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Impacts of eelgrass (*Zostera marina*) on pore-water sulfide concentrations in
intertidal sediments of Casco Bay, Maine

An Honors Paper for the Department of Biology

By Sabine Berzins

Bowdoin College, 2016

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Abstract

Eelgrass (*Zostera marina*) is a perennial seagrass that provides many vital ecosystem services including stabilizing sediments, maintaining water clarity, and providing complex habitat in the intertidal and shallow subtidal coastline. Historically, Maine supported dense eelgrass beds in shallow waters surrounding islands and along the coastal mainland. However, in 2012, high population densities of European green crabs (*Carcinus maenas*), which physically disturb and remove eelgrass as they forage, were correlated with widespread eelgrass declines. Over 55% of the area of eelgrass in Casco Bay was lost, mainly between 2012 and 2014. Eelgrass typically grows in low-oxygen sediments that produce a chemically reducing environment. Sulfate-reducing bacteria in these reduced sediments produce hydrogen sulfide, a toxin that can intrude into eelgrass tissues and impair the plants' ability to photosynthesize. When eelgrass is not present, sulfide can build up in the pore-water. When eelgrass is present, it can oxygenate the sediments through its roots, thereby preventing the intrusion and buildup of toxic hydrogen sulfide. However, if the substrate is de-vegetated, oxygen levels drop as sedimentary organic matter is decomposed, and the accumulation of sulfides to harmful concentrations in the pore-water may make recolonization of eelgrass difficult or perhaps impossible even in the absence of green crabs. In an effort to monitor characteristics of Casco Bay eelgrass beds and determine spatially where eelgrass may be more likely to recover, four Casco Bay sites with varying degrees of vegetation loss were sampled in 2015 for pore-water sulfide concentration, sediment carbon and nitrogen content, and sediment grain size analysis. Measurements of sulfide concentrations showed correlations with the timing of eelgrass loss, such that vegetated sites had low pore-water

sulfide concentrations and sites that had been de-vegetated for longer periods of time had high sulfide concentrations. Carbon and nitrogen content in the sediment was higher at de-vegetated sites, likely due to a higher percentage of finer sediments at those locations. Coarser sediments were more highly vegetated than finer sediments, perhaps displaying a preference of green crabs to forage in finer sediments. Catastrophic loss of eelgrass in Casco Bay has likely led to differences in sulfide levels, carbon and nitrogen content in the sediment, and grain size distribution, depending on degree of vegetation. Eelgrass restoration in Casco Bay will likely be limited by high pore-water sulfide concentrations.

Introduction

Eelgrass (*Zostera marina* L., family Zosteraceae) is a perennial seagrass that is widely distributed along the intertidal and shallow subtidal coastline of the Atlantic United States and Canada (Short and Short 2003). This monoecious flowering plant species with vegetative rhizomes and sexually reproductive stems forms extensive meadows that comprise highly productive coastal ecosystems (Duarte 2002, Moore and Short 2006). A keystone species, eelgrass is a good bioindicator of overall ecosystem health that maintains ecosystem function by stabilizing sediments, preventing shoreline erosion, absorbing nutrients, and providing complex habitat for a wide variety of animals including commercially important species such as soft shell clams and blue mussels (Fonseca and Uhrin 2009, Malyshev and Quijón 2011, Moore and Short 2006, Orth *et al.* 2006, Short and Short 2003). Loss of eelgrass can therefore lead to degraded water quality, increased turbidity, reduced ability to mitigate coastal acidification, shoreline erosion, and reduced fish and wildlife populations (Duarte 2002, Hendriks *et al.* 2014, Lazzari 2013, Waycott *et al.* 2009).

In New England, eelgrass is the dominant form of submerged aquatic vegetation and usually grows as a monoculture (Short and Short 2003). In particular, the Maine coast historically supported extensive eelgrass beds, notably in Casco Bay, a southern inlet of the Gulf of Maine and the state's second largest embayment. Mapping in 2001 and 2002 showed 3338 ha of eelgrass in Casco Bay (Casco Bay Estuary Partnership 2010). However, between 2012 and 2013, eelgrass distribution in Casco Bay declined in area by over 55%. Aerial photographs taken in 2013 revealed that only 1477 ha of eelgrass in Casco Bay remained (Maine Department of Marine Resources 2013), with

nearly complete de-vegetation of the upper Bay. This decline in eelgrass distribution coincides with a regional population explosion of European green crabs (*Carcinus maenas*), an invasive species that has been implicated in eelgrass loss as the crabs physically disturb eelgrass while foraging for prey (Garbary *et al.* 2014, Neckles 2015). The species first arrived in Maine in 1905 in ballast water (Bravo *et al.* 2007); its range now includes much of the Atlantic coast from Virginia to Maritime Canada (Leignel *et al.* 2014). Small (<10cm) shore crabs which inhabit the intertidal and subtidal zones, green crabs are formidable invaders: they can survive without food for three months, live out of water for ten days, and tolerate a wide range of temperatures and salinities (Tepolt and Somero 2014). They are highly fecund. A two-inch long female lays as many as 165,000 eggs per reproductive season (Leignel *et al.* 2014). Mud, sand, rock, and eelgrass beds make ideal habitats. Green crabs have a diverse diet; blue mussels (*Mytilus edulis*) and soft-shell clams (*Mya arenaria*) are preferred foods (Davis *et al.* 1998). Juvenile green crabs utilize eelgrass beds as nursery habitat (Malyshev and Quijón 2011). Adult and juvenile crabs are capable of ripping up vast quantities of eelgrass as they forage for benthic prey, and juveniles may cut off eelgrass shoots as they graze on the meristems (Malyshev and Quijón 2011).

High population densities of green crabs have been shown to cause dramatic loss of eelgrass from Benoit Cove in Nova Scotia, Canada (Garbary *et al.* 2014). Previous population explosions of green crabs were correlated with periods of warm sea-surface temperatures, which led to declines in soft-shell clam populations (Welch 1969). Cold winters following these periods of high population density led to population declines of green crabs. Exceptionally warm waters in Casco Bay in 2012 similarly brought

increased abundance in green crab numbers (Beal 2014, Maine Department of Marine Resources 2009). Exclosure experiments performed in Casco Bay in 2013 demonstrated that eelgrass transplanted into exclosures preventing access of green crabs in formerly vegetated areas of Casco Bay increased survivorship 400% compared to transplanted shoots planted outside the exclosures (unprotected from crab disturbance). Because all transplanted shoots grew at approximately the same rate, it is likely that green crab disturbance was the cause of the difference in survival rather than an unknown environmental factor (Neckles 2015).

While bioturbation, or physical disturbance and reworking of the sediment by green crabs, has been heavily implicated in eelgrass loss in Casco Bay, there are many other factors which impact eelgrass distribution. Fall grazing by migratory waterfowl such as Canada geese (*Branta canadensis*) has the ability to significantly reduce plant biomass, though this effect is usually seen only in the short term and beds recover during the next growing season (Rivers and Short 2007, Seymour *et al.* 2002). Wasting disease, caused by *Labyrinthula zosterae*, a pathogenic slime mold that is visible in dark necrotic lesions on eelgrass leaves and is transferred by contact, was responsible for a large-scale die-off of eelgrass in the 1930s all along the east coast (Short *et al.* 1987). Beds had rebounded by the 1940s, and the presence of wasting disease in Maine today, while a concern for managers hoping to transplant eelgrass shoots, is not significant (Neckles 2015). Additional factors include human disturbance, light attenuation, temperature, salinity, substrate type, physical disturbance by wave action, nutrient availability, disease, and pore-water sulfide concentration (Duarte 2002, Holmer *et al.* 2005, Moore *et al.* 2011, Neckles *et al.* 2005, Orth *et al.* 2006). This study focuses on pore-water sulfide, a

factor that varies with degree of vegetation, substrate type, and organic matter content. Pore-water sulfides can be toxic to plant life and prevent establishment of eelgrass and other aquatic plant species.

Eelgrass grows in highly reduced sediments, where microbes break down organic matter in often anoxic conditions (Goodman *et al.* 1995, Terrados *et al.* 1999). Near the surface of the sediment, oxygen is readily available for bacteria to use as an electron acceptor during respiration. However, just below the surface of the sediment, oxygen is quickly depleted and bacteria must use alternate, albeit less energetically favorable, electron acceptors as oxidizing agents as they decompose organic matter (Jørgensen 1977). Sulfate is one such oxidizing agent. When sulfate-reducing sediment bacteria (e.g. *Desulfovibrio*) use sulfate as their primary electron acceptor, free sulfides (H_2S and HS^-) are produced due to the presence of reduced iron sulfides, FeS and FeS_2 (Jørgensen 1977). While sulfur is one of the six macronutrients necessary for plant growth, an excess of sulfur as H_2S due to oxygen-limited conditions is problematic to aquatic vegetation as it is a toxin (Lamers *et al.* 2013). Free sulfides can invade the plant through the roots and accumulate in roots and tissues (Frederiksen *et al.* 2006, Holmer *et al.* 2005). Experimentally raising sulfide levels has demonstrated that the maximum rate of photosynthesis was lowered and more light intensity was required for eelgrass plants to reach the point where gross photosynthesis equals respiration (Goodman *et al.* 1995, Holmer and Bondgaard 2001). Treating eelgrass with high levels of sulfide and low levels of oxygen decreased the rate of photosynthesis and reduced rates of shoot elongation and decreased above-ground biomass (Holmer and Bondgaard 2001). High concentrations of sediment sulfide have been shown to cause photosystem and tissue

degeneration that is not recoverable (Dooley *et al.* 2013, Korhonen *et al.* 2012). Inhibition of cytochrome oxidase has also been recorded (Raven and Scrimgeour 1997). Adult plants and seedlings are vulnerable to high levels of sulfide, with concentrations greater than 680 μM proving fatal to seedlings and depressing the rate of photosynthesis in adult plants (Dooley *et al.* 2013). Eelgrass re-vegetates naturally through survival of seedlings and germination of new seedlings to form patches, which expand laterally (Greve *et al.* 2005). High levels of sediment sulfide can therefore damage existing eelgrass beds and effectively prevent natural recruitment of new patches.

Eelgrass plants have the ability to transport oxygen produced from photosynthesis during the day down into the roots at night, oxidizing the sediments and preventing sulfide accumulation (Holmer and Bondgaard 2001, Holmer *et al.* 2005). However, if oxygen becomes limited and the photosynthetic capacity of eelgrass is reduced, sulfide can invade the tissues and kill the plants (Korhonen *et al.* 2012). When eelgrass is lost, the sediment is rendered completely bare as eelgrass is usually the only form of submerged aquatic vegetation growing in the intertidal/shallow subtidal zone (Short and Short 2003). This phenomenon describes an alternative stable state (Scheffer *et al.* 2001), where the structure and function of the ecosystem is profoundly altered with little chance of returning to its previous, often more desirable, condition. Dead shoots and senescent biomass increase the organic matter content of sediment, while bacteria increase their production of H_2S as a byproduct of decomposing that organic matter. Additionally, iron in the sediment is not reactive with H_2S , allowing H_2S concentrations to build up in the pore-water (Jørgensen 1977). H_2S then accumulates to toxic levels such that eelgrass can no longer survive in previously vegetated areas (Jeremy *et al.*

2001). De-vegetated substrates are chemically stable, as there is no oxygenation of the sediments by eelgrass to remove the free sulfides, so eelgrass is unlikely to recolonize. Conversely, vegetated substrates are also chemically stable as they promote the oxygenation of sediments and healthy eelgrass growth. A catastrophic shift in the ecosystem's state, however, such as decimation by European green crabs, can force the system into the alternative, i.e., de-vegetated, state (Jeremy *et al.* 2001). Even in eelgrass beds that have not been lost, eelgrass plants that have experienced damage to their photosystems are also unlikely to recover to their prior rates of photosynthesis (Korhonen *et al.* 2012, Pulido and Borum 2010). These permanently damaged beds are more vulnerable to disturbance, making them more likely to shift over to the less desirable de-vegetated state should a disturbance occur. Following de-vegetation events, it may be difficult for eelgrass to return to its original abundance.

The sedimentary environment can also have effects on both sulfide production and eelgrass growth. The sulfur cycle is the main process by which organic matter is degraded (mineralized) in coastal environments, and organic carbon is worked into the sediment organic matter is mineralized (Jorgensen *et al.* 1990, Jørgensen 1982). Particulate organic matter is deposited mainly in shallow water, and is found in the sediment at 10,000-100,000 times the concentrations found in sea water (Jørgensen 1983). Derived mainly from primary production by phytoplankton in surface waters, this organic matter eventually settles on coastal shelves and is broken down by sulfur-reducing benthic microorganisms (Jørgensen 1977). As highly productive coastal habitats, eelgrass beds also provide a source of organic matter. C:N ratios tend to increase with increasing sediment depth as rates of organic carbon burial are higher than

for nitrogen, and nitrogen tends to mineralize higher in the water column than carbon (Jørgensen 1983). Carbon and nitrogen content in the sediment tend to correlate with each other as they are both bound up as organic compounds in organic matter, which is then mineralized. The presence of HS^- , which is in equilibrium with H_2S , can reduce rates of nitrification (Joye and Hollibaugh 1995). Grain size also can impact sediment composition, as finer-grained sediments can hold more organic carbon and have more surface area available for ion exchange (Horowitz and Elrick 1987). Varying inputs of organic matter could alter carbon content, nitrogen content, and sulfide production in the sediments.

This study examines pore-water sulfide concentrations in Casco Bay, which offers a broad geographic range in eelgrass habitat with varying degrees of vegetation and de-vegetation. Eelgrass was nearly lost from the upper reaches of the Bay between 2012 and 2013, but the lower Bay maintained patches that have been historically vegetated (see Fig. 1). As part of a larger-scale feasibility test conducted by the Ad hoc Casco Bay Eelgrass Consortium (CBEC) for restoration of eelgrass in Casco Bay in summer 2015, sediment sulfide analysis in regions where eelgrass was or was not lost will aid in identifying sites with appropriate sediment conditions for eelgrass restoration. This work builds on three previous summers (2013-2015) of CBEC research to identify and characterize the green crab threat and factors that confer resilience to eelgrass even in the presence of green crabs. In 2013, exclosure experiments in upper Casco Bay determined that eelgrass transplants protected from green crab disturbance survived at significantly higher rates than unprotected shoots (Neckles 2015). In 2014, eelgrass transects were established at five sites throughout Casco Bay to monitor eelgrass growth in either coarse

or fine sediments and determine other environmental factors that may contribute to changes in eelgrass distribution and abundance. Each site had variation in sediment type, eelgrass shoot density and size, green crab population density, and other environmental stressors such as light limitations, temperature, physical disturbance, and nutrient availability. In 2015, two pilot sites for eelgrass restoration were identified, testing the feasibility of transplanting eelgrass using a variety of different planting methods. These plots were monitored throughout the summer; additional monitoring will be completed in June 2016. This study builds on these prior eelgrass restoration efforts by examining pore-water sulfide concentrations along a gradient of vegetation to determine the feasibility of transplanting eelgrass to these sites and the impacts that eelgrass has on pore-water sulfide concentrations in Casco Bay.

Methods

Field-site description

Sites were chosen in Casco Bay between Cape Elizabeth and Cape Small, Maine, to represent a gradient of eelgrass cover (Fig. 2). Historically, the intertidal and subtidal flats of Casco Bay contained Maine's greatest extents of eelgrass and some of the largest eelgrass beds in the western North Atlantic (Short and Short 2003). The bottom substrate ranges from predominately sand to predominately mud (clay/silt), with finer sediments in the upper Casco Bay and coarser sediments in the lower Bay (Kelley *et al.* 1987). Green crab density was very high in 2012-2014—in 2013, a one-day (24hr) green crab trapping survey of twenty-eight Maine towns found crabs in 193 out of 208 sample sites, most of which trapped counts of over 100 crabs (Kanwit 2013). Cold winter temperatures in 2014-2015 reduced green crab numbers such that very few were observed at the Casco Bay sampling locations in summer 2015. Loss of eelgrass beginning in 2012 was correlated with green crab abundance; the upper regions of Casco Bay were largely de-vegetated at this time (Maine Department of Marine Resources 2013). Loss of vegetation and reductions in percent cover continued through the summer of 2014.

Sampling locations

Sediment cores were sampled from four sites characterized by varying degrees of vegetation loss. Broad Cove (BC) in Cumberland/ Foreside contains a dense, historically vegetated eelgrass bed that had not experienced significant declines in density or eelgrass area (Fig. 3A). Eelgrass plants were harvested from this location for transplant feasibility tests in summer 2015. A 100-m transect was established in the continuous eelgrass bed

parallel to the shore, avoiding places where shoots had been harvested earlier; twelve sediment core samples were taken at random points along this transect on August 8, 2014. Flying Point (FP) in Freeport lost eelgrass in 2012-2013, but is experiencing a high natural rate of recruitment of eelgrass (Fig. 3B). Substrate is soft, easily suspended, and smells of sulfide (pers. obs.). This site is one of the pilot sites for eelgrass restoration. Sediment core samples were taken from twelve transplant plots parallel to the shore on August 14, 2015. Simpson's Point (SP) in Brunswick experienced total eelgrass loss in 2012-2013, and the substrate is soft, extremely flocculent, and smells strongly of sulfide (Fig. 3C). This is the second 2015 pilot plot site for eelgrass restoration. Twelve plots on the shore side were sampled on August 15, 2015. Cousin's Island (CI) was completely de-vegetated in 2014; in 2015, there were still dead rhizomes visible on the surface of the sediment (Fig. 3D). Sediments were soft and muddy; a 100-m transect was established among the dead rhizomes and twelve random points were sampled on September 2, 2015.

Sample collection

Sediment corers were constructed by slicing off the tips of 60-mL syringes. Cores were extracted from twelve randomly selected locations along each transect, taking care to avoid severing roots when possible. The first two cores were extruded slightly and the tip of the core was removed to get a 10 cm long core to represent the root depth of eelgrass (Frederiksen *et al.* 2006). These two cores were placed in separate, sealed bags for sediment character analyses and placed on ice for transport, then immediately frozen until analysis. A final core for sulfide analysis was taken to the full length of the syringe

without extruding as extra protection against oxidation; the tips of the corers were additionally covered with Parafilm and aluminum foil, and rubber banded so as not to expose the samples to air. The syringes were placed on ice for transport. Samples were processed immediately to prevent loss of sulfides.

Sample processing for sediment sulfide analysis

In a glovebox flushed with nitrogen, cores were extruded from the syringes to 10 cm and the excess discarded. The remaining 10 cm sediment cores were homogenized and centrifuged at 3200 rpm for 8 minutes to extract pore-water. Pore-water was removed (0.5 mL) and immediately placed into a vial containing 12 mL 2% zinc acetate. Sample vials were capped, then frozen until spectrophotometric analysis at 670 nm using a method of (Cline 1969).

CHN analysis

Each sediment sample was dried and homogenized by hand using a mortar and pestle. A subsection of the sample was broken down further using a ball mill for CHN analysis. A portion of this pulverized sample was also saved to measure loss on ignition. Total carbon, total organic carbon, and total nitrogen of each sediment sample were determined using a CHN analyzer (Costech ECS 4010 Nitrogen / Protein Analyzer). Combustion temperature was 980°C. Standards were acetanilide (C=71.09%, H=6.71%, N=10.36%, O=11.84%). Half of each sediment sample was acidified in 10% HCl to remove carbonates. Acidification caused small losses of C and N, likely caused by leaching of organic matter than a true loss of carbonates.

Grain size analysis

Homogenized sediment samples were sieved through a 2 mm sieve to remove large material, such as shells and large pebbles. Samples were sieved further to separate coarse, medium, and fine sand, as well as silt/clay by percentage of total mass (Table 2).

Loss on ignition

A muffle furnace (Fisher Scientific Isotemp) was used to measure loss on ignition to corroborate the values from CHN analysis. Samples were dried overnight at 100°C in a drying oven to remove water. Samples were cooled, weighed, and heated in the muffle furnace for 4 hours; the percent difference in weight represents organic matter loss.

Statistical analyses

Differences in pore-water sulfide concentration, carbon content, nitrogen content, and sediment grain size between sites were analyzed using ANOVA followed by Tukey's multiple comparisons test in GraphPad Prism 6 (GraphPad Software 1993). Linear regressions between sulfide, carbon content, and grain size were also computed in GraphPad Prism 6.

Results

Pore-water sulfide

Concentrations of pore-water sulfide varied significantly among sites (Fig. 4; one-way ANOVA, $p < 0.0001$). The lowest concentrations of sulfide, below 0.15 mM on average, occurred at Broad Cove (BC), the historically vegetated site, and Flying Point (FP), a de-vegetated site experiencing high levels of natural recruitment of eelgrass. Higher levels of sulfide were observed at Simpson's Point (SP), a de-vegetated site where eelgrass was lost between 2012 and 2013, and Cousin's Island (CI), where eelgrass was completely lost recently in 2014. Sulfide levels were significantly higher at SP and CI than at BC and FP.

CHN analysis and Loss on ignition

The percentage of total carbon was lowest at BC and FP, and significantly higher at CI and SP (Fig. 5a; one-way ANOVA, $p < 0.0001$). Total organic nitrogen was significantly lower at FP than at other sites (Fig. 5b; one-way ANOVA, $p < 0.0001$). The ratio of C:N was significantly higher at FP than at other sites (Fig. 5c; one-way ANOVA, $p = 0.0226$). Nitrogen content was relatively well-correlated with carbon content (Fig 5d; linear regression, $R^2_{BC} = 0.90$, $R^2_{FP} = 0.77$, $R^2_{CI} = 0.54$, $R^2_{SP} = 0.36$). For acidified samples, there were significant differences in organic carbon content between all sites (Fig. 6a; one-way ANOVA, $p < 0.0001$). The site with the lowest amount of organic carbon was BC, followed by FP, then SP; CI had the highest percentage of organic carbon on average. Acidified samples were significantly higher in nitrogen at CI and SP (Fig. 6b; one-way ANOVA, $p < 0.0001$). The C:N ratio was significantly higher at FP than at other

sites (Fig. 6c; one-way ANOVA, $p < 0.0001$). Correlations between carbon and nitrogen content were reduced compared to samples that were not acidified (Fig. 6d; linear regression, $R^2_{BC}=0.20$, $R^2_{FP}=0.25$, $R^2_{CI}=0.28$, $R^2_{SP}=0.$). Acidification of samples to remove carbonates significantly reduced the percentage of carbon found at BC and FP (Fig. 7a; two-way ANOVA, $p < 0.0001$). Acidification also significantly reduced the percentage of nitrogen found at FP (Fig 7b; two-way ANOVA, $p < 0.0001$). Carbon content and sulfide concentration were not well-correlated at any of the sites, but correlations were weaker at high-sulfide sites CI and SP (Fig. 8; $R^2=0.0983$ and 0.0877 , respectively) than at low-sulfide sites BC and FP ($R^2=0.304$ and 0.137 , respectively).

Loss on ignition analysis on a subset of the samples showed that organic matter increased with increasing vegetation. There was the least amount of organic matter present at BC, and the most at SP (Table 3).

Grain size analysis

Sites were predominately sand (Fig. 9). All sites had at least 85% sand, with the highest percentage of sand at BC and the lowest at FP. There were significant differences between percentage of sand at each site (one-way ANOVA, $p < 0.0001$). Breaking down the sand into categories of coarse, medium, or fine resulted in significant differences as well (Fig. 10; two-way ANOVA, $p < 0.0001$). There was significantly less coarse sand at CI than at BC, and significantly less coarse sand at SP than at BC (Tukey test). There were no significant differences in the amount of medium sand between any of the sites. Fine sand was comparable between BC, FP, and SP, but significantly higher at CI.

Percent silt/clay was significantly different between sites (Fig. 11; one-way ANOVA, $p < 0.0001$); there was more silt/clay in the sites that had been de-vegetated longest.

Discussion

Clear site differences were visible in pore-water sulfide concentration, carbon and nitrogen content, and grain size. The sites were also markedly different in the degree of eelgrass vegetation and cover. Comparing these sites could give an indication of factors that could bolster the success of eelgrass recovery via transplants, and help provide insight on how eelgrass impacts the sedimentary environment in which it grows.

Relationships between pore-water sulfide concentration and eelgrass growth and health have been observed in the field and manipulated in the laboratory; therefore thresholds are known for sulfide toxicity levels. Terrados *et al.* (1999) found that average pore-water sulfide concentrations in a dense, healthy Florida eelgrass bed ranged from 0.59-0.71 mM, suggesting that those concentrations were not limiting for eelgrass growth. They also found that seagrass sediments were only moderately reduced for sulfide levels under 0.1mM in the top 10cm of sediment (Terrados *et al.* 1999). Eelgrass shoots growing in enriched sulfide conditions showed reduced rates of photosynthesis, particularly in low light conditions; the higher the sulfide and the lower the light, the slower the rate of photosynthesis (Goodman *et al.* 1995). Photosynthesis was still observed, albeit at depressed rates, at sediment sulfide concentrations greater than 0.8mM. Holmer and Bondgaard (2001) showed that exposure to high (0.1-1 mM) sulfide concentrations completely stopped photosynthesis in eelgrass after just six days and halted leaf elongation ten days later. Concentrations of 0.5-1.0 mM showed a three-fold reduction in photosynthetic output. Low oxygen conditions also gave way to reduced photosynthetic activity. Dooley *et al.* (2013) found that sediment sulfide concentrations greater than 0.68 mM consistently killed eelgrass seedlings and decreased photosynthetic

output in adult plants; LD₅₀ for seedlings was 0.334 mM in the lab. These previously established values can help contextualize the sulfide concentrations that were measured in this study.

Sediment sulfide concentrations at BC and FP were well below previously described thresholds that cause damage to growth and photosynthetic systems in eelgrass. BC has had a dense, historically vegetated eelgrass bed in place since at least 1997; FP, which had a dense eelgrass bed that was de-vegetated in 2012, has a high degree of natural eelgrass recruitment. While sulfate reduction rates have been shown to be higher in vegetated areas than outside of them due to decomposing organic matter, eelgrass and other seagrasses are somewhat adapted to grow in reduced, high-sulfide, low-oxygen environments by shunting oxygen produced during daytime photosynthesis down into the roots and rhizosphere (Holmer *et al.* 2005, Holmer and Nielsen 1997, Pedersen *et al.* 2004). These oxic microzones around the eelgrass roots are more prevalent in the daytime when the plants are photosynthesizing, but may be enough to prevent sulfide intrusion if concentrations of sulfide are low (Pulido and Borum 2010, Raven and Scrimgeour 1997). At BC, the very low sulfide concentrations indicate that the healthy eelgrass bed that has existed there for decades is oxygenating the sediments to a degree that prevents the buildup of pore-water sulfide. While the 0.11 mM concentrations of sulfide that were observed on average at FP have been considered potentially damaging in other studies (Holmer and Bondgaard 2001), the fact that eelgrass has successfully recolonized the area naturally following devegetation by green crabs suggests that the concentration was not so high as to prevent natural recruitment of seedlings. Additionally, eelgrass has been known to grow healthily in the field in higher sulfide

conditions (Terrados *et al.* 1999). Indeed, the concentration is well below the 0.68 mM shown by Dooley (2013) to kill eelgrass seedlings. FP was on the front line of eelgrass loss in Casco Bay in 2012, meaning that vegetated sites nearby facilitated natural recruitment via seeding of new plants. It is also possible that the sulfide levels at FP today are lower than they were directly following de-vegetation (like the elevated sulfide levels observed at CI, which was recently de-vegetated), and that the growth of eelgrass has helped to reduce sulfide levels.

The high sulfide sites, CI and SP, were completely absent of eelgrass at the time of sampling. CI was recently de-vegetated in 2014, and dead rhizomes were still visible on the substrate surface in the spring of 2015. The mean CI pore-water sulfide concentration of 0.69 mM is above Dooley's threshold for eelgrass seedling death, and double the LD₅₀ for eelgrass seedlings in the lab of 0.334 mM (Dooley *et al.* 2013). However, there has not been enough time since the site was de-vegetated to observe whether natural recruitment is a possibility, though the high sulfide concentrations make seedling survival particularly difficult even though the site is located nearby established beds that could facilitate recruitment of new seedlings. The 1.26 mM mean sulfide concentration observed at SP is well above thresholds that have been shown to kill adult plants, much less allow the growth of seedlings (Dooley *et al.* 2013, Holmer and Bondgaard 2001). These high sulfide concentrations suggest that eelgrass recovery in these areas will be unlikely, and efforts to restore eelgrass via transplant may be less likely to be successful due to the negative effects of sulfide toxicity.

Loss of eelgrass in Casco Bay is of great local concern, and restoration efforts beginning in 2013 have so far largely been to ascertain the feasibility of transplanting

eelgrass in the upper part of the bay. In the summer of 2015, donor plants were harvested from BC and transplanted using several planting methods to FP—a site where restoration seemed likely to be successful due to the high degree of natural recruitment—and SP, a completely bare site that had been de-vegetated since 2012. Preliminary results show that FP appears to be a good site for transplanting eelgrass; at eight weeks post-transplant, the number of shoots at the transplant plots had increased three to four times the initial number. SP, however, showed either a loss of transplanted shoots or a failure of the transplants to produce new shoots (Ad hoc Casco Bay Eelgrass Consortium, personal communication, 2016). The extremely high sulfide concentrations found at the plot sites at SP indicate that sulfide is one factor preventing the success of those transplanted shoots. In fact, sulfide concentration matched the timing of de-vegetation of each site: BC, which was historically vegetated, had the lowest pore-water sulfide concentration, while SP, which was de-vegetated earliest and never recovered eelgrass, had the highest. The extremely high concentrations of pore-water sulfide found at SP suggest that concentrations increased after or near the time that eelgrass was lost, as eelgrass would not have been able to survive at those levels.

Pore-water sulfide concentrations that were measured in this study may not be representative of the entire growing season for eelgrass; higher temperatures in late summer increase biological oxygen demand and pH. The equilibrium between S^{2-} , HS^{-} and H_2S is pH dependent; at pH 6, 90% will be H_2S , while at pH 7, that number falls to 50%, and at pH 8 it falls further to 20% (Korhonen *et al.* 2012). In Casco Bay, the average pH from 2010-2015 was 7.84, indicating that the pore-water sulfide measured could represent less than half of the available sulfide pool (Casco Bay Estuary

Partnership 2015). Additionally, heightened growth during the summer could stimulate anaerobic metabolism and elevate pore-water sulfide concentrations (Joye and Hollibaugh 1995, Koch *et al.* 2007). Measuring summer sulfide concentrations gave an indication of what sedimentary conditions were like during the growing season for eelgrass, but concentrations may be slightly variable during other times of the year, such as late spring when eelgrass plants are flowering and seeding.

Other systems have experienced eelgrass loss due to increased sulfide conditions (Holmer and Bondgaard 2001, Koch *et al.* 2007). In contrast, a catastrophic removal of eelgrass in Casco Bay by green crabs rather than gradual environmental or geophysical changes precipitated eelgrass loss, but the subsequent changes to the sedimentary environment caused by de-vegetation continue to be important factors when considering eelgrass recovery today. Similar die-offs have been recorded in *Thassia testudinum* (turtlegrass) due to increased sulfide concentrations; increased temperatures paired with increased salinity and higher microbial sulfate reduction rates have been shown to cause die-back or complete die-off (Koch *et al.* 2007). However, Korhonen (2012) suggests that once seagrass has been impacted by sulfide intrusion, the bed will never be able to return to its previous capacity if sulfide-free conditions are re-established. Damage to the photosynthetic tissue and reduced sediment oxidation capacity render eelgrass with a lowered ability to photosynthesize or to support growth and transport of sugars throughout the plant (Holmer and Bondgaard 2001, Korhonen *et al.* 2012). Dooley (2013) observed eelgrass beds that were failing to recover in the field following decline had sulfide concentrations that were higher than the LD₅₀ observed in laboratory experiments. Sediment sulfide can be a factor that prevents the recovery and

recolonization of eelgrass and other seagrasses. Even in the presence of low concentrations of oxygen, sulfides can persist in the water column and accumulate in pore-water as stable metal-sulfide complexes are formed (Kuwabara *et al.* 1999). A healthy eelgrass bed can oxygenate the sediments and keep sulfide levels below a toxic threshold. When eelgrass is lost, increasing sediment sulfide levels due to decaying plant material can damage the remaining eelgrass and lessen its ability to reduce sulfide concentrations, or prevent new eelgrass shoots from recolonizing the area. The question is, of course, how well eelgrass transplants might be able to oxygenate the sedimentary environment and facilitate conditions that make growth possible. The transplant sites at FP and SP will be sampled again in Summer 2016. If the shoots at SP have gone on to propagate, it is likely that the plants have altered their sedimentary environment such that it will be easier for eelgrass to grow within the transplant plots than outside of them. Further transplant efforts in that case may be worthwhile in helping to restore eelgrass to SP. If, however, shoots have been lost or failed to expand vegetatively, perhaps larger-scale restoration efforts at SP would not be successful, or perhaps the shoots need more time than just one year of growth to begin oxygenating the sediments in a significant way in this high-sulfide site.

The internal oxygen status in an eelgrass plant, which can be altered by other factors such as light attenuation and temperature, influences sulfide invasion (Frederiksen *et al.* 2006). A combination of additional stressors including light reduction, increased salinity, or anoxia can initiate die-back events (Holmer *et al.* 2005, Korhonen *et al.* 2012). Other sedimentary factors can also play a role in the production of hydrogen sulfide within the sulfur cycle. Carbon content, nitrogen content, and grain size distribution of the sediment

are interconnected factors that can give an indication of how eelgrass beds are related to the quality of the sediment in which they grow.

Percent carbon and nitrogen were lower at FP than at other sites, which in turn increased the C:N ratio found at FP. Acid-treated samples, meant to remove carbonates, reduced carbon concentrations, displayed a different pattern of sediment carbon and nitrogen content. BC and FP showed significant differences in percent carbon before and after acidification, indicating that those sites contained a lot of carbonates. Younger or more recalcitrant forms of carbon at FP could account for the spike in C:N following acidification. Small reductions in carbon and nitrogen content following acidification were likely due to leaching of organic matter rather than a true loss in material. Acid treatment may have removed small amounts of organic matter in addition to carbonates. Linear correlations between sulfide concentration and percentage of organic carbon were not statistically significant, suggesting that while sulfides were highly variable between sites, total organic carbon was variable in a different pattern. Linear correlations between carbon and nitrogen were stronger before acidification than after. The increased amount of carbon and nitrogen seen at the de-vegetated sites, CI and SP, could be indicative of the legacy of de-vegetation, in which the increased mineralization of organic matter following the death of roots and rhizomes in 2012 led to higher levels of nitrogen and carbon in the sediment today. However, since there is no way to compare the amounts of carbon and nitrogen that were in the sediments at these sites prior to de-vegetation, it is also possible that these sites simply had more organic matter to begin with, which may be related to the grain size distribution of these sites.

Grain size and degree of vegetation are also related to carbon and nitrogen content in the sediment. Seagrasses store organic carbon and are known as carbon sinks (Kennedy *et al.* 2010). Generally, more nitrogen and more silt/clay are found within seagrass beds than outside of them (Kenworthy *et al.* 1982). Silt/clay also generally contains more organic matter than larger-grained sediments, which is corroborated here by loss on ignition data. Seagrass shoots and leaves decrease the flow velocity of the seawater, and roots and rhizomes help to trap sediment within the beds, and the diverse community of benthic organisms that live within seagrass beds add to the volume of the total nitrogen pool (Kenworthy *et al.* 1982). Additionally, higher rates of sedimentation and sulfide production are more typically observed within seagrass beds than outside of them (Holmer and Nielsen 1997). Rates of nitrification are reduced when HS^- is present; Joye and Hollibaugh (1995) found that 60 μM concentrations of HS^- reduced rates of nitrification by 50%, and 100 μM concentrations dropped nitrification rates by 100%. Since CHN analysis measures total organic nitrogen, we see higher percentages of nitrogen at the de-vegetated sites SP and CI, where fewer organisms are living to decompose organic matter into biologically usable forms of nitrogen. Increasing grain size tends to correlate with increasing surface area, and total organic carbon content increases with increasing surface area (Horowitz and Elrick 1987).

Large-resolution sediment grain size analysis of Casco Bay show that the first 5m of sediment depth throughout the bay are uniformly mud, or a combination of sand, silt, and clay (Kelley 1992). The results of this study showed a finer resolution from site to site; there was variation in the percentage of sand and silt/clay between sites, and differences in the breakdown between coarse, medium, and fine sand. Interestingly, there

was a higher percentage of sand at the sites that had been vegetated the longest, and a higher percentage of silt/clay at the sites that had been de-vegetated the longest. While this study did not compare sediment grain size inside and outside of current and former beds, these results could indicate that eelgrass may be good at trapping coarser sediments while finer sediments are better able to settle and accumulate on bare substrate. This is in direct contrast to Kenworthy *et al.* (1982), who showed that the finest grained sediments in an area were found within a seagrass bed. Alternatively, green crabs may prefer fine-textured sediments, thereby removing more eelgrass from sites with more silt/clay and less coarse sand. Paired transect experiments in Casco Bay in 2014 showed that green crabs removed more eelgrass from finer sediments than from coarser sediments. Baeta *et al.* (2005) also found that green crabs were associated with finer sediments. In Casco Bay, trapping data from 2014 indicates that there were more green crabs at CI than at BC, and the initial removal of eelgrass from the finer substrates of the upper Bay could indicate that green crabs forage preferentially in finer sediments. Indeed, the distribution of soft-shell clams and blue mussels shows that these green crab prey items are concentrated in upper Casco Bay (Maine Department of Marine Resources 2010).

The success of eelgrass restoration efforts in Casco Bay may therefore depend on the careful selection of locations in which sediment sulfide levels are low enough to promote growth and the sediments are coarser. Measuring sulfide levels is essential for managers looking to transplant eelgrass to a site. Measuring the carbon and nitrogen content of the sediment could also give an idea of the rates of decomposition and nitrogen availability at those sites. Additional measurements that were not taken in this study but which could be useful in predicting eelgrass transplant success would include factors that

potentially limit eelgrass growth. These factors are interrelated with the variables that were measured in this study: light attenuation, salinity, pH, dissolved oxygen, and ammonium concentrations, for example. Eelgrass, when present, is considered to be an ecosystem engineer; the loss of eelgrass also has clear impacts on the sedimentary environment in which it once grew.

Conclusions

The relationships between eelgrass and its sedimentary characteristics are complex. Pore-water sulfide concentrations were correlated with eelgrass density, with vegetated sites claiming lower sulfide concentrations, and de-vegetated sites showing high sulfide concentrations. The timing of eelgrass loss/recovery could also play a role in sulfide concentrations, with increased sulfide levels at sites that had been de-vegetated earlier. While many studies have examined eelgrass beds that have been lost due to increased levels of sediment sulfide, the role of sulfide in habitats where eelgrass was lost due to widespread damage due to invasive green crabs has not been as well characterized. The catastrophic loss of eelgrass in Casco Bay from 2012-2014 has likely led to differences in sulfide levels, carbon and nitrogen content in the sediment, and possibly grain size. Increased sulfide levels that are above known thresholds for toxicity at de-vegetated sites will likely prevent recruitment of new eelgrass plants.

Implications for eelgrass restoration in Casco Bay, ME

The extremely high concentration of pore-water sulfide found at SP, a site that has been de-vegetated since 2012, suggests that sulfide concentrations increased following the loss of eelgrass, since eelgrass could not have survived at those levels. Since eelgrass can oxygenate the sediments and prevent the buildup of sulfide in the sediment, the loss of eelgrass represents a feedback loop in which it is difficult for eelgrass to recolonize the de-vegetated area. Even partial de-vegetation of an eelgrass bed by green crabs could trigger complete die-back due to added stressors. Imagine if a damaged bed with reduced percent cover went anoxic due to the increased oxygen demands of decomposing the

eelgrass tissue, or if the bed had a lessened capacity to hold down sediments leading to reduced light availability. Such a bed would be experiencing feedback that would make complete collapse more likely. Restoration of eelgrass via transplants may prove to be difficult due to a similar feedback loop: high concentrations of sediment sulfide would make photosynthesis and growth difficult on the transplanted shoots. Bare substrate without eelgrass roots and rhizomes to hold it down also increases turbidity and reduces the amount of light and protection from bioturbation and wave action for the transplants. Green crabs, though their populations have gone down in the past year, also remain as a possible threat to the survival of eelgrass beds.

Alternatively, if transplanted shoots are established in high-sulfide sites, such as what is occurring in transplant feasibility experiments at SP currently, the growth of the transplanted shoots could eventually serve to re-oxygenate the sediments and facilitate a healthy environment for eelgrass growth. This process takes time—it took nearly two decades following widespread die-off of eelgrass in the 1930s for beds to recover (Short *et al.* 1987). However, it is possible that transplants may have the ability to jump-start eelgrass recovery if ecosystem managers are conscientious about choosing sites for restoration. Planting methods that also take into account the patch size of eelgrass transplant plots could bolster the success of restoration efforts.

In this study, four sites were used to compare eelgrass growth and sediment sulfide levels across a gradient of de-vegetation. In lieu of sampling eelgrass beds throughout a decline, this method of substituting spatial differences for measuring a single location over time certainly results in some confounding variables—as seen in differences in sediment characteristics in this study. Ideally, adding sampling sites and

sampling the transplant sites inside and outside of the beds as they grow would allow more comparisons and stronger conclusions to be drawn about the complicated relationships between eelgrass and pore-water sulfide concentrations.

Factors that may contribute to successful eelgrass restoration

It has been shown that eelgrass can survive in Casco Bay successfully if green crabs are removed from the system (Neckles 2015). We were lucky that the winter of 2014-2015 was extremely cold, which greatly reduced green crab numbers. However, as the effects of global climate change become more prevalent, we cannot count on cold winters to regularly control green crab populations. Cold-resistant genotypes of green crabs from Atlantic Canada are also moving south, perhaps spelling an additional threat to Maine's eelgrass (Roman 2006). Therefore, restoration cannot rely on cold winters alone, and managers will have to make decisions about where transplanting of eelgrass will be the most likely to succeed. Continuous green crab trapping, planting methods that reduce the rate of sediment suspension, and careful considerations about thresholds sediment sulfide concentrations and light availability will help to bolster the success of eelgrass restoration efforts in Casco Bay.

Figures

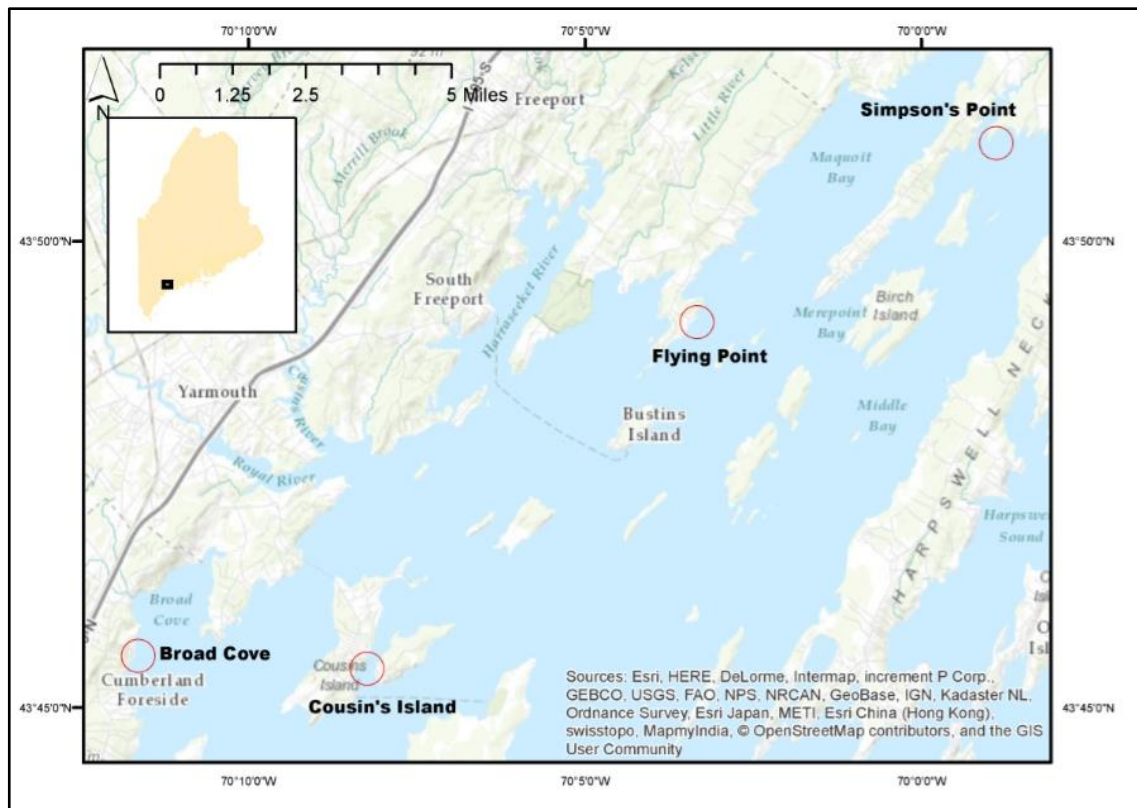


Fig. 1 Red circles outline sampling sites for sediments in Casco Bay, Maine. From West to East: Broad Cove (BC) in Cumberland/Forside, ME is a historically vegetated site with dense, persistent eelgrass; Cousin's Island (CI) in Yarmouth, ME lost eelgrass in 2014-15; Flying Point (FP) in Freeport, ME lost eelgrass in 2012 but has a high rate of natural recruitment that began in 2015; Simpson's Point (SP) in Brunswick, ME was de-vegetated in 2012 and had no natural recruitment of eelgrass as of 2015.

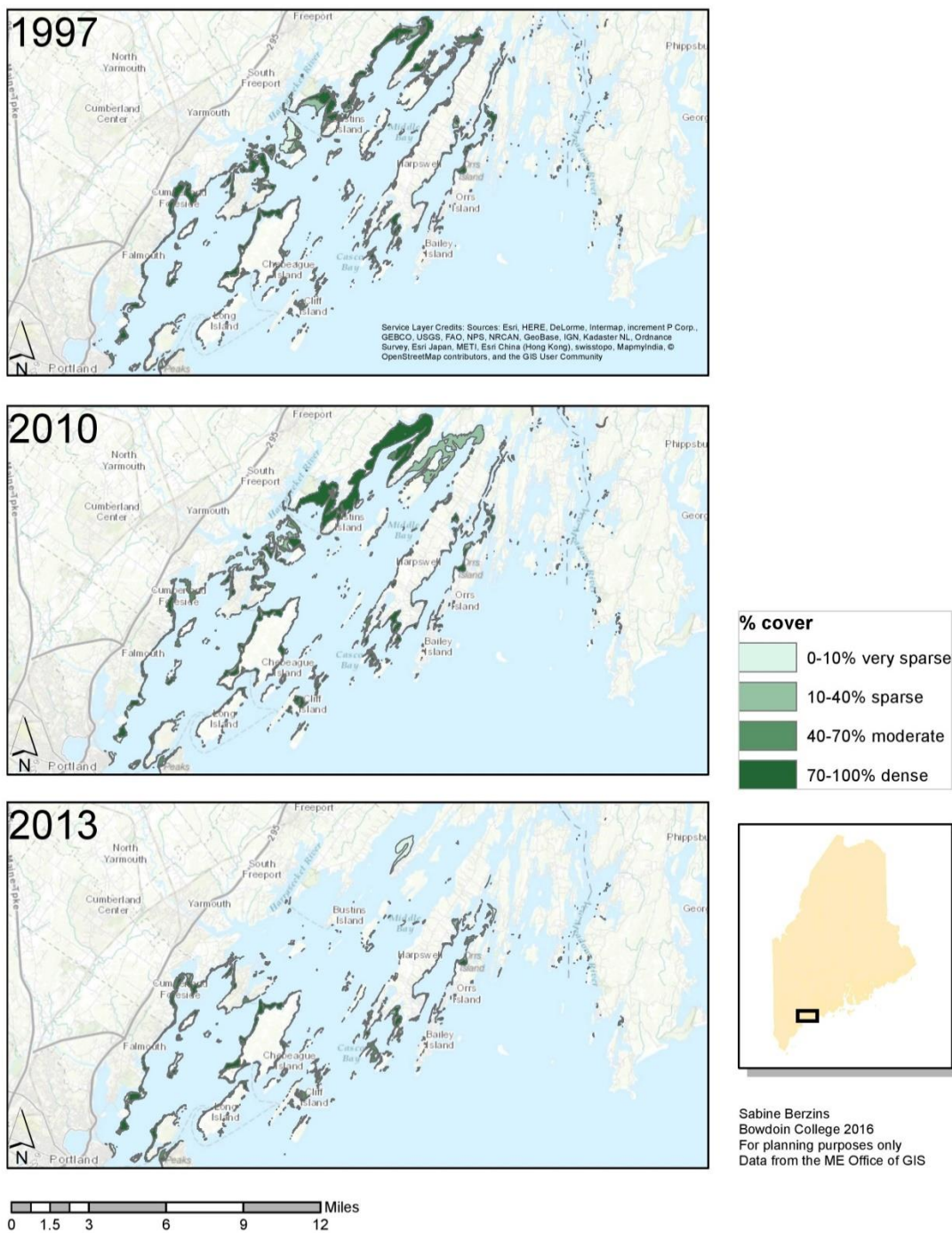


Fig. 2 Eelgrass (*Zostera marina*) cover in Casco Bay, ME in 1993, 2010, and 2013. There is dramatic eelgrass loss in upper Casco Bay between 2010 and 2013 representing a >55% loss in eelgrass area and a reduction in percent cover. Eelgrass cover represents 3,338 ha in 2001 and 1,478 ha in 2013. Data from MDEP and the Maine Office of GIS.

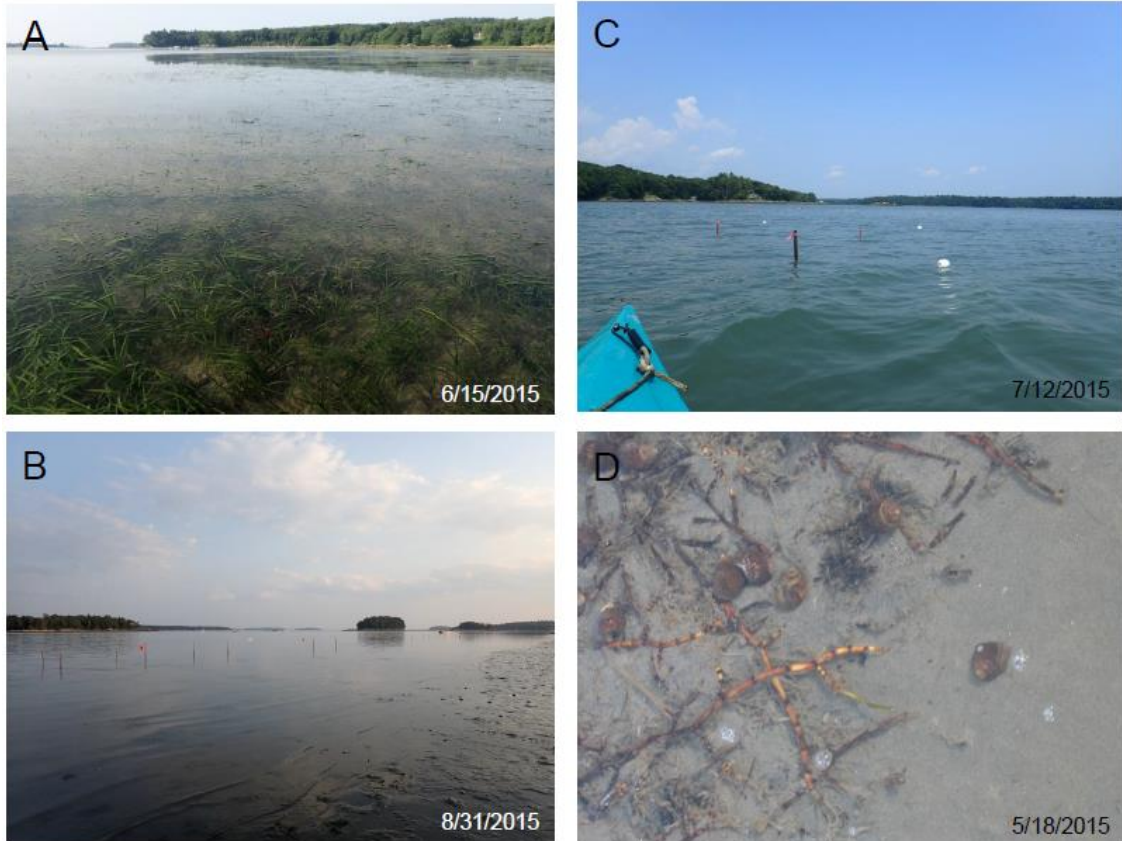


Fig. 3 Photographs from four sampling sites in Casco Bay, ME. (A) Broad Cove, a continuous historically vegetated eelgrass bed. (B) Submerged transplant plots at Flying Point. (C) Submerged transplant plots at Simpson's Point. (D) Dead eelgrass rhizomes visible on bare substrate at Cousin's Island. Photos A-C by Hilary Neckles; Photo D by Fredrick Short.

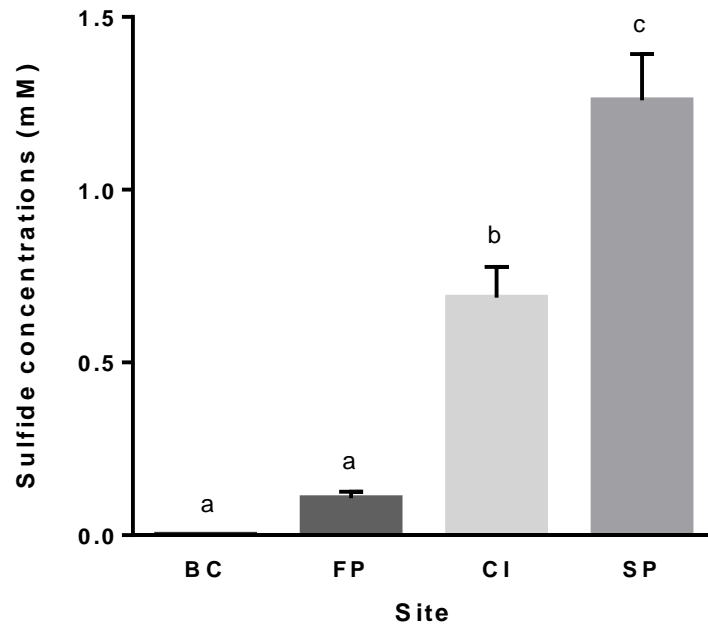


Fig. 4 Mean pore-water sulfide concentrations in four eelgrass beds in Casco Bay, ME were significantly different between sites (one-way ANOVA, $p < 0.0001$). BC=Broad Cove, historically vegetated; FP=Flying Point, de-vegetated in 2012 with natural recruitment; CI=Cousin's Island, de-vegetated in 2014; SP=Simpson's Point, de-vegetated in 2012. Error bars represent ± 1 SEM. $N=8$.

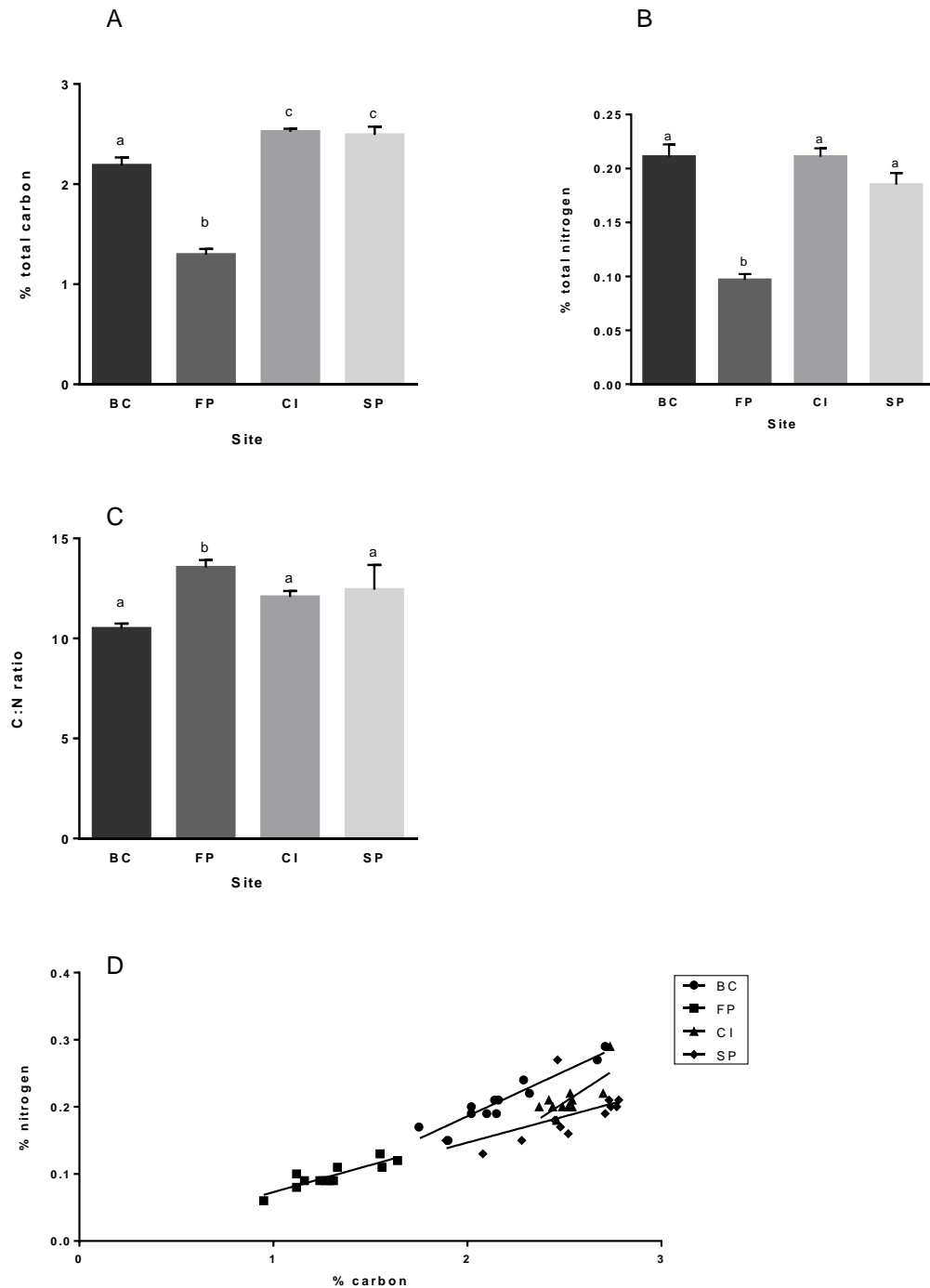


Fig. 5 Percent of total carbon and nitrogen by weight, and the ratio of C:N for four sites in Casco Bay, ME. (A) BC and FP were significantly lower in total carbon content on average than CI and SP (one-way ANOVA, $p < 0.0001$). (B) Total mean nitrogen content was significantly lower at FP than at other sites (one-way ANOVA, $p < 0.0001$). (C) C:N ratios. C:N was significantly higher at FP than at other sites (one-way ANOVA, $p = 0.0226$). (D) Correlations between % carbon and % nitrogen (linear regression, $R^2_{BC} = 0.90$, $R^2_{FP} = 0.77$, $R^2_{CI} = 0.54$, $R^2_{SP} = 0.36$). Error bars represent ± 1 SEM. $N = 12$.

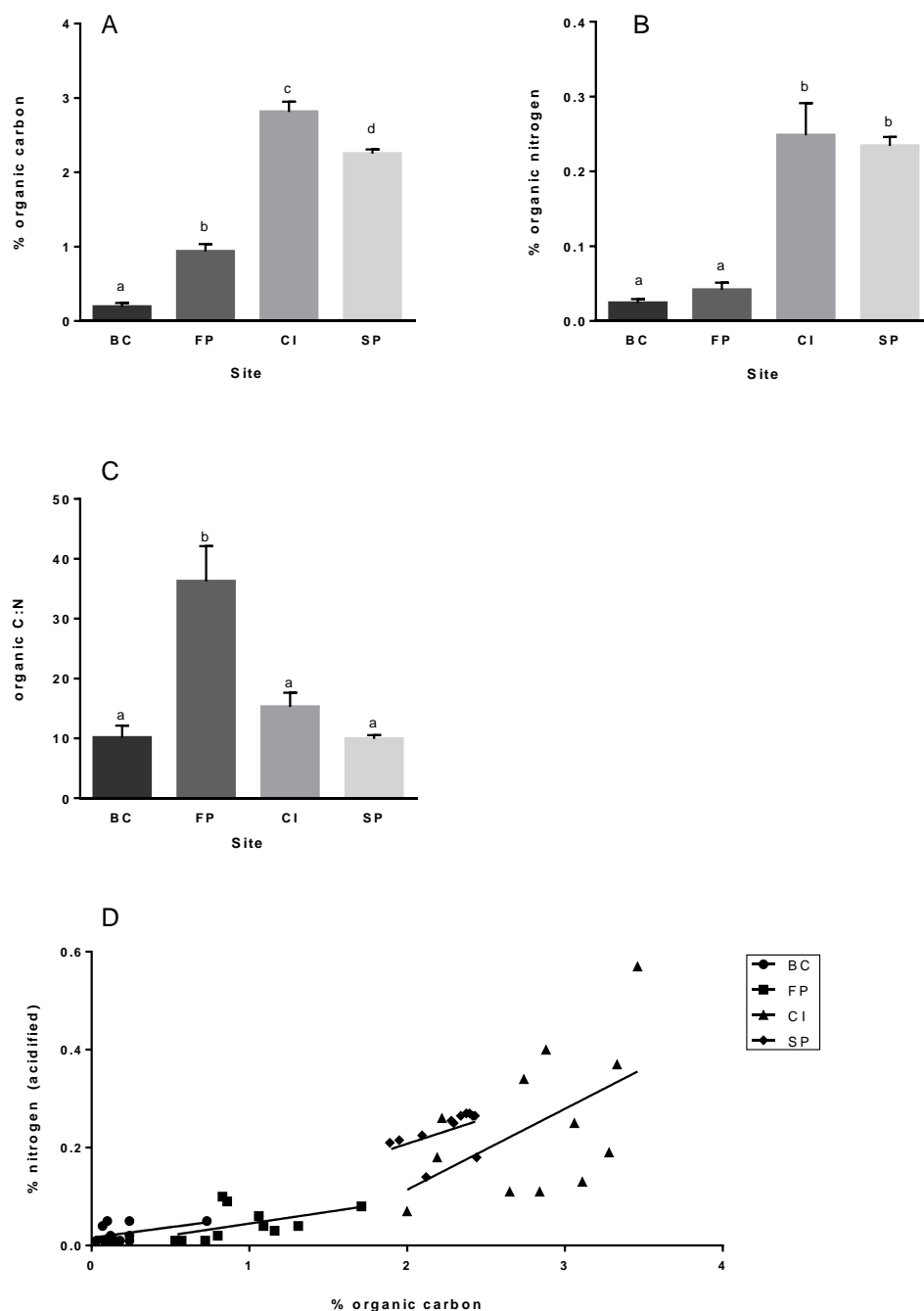


Fig. 6 Percent of carbon and nitrogen by weight, acidified in 10% HCl to remove carbonates, and the ratio of acidified C:N for four sites in Casco Bay, ME. (A) Percent of total organic carbon was significantly different at each site (one-way ANOVA, $p < 0.0001$). (B) Total mean organic nitrogen content was significantly lower at BC and FP (one-way ANOVA, $p < 0.0001$). (C) Acidified C:N ratios. C:N was significantly higher at FP than at other sites (one-way ANOVA, $p < 0.0001$). (D) Correlations between % carbon and % nitrogen (linear regression, $R^2_{BC}=0.20$, $R^2_{FP}=0.25$, $R^2_{CI}=0.28$, $R^2_{SP}=0.23$. Error bars represent ± 1 SEM. $N=12$.

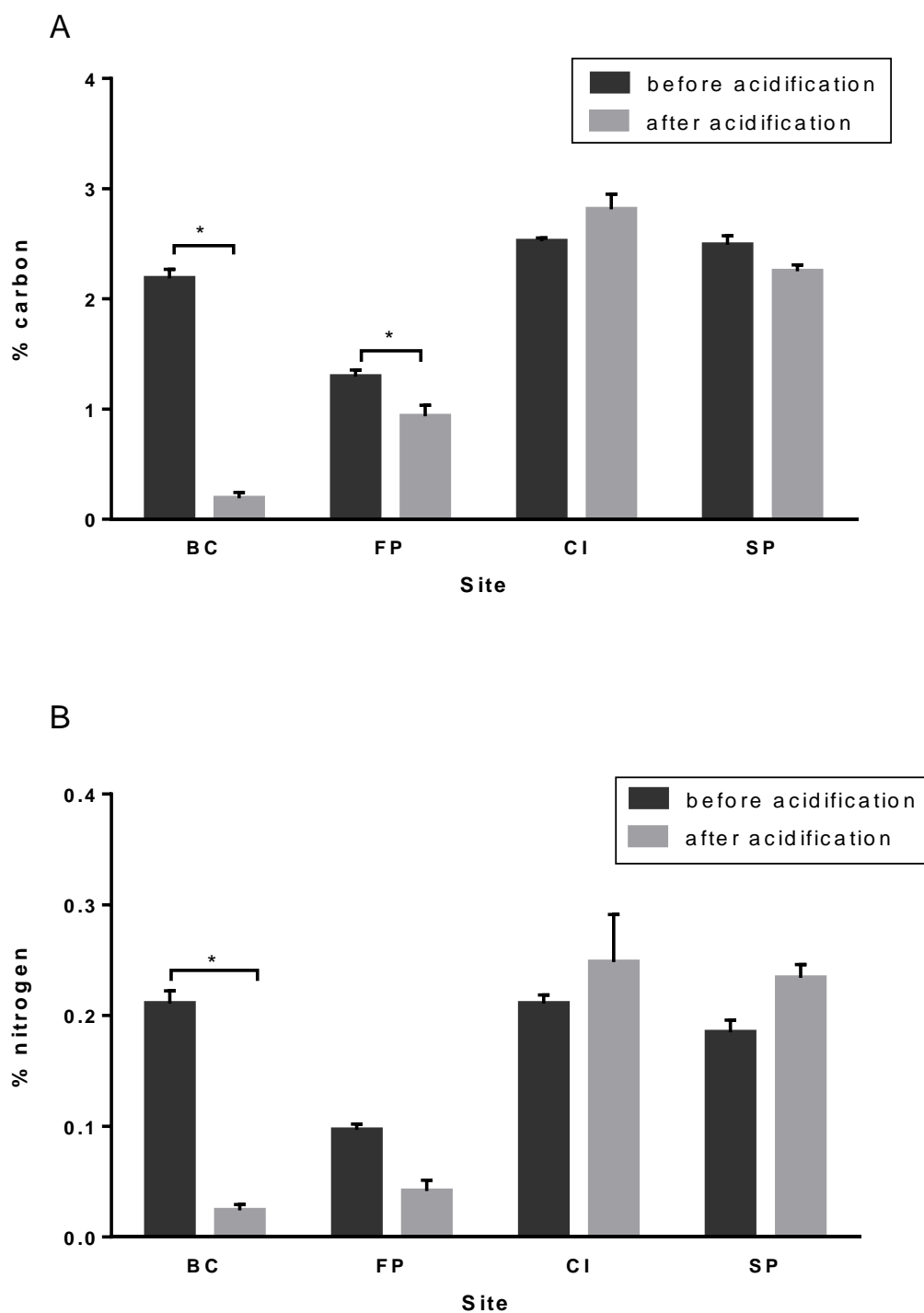


Fig. 7 (A) Differences in mean percent carbon content in sediment samples before and after acidification. The percent carbon was significantly reduced at BC and FP following acidification (two-way ANOVA, $p < 0.0001$). (B) Differences in mean percent nitrogen content in sediment samples before and after acidification. Percent nitrogen was significantly reduced following acidification at BC only (two-way ANOVA, $p < 0.0001$). Error bars represent ± 1 SEM. $N=8$.

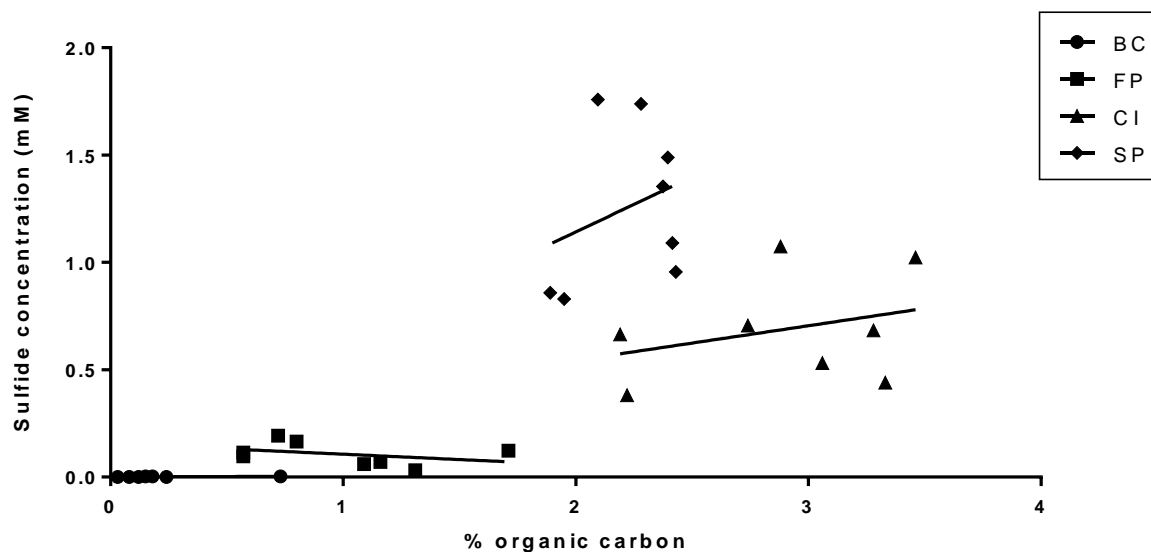


Fig. 8 Sulfide concentration vs. percentage of organic carbon. No significant linear relationships exist at high-sulfide sites CI (linear regression, $R^2=0.098$) and SP ($R^2=0.0877$), or at low-sulfide sites BC ($R^2=0.303$) or FP ($R^2=0.137$). Each point represents one sampling site. $N=8$.

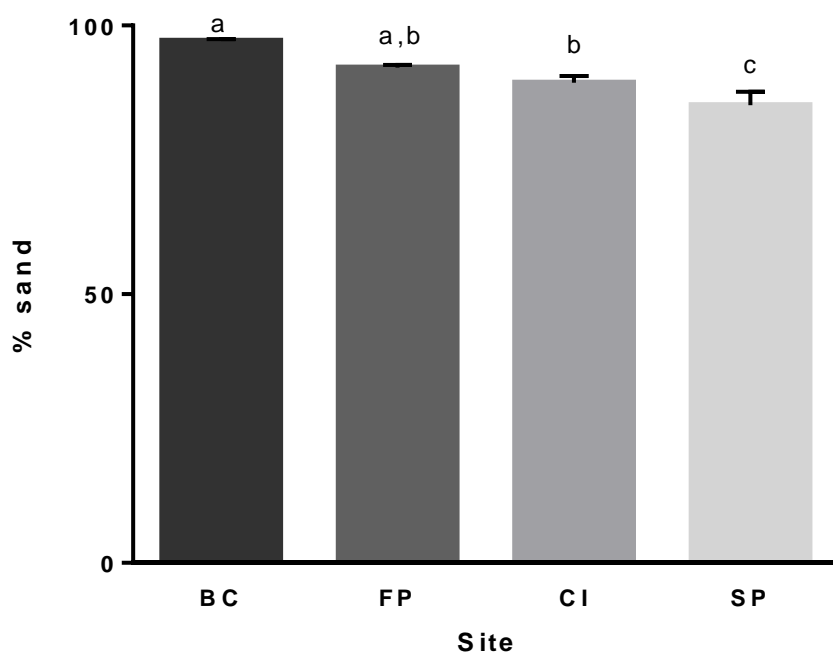


Fig. 9 Mean percent sand (grain size $>63 \mu\text{m}$) of each sample by weight is significantly different between sites (one-way ANOVA, $p<0.0001$). All sites were at least 85% sand in total. Error bars represent ± 1 SEM. $N=12$.

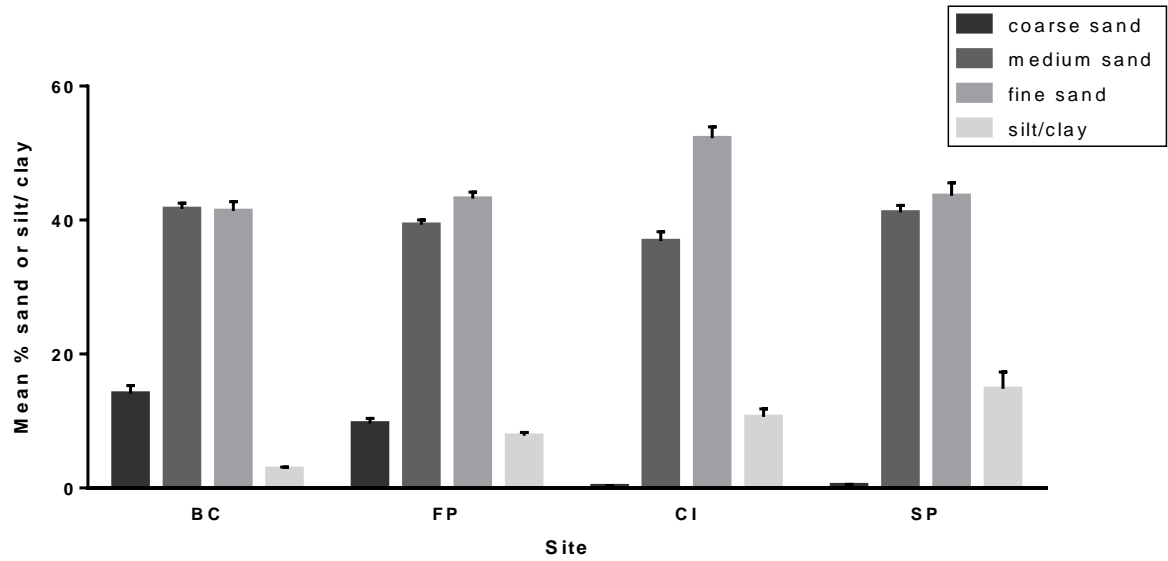


Fig. 10 Mean percentage of sand and silt/ clay of each sample by weight. Coarse sand is $>500\mu\text{m}$ in diameter, medium sand is between 500 and $250\mu\text{m}$ in diameter, fine sand is between 250 and $63\mu\text{m}$ in diameter, and silt/clay is $<63\mu\text{m}$. Error bars represent ± 1 SEM. $N=12$.

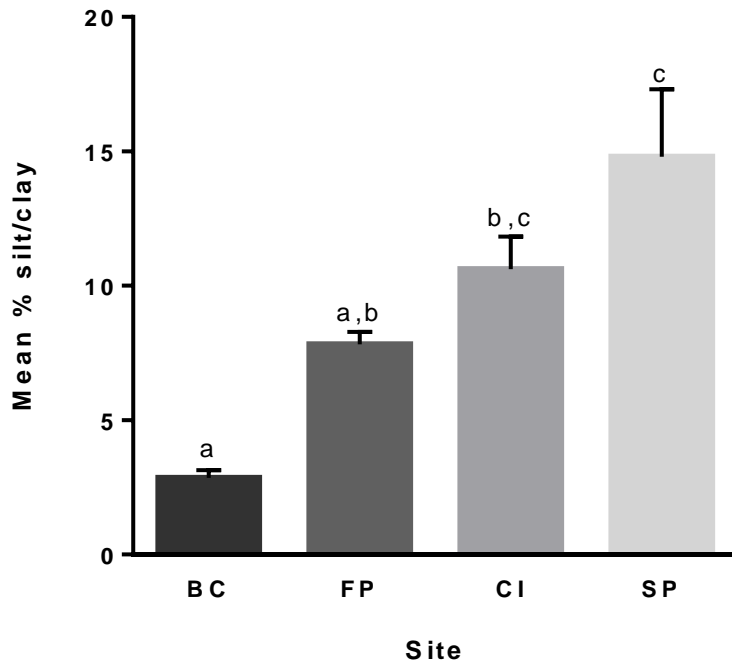


Fig. 11 Mean percentage of silt/clay ($<63\mu\text{m}$) of each sample by weight is significantly different between sites (one-way ANOVA, $p<0.0001$). Error bars represent ± 1 SEM. $N=12$.

Tables

Site Name	Town	Coordinates (dd)	Temp. (°C)	Salinity (ppt)	Eelgrass density 2010	Eelgrass density 2013	Year de-vegetated
Broad Cove (BC)	Cumberland/Foreside	43.7992073°, -70.1190376°	no data	no data	70-100%	40-100%	N/A
Flying Point (FP)	Freeport	43.81892°, -70.05569°	18 (8/14/15)	29.7	70-100%	0%	2012 (natural recruitment in 2015)
Cousin's Island (CI)	Yarmouth	43.75696°, -70.1374°	no data	no data	70-100%	40-70%	2014-2015
Simpson's Point (SP)	Brunswick	43.85086°, -69.98153°	20.8 (8/15/15)	29.6	10-40%	0%	2012

Table 1. Sampling sites in Casco Bay, Maine. Eelgrass density from Maine Department of Environmental Protection mapping in 2010 and 2013.

Sediment Type	Standard sieve number	opening (um)	Mean % at site			
			BC	FP	CI	SP
Coarse sand	35	500	14.1	9.66	0.285	0.419
Medium sand	60	250	41.7	39.3	36.9	41.1
Fine sand	230	63	41.4	43.2	52.2	43.6
Silt/clay		<63	2.85	7.82	10.6	14.8

Table 2. Mean percentage of coarse sand, medium sand, fine sand, and silt/clay by weight found in each site.

Site	mean % loss on ignition (N=3)
BC	1.70
FP	3.35
CI	6.22
BC	7.25

Table 3. Mean percentage of loss on ignition at 550°C to remove organic matter.

Appendix

Black-and-white copies of color figures

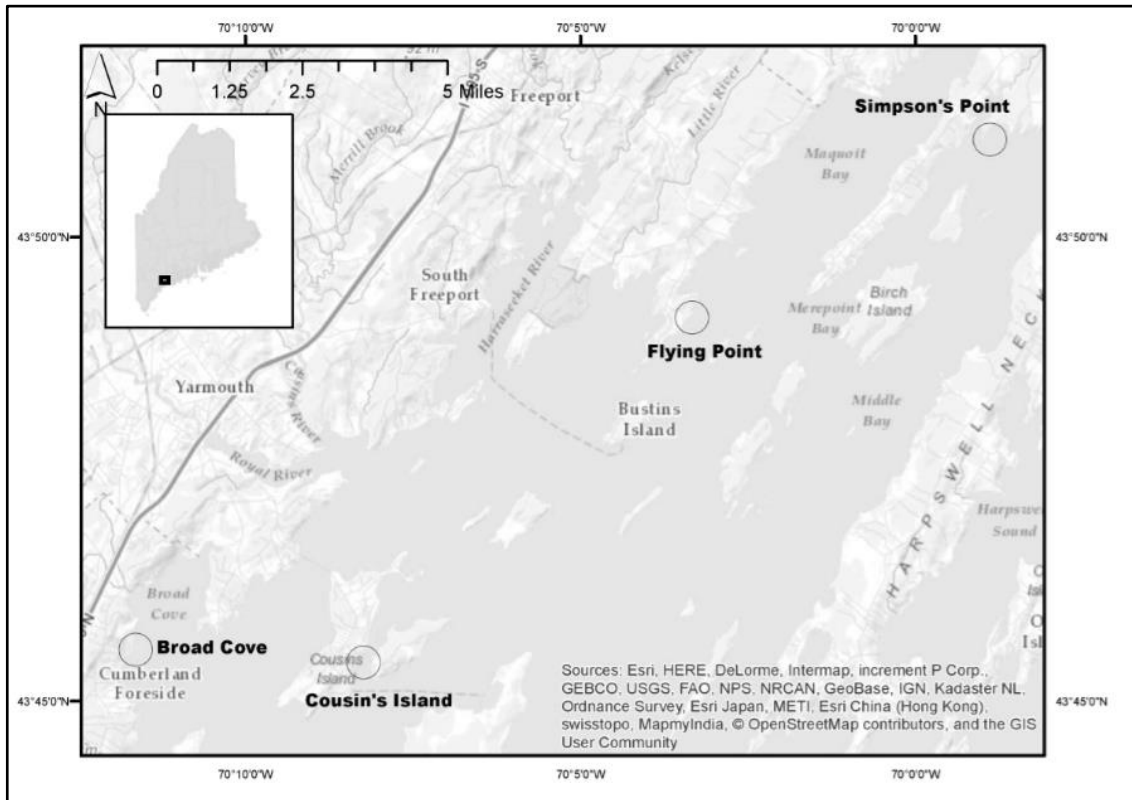


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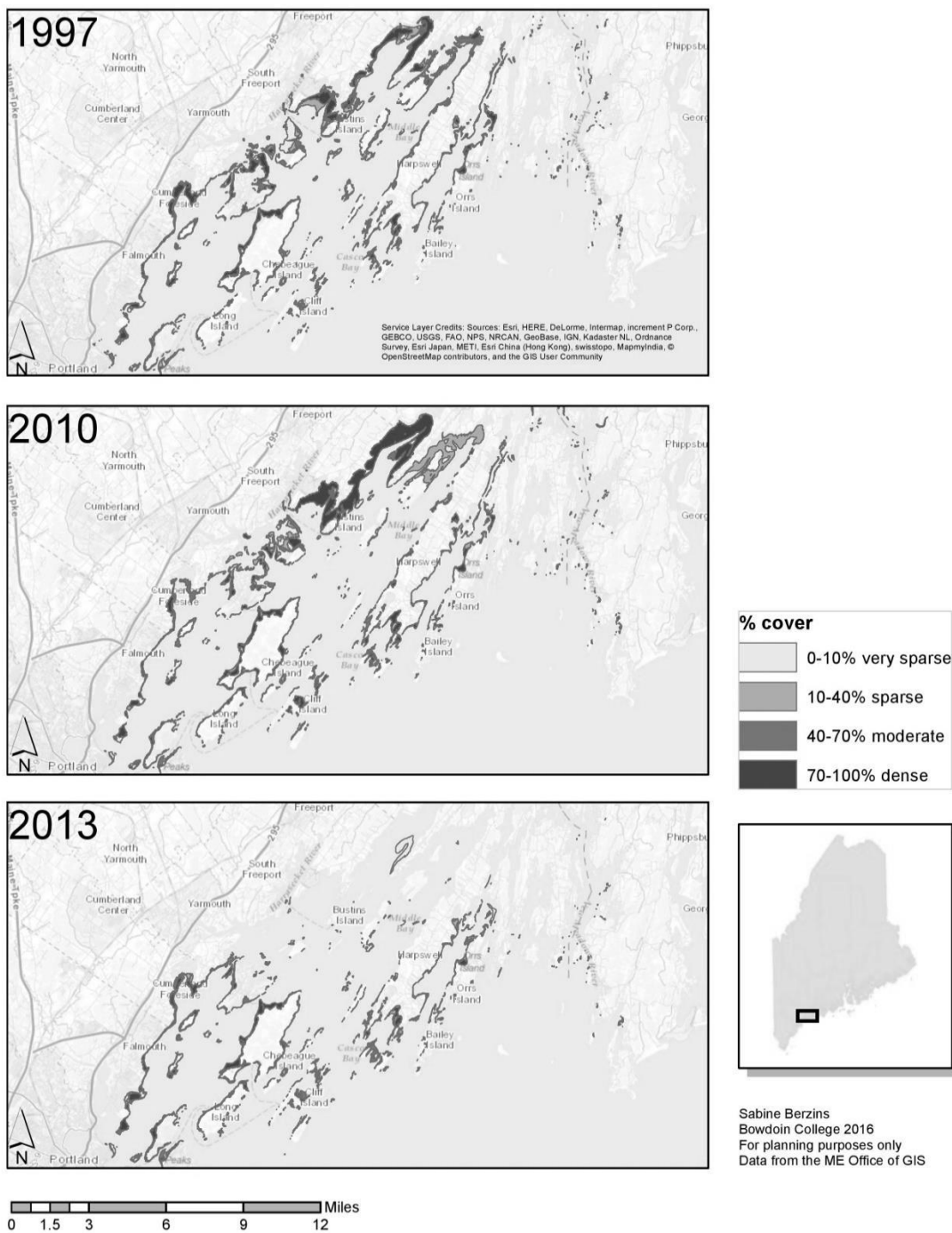


Fig. 2A Eelgrass (*Zostera marina*) cover in Casco Bay, ME in 1993, 2010, and 2013. There is dramatic eelgrass loss in upper Casco Bay between 2010 and 2013 representing a >55% loss in eelgrass area and a reduction in percent cover. Eelgrass cover represents 3,338 ha in 2001 and 1,478 ha in 2013. Data from MDEP and the Maine Office of GIS.

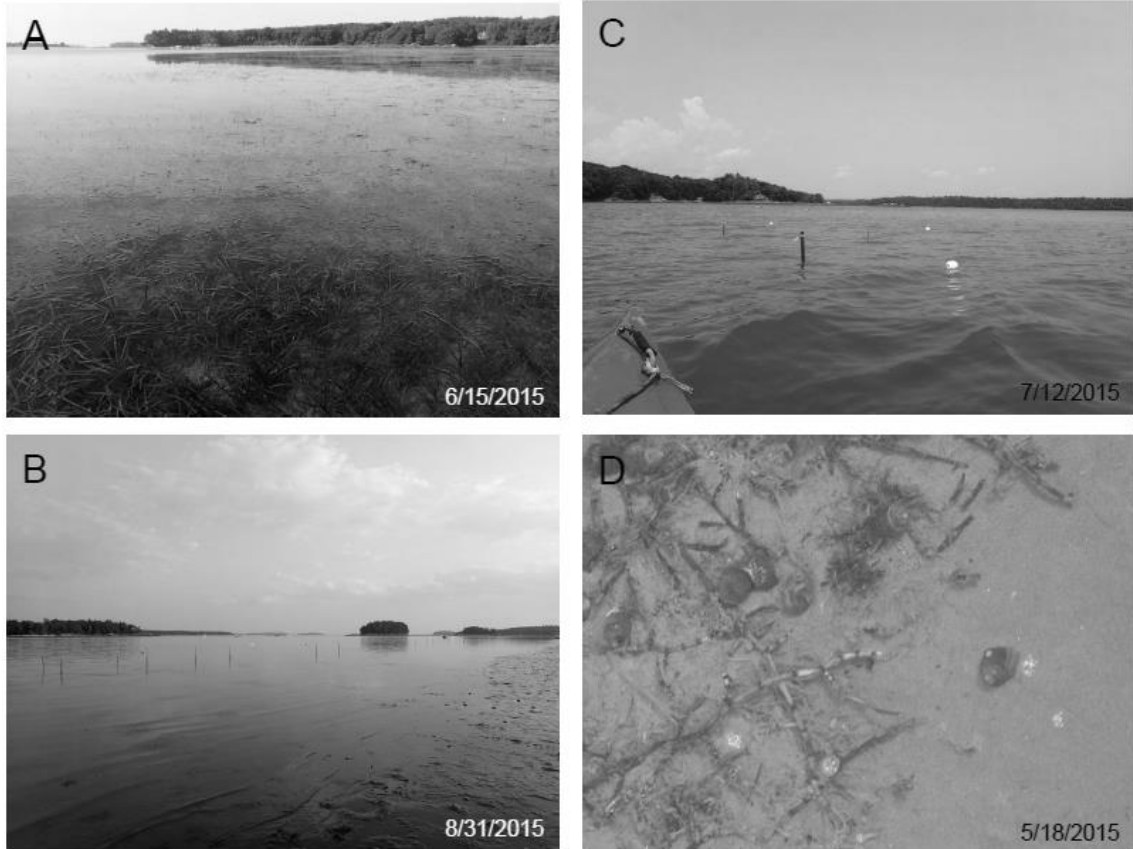


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