Title: Depth affects seagrass restoration success and resilience to marine heat wave disturbance

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Abstract

Accelerating losses of seagrass meadows motivate the restoration of these highly productive and beneficial ecosystems. Understanding how environmental parameters including depth and temperature affect restoration trajectories through time is key to conserving and restoring seagrass meadows. We used a long-term (12-year), landscape-scale experiment to test the effect of depth on eelgrass (*Zostera marina)* restoration success and resilience to a marine heat wave (MHW) disturbance. We found that depth was a critical determinant of seagrass restoration success, with no long-term success at sites deeper than 1.5 m below mean sea level (MSL) or shallower than -0.8 m MSL. Seeds germinated below -1.5 m MSL but shoots did not persist, confirming earlier predictions from a hydrodynamic-vegetation model. Depth was also a significant predictor of seagrass resilience following MHW disturbance. Our results suggest that areas of restored seagrass that are resilient to temperature stress exist across an intermediate depth range, excluding the shallowest and deepest portions of the full habitable depth range for restored seagrass. Over the next decades, sea-level rise will likely affect both the habitable area, and the resilient area, available for seagrass restoration. However, seagrass enhancement of sediment accretion may at least partially offset sea-level rise rates. As ocean temperatures warm and MHWs occur more frequently, the resilience of seagrass meadows to temperature stress will be of increasing concern. These results suggest that depth is a critical parameter that will help determine what areas are most resilient and therefore most suitable for conservation and restoration.

Keywords: restoration, seagrass, *Zostera marina*, marine heat wave, depth limit

Introduction

Seagrass meadows are important yet threatened coastal ecosystems; more than a third of historic seagrass meadow extent has been lost since the late 1800s, with losses accelerating through the end of the 20th century (Waycott et al. 2009). Many factors contribute to seagrass loss, including degraded water quality and impacts from coastal development (Orth et al. 2006). Restoration is a critical tool to recover lost meadow area and to reinstate the many ecosystem functions and services that seagrass meadows provide (McGlathery et al. 2012; Unsworth et al. 2018).

A recent meta-analysis of seagrass restoration emphasized the importance of careful site selection in the dynamic and stressful coastal environment, where environmental parameters limit the suitable habitat for seagrass restoration (van Katwijk et al. 2016). One essential parameter is depth, as seagrass growth is limited by light availability at the benthos. Worldwide, seagrass may exist at depths ranging from the intertidal to 90 m below sea level, depending on water clarity (Duarte 1991); the lower depth limit at a given site depends on the availability of sufficient light to support seagrass metabolism. Temperature also plays a critical role in controlling seagrass growth rates (Lee et al. 2005). As in other higher plants, seagrass photosynthesis achieves peak rates at an optimum temperature; above that temperature, photosynthetic efficiency declines rapidly. In contrast, respiration continues to increase with increasing temperature, potentially leading to a metabolic imbalance and impaired growth above a temperature threshold (Bulthuis 1987; Collier et al. 2011). Sufficiently high temperatures, and for sufficient amounts of time, can cause plant mortality (Abe et al. 2008; Hammer et al. 2018). Even short durations (1-2 h) of high temperatures have been shown to damage photosynthetic capacity and cause tissue necrosis and shoot mortality (Seddon and Cheshire 2001; Collier and

Waycott 2014). Temperature also interacts with depth, with seagrass at shallow (intertidal or near intertidal) depths experiencing warmer temperatures compared to deeper depths, leading to increased temperature stress as well as desiccation for exposed plants (Lee et al. 2007). The depth range for successful seagrass growth in a given system is thus limited by the interaction between light availability and temperature stress. Identifying a suitable depth range is an important step in successful restoration.

Long-term seagrass success also depends on the establishment of key positive feedbacks between a dense seagrass canopy and the surrounding environment (van Katwijk et al. 2016). The seagrass canopy attenuates wave energy and slows currents, leading to deposition of particulates from the water column, and the roots and rhizomes stabilize sediments, preventing resuspension (van der Heide et al. 2011; Hansen and Reidenbach 2012). These effects create greater light penetration to the benthos, supporting seagrass productivity, while also sheltering smaller shoots. These feedbacks may be critical to seagrass restoration success and to seagrass recovery trajectories following disturbance (Nyström et al. 2012). Given the highly variable nature of the coastal zone, as well as increasing stresses from climate change, it is important to understand how restored meadows persist, and how variability in environmental parameters, including depth and temperature, affect restoration success over time.

Seagrass restoration occurs against a dynamic background of climate change impacts in shallow coastal waters. As baseline ocean temperatures rise, seagrass experience increased stress when temperatures exceed their thermal tolerance (Short and Neckles 1999; Moore et al. 2012). This stress is especially important for species near the thermal limit of their geographic range (Koch et al. 2013). Marine heat waves (MHWs), defined as periods of at least 5 consecutive days when water temperatures exceed the 90th percentile according to local, long-term climatology

(Hobday et al. 2016), have increased in frequency and duration over the last century (Oliver et al. 2018), with negative effects on seagrass, as well as other coastal foundation species (Smale et al. 2019). Previous studies have not always applied the climatological definition of a MHW; however, it is clear that periods of extreme water temperatures impact seagrass ecosystems. In Shark Bay, Australia, a MHW in 2010/2011 caused widespread shoot losses, with declines of more than 90% of seagrass cover at some sites, altering the community composition and ecosystem dynamics (Thomson et al. 2015; Kendrick et al. 2019). Elevated water temperatures have also caused extensive seagrass declines in the western Mediterranean (Marbà and Duarte 2009) and in Chesapeake Bay, USA (Lefcheck et al. 2017). Simultaneously, rising sea levels can alter the light environment in shallow coastal areas through increased depth and changes in turbidity due to altered tidal range, potentially altering the suitability of seagrass habitat (Short and Neckles 1999; Pergent et al. 2015; Short et al. 2016). These climate change trends are accelerating (Pachauri et al. 2014). Successful seagrass restoration and conservation will require understanding not only of habitat requirements but also of the long-term response to sea-level rise and resilience of seagrass to biophysical perturbations, such as disturbance from MHWs (Unsworth et al. 2015).

In this study, we used a long-term, landscape-scale experiment to understand the effect of depth on seagrass restoration and resilience trajectories. The experiment took place along the mid-Atlantic coast of North America, in the Virginia coastal bays. Located between ~36-38ºN, the Virginia coastal bays are close to the southern geographical limit of 35ºN for eelgrass (*Zostera marina*) (Moore and Short 2006) and are the site of a landscape-scale (>30 km^2) eelgrass restoration project (Orth et al. 2012). We examined the direct effect of depth by monitoring the long-term (12-year) success of eelgrass restoration plots seeded along a depth gradient. In July 2012, six years into the monitoring period, unusually high temperatures caused a meadow-wide decline in seagrass shoot densities. We used this high-temperature event as a natural experiment and tracked the recovery of the seagrass over the next five years to understand the effect of depth on seagrass resilience to and recovery from high-temperature disturbance.

Methods

Site description

Eelgrass meadows were historically present in the Virginia costal bays, but the meadows were lost in the 1930s due to the effects of an infectious pathogen (*Labyrinthulae* spp.) and a hurricane in 1933 (Orth and McGlathery 2012). Eelgrass did not recover naturally in the bays due to seed limitation, and restoration via seeding was begun in 1999 (Orth et al. 2012). As of 2018, over 30 km^2 of seagrass meadow have been established through seeding (1.25 km^2 seeded from 1999-2010) and natural expansion. Much of the restoration was designed as a landscape-scale experiment, with replicated 0.4 and 0.2 hectare plots seeded across successive years. In Hog Island Bay, the site of this study, replicate plots were seeded along a depth gradient to determine the depth limit of seagrass in this system.

Hog Island Bay experiences semi-diurnal tides across a tidal range of 1.23 m. At the closest NOAA station in Wachapreague, VA (station 8631044, approximately 21 km from the Hog Island Bay meadow), mean higher high water is 0.68 m above mean sea level (MSL), mean low water is -0.65 m, and mean lower low water is -0.70 m below MSL. The rate of relative sea-level rise is high in this region, 5.37 ± 0.72 mm y^{-1} at the Wachapreague station (1978-2018, tidesandcurrents.noaa.gov). The restoration plots in Hog Island Bay were located across a depth gradient of -0.80 to -1.6 m MSL: 6 plots at depths of -0.8 to -1.0 m MSL, 21 plots at depths of -

1.0 to -1.2 m MSL, 24 plots at -1.2- to -1.5 m MSL and 7 plots at -1.5 to -1.6 m MSL. Sediments in the area of Hog Island Bay that was targeted for restoration are dominated by fine sands (80% of sediment grain size 63-250 µm, McGlathery et al. 2012); sandy sediments benefit seagrass by enhancing porewater flow and limiting the accumulation of toxic sulfides in the porewater relative to more muddy sediments (Koch 2001).

Seagrass parameters

Seagrass shoot densities were monitored at 58 restoration plots in Hog Island Bay (37.4138857ºN, 75.7225221ºW) from 2007-2018. These plots were seeded across three years (2006-2008) using a crossed design where plots were either 0.4 or 0.2 hectares in size and seeds were broadcast at densities of 50 or 100 thousand seeds per acre (Orth et al. 2012). These elements of the crossed design had no effect on plant characteristics (e.g. canopy height, blade width, shoot density) after seeding and therefore plots of the same age were pooled (McGlathery et al. 2012). Seagrass shoot densities at each plot were monitored annually beginning one year after seeding. In July of each year, a transect (50 or 25 m for the 0.4 and 0.2 hectare plots respectively) was laid along a north-south heading from the approximate center of each plot (transect locations were identified using GPS coordinates and were not permanently marked in the meadow). Shoot densities were counted by hand in ten 0.25 m^2 quadrats evenly spaced along the transect. Previous analysis of seagrass samples showed that shoot-specific aboveground and belowground biomass were consistent over the 12-year monitoring period, with a consistent ratio of ~1.8 (aboveground:belowground); restoration trajectories were therefore driven by the changes in seagrass density (McGlathery et al. 2012; McGlathery 2017).

We categorized individual restoration plots as “resilient”, “vulnerable”, or “unsuccessful” based on long-term patterns in seagrass shoot densities. Plots where seagrass densities did not exceed 10 shoots m^{-2} at 10 years after seeding were categorized as unsuccessful. At the other sites, where seagrass did establish after seeding, densities peaked in 2012 and then declined dramatically in 2013 and gradually recovered in the following years. We considered the plots to be resilient if, by 2017, the shoot densities had recovered to the 2012 peak, i.e. to the peak densities before the disturbance in July 2012. We considered plots to be vulnerable if, by 2017, the shoot density remained below the 2012 peak.

Temperature

Hourly temperature data collected at the Wachapreague, VA NOAA station were analyzed to determine whether the high temperatures in July 2012 qualified as a MHW according to Hobday et al. (2016), i.e., when water temperatures exceed the 90th percentile relative to local, long-term climatology for 5 consecutive days. Local climatology was based on an 11-day running average of the 24-year record of hourly water temperatures from 1994-2018. This long-term record showed that throughout July, the 90th percentile threshold value had a mean of 29.9ºC and a peak value of 30.1ºC occurring on July 25th. This threshold represents the most extreme temperatures that occurred in the meadow, and the climatological definition of a MHW provides a framework for comparing the ecological impacts of individual high-temperature events occurring in different ecosystems and locations. However, eelgrass can experience negative effects from high temperatures that are below the climatological threshold for MHWs (Moore and Jarvis 2008).

In laboratory experiments, *Z. marina* exhibit physiological stress at water temperatures >25ºC (Abe et al. 2008; Nejrup and Pedersen 2008; Hammer et al. 2018), and in neighboring Chesapeake Bay, episodic temperatures above 28ºC have been linked to eelgrass diebacks (Moore and Jarvis 2008; Lefcheck et al. 2017). We therefore considered 28ºC to be the appropriate stress threshold for the local population of *Z. marina.* In July 2012, we measured in situ water temperatures in the meadow using HOBO temperature loggers in order to compare exposure to high temperatures across the depth gradient. The temperature loggers were deployed at 20 restoration plots from July 16-July 31. These plots were distributed across the entire restoration area and captured the full depth gradient from -0.80 to -1.6 m MSL (Figure 1). The loggers were fixed to PVC poles at 30 cm above the sediment surface, i.e. above the seagrass canopy, and recorded water temperatures every 15 minutes. To discern whether depth provided a refuge from high temperatures, we regressed mean, maximum, and minimum temperatures at each plot against depth. Mean, maximum, and minimum temperatures were calculated over the entire 2-week period of the logger deployment. In situ water temperatures were further analyzed by binning temperatures compared to two thresholds, 28ºC (seagrass stress threshold) and 29.9ºC (mean climatological MHW threshold).

Depth

Depths of the seagrass plots relative to mean sea level were extracted from a bathymetric survey of Hog Island Bay conducted by Oertel et al. (2000). The survey used an Innerspace Technologies digital depth sounder to measure depth soundings and a GPS unit with real-time NAVbeacon corrections (estimated horizontal accuracy of 5 m) to provide locations. Depths were corrected for tidal fluctuations to be relative to mean sea level.

Statistical analysis

We modeled recovered seagrass shoot density (density in 2017, 5 years after the dieback) as a function of bathymetric depth and initial shoot density (density in 2012, before the dieback), using generalized least squares regression (GLS). We did not include a temperature component in the model, since our in-situ temperature data were limited to 15 of the seagrass monitoring sites with only 2 weeks of data over the entire 5-year recovery period. We also excluded the unsuccessful sites from the GLS model, as those sites failed before the 2012 die-off and did not show any relevant recovery patterns. Following the model selection protocol outlined by Zuur et al. (2009), we included a variance structure (constant plus power of the variance covariate) to account for increasing spread of residuals at higher initial densities, and an exponential correlation structure using latitude and longitude to account for spatial correlation between sampling plots. Starting with a full model (two fixed effects and the interaction term) we applied backward model selection. Assumptions of normality and homogeneity of residuals were validated graphically for the final model. All analyses were conducted in R v3.5.2 (R Core Team 2018).

Results

Temperature

Analysis of water temperature from the NOAA tide station located in Wachapreague, VA (21 km from the Hog Island Bay meadow) showed that water temperatures in July were above the seagrass stress threshold of 28ºC on average 44% of the time between 2007 and 2018 (Figure 2a). In July 2012, the tide station recorded water temperatures above 28ºC for 71% of the time. The high temperatures in early July 2012 did qualify as a climatological MHW, with temperatures above the 90th percentile threshold for 5 consecutive days (Figure 2b). Later in the month, temperatures at the tide station exceeded the 90th percentile threshold for 4 consecutive days and for multiple partial days. Both the MHW in early July 2012 and the elevated temperatures later in the month likely caused temperature stress and contributed to the decline in shoot densities the following year.

The in-situ temperature loggers deployed during the last two weeks of July 2012 showed that the entire meadow was exposed to elevated temperatures. During this period, all sites experienced water temperatures >28ºC for multiple days at a time, across consecutive tidal cycles. Temperatures were above the 28ºC stress threshold more than 60% of the time at all sites, across the depth gradient (Table 1); however, the shallow sites experienced the highest maximum temperatures (Figure 3). In addition to exceeding the 28ºC stress threshold in late July, all plots in the meadow achieved temperatures >29.9ºC, the average of the 90th percentile of the long-term climatology at the NOAA tide station. During the 2-week monitoring period, temperatures were >29.9ºC for 117 h on average at the shallow sites, 88 h on average at the moderate-shallow sites, 69 h on average at the moderate-deep sites, and 38 h on average at the deep sites. Thus, although all sites in the meadow experienced elevated temperatures, the deeper sites were exposed to very high temperatures for a shorter total duration of time.

Daily temperatures showed a diurnal pattern, with peak daily temperatures occurring in the afternoon. When low tides occurred in the afternoon, near maximum daily temperatures occurred for roughly an hour before and after the low tide; during afternoon high tides, near maximum temperatures occurred for shorter periods of time. Mean, maximum, and minimum temperatures recorded across the sites during the entire 2-week period were all significantly correlated with depth (Figure 4). Deeper sites had lower mean and maximum temperatures. Minimum temperature had the weakest relationship with depth, and shallower sites had lower minimum temperatures than deeper sites, likely due to stronger influence of air temperature on water temperature at the shallower sites. Overall, temperature relief at the deeper sites occurred mainly through cooler daily maximum temperatures.

Seagrass

Seagrass seeds germinated at all 58 plots to a depth of -1.6 m (Figure 5); however, seagrass success over the long-term varied by depth (Figure 6). At 19 sites, seagrass shoots did not persist over the long-term; we categorized these sites, with densities <10 shoots m^{-2} after 10 years, as “unsuccessful”. At sites where seagrass shoots did establish, shoot densities increased rapidly, following an initial ~2-year lag, to peak densities in 2012 (4-6 years after seeding) measured in mid-July, partway through the high-temperature period. After the prolonged high temperatures in July 2012, shoot densities declined dramatically across the meadow in 2013. Following the dieback, shoot densities at 23 sites recovered rapidly and equaled or surpassed the 2012 peak densities by 2017 (five years after the dieback). We identified these sites as “resilient” (mean of 274 shoots m^{-2} in 2017). At the remaining 16 sites, densities did not recover to the pre-dieback levels. We identified these sites as “vulnerable” (mean of 82 shoots m^{-2} in 2017). The vulnerable and resilient trajectories diverged approximately 4 years after the die-off (Figure 7).

Depth

There was no long-term seagrass success at plots deeper than -1.5 m MSL, indicating a light limitation below that depth for seedlings (McGlathery et al. 2012). The resilient and vulnerable plots had the same mean depth (-1.2 m MSL), but the resilient plots were clustered more tightly from -1.0 to -1.3 m MSL while the vulnerable plots occurred across the full depth gradient from -0.8 to -1.5 m MSL (Figure 8a). The unsuccessful plots occurred below -1.2 m MSL, with a mean depth of -1.5 m MSL. In this lagoon, depth was correlated with longitude, with deeper depths towards the eastern edge of the meadow. This correlation created spatial patterns of seagrass recovery in the meadow, with the unsuccessful plots clustered in the deeper east side of the meadow, the resilient plots throughout the northern and southwestern areas of the meadow, and the vulnerable plots along the edges of the resilient areas (Figure 8b).

Model results

Results of the GLS regression are shown in Table 2. Depth was significant (p=0.0057) whereas initial shoot density was not significant (p=0.19) but was kept in the model to maintain the variance structure. The interaction term was eliminated during model selection. These results suggest that depth was an important factor in determining the recovery of the seagrass shoot densities. Fitted results from the model are plotted against measured shoot densities in 2017 in Figure 9; the correlation was 0.79, indicating a moderately strong explanatory value of the model.

Discussion

Depth limitation of restored eelgrass

The seagrass recovery patterns over the long-term (10+ years after seeding) showed a clear depth limitation in this system below -1.5 m MSL. Depth limitations for seagrass restoration are site specific, depending on the water clarity (van Katwijk et al. 2009). At this site, long-term monitoring of water quality has shown that nutrient concentrations and phytoplankton abundance in the water column are low (McGlathery and Christian 2017). However, high levels of turbidity occur episodically through the resuspension of sediments by wind and waves, leading to rapid light extinction at deeper depths (Lawson et al. 2007). The failure of seagrass restoration at all sites below -1.5 m MSL suggests that light availability below this depth was insufficient to support the metabolic demands of the eelgrass (McGlathery et al. 2012).

The depth limit for restored seagrass may be shallower than the depth limit for natural meadows because of differences in plant feedbacks on sediment suspension. The seagrass canopy creates a positive feedback with light availability by attenuating wave energy and slowing currents, leading to the deposition of fine particles and greater light penetration to the benthos (Hansen and Reidenbach 2012). This feedback requires a dense canopy; sparse patches of seagrass have been shown to increase scour rather than attenuate wave energy (Lawson et al. 2012). In this restoration project, eelgrass seeds were broadcast onto the bare sediment; the initial seedlings therefore did not have the advantage of an intact seagrass canopy that could increase light availability at the benthos and stabilize sediments. Seagrass seeds did germinate at all sites across the depth gradient, but were unable to persist at the deepest sites. In contrast, in an established, dense meadow, the canopy would enhance the light availability, and seagrass shoots would be able to persist at deeper sites (van der Heide et al. 2007; Nyström et al. 2012).

Previously, Carr et al. (2012) modeled this dynamic for Hog Island Bay using a coupled vegetation-hydrodynamic model. The coupled model showed that between -1.6 and -1.8 m MSL, seagrass success depended on initial conditions. Starting from a dense canopy state, the model predicted that the meadow would stabilize and persist. Starting from a sparse canopy state, the seagrass densities would not stabilize within this depth range and would decline over time. The empirical depth limit of -1.5 m MSL, derived in our study from more than a decade of monitoring, is in general agreement, though slightly shallower than the prediction from the model. These results validate the model predictions in that seeds were able to germinate below the depth limit but the shoots did not persist over the long-term.

Vulnerability to temperature

Widespread losses of seagrass shoots after 2012 suggest the vulnerability of the restored meadow to disturbance from high temperatures. The water temperatures of the Virginia coastal bays during July 2012 were the warmest July temperatures over the 12 years of monitoring, with a climatological MHW occurring in early July and temperatures above the biological stress threshold of 28ºC throughout the month. The in-situ temperature loggers deployed in Hog Island

Bay showed that these high temperatures were sustained over at least four days in late July in all areas of the seagrass meadow, leading to continuous temperature stress for the seagrass. The direct effects of temperature stress likely caused the seagrass shoot losses, with the high temperatures increasing seagrass respiration rates disproportionately over photosynthesis (Greve et al. 2003). Temperature stress may have also caused indirect effects. Metabolic imbalance that decreases internal oxygen content of seagrass tissue enables sulfide intrusion, which played a role in rapid seagrass dieback in Florida Bay (Borum et al. 2005). Furthermore, as the die-off progressed, greater sediment resuspension following shoot losses could have affected light availability, as was the case in Shark Bay, Australia (Kendrick et al. 2019). These indirect effects were not measured, so it is not clear how much of the dieback was due to temperature stress alone. However, it is clear that the meadow was highly disturbed following the MHW in July 2012.

Declines in seagrass shoot density are likely to become more common as MHWs increase in frequency throughout the world oceans (Smale et al. 2019). In the mid-Atlantic region, average water temperatures are predicted to increase by 2-6ºC by 2100, and MHWs are predicted to occur more frequently (Najjar et al. 2010; Oliver et al. 2018). Eelgrass in the Virginia coastal bays will be particularly sensitive to these changes, given that summer temperatures already routinely exceed the 28ºC stress threshold for hours to days at a time during July and August. In the Hog Island Bay meadow, shoot densities at the resilient plots did not recover to pre-dieback levels until 5 years after the 2012 MHW disturbance, and at the vulnerable plots, densities remained below pre-dieback levels through 2018. Densities also declined at some plots in 2018 relative to 2017 (see Figure 7). July 2017 had the second-most hours above 28ºC during the monitoring period (see Figure 2a), but additional analysis has shown that these high temperatures did not qualify as a MHW, as the high temperatures were not sustained above the 90th percentile threshold over 5 days (P. Wiberg, unpublished data). Nevertheless, those sporadic high temperatures may have caused the moderate declines in density observed in 2018.

In the event of future MHWs, warmer background water temperatures are likely to inhibit rapid recovery and restrict the areas where seagrass restoration can succeed. Previous modeling work in this system predicted that increased frequency of high-temperature events would lead to significant summer die-backs and eventual meadow collapse at sites deeper than -1.6 m MSL (Carr et al. 2012). Our empirical dataset suggests that disturbance from high temperatures will lead to losses at shallow depths as well; sites shallower than -1.0 m MSL experienced drastic shoot losses and limited recovery (Figure 6). Loss of eelgrass from the Virginia coastal bays due to temperature stress might offer opportunities for colonization by more heat-tolerant species, such as *Halodule* spp. However, there is not yet any evidence of non-*Zostera* seagrass present in the Virginia coastal bays. For now, the landscape-scale eelgrass restoration remains vulnerable to disturbance from MHWs.

Shift in depth limit following disturbance

The resilient and vulnerable plots showed different distributions across the depth gradient, highlighting the importance of depth in determining recovery trajectories (Figure 8a). The depth range of the resilient plots suggests a contraction of the lower depth limit following disturbance; 19 out of 23 resilient plots occurred above -1.3 m MSL. The four deepest resilient plots, with depths from -1.35 to -1.40 m MSL, were technically resilient (i.e., shoot densities had recovered within 5 years of the dieback); however, these deeper sites had average densities of only 128 shoots m^{-2} in 2017 compared to an average of 305 shoots m^{-2} at the 19 resilient plots above -1.3 m MSL. These deeper sites also had lower shoot specific biomass in 2017, 0.11 g shoot^{-1} compared to 0.21 g shoot^{-1} at the other resilient plots (McGlathery 2017). The plots between -1.0 and -1.3 m MSL clearly recovered to a more robust meadow state, suggesting that resilient plots had a shallower lower depth limit compared to all restored plots in the meadow. Resilient plots also appear to have a deeper upper depth limit compared to the total meadow. Five out of the 6 shallow plots between -0.8 and -1.0 m MSL were vulnerable (i.e. shoot densities did not recover within five years); the sixth shallow site had a depth of -0.98 m MSL and was resilient.

This contraction of the resilient depth range (-1.0 to -1.3 m MSL) compared to the habitable depth range (-0.8 to -1.5 m MSL) can be explained by interactions between depth and temperature. At the deeper sites, high temperatures would increase the light compensation point, as increased respiration would require greater rates of photosynthesis, thus causing seagrass growing below -1.3 m MSL to become light limited (Lee et al. 2007). Although we know that light extinction occurs rapidly with depth in these bays (Lawson et al. 2007), and we observed a clear depth limitation prior to the MHW disturbance, we do not have light data from across the depth gradient during the recovery period to demonstrate this expected interaction between depth and temperature at the deeper sites. At the shallow sites, seagrass would experience the highest maximum temperatures, leading to extreme metabolic imbalance. The in situ temperature data show partial evidence of greater temperature stress at shallow sites. Although mean temperatures during the two-week monitoring period were similar (within 0.5 ºC) across the entire depth gradient, the shallow sites experienced higher maximum temperatures, on average 34.4ºC compared to 31.5ºC at the deep site. These maximum temperatures occurred during afternoon low tides, when the shallowest sites have water levels of only 10 cm and seagrass blades can be partially exposed (pers. obs.), possibly leading to desiccation. Short term (2.5 h) spikes in temperature have been shown to cause tissue necrosis and shoot mortality in laboratory experiments (Collier and Waycott 2014); here, the peak temperatures occurred during days when the eelgrass had no relief from stressful temperatures >28ºC. Thus, the combination of high maximum temperatures and possible desiccation during the approximately 2 hours of the low tide could potentially explain the greater vulnerability of the shallowest sites.

While these depth and temperature interactions likely played a role in the recovery patterns, it is also clear that other factors were involved, as some vulnerable plots occurred at comparable depths to resilient plots. Furthermore, the in situ temperature data were limited to the period of the disturbance, i.e. when high temperatures caused the dieback. The recovery occurred over the next 5 years, and the interactive effects of depth and temperature on seagrass growth over that time period were certainly more complex than presented here. Importantly, the resilient and vulnerable plots showed similar declines after the MHW disturbance, and the recovery trajectories did not diverge until 4 years into the recovery period (Figure 7). What these trajectories clearly show is that although all sites were exposed to high temperatures during the MHW disturbance, the sites that recovered best fell within a narrow range of depths.

Predicting seagrass recovery

The model results show that depth was an important factor but not the only predictor of recovery after the MHW disturbance. Using a simple model based on depth and initial density, the predicted recovered densities were moderately correlated with the observed recovered densities (Figure 9). However, the model under-predicted the recovered densities at resilient sites with very high recovery (>400 shoots m^{-2}). This result is likely due to spatial patterns in the seagrass recovery that were not captured by the simple model. As shown in Figure 8b, the resilient plots generally clustered in two sub-regions of the sampling area, the northern and the southwestern regions. The resilient plots in the northern region included all 8 plots with recovered densities >400 shoots m^{-2}, i.e. the plots where recovery was underestimated by the model. These 8 plots in the northern region encompassed the full resilient depth range, from -1.0 to -1.3 m MSL, whereas the resilient plots in the southwestern region covered a narrower depth range (-1.04 to -1.18 m MSL) and had lower recovered shoot densities (150 shoots m^{-2}). In other words, the seagrass recovery was more successful across a greater range of depths in the northern sampling region compared to the southwestern sampling region. Differences in environmental parameters besides depth likely affected this spatial pattern. For example, the two regions are separated by an intertidal sandbar, which may subtly affect hydrodynamic variables such as flow rates and residence time in each region. Seagrass expansion in the southwestern region is also limited by the presence of a deep channel (Machipongo channel) near the meadow border whereas in the northern region, the meadow has continued to expand. Given the importance of large meadow area for establishing positive feedbacks, the larger meadow area in the northern region may have helped to support nearby recovery. Additional measurements of environmental parameters are needed to understand which variables besides depth and temperature affected the spatial patterns in seagrass recovery.

In the model, pre-dieback density was not a significant predictor of recovered density. This result suggests that plot-specific restoration success was not necessarily a good indicator for resilience to MHW disturbance. The difference in recovery between the resilient and vulnerable plots suggests that the full range of environmental conditions, including depth, residence time, and sediment grain size, that define the habitable niche for eelgrass may not support recovery of eelgrass following high levels of shoot losses. For future restoration projects, optimizing long-term seagrass success will require consideration of a subset of environmental conditions that maximize seagrass resilience within the habitable range.

Resilient vs habitable depth range for seagrass

In this seagrass meadow, we observed a contraction of the habitable depth range to a narrower resilient depth range following MHW disturbance. This result has implications for restoration planning. The difference between the habitable depth limit of -1.5 m MSL and the resilient depth limit of -1.3 m MSL is small in absolute terms but it indicates a steep environmental gradient that strongly affects long-term success of seagrass restoration at the landscape scale. One implication of this gradient is that relatively flat areas within the habitable depth range may be more suitable for seagrass restoration than areas with steeper slopes or variable bathymetry. Furthermore, in shallow coastal bays, the difference in the habitable and resilient depth limit may exclude large areas of seafloor from restoration. Depth is not the only factor to determine habitat suitability, but our data show that depth limits affect both the initial success of seagrass restoration and the recovery following disturbance.

Sea-level rise is likely to affect the spatial distribution of both the habitable and resilient areas in this system over the coming decades. The mid-Atlantic coast of the United States is a hotspot for relative sea level rise (Sallenger et al. 2012), and the local rate of 5.37 mm y^{-1} is among the highest along the Atlantic Coast. Over the 12-year monitoring period, sea-level rise may have increased bathymetric depth in the seagrass meadow by 6.5 cm; by 2050, an additional 16.6 cm of sea-level rise could shift sites currently in the resilient depth range of -1.0 to -1.3 m MSL to the more vulnerable -1.2 to -1.5 m MSL. In that case, we might expect much of the meadow to become more sensitive to effects of temperature stress. With rapid rates of sea-level rise, we might also expect migration of the meadow area to maintain the habitable depth range

(Short et al. 2016). This suggests that meadows situated on gently sloping flats with room for seagrass to migrate, and without barriers to migration, are likely to have greater potential for long-term success.

Predicting long-term seagrass success is complicated by the many interacting factors affecting the persistence of the meadow. For example, concurrently with sea-level rise, the seagrass canopy increases sedimentation of fine particles, leading to enhanced accretion rates that may be sufficient to balance out sea-level rise. We have not measured accretion in the Hog Island Bay meadow, but in a neighboring eelgrass meadow that was restored as part of the landscape-scale restoration project in the Virginia bays, surface accretion rates 10 years after restoration were approximately 6.0 mm y^{-1} (Greiner et al. 2013; Oreska et al. 2017). Accretion rates in Hog Island Bay are likely lower, due to the low shoot densities between 2012-2017. However, in the fully recovered meadow, the seagrass-enhanced accretion may be sufficient to ameliorate the direct effects of sea-level rise on depth in the near future. Long-term accretion rates (i.e. over century timescales) will likely be lower, due to the compaction of deep sediments (Santos et al. 2012), such that continued and accelerating and sea-level rise may eventually overwhelm any seagrass effect on sediment accretion.

In addition to sea-level rise and MHWs, seagrass meadows continue to face pressure from eutrophication and other anthropogenic stressors (Valle et al. 2014; Lefcheck et al. 2017). Managing seagrass resilience in the face of these interacting stressors remains challenging. The results of this long-term, landscape-scale experiment can be used to maximize resilience to MHW disturbance. The particular depth limits observed here are likely site-specific, but the finding that plots within the middle of the depth range are most resilient can be applied across sites. Through consideration of depth, among other factors such as water residence time, managers can identify areas more likely to be resilient to MHW disturbance, helping to maximize the benefits of restoration effort.

Acknowledgements

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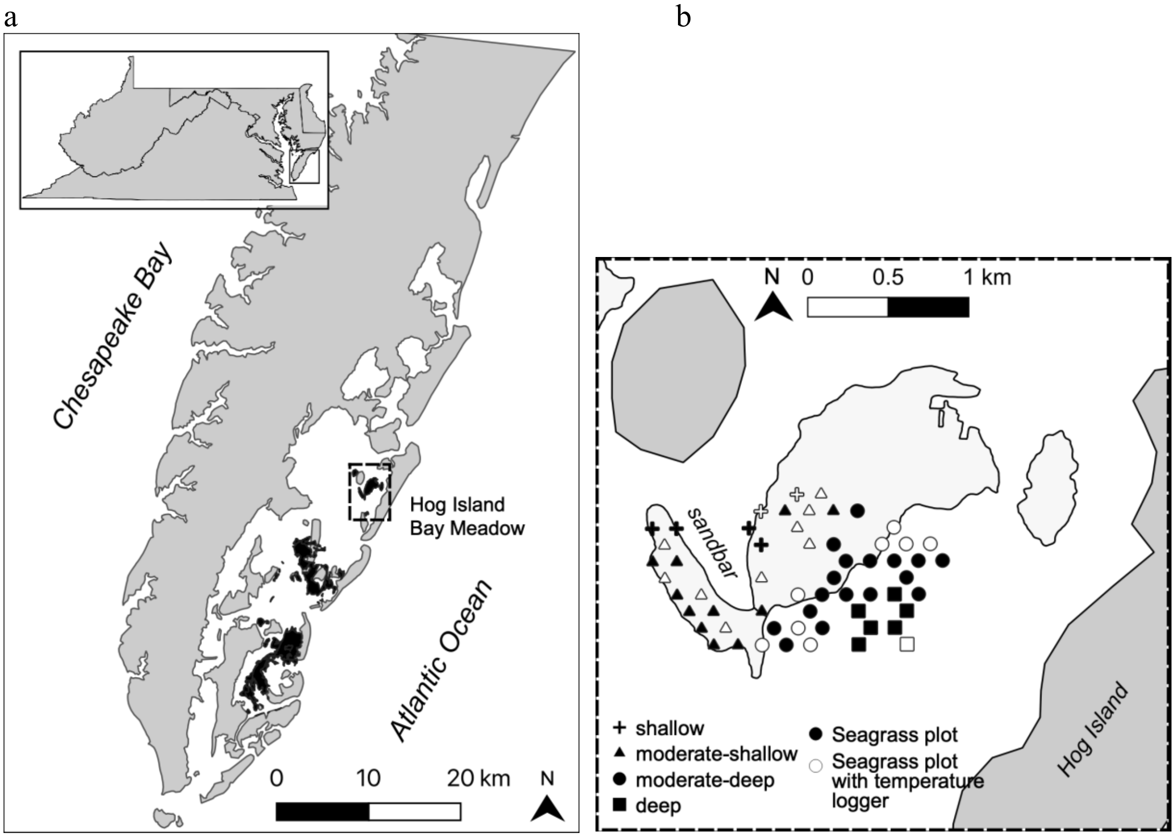

Figure 1. (a) The full extent of the seagrass restoration in the Virginia coastal bays as of 2017, with the restored seagrass area in black (areal extent supplied by the VIMS SAV survey, http://web.vims.edu/bio/sav) (b) The Hog Island Bay meadow and individual plots monitored in this study, with the seagrass area in 2017 in light gray. Symbol shapes show the depth gradient of restoration plots (shallow sites were 0.8-1.0 m below MSL, moderate-shallow sites were 1.0-1.2 m below MSL, moderate-deep sites were 1.2-1.5 m below MSL, and deep sites were 1.5-1.6 m below MSL). Open symbols represent seagrass plots where HOBO temperature loggers were deployed during July 2012.

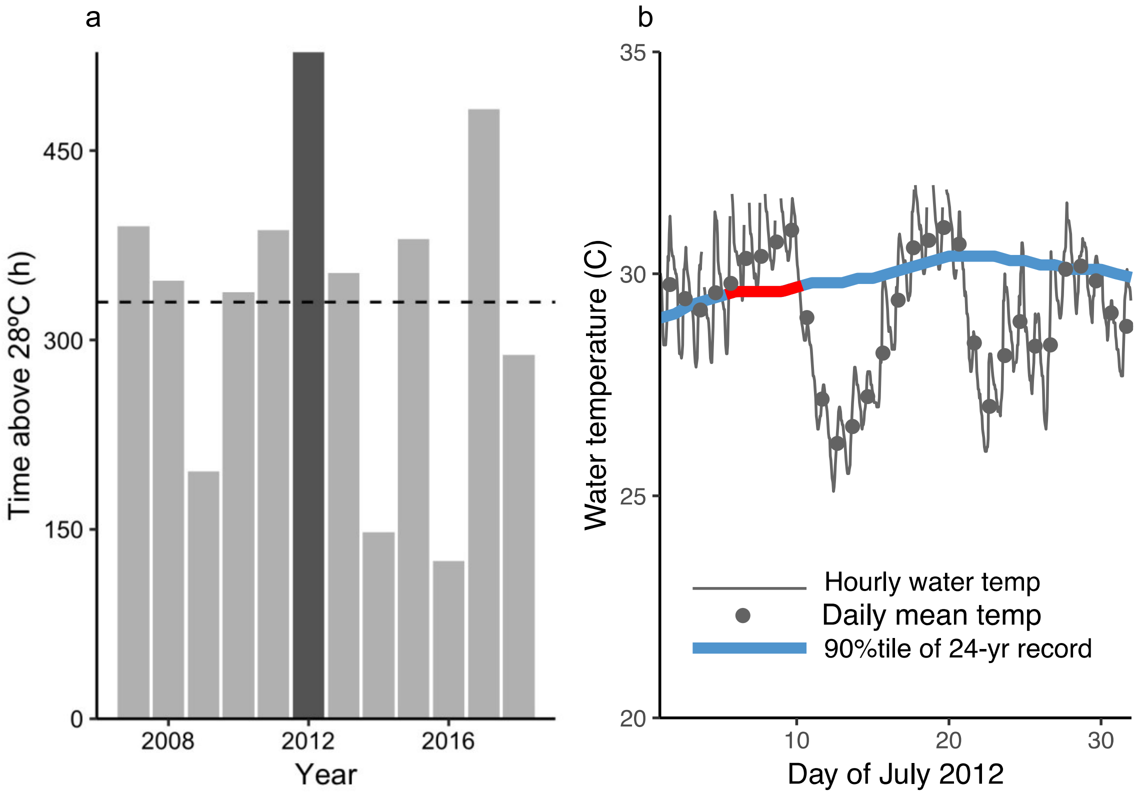

Figure 2. (a) July water temperatures measured at the NOAA tide station in Wachapreague, VA, show that during July 2012 (highlighted in dark grey), more hours were spent above 28ºC than in July of any other year during the 12-year monitoring period (although temperatures were also high in July 2017). The dashed line shows the 12-year average (330 hours above 28 ºC). (b) Sustained high water temperatures in early July 2012 qualified as a marine heatwave (MHW), when temperatures exceeded the 90th percentile relative to local, long-term climatology for at least 5 consecutive days (red shading). Temperatures later in July also exceeded the 90th percentile for shorter periods of time.

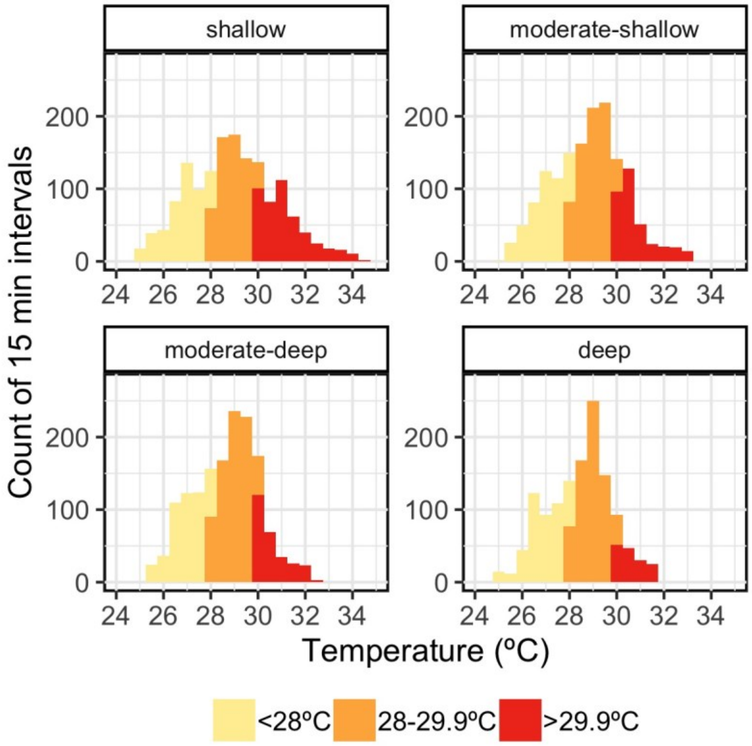

Figure 3. The distributions of water temperatures monitored at 20 sites in Hog Island Bay during July 2012 showed that all areas of the meadow experienced temperatures above 28ºC (seagrass stress threshold) and above 29.9ºC (mean 90th percentile threshold for MHW). The shallower sites experienced higher peak temperatures more often than deeper sites. Each histogram shows the average distribution recorded by temperature loggers deployed at each depth interval: shallow (0.8-1.0 m below MSL, n=2), moderate-shallow (1.0-1.2 m below MSL, n=9), moderate-deep (1.2-1.5 m below MSL, n=8), deep (1.5-1.6 m below MSL, n=1).

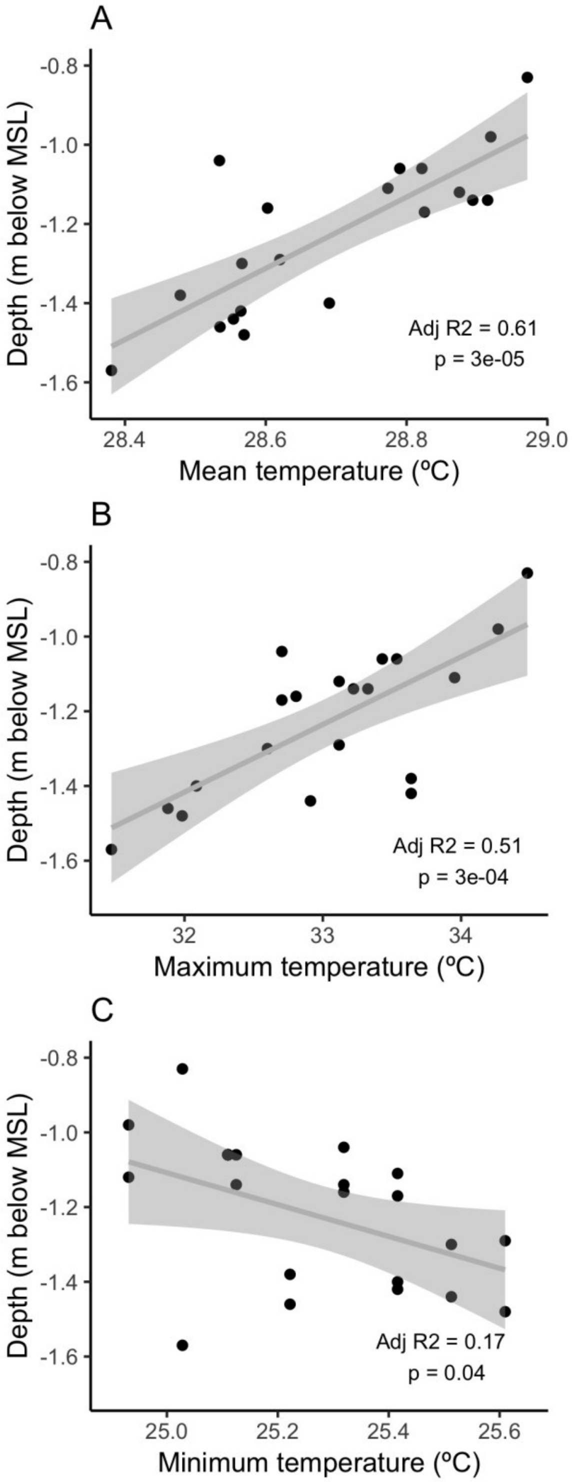

Figure 4. Temperature values from the 20 monitoring sites during July 2012 showed significant relationships with depth; deeper sites had lower mean and maximum temperatures (A and B). Minimum temperatures showed the inverse pattern with depth (C) and had the weakest relationship with depth. Shaded areas indicate 95% confidence intervals around the linear regression.

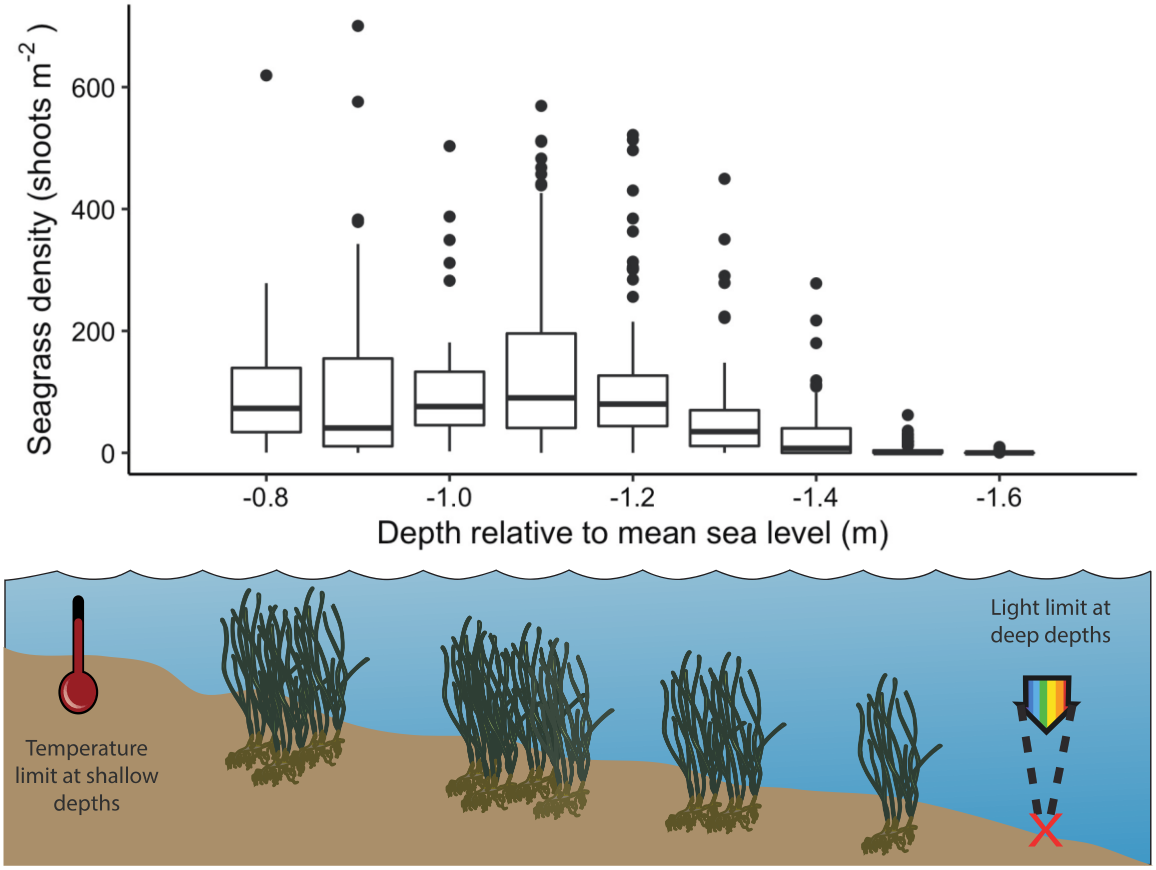

Figure 5. Boxplots show the distribution of seagrass shoot densities across the depth gradient of -0.8 to -1.6 m MSL over the 12-year restoration period (including during the die-off and recovery from 2012-2017). Eelgrass seeds germinated at all depths but shoot densities were limited below -1.4 m MSL and did not persist below -1.5 m MSL. The lower panel shows a conceptual model of the seagrass depth distribution; images courtesy of the Integration and Application Network (https://ian.umces.edu/)

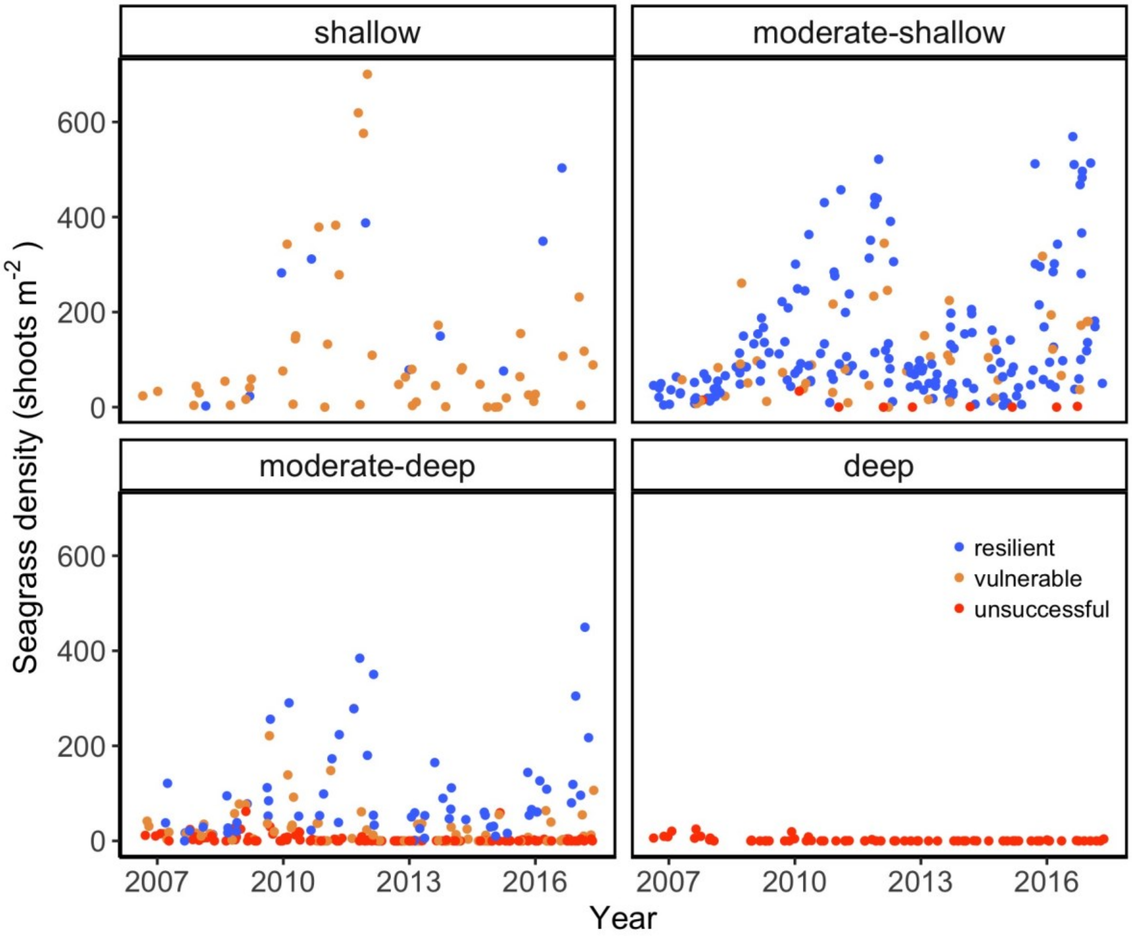

Figure 6. The long-term record of shoot densities showed that plots at moderate depths were more successful than shallow and deep plots. Unsuccessful plots did not establish seagrass, resilient plots established seagrass by 2012 and recovered to 2012 densities by 2017, and vulnerable plots established seagrass by 2012 but did not recover by 2017. Shallow sites were 0.8-1.0 m below MSL, moderate-shallow sites were 1.0-1.2 m below MSL, moderate-deep sites were 1.2-1.5 m below MSL, and deep sites were 1.5-1.6 m below MSL. Each dot represents the mean density across 10 replicate quadrats at an individual plot; points are jittered for improved visualization.

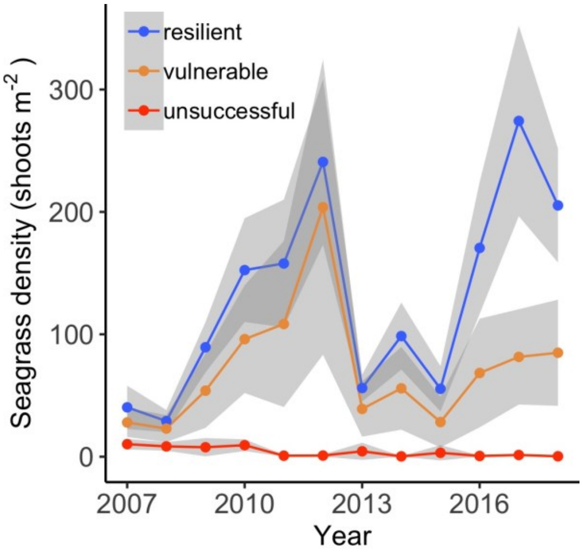

Figure 7. The resilient and vulnerable plots followed similar trajectories from seeding through the peak densities in 2012 and the decline after the 2012 MHW but diverged 4 years after the MHW disturbance. Grey shading indicates the area of +/- 2 x standard error for the plots in each trajectory.

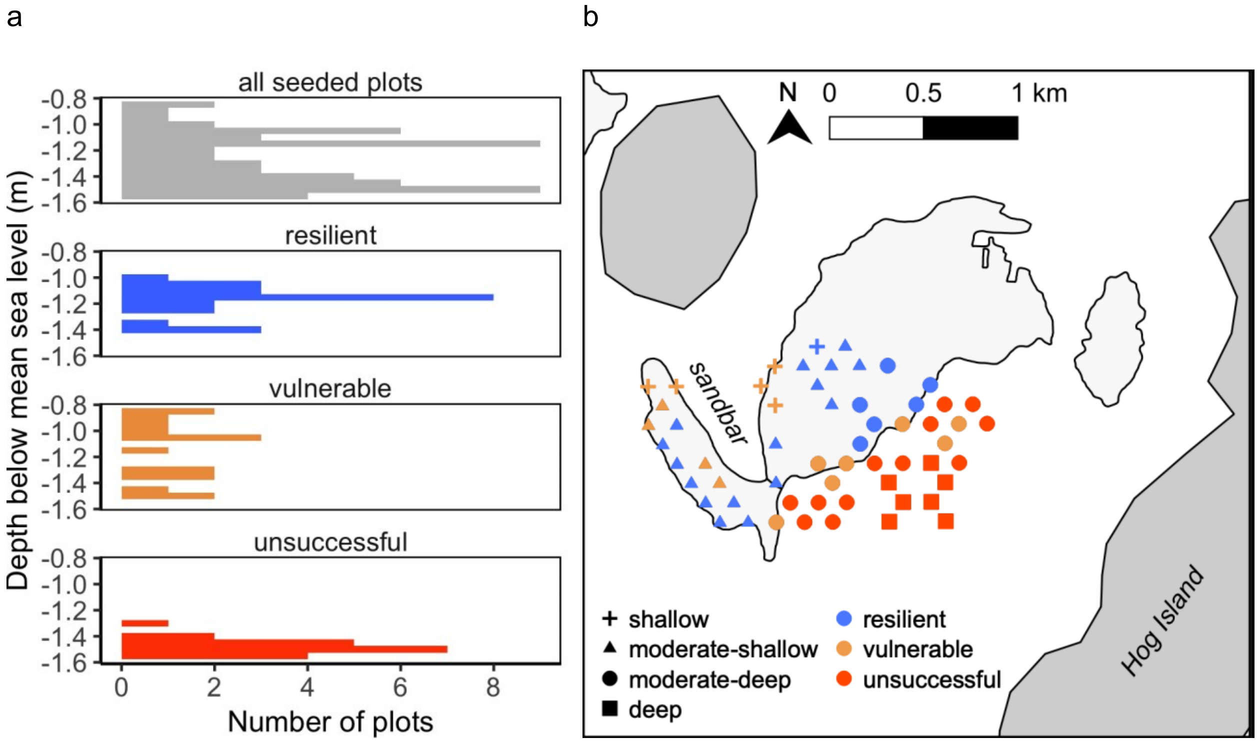

Figure 8. (a) Unsuccessful plots occurred primarily at deep depths, resilient plots occurred at moderate depths, and vulnerable plots occurred across the depth gradient. (b) Spatial patterns in bathymetry (deeper sites toward the eastern edge of the meadow) created spatial patterns in seagrass resilience. Meadow area in 2017 is shown in light grey. Resilient plots (in blue) were clustered in the northern and southwestern portions of the monitoring area, unsuccessful plots (in red) were clustered to the southeast, and vulnerable plots (in orange) were located along the edges of the resilient areas. Depth of each plot is indicated by the shape of the symbol; shallow sites were 0.8-1.0 m below MSL, moderate-shallow sites were 1.0-1.2 m below MSL, moderate-deep sites were 1.2-1.4 m below MSL, and deep sites were 1.4-1.6 m below MSL.

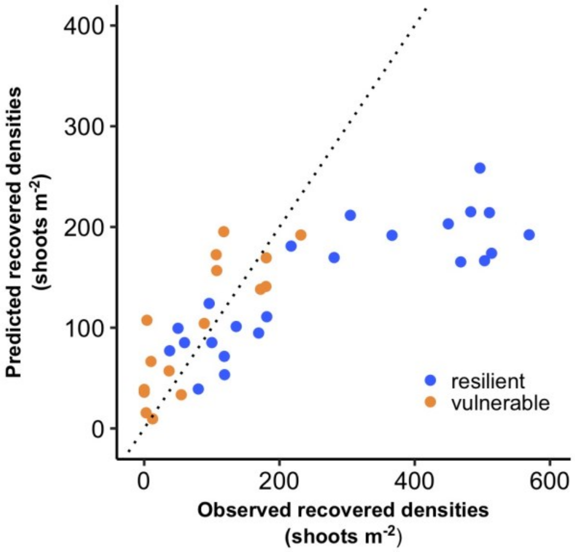

Figure 9. Shoot densities in 2017 predicted by the GLS model plotted against the observed densities in 2017 had a correlation coefficient of 0.79, indicating moderate explanatory power of the model. The dotted line shows the 1:1 line.

Table 1. Proportion of time spent at and below the seagrass stress threshold (28ºC) and the mean MHW threshold (29.9ºC)

Depth interval (m below MSL)	Proportion of time spent <28ºC	Proportion of time spent 28-29.9ºC	Proportion of time spent >29.9ºC
-0.8 to -1.0	0.31	0.39	0.31
-1.0 to -1.2	0.30	0.47	0.23
-1.2 to -1.5	0.31	0.51	0.18
-1.5 to -1.6	0.35	0.53	0.12

Table 2. In the final GLS model, only depth had a significant effect on recovered shoot densities.

Fixed effects	Estimate	Standard error	t-value	p-value
Depth	177.32	60.30	2.9408	0.0057
Initial density	0.33	0.25	1.3117	0.1979