastal bay within the Virginia Coast Reserve, USA, where eelgrass restoration efforts are ongoing. The results indicate that while extant eelgrass meadows are likely to tolerate sea-level rise, an increase in the frequency of days when summer water temperature exceeds 30°C will cause more frequent summer die-offs. This increase in the number of higher temperature disturbance events is likely to push a dense meadow initially located within the bistable depth range (1.6 to 1.8 m mean sea level) toward and eventually past a critical bifurcation point, from which recovery is not possible. We identified 2 leading indicators of a meadow nearing this bifurcation point, both associated with the number of leaves per shoot: 'flickering,' which reflects conspicuous fluctuations from one attractor to the other across the threshold, and 'slowing down,' which is the decreased recovery from perturbations as a system gets close to a threshold. Our model indicates that the eelgrass in these coastal bays has limited resilience to increases in water temperatures predicted from current climate change models.

**KEY WORDS:** *Zostera marina* · Seagrass · Climate change · Bistable dynamics · Ecosystem shifts · Leading indicators

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## INTRODUCTION

While subtidal seagrass meadows and the surrounding salt marshes are highly productive ecosystems that provide critical ecosystem services, they are also susceptible to changes in nutrient, sediment, and freshwater delivery due to changes in anthropogenic land use and population density (Viaroli et al. 1996, Havens et al. 2001, Nixon et al. 2001). Increased water temperature and depth associated

with climate change also pose potential threats to the health of seagrass meadows (Najjar et al. 2000, Cazanave et al. 2008, Keller 2009). The shallow depths of coastal bays provide a good environment for benthic primary productivity of species with high light requirements, such as seagrasses (Duarte 1991, Dennison et al. 1993, Zimmerman et al. 1995). However, these shallow depths also increase the vulnerability of the bed sediment to resuspension by waves and currents, which increases water column turbidity and

\*Email: jac6t@virginia.edu

adversely affects the light environment. In coastal bays that lack a significant river sediment supply, internal sediment resuspension is typically the dominant control on water column turbidity (Lawson et al. 2007).

The presence of seagrass reduces the wave and current shear stresses acting on the underlying bed sediment, thereby reducing resuspension and enhancing deposition of fine sediment (Folkard 2005). This can lead to less turbid waters and a more favorable light environment. This positive feedback, whereby a decrease in seagrass density decreases favorable light conditions, causing further loss of seagrass, has been shown to induce bistable dynamics (van der Heide et al. 2007, Carr et al. 2010). The emergence of bistability in ecosystems has important implications. Bistable systems exhibit nonlinear behavior in their response to environmental drivers, and can undergo rapid shifts between states due to only small changes in environmental conditions. Similarly, bistable systems are associated with limited resilience in that if the system is perturbed past a critical threshold, the system collapses to the alternate state (Wilson & Agnew 1992). In this case, a seagrass meadow undergoing a disturbance exceeding a certain magnitude, duration, or frequency may pass into a state from which it cannot recover, resulting in loss of the seagrass meadow. The high turbidity environment of the now bare sediment inhibits establishment of new meadows, and the system remains locked in the alternate stable state, even if the disturbance is removed. Because of the abrupt and often irreversible character of these transitions, ecosystem managers need to be able to recognize whether a system is about to shift from a seagrass-covered bottom to a bare sediment state. However, such ecosystem shifts may or may not be associated with early warning signs (Hastings 2010). As such, it is important not only to identify the threshold between the 2 attraction domains (Brock & Carpenter 2010), but also to recognize early warning signs, if they exist, that the system is about to cross the threshold between regimes.

It is difficult to predict these transition points because the state of the system may display little change prior to the transition (Scheffer et al. 2009). In some systems there is a critical slowing down effect (van Nes & Scheffer 2007), whereby as the system approaches the bifurcation point, its response to small perturbations (i.e. small displacement from stable equilibrium) becomes increasingly slow. This slowing down results in an increased autocorrelation of the state variable and possibly an increase in variance

when nearing the critical threshold (van Nes & Scheffer 2007). Critical slowing down, increase in variance, or increase in autocorrelations have been shown to be suitable early warning signals of state change in some ecosystem and population dynamics (Litzow et al. 2008, Drake & Griffen 2010, Carpenter et al. 2011) as well as in geophysical records (Dakos et al. 2008). Another possible symptom of being near the critical bifurcation point is flickering, which occurs when environmental or stochastic drivers are strong enough to induce relatively rapid oscillations between attraction domains of a system near the critical bifurcation point (Scheffer et al. 2001, 2009, Brock & Carpenter 2010; Fig. 1). Regardless of the specific metric, evidence of a system being near catastrophic collapse is important for mitigation management practices, and also provides insight to guide and monitor successful restoration efforts. However, the sensitivity of these systems to natural and anthropogenic drivers complicates the development and implementation of successful management strategies.

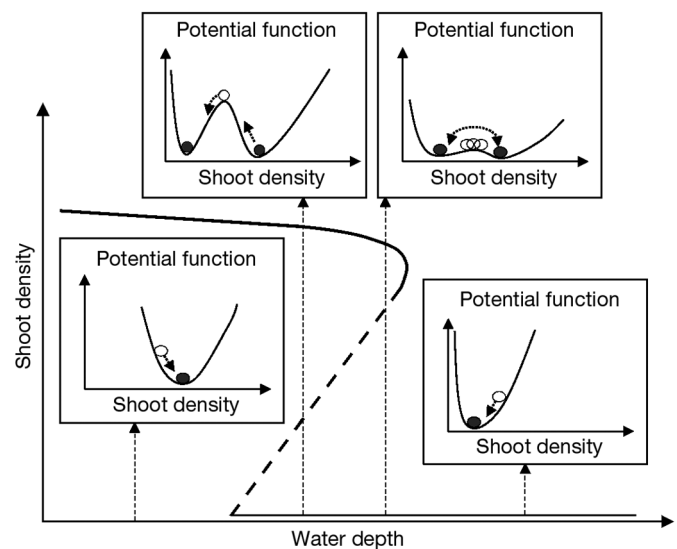


Fig. 1. Schematic representation of the 'fold-type' bifurcation of seagrass dynamics. Stable (solid) and unstable (dashed) states of the system are shown as a function of water depth. In shallow waters, the light environment is sufficient for seagrass establishment and survival regardless of the initial existence of a seagrass canopy stabilizing the benthic sediments. In relatively deep waters, the light penetrating the water column is insufficient for seagrass growth. In intermediate conditions, the system may be stable either with or without a seagrass bed. These stability and bistability conditions are shown in terms of minima of the potential function which is qualitatively plotted in the insets. Close to the critical fold bifurcation point, the potential barrier between the stable states is small, and the system may repeatedly fluctuate between these 2 states ('flickering'). (●) stable states; (○) unstable states



To guide restoration and management efforts as well as to improve our understanding of the susceptibility of shallow coastal bays to changes in climate drivers and disturbances, including sea-level rise and temperature increase, we applied a coupled eelgrass growth and hydrodynamic model (Carr et al. 2012) to a set of scenarios reflecting expected future increases in sea level and water temperature in a shallow coastal bay. This model was used to investigate the strength of the feedback between water quality and eelgrass ecosystems, the impact of these feedbacks on the stability and resilience of estuarine seagrass meadows (van der Heide et al. 2007), and possible leading indicators of an ecosystem shift.

## MATERIALS AND METHODS

### Study area

The model was applied to coastal bays of the Virginia Coast Reserve Long Term Ecological Research (VCR LTER) site, located on the Atlantic side of the Delmarva Peninsula, USA (37° 25' N, 75° 46' W). The coastal bays in this system were dominated by the seagrass *Zostera marina* (eelgrass) until the 1930s, when the eelgrasses, under stress from disease, were extirpated by a hurricane (Orth et al. 2006). Restoration efforts were prompted when small natural

patches of eelgrass were discovered in the late 1990s. Eelgrass meadows now cover some 1700 ha in the VCR coastal bays (Orth et al. 2012, this Theme Section). Nutrient loading of the coastal bays is low and water column chlorophyll levels are also very low (Cole & McGlathery this Theme Section; see also VCR LTER data base: [www1.vcrlter.virginia.edu/home1/?q=data\\_wq](http://www1.vcrlter.virginia.edu/home1/?q=data_wq)).

Hog Island Bay is representative of the shallow bays in the VCR, and is typical of coastal bay systems along the Atlantic and Gulf coasts, and served as our study site. Hog Island Bay has a tidal range of roughly 1.2 m, with half of the bay <1 m deep at mean low water (Oertel 2001). The sediment at the bay bottom ranges from fine sand to fine silt. Wind-driven resuspension dominates sediment transport in the bay, with dominant storms occurring from October to April (Lawson et al. 2007). Restored eelgrass meadows exist in Hog Island Bay between 0.6 and 1.6 m depths at mean sea level (MSL; McGlathery et al. 2012, this Theme Section). Locally, climate change is expected to generate a 30 yr increase in MSL of 12 cm and an increase of 1.3°C in mean water temperature (Najjar et al. 2000).

### Modeling framework

The model couples a hydrodynamic/light model (Carr et al. 2010) with a daily growth model (Carr et

Table 1. Eelgrass growth model, where ecosystem dynamics are represented through the following state variables:  $N$  (total number of shoots),  $P_{n,l}$  (biomass of leaf  $l$  belonging to shoot  $n$ ), and  $R$  (rhizome biomass). The dynamics of these state variables are expressed by the following equations, with functions and parameters reported in Tables 2 & 3

Equation	Description
$\frac{dN}{dt} = N(\max_{N_{\text{grow}}} F_1(I) F_{\text{phot}}(T) N_{\text{lim}} R_{\text{lim}} - N_{\text{loss}})$	Change in the total number of shoots $N = \sum n$ , where $n$ is an individual shoot, is a product of the total number of shoots, and the difference between recruitment and loss
$\frac{dP_{n,l}}{dt} = P_{n,l}(\max_{\text{phot}} F_1(I) F_{\text{phot}}(T) F_{\text{age}} P_{\text{lim}} - \max_{\text{resp}} F_T(T))$	Change in biomass of leaf $l$ belonging to shoot $n$ , modeled as a collection of up to 4 leaves ( $l = \text{leaf number}$ ), with $P_{n,0}$ being the biomass of the stem
$\frac{dR}{dt} = k_{\text{transfer}} \sum_{n,l} \frac{dP_{n,l}}{dt} - \gamma F_T(T) R - \frac{dN_n}{dt} P_{n_{\text{new}},0} + k_{\text{uproot}} \frac{dN_n}{dt} -$	Belowground biomass associated with the rhizome structure of eelgrass, $R$ , is modeled as a bulk quantity with rhizome growth due to translocation of above-ground production, and loss due to respiration, translocation of biomass to a new shoot, and loss of biomass to uprooting when a shoot is lost
$\frac{dL_{n,l}}{dt} = \max_{\text{elong}} F_1(I) F_{\text{phot}}(T) L_{\text{lim}}$	Width and thickness are held constant, and change in the length of leaf $l$ of shoot $n$ , $L_{n,l}$ is modeled as a linear function of maximum leaf elongation rate, modulated by light, and photosynthetic productivity as a function of temperature up to some maximum leaf length

Table 2. Limitation functions, description, and sources for the eelgrass growth model

Limitation function	Description and values	Source
$I = I_{\text{surface}} e^{-K_d h_d}$	Irradiance at depth. $h_d$ is the distance from the water surface to the deflected canopy. $K_d$ is the light attenuation coefficient	Lawson et al. (2007)
$I_K = I_{K20} \theta_K^{T-20}$	Saturation irradiance as a function of temperature. $I_{K20}$ is saturation value at 20°C set to 25.5 and $\theta_K$ is shape value set to 1.04	Zharova et al. (2001)
$I_C = I_{C20} \theta_C^{T-20}$	Compensation irradiance as a function of temperature. $I_{C20}$ is compensation value at 20°C set to 2.4 and $\theta_C$ is shape value set to 1.17	Zharova et al. (2001)
$F_1(I) = 0$ for $I \leq I_C$ $F_1(I) = 1$ for $I \geq I_K$ $F_1(I) = \frac{I - I_C}{I_K - I_C}$ for $I_C \leq I \leq I_K$	Light limitation function	Zharova et al. (2001)
$F_{\text{phot}}(T) = K_{\text{Ophot}} \left( \frac{T_{\text{opt}} - T}{T_{\text{opt}}} \right)^2$ for $T \leq T_{\text{opt}}$ $F_{\text{phot}}(T) = K_{\text{mphot}} \left( \frac{T - T_{\text{opt}}}{T_{\text{max}} - T_{\text{opt}}} \right)^2$ for $T > T_{\text{opt}}$	Temperature photosynthesis limitation function. $T_{\text{opt}}$ is the optimum temperature for photosynthesis set to 21.5°C. $T_{\text{max}}$ is the maximum temperature for photosynthesis set to 34°C. $K_{\text{Ophot}}$ is a shape coefficient set to 0.01, and $K_{\text{mphot}}$ is a shape coefficient set to 0.00001	Zharova et al. (2001)
$F_{\text{age}} = 1$ for ages $\leq \text{min}_{\text{age}}$ days and $P_{n,0}$ $F_{\text{age}} = 1 - 0.99(\text{age} - \text{min}_{\text{age}}) / (\text{max}_{\text{age}} - \text{min}_{\text{age}})$ for $\text{min}_{\text{age}} < \text{ages} \leq \text{max}_{\text{age}}$ days $F_{\text{age}} = 0.01$ for ages $> \text{max}_{\text{age}}$ days	Leaf age limitation function. $\text{min}_{\text{age}} = 70$ , $\text{max}_{\text{age}} = 175$	Verhagen & Nienhuis (1983), Hemminga & Duarte (2000)
$F_T(T) = \theta_{\text{loss}}^{T-20}$	Temperature respiration scaling function. $\theta_{\text{loss}}$ is shape value set to 1.05	Zharova et al. (2001)

al. 2012; Tables 1–3) and is used to estimate the annual and interannual morphology of a 1 m<sup>2</sup> patch of a eelgrass meadow 1 m in from the edge of the meadow. Site measurements of peak shoot density, leaves shoot<sup>-1</sup>, leaf length, and above- to below-ground biomass ratios were used to constrain model parameters (Table 3). The eelgrass growth model is built upon prior efforts to model eelgrass dynamics (Verhagen & Nienhuis 1983, Zharova et al. 2001). The primary difference in our approach is that biomass is allocated and tracked by accounting for the distinct structural components (e.g. leaves and stem, where stem is defined as the rigid base of the shoot) for each shoot (Carr et al. 2012).

The hydrodynamic model (Carr et al. 2010) simulates the 1-dimensional dynamics (in the vertical direction) of sediment entrainment and settling within the water column on an hourly basis. It accounts for

the effect of eelgrass vegetation on these dynamics, including its effect on the velocity profile, eddy diffusivity, shear-flow dispersion, and wave and current shear stress. At the same time it accounts for the effect of flow on the height of the deflected eelgrass canopy. Values of suspended sediment concentrations calculated by the model using a 1-dimensional advection diffusion equation are used to determine the degree of light attenuation within the water column (from the water surface to the top of the canopy, as in Lawson et al. 2007) and to calculate light availability for the eelgrass meadow.

Growth of the eelgrass meadow was modeled (Carr et al. 2012) based on available eelgrass data (Table 3), irradiance, and water temperature conditions in the VCR coastal bays. Eelgrass biomass is allocated among individual leaves and shoots, which transfer a fraction of aboveground production to a combined

Table 3. Parameters and sources for the eelgrass growth model

Parameter	Description and values	Source
$\tau_{cr}$	Critical shear stress to erode sediment: 0.04 Pa	Lawson et al. (2007)
$\max_{resp}$	Respiratory loss rate: 0.014 d <sup>-1</sup>	Bach (1993)
$\max_{phot}$	Maximum specific growth rate: 0.095 d <sup>-1</sup>	Bach (1993)
$\max_{elong}$	Maximum elongation rate: 0.00164 m h <sup>-1</sup>	Kemp et al. (1987)
$\max_{N_{grow}}$	Maximum shoot recruitment: 0.028 d <sup>-1</sup>	Zharova et al. (2001)
$N_{loss}$	Shoot mortality rate: 0.0065 d <sup>-1</sup>	Zharova et al. (2001)
$L_{lim}$	Maximum leaf length limit, set to 1 if leaf length is <1 m, otherwise set to 0	
$R_{lim}$	Above- to below-ground biomass ratio limitation, set to 1 if the ratio is <4.0, otherwise set to 0	Site-specific measurements
$N_{lim}$	$N_{lim} = 1 - \left( \frac{N}{N_{cc}} \right)^2$ for $N$ less than $N_{cc}$ , otherwise set to 0. Maximum shoot density, $N_{cc}$ set to 1000 shoots m <sup>-2</sup>	
$P_{lim}$	Maximum biomass of a single leaf set to 0.12 g C corresponding to a maximum above-ground single shoot biomass of 0.5 g C with 4 leaves of equal biomass	Zharova et al. (2001)
$P_{new}$	Biomass of a new shoot and single leaf set to 0.0024 g C	Zharova et al. (2001)
$\gamma$	Rhizome respiration rate set to 0.009 d <sup>-1</sup>	Zharova et al. (2001)
Sparse meadow	3 shoots m <sup>-2</sup> , $R = 2$ g C, 3 leaves shoot <sup>-1</sup> , $P = 0.08$ g C leaf <sup>-1</sup>	
Dense meadow	640 shoots m <sup>-2</sup> , $R = 45$ g C, ~3 leaves shoot <sup>-1</sup> , $P = 0.09$ g C leaf <sup>-1</sup>	Shoot density, leaves shoot <sup>-1</sup> are site-specific measurements

rhizome biomass. The growth model utilizes rhizome biomass storage to form a new shoot and leaf. Based on leaf productivity of that shoot, biomass is then transferred into new leaf growth, leaf elongation, and back to rhizome storage. Senescence of aboveground biomass is due to (1) leaves reaching a maximum age, (2) respiratory losses, and/or (3) shoot mortality. Rhizome biomass is lost due to (1) respiration, (2) new shoot recruitment, or (3) uprooting when a shoot is lost. The growth model is run on a daily basis with the modeled density of shoots and leaves above the sediment surface directly incorporated into the hydrodynamic model, influencing both the shear stress acting on the sediment surface as well as the mixing of sediment within the water column. As productivity is a direct function of water temperature and available irradiance (which, in turn, depends on suspended sediment concentration), the model was used to explore the effects of annual and interannual temperature and wave–current conditions on the resilience and stability of a eelgrass meadow.

For the eelgrass growth model, the parameter values used, descriptions, and limitation functions can be found in Tables 1 to 3. Model calibration was

obtained by increasing the maximum shoot growth rate until maximum modeled daily shoot recruitment (Table 3) for repeated runs of the year 2001, which possessed the best growth characteristics, matched the maximum reported value of 14.4 shoots m<sup>-2</sup> d<sup>-1</sup> (Olesen & Sand-Jensen 1994, Zharova et al. 2001). In general, the model was not sensitive to the calibration parameter (Carr et al. 2012), and the modeled maximum daily shoot recruitment for all years fell within the reported maximum range of 7 to 14.4 shoots m<sup>-2</sup> d<sup>-1</sup> (Olesen & Sand-Jensen 1994).

Modeled peak shoot densities for dense meadows during randomized runs match observed values well, with measured dense meadows exhibiting peak modeled densities of around 650 shoots m<sup>-2</sup>. The model also captures well both the mean and variability in the measured peak shoot densities for re-establishing meadows (Carr et al. 2012). Similarly, modeled blade lengths correspond well to photographic measurements. In general, both modeled shoot densities and depth ranges align well with field observations in the restored seagrass meadows in Hog Island Bay (McGlathery et al. 2012).

### Model simulations

For any given year, hourly measured tides, winds, and water temperatures from the NOAA Wachapreague Station (WAHV2, 37° 36' 24" N, 75° 41' 12" W) and photosynthetically active radiation (PAR) from the VCR-LTER flux tower at Fowling Point (37° 24' N, 75° 50' W) were used as external drivers for the model. For each 1 h time step, PAR reaching the canopy was calculated following Carr et al. (2010). Average daily water temperature and net daily PAR reaching the canopy were then used to drive the growth model on a daily time step. Summary statistics of growth conditions of the various years can be found in Table 4.

Supplied with a measured sediment grain size distribution, water depth relative to MSL, and a site-representative initial eelgrass meadow state of above- and below-ground biomass and shoot and leaf densities (Table 3), the model calculated annual growth or senescence of the eelgrass within a meadow. The modeled meadow state at the end of any given year was used as the input meadow state for the following year, allowing us to model long-term changes in the meadow, investigate the ecosystem's stability and emergent bistability due to the hydrodynamic feedback, and explore how an expected 30 yr increase in MSL of 12 cm and mean water temperature of 1.3°C (Najjar et al. 2000) will affect the stability of eelgrass meadows.

Years of forcing conditions (winds, tides, water temperature, and PAR) were selected from data records from 1996 through 2004 to form randomized 150 year-long sequences. Using these sequences at initial water depths ranging from 1.5 to 2.0 m MSL in 0.1 m increments, and by starting a meadow

from both an initial dense and sparse meadow state (Table 3), we explored the attractors of the system (eelgrass meadow or bare sediment state) as a function of water depth and meadow state. To investigate the sensitivity of these attractors to sea-level rise and warming, 5 randomized 30 yr sequences of forcing conditions were used to drive the model from an identical initial dense meadow of 640 shoots m<sup>-2</sup>, for the same depth range. Two of the five 30 yr sequences were the same for all water depths to allow for direct behavioral comparison of how meadows initiated at different water depths react to identical sequences of environmental drivers. Averaging the 5 runs allowed for exploring the general behavior of the meadow. Three scenarios were explored in these simulations: (1) sea-level rise of 4 mm yr<sup>-1</sup> for 30 yr, (2) increase of 0.043°C yr<sup>-1</sup> in water temperature for 30 yr, and (3) combined sea-level rise and temperature increase for 30 yr (Najjar et al. 2000).

## RESULTS

### Overview

Meadows initiated at depths between 1.6 and 1.8 m at MSL were found to display bistable behavior. Meadows initiated from a sparse canopy state were unable to grow in waters deeper than 1.6 m MSL. Shallower than 1.8 m MSL, meadows initiated from a dense meadow state were able to maintain a favorable light environment. Deeper than 1.8 m MSL, meadows initiated from both the sparse and dense states collapsed to the bare sediment state (Fig. 2). Meadows within the bistable range from 1.6 to 1.8 m MSL exhibited limited resilience such that a sequence of disturbances (years with poor light and temperature conditions) could shift a meadow into the attraction domain of the bare sediment state (Fig. 2). Thus, simulations aimed at exploring the effect of sea-level rise and increasing water temperatures were expected to show that meadows initialized at 1.5 and 1.6 m MSL remain dense under an increase in MSL of 4 mm yr<sup>-1</sup> for 30 yr (total increase of 0.12 m), whereas meadows initiated at 1.8 m MSL or deeper should show slow decline in shoot density. We also expected the meadow initiated at 2.0 m to decline, as at that water depth, the only attraction domain

Table 4. Comparison of days of above-ground biomass growth, loss, and days for exceeding 30°C under measured, and expected climate change conditions with an increase of 1.3°C based on temperature records for the years 1996 to 2004 for *Zostera marina*

Year	Days of growth		Days of loss		Days exceeding 30°C	
	Measured	+1.3°C	Measured	+1.3°C	Measured	+1.3°C
1996	247	232	119	133	15	47
1997	288	271	77	94	18	47
1998	285	265	80	100	11	43
1999	302	292	63	73	5	37
2000	334	311	31	54	0	9
2001	309	307	56	58	0	0
2002	301	283	64	82	11	39
2003	276	269	89	96	0	19
2004	280	246	85	119	2	30

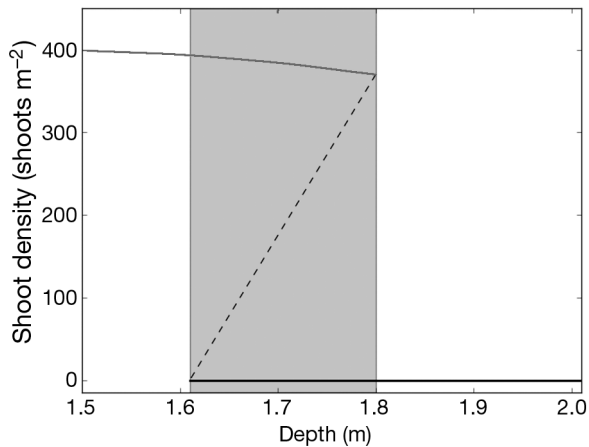


Fig. 2. *Zostera marina*. Fold bifurcation plot exhibiting the bistable depth range for *Z. marina* as a function of water depth and wintering shoot density (shoots  $\text{m}^{-2}$ ), under random drivers. Meadows at water shallower than 1.6 m mean sea level (MSL) exhibit a single attraction domain of a dense meadow state (gray line). Meadows initiated deeper than 1.8 m MSL display a single attraction domain of the bare sediment state (black line). In between 1.6 and 1.8 m MSL, depending on shoot density, depth and environmental forcing conditions, a meadow may be within the attractive domain of either the dense meadow state or the bare sediment state (shaded area)

of the dynamics is bare sediment. Similarly, across all water depths an increase in water temperature was expected to decrease net productivity of the eelgrass meadows with catastrophic losses when summer temperature exceeded  $30^{\circ}\text{C}$  for weeks at a time.

### Eelgrass stability and resilience under sea-level rise and warming scenarios

Annual shoot densities were averaged for the 5 random 30 yr simulations to examine the general effect of sea-level rise, increased temperature, and the combined effects of sea-level rise and temperature as a function of initial starting depth. Under sea-level rise only, for water depths of 1.5 and 1.8 m MSL, an extant meadow was present on average at the end of the 30 yr (Fig. 3a). Meadows initiated in deeper water on average showed a general slow decline, with 2 of the 5 meadows initiated at 2.0 m MSL collapsing to the bare sediment state by

the end of the 30 yr period. The meadows initiated at 1.9 m MSL did not begin to show decline until after 20 yr, with an extant, albeit declining, meadow for each of the 5 runs at the end of the 30 yr period (data not shown).

Increased water temperature caused meadows to experience sudden collapses sometime after 20 yr due to the increased frequency of high summer temperature events (Table 4, Fig. 3b); this occurred for all water depths including some sequences at 1.5 m MSL (Fig. 4b). These collapses resulted in the abrupt loss of approximately 100 shoots in shallower water, with wholesale loss possible in water deeper than 1.7 m MSL. In 1 run (Fig. 4b), shoot loss at 1.5 m MSL occurred as a result of encountering 2 significant disturbance events (years with extremely poor growing conditions either due to storms and/or temperature) with only 3 yr of recovery in between. These rapid losses are in contrast to the slow declines under just sea-level rise alone (Figs. 3a & 4a). Initial depths less than 1.7 m MSL all displayed some residual meadow at the end of 30 yr, indicating still favorable growth conditions (i.e. the meadow remains in the attraction domain of a dense meadow). However, with increasing frequency and magnitude of disturbance due to increased temperature alone, it is unlikely that a dense meadow would be able to reestablish. Meadows initiated at 1.7 m MSL and deeper exhibited collapse to bare sediment after a

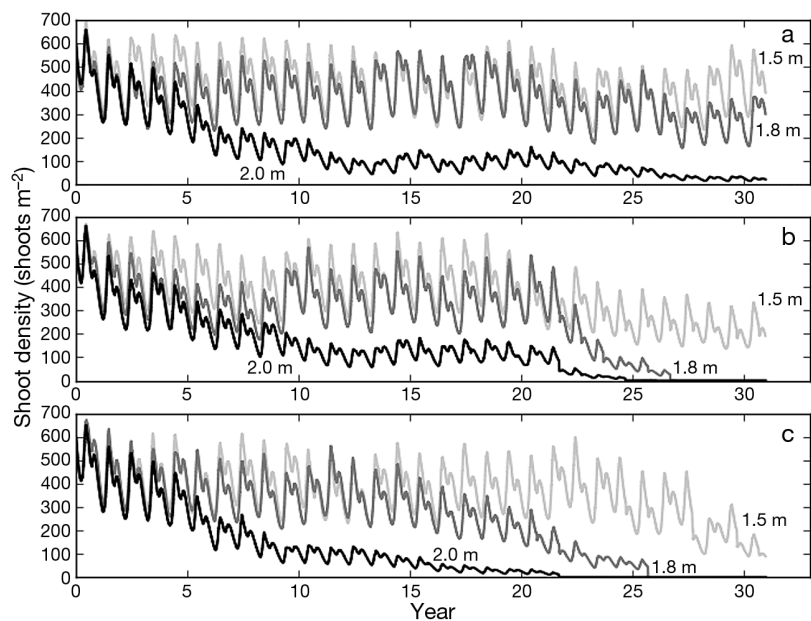


Fig. 3. *Zostera marina*. Shoot densities averaged over all runs initiated at water depths of 1.5, 1.8, and 2.0 m mean sea level, under (a) increase in sea level (b) increase in temperature, and (c) both increased temperature and sea level

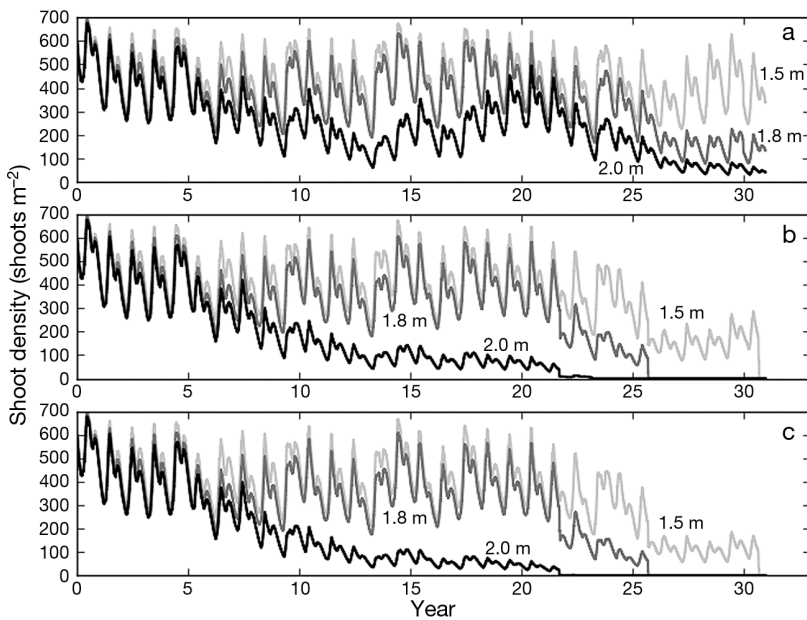


Fig. 4. *Zostera marina*. Shoot densities for run 4 initiated at water depths of 1.5, 1.8, and 2.0 m mean sea level, under (a) increase in sea level (b) increase in temperature, and (c) both increased temperature and sea level

Table 5. Years to collapse as a function of water depth (mean sea level, MSL) for the 5 model runs under combined conditions of sea-level rise and increased water temperature

Run	MSL depth:	Years to collapse					
		1.5 m	1.6 m	1.7 m	1.8 m	1.9 m	2.0 m
1		–	28	28	24	21	17
2		27	30	30	26	25	18
3		30	27	26	26	24	22
4		30	26	26	26	26	22
5		–	26	26	24	23	23
Mean		29	27.4	27.2	25.2	23.8	20.4

single disturbance event. In all cases, this significant disturbance event corresponded to years under increased temperatures where the duration of extremely high water temperatures in the summer months (Table 4) results in significant loss of leaves and shoots (Fig. 3b)

Under the combined increase in MSL and water temperature, meadows which fell into the bistable range between 1.6 and 1.8 m MSL when sea-level rise alone was considered, all collapsed to the bare state as a result of limited resilience to the now more frequent high-temperature events (Figs. 3c & 4c). Time to collapse varied but was predominantly a function of initial meadow depth, with meadows in deeper waters being more susceptible (Table 5).

### Leading indicators of ecosystem shift

We explored whether leading indicators of an ecosystem shift can be found in (1) the change in standard deviation of the number of leaves shoot<sup>-1</sup> for each day of the year and (2) trends in the mass distribution of empirical distribution functions (EDFs; Fig. 5) approaching the bifurcation point. This was done by examining the state variables and mass distribution of the number of leaves shoot<sup>-1</sup> during the expected slow decline (Fig. 6a) of a meadow initiated at 2.0 m MSL undergoing sea-level rise. Unlike other authors (Carpenter 2006, Guttal & Jayaprakash 2008), we did not find any leading indicators of state shift in standard deviation, skewness, or autocorrelation in the system state variables. While standard deviation and suitable metrics of multimodality increase as the system temporarily enters into the domain of attraction of bare sediment, these metrics were not good leading indicators of state change in that they did not exhibit any symptomatic changes as the system approached the bifurcation point. However, close to this point, eelgrass meadow behavior exhibited conspicuous fluctuations from one attractor to the other across the threshold, a phenomenon known as ‘flickering’ (Scheffer et al. 2009; our Figs. 1, 5, 6b) in the mass distribution of less than 2 leaves shoot<sup>-1</sup>. Every time the system shifted

into the domain of attraction of bare sediment, the probability distribution of the number of leaves shoot<sup>-1</sup> exhibited an increase at the lower leaf-per-shoot end of the distribution (Figs. 5 & 6b). This increase was due to leaf loss and/or high new shoot mortality. This conspicuous flickering was evident in collapsing meadows under increased temperature (Fig. 7) as well as those undergoing the combined effects of sea-level rise and temperature (Fig. 8). In comparison to a meadow in the stable attraction domain of the dense meadow state, a meadow near the critical bifurcation point, an increase the number of shoots with less than 2 leaves was not only larger but became more persistent until the meadow collapsed (Figs. 7b & 8b). This increasing persistence is

a typical indicator of a system slowing down close to a threshold due to its inability to recover (Scheffer et al. 2009). Under these conditions, a meadow as it approached the critical bifurcation point initially began to oscillate back and forth across the threshold. As the meadow neared the critical bifurcation

point, it began to remain more frequently in the attractive domain of the bare sediment state (Figs. 7b & 8b) with a relatively persistent increase in the mass distribution of shoots with <2 leaves (Figs. 7b & 8b). From this condition, the meadow was susceptible to fully crossing into an unrecoverable bare sediment state. Therefore, the emergence of substantial flickering (evidenced by oscillation in the mass distribution <2 leaves shoot<sup>-1</sup>) followed by slowing down (evidenced by persistence and increase in the mass distribution <2 leaves shoot<sup>-1</sup>) appear to be leading indicators of proximity to a fold bifurcation.

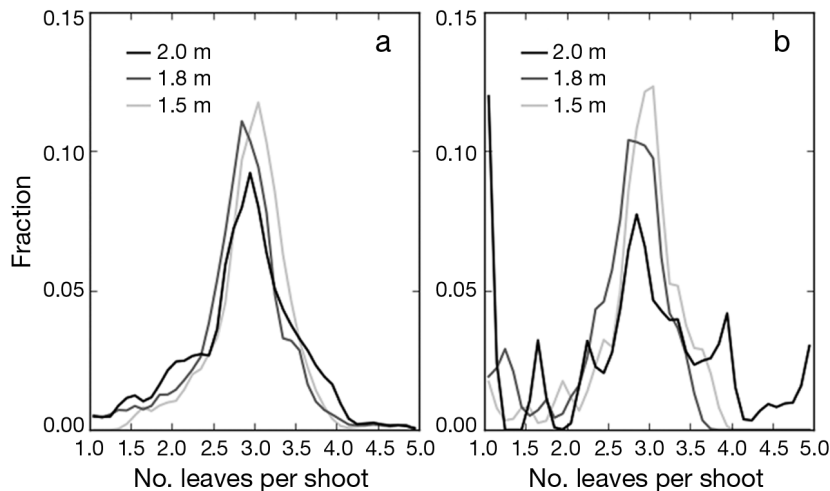


Fig. 5. *Zostera marina*. (a) Empirical distribution function (EDF) for number of leaves shoot<sup>-1</sup> across all 5 sequences ( $n = 1\,357\,800$ ) with increased sea-level rise for meadows initiated at 1.5, 1.8, and 2.0 m mean sea level (MSL). (b) Multimodal behavior and increase in the mass fraction <2 leaves shoot<sup>-1</sup> in the EDF for meadows initiated at 1.5, 1.8, and 2.0 m MSL for an individual year ( $n = 8\,760$ ) with poor growing conditions due to elevated temperatures

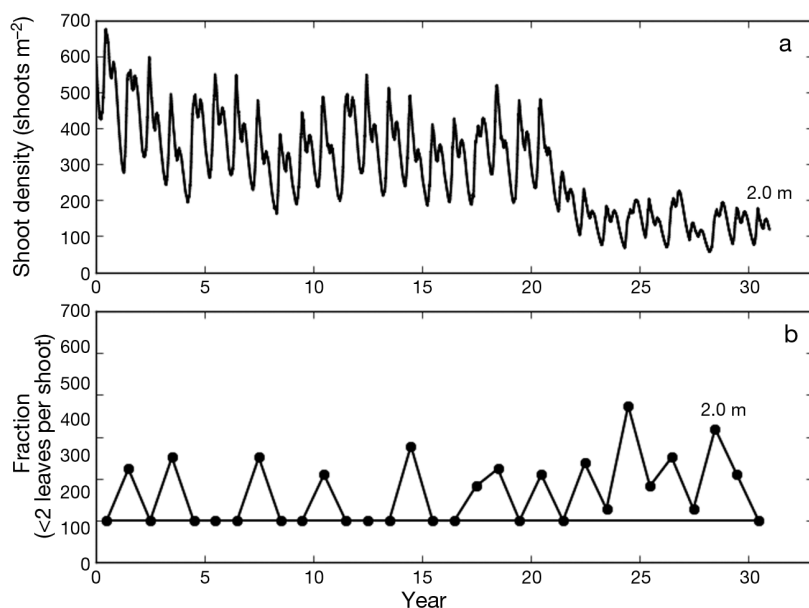


Fig. 6. *Zostera marina*. (a) Shoot densities for meadow initiated at 2.0 m mean sea level (MSL) under sea-level rise already within the attractive domain of the bare sediment state. (b) The evidence of flickering, in the fraction of mass <2 leaves per shoot in the meadow initiated at 2.0 m MSL under increasing sea-level rise

## DISCUSSION

Prior efforts have examined the loss of resilience, the existence of alternate stable states, and possible leading indicators of ecosystem shift in various systems (Gunderson 2000, Scheffer & van Nes 2004). Here we demonstrate that the emergence of bistable dynamics due to the prior established positive feedback between seagrass and its light environment (de Boer 2007, van der Heide et al. 2007, Carr et al. 2010) plays an important role in the resilience of these meadows to the increased stresses of predicted climate change, namely sea-level rise and higher temperatures. The results indicate that while meadows shallower than 1.6 m MSL for our study site are currently stable and can be expected to remain within the stable light growth environment, the onset of increased water temperatures is likely to cause significant, but reversible collapse of these meadows. The expected effect of increased sea level is to push a meadow initially located within the attraction domain of a dense meadow towards the bistable depth range where the meadows will possess limited resilience. Frequent disturbances (e.g. years with poor growing conditions due to storms and/or high temperatures) may push the meadow into the attraction domain of the bare sedi-

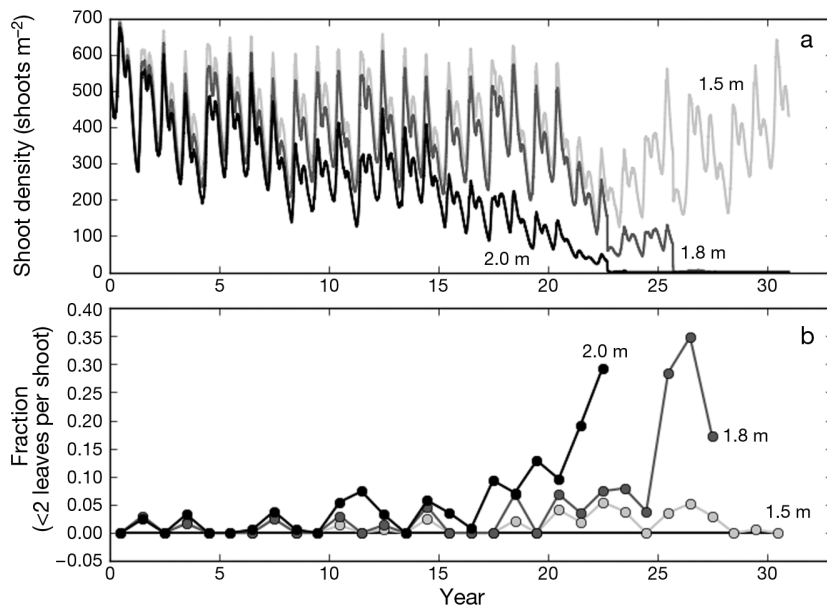


Fig. 7. *Zostera marina*. (a) Shoot densities for meadows initiated at 1.5, 1.8, and 2.0 m mean sea level (MSL) under increased temperature. (b) The appearance of flickering and slowing down prior to loss for the meadows initiated at 1.8 and 2.0 m MSL. Flickering, slowing down, but resilience of the meadow initiated at 1.5 m MSL

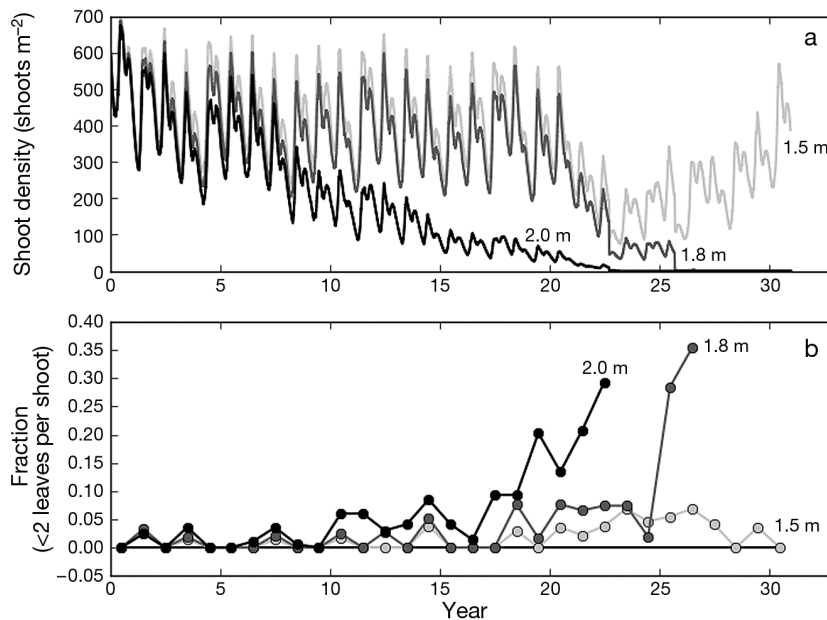


Fig. 8. *Zostera marina*. (a) Shoot densities for meadows initiated at 1.5, 1.8, and 2.0 m mean sea level (MSL) under increased temperature and sea level. (b) The appearance of flickering and slowing down prior to loss for the meadows initiated at 1.8 and 2.0 m MSL. Flickering, slowing down, but then recovery of the meadow initiated at 1.5 m MSL

ment state. Thus, as sea level increases, these meadows will begin to show limited resilience to disturbance events. Increases in water temperature (Moore & Jarvis 2008) effectively increase the frequency of

disturbances (e.g. years with poor growing conditions), as more years exhibit extended periods of high summer temperatures (Table 4) associated with significant die offs. As such, the more frequent occurrence of high-temperature events in the summer can rapidly push a meadow located within the bistable depth range past the critical bifurcation point and into the attraction domain of the bare sediment state from which irreversible collapse occurs. Sea-level rise alone can push a meadow into the attractive domain of the bare sediment state for depths that increase below the bistable range; however, collapse of these meadows is slow (Figs. 3a & 4a) in contrast to the rapid losses brought about by summer high temperature events (Figs. 3b,c & 4b,c).

Our results indicate that extant eelgrass meadows in the VCR LTER are unlikely to fail due to increases in sea-level rise alone, as rhizome branching rates and seed dispersal should be able to maintain migration of the meadows toward shallower waters. A benefit of sea-level rise for the eelgrasses in Hog Island Bay, which is a relatively undeveloped system with no significant riverine input (Oertel 2001), is that the area of suitable habitat (area deeper than 0.6 m MSL and shallower than 1.6 m MSL) will increase over the next 30 yr based on hypsometry (Fig. 9). The area of the bay exhibiting bi-stable behavior (area between 1.6 and 1.8 m MSL) will also increase. However, the results also indicate that the eelgrass meadows of the VCR LTER are likely to undergo severe die-offs due to extended summer periods with increased water temperatures. This is consistent with observed high temperature effects on *Zostera marina* (Duarte 2002, Moore & Jarvis 2008, Moore et al. 2012, this Theme Section). The temperature is projected to increase from 1 to 1.5°C in 30 yr with a projected 2.7 to 5.5°C increase by 2095 (Najjar et al. 2000). Even a modest



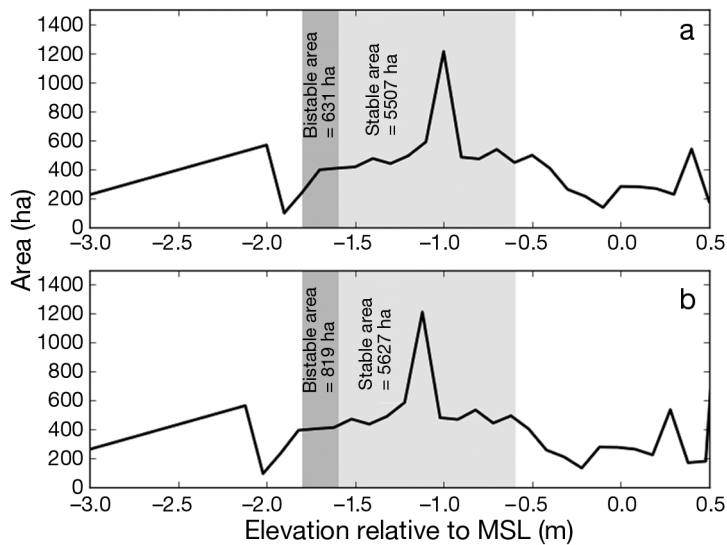


Fig. 9. (a) Current area of Hog Island Bay suitable for eelgrass establishment or maintenance. (b) Increase in suitable area under an increase in sea level of 0.12 m in 30 yr

increase in water temperature can result in significant changes in compensation light requirements (Moore et. al. 2012) and can result in large changes in the trajectories of *Z. marina* expansion. As such, with the projected continued increase in water temperature, eventually the coastal bays of the VCR will not be suitable habitat for *Z. marina*. Thus, while the meadows are able to moderate their light environment and increasing sea level increases the total area of suitable habitat, an increase in water temperature is likely to have significant and catastrophic effects.

From the ecosystem management standpoint, knowledge of whether seagrass meadows are approaching a bifurcation point is crucial to timely seagrass conservation efforts. A bifurcation point is not readily measurable or identifiable, as it is a function of both the current meadow state and the environmental drivers. As such, we attempted to identify what traits an eelgrass meadow exhibits when it approaches or crosses the transition point to the bare sediment state. We focused on meadow characteristics that are non-destructive and easily measured, such as leaf density, shoot density, and number of leaves shoot<sup>-1</sup>. We found that while density metrics are not adequate to predict the location of the critical bifurcation point, the number of leaves shoot<sup>-1</sup> for a meadow may be a useful indicator.

Comparing simulations of eelgrass meadows initiated at depths of 1.5, 1.8, and 2.0 m MSL experiencing the same sequence of randomized forcing under varying temperature and sea-level conditions

allowed us to examine the dynamics of shoot and leaf populations in stable meadows, bistable beds, and stable bare sediment regimes. Similarly, this allowed for investigation of leading indicators of an ecosystem shift as the meadows approach the depth for which they fall within the attractive domain of the bare sediment state. The EDFs of the number of leaves shoot<sup>-1</sup> calculated in an individual year exhibited multimodal behavior, particularly when a meadow was under duress (Fig. 4b). Multimodality tended to disappear in the case of dense meadows. This is comparable to a change in properties of the state variables' distribution, such as variance or skewness, witnessed in some systems (Carpenter 2006, Guttal & Jayaprakash 2008). Similarly, the emergence of significant multimodality can be considered as an indicator of eelgrass stress. This multimodality may happen for various reasons: in some cases, it is due to a year when all the young shoots established but were continuously being lost, while the 'middle-aged' shoots were able to increase leaf density, thereby creating a multimodal distribution. Alternatively, the occurrence of multiple modes may be due to patterns of eelgrass loss and regrowth associated with the seasonal cycle. The presence of multimodal distributions in the number of leaves shoot<sup>-1</sup> generally resulted in a larger standard deviation in leaves shoot<sup>-1</sup>.

The measures of stability investigated herein allow for the possibility of non-destructive monitoring of the viability of extant eelgrass meadows, through repeated sampling to assess leaf and shoot counts. The emergence of annual multimodality and a significant decrease in the variability of number of leaves shoot<sup>-1</sup> are indicators of stress and possible future collapse. Synoptic sampling of these parameters within a year and cross comparison of years would allow for identification of the flickering or persistent increase in the number of shoots with low leaf counts that may be indicative of being close to the threshold of collapse.

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Our approach assumed a constant linear increase in both local relative sea-level rise and water temperature. For the 30 yr considered in this study, a linear increase is an acceptable approximation. However, the omission of possible increases in storminess must be noted. Increased storminess (Hayden 1999) is likely to increase light stress due to enhanced re-suspension, especially since eelgrass has less of an effect on the attenuation of near bed wave orbital velocities than current velocities (Luhar 2010). Re-

garding the effects of climate change and the identification of leading indicators of collapse, the model is primarily limited by the length of available data sets of environmental drivers; however, the strong symptoms of flickering and slowing down were robust across all model simulations.

While the detailed coupled model used in this study provides many insights into the stability, bistability, and indicators of meadow stress and collapse, there are limitations. The vegetation growth model is sensitive to temperature (Zharova et al. 2001, Pastres et al. 2004), due to the involvement of temperature both in the saturation irradiance compensation curves as well as photosynthetic and respirations rates. Summer die-offs due to high temperature are typically observed when the water temperatures exceed 30°C for a long period of time in July and August (Moore & Jarvis 2008). The modeled values indicate this temperature threshold to be around 28.5°C, and as such, effects of temperature modeled herein may be overestimated. If so, however, these results would hold for a period in time further in the future in terms of increased water temperature. Other vegetation growth parameters in the model such as leaf elongation rates, labile to structural carbon ratios, and biomass characteristics of the various plant structures could be adapted to be more site specific. Regardless, modeled shoot densities and depth ranges align well with field observations in the restored eelgrass meadows in Hog Island Bay where the maximum depth limit of eelgrass was found to be 1.6 m MSL, matching the limit of the stable distribution predicted by the model (McGlathery et al. 2012).

## CONCLUSIONS

Both water temperature and the emergence of bistable dynamics due to the positive feedback between eelgrasses and their light environment play important roles regarding the survivability of eelgrass meadows. Eelgrass that fall within the bistable depth range display limited resilience to increased environmental stressors associated with climate change. Eelgrass meadows shallower than this bistable range can withstand the increased sea-level projections for the next 30 yr, as will meadows at the shallower end of the bistable range. Meadows at the deeper end of the bistable range will be pushed into the attraction domain of the bare sediment state. Increases in water temperature will likely result in more frequent and severe high summer temperature stress resulting in significant summer die-offs for

meadows at all depths. Meadows within the bistable depth range displayed limited resilience, and as they decreased in density, they approached the critical bifurcation point beyond which the meadows were unable to recover. Both flickering and slowing down are seen as leading indicators of collapse.

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