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Assessment and Monitoring of Ecological Characteristics of *Zostera marina* L beds in Elkhorn Slough, California

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NERR SYSTEM-WIDE MONITORING

The National Estuarine Research Reserve conducts System-Wide Monitoring (NERR SWMP) according to nationally consistent protocols, and archives and disseminates data via their Central Data Management Office. This report describes the results of implementation of NERR SWMP vegetation monitoring protocols in 2010-2011 conducted with funds provided by the Estuarine Reserve Division of NOAA, in a grant to the Elkhorn Slough Foundation on behalf of the Elkhorn Slough National Estuarine Research Reserve, a partnership between NOAA and California Department of Fish and Game.

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ABOUT THE ELKHORN SLOUGH TECHNICAL REPORT SERIES

The mission of the Elkhorn Slough Foundation and the Elkhorn Slough National Estuarine Research Reserve is conservation of estuarine ecosystems and watersheds, with particular emphasis on Elkhorn Slough, a small estuary in central California. Both organizations practice science-based management, and strongly support applied conservation research as a tool for improving coastal decision-making and management. The Elkhorn Slough Technical Report Series is a means for archiving and disseminating data sets, curricula, research findings or other information that would be useful to coastal managers, educators, and researchers, yet are unlikely to be published in the primary literature.

Abstract

Seagrass meadows are one of the most productive habitats in the world. Because seagrasses grow in shallow water, they are found in coastal locations, close to sources of anthropogenic disturbance. In the past few decades not only has aerial coverage declined worldwide, but the pace of decline is increasing. Shoreline development, erosion, eutrophication, and global climate change are just a few of the disturbances to which seagrasses are exposed. Despite the importance of seagrass habitat and the increasing threats to seagrass persistence, in many places in the world very little is known about local seagrass abundance and distribution. Establishment of seagrass monitoring programs enable scientists and managers to understand these dynamic habitats and create policies designed to protect and conserve them.

Eelgrass, *Zostera marina* L., is the dominant seagrass species in the Elkhorn Slough watershed. It forms monospecific meadows in the lower main channel of Elkhorn Slough. The earliest surveys of Elkhorn Slough in the 1930s indicated that eelgrass was plentiful. Aerial surveys revealed that eelgrass cover declined dramatically in the 1950s after the opening of a permanent harbor mouth at Moss Landing. Declines continued through the 1980s, when a shoal formed in the Seal Bend region and was colonized by eelgrass. Since that time aerial surveys have demonstrated increasing eelgrass cover in the lower slough. Yet despite the dramatic changes in areal cover, there has been only one survey in which researchers measured biological properties of eelgrass. Higher resolution surveys, in which characteristics such as percent cover, shoot density, and biomass are measured, are necessary to tie remote-sensing based maps to population-scale characteristics relevant to management objectives. For this reason, the National Estuarine Research Reserve System created a protocol for long-term monitoring of estuarine submerged and emergent vegetation communities. The Elkhorn Slough NERR was selected for implementation of the monitoring protocol in 2010.

Three surveys of Elkhorn Slough eelgrasses were conducted in August 2010, January 2011, and August 2011. Two sites were chosen to capture the range of light, current, and sediment conditions in which eelgrasses grow in Elkhorn Slough. We estimated eelgrass and macroalgae percent cover and recorded eelgrass shoot density, flowering density, canopy height, and presence of grazers and wasting disease, as well as collecting quantitative samples for eelgrass biomass, seed bank density, and sediment grain size along up to five transects at each site. General patterns followed those observed in most seagrass ecosystems, with percent cover, flowering density, canopy height and biomass tending to be higher in summer months and lower in winter months. In contrast, shoot density increased in the winter survey and decreased in the summer surveys, possibly due to density-dependent self-thinning. In addition to continued seasonal monitoring, we suggest ground-truthing and collection of additional demographic and environmental data that would help to explain observed patterns in eelgrass distribution and abundance.

Introduction

Background

Seagrass habitat is some of the most productive coastal habitat in the world, on par with many other marine and terrestrial habitats (Costanza et al. 1997). Seagrass meadows enhance biodiversity, providing nursery and feeding areas for many species of fish, shellfish, birds, and mammals. Seagrasses act as ecosystem engineers by reducing flow velocities, filtering sediment out of the water column and preventing sediment resuspension, attenuating waves, and buffering the nearshore environment from the effects of storms (van der Heide et al. 2011). Seagrass meadows cycle nutrients, serving as sinks for organic carbon and also exporting organic carbon to adjacent ecosystems (Mateo et al. 2006). The value of ecosystem services provided by seagrasses has been estimated to be $34,000 per hectare per year (Short et al. 2011).

Like other coastal species, seagrasses are under increasing threats from natural and anthropogenic disturbance (Duarte 2002, Moore & Short 2006, Orth et al. 2006, Crain et al. 2009, Short et al. 2011). Not only has seagrass areal cover declined worldwide, the rate of loss is accelerating, and 29% of the known areal extent has been lost during the last 127 years (McKenzie et al. 2003, Waycott et al. 2009). Mechanical damage, eutrophication, salinity changes, increased siltation, seawater temperature rise, global climate change and the associated sea level rise, shoreline development, increased wave action and storms can all negatively impact seagrass extent and habitat function (Duarte 2002 and Orth et al. 2006 and references therein). Common consequences of these direct and indirect forces include physical disturbance of seagrasses, sedimentation, and decreased water quality and clarity. Shoreline development and increased wave action and storms increase sedimentation and turbidity, both of which cause greater light attenuation in the water column (Moore et al. 1997, Kirk 1994). Eutrophication may reduce water clarity by triggering increased phytoplankton abundance or decrease seagrass photosynthesis by increasing epiphyte loading on leaves (Ralph et al. 2006 and references therein).

Importance of Monitoring

Researchers have written about the importance of establishing monitoring programs to track changes in seagrass habitat (Short et al. 2006, Neckles et al. 2012). Recent attempts have been made to standardize monitoring in disparate locations in order to compare data across broad spatial scales (, Fourqurean et al 2003a, McKenzie et al. 2003, Short et al. 2006.). A recent publication in Ecological Applications (Fourqurean et al. 2003b) reported on a model predicting the change in abundance and distribution of seagrass species as a result of changes in freshwater flow into Florida Bay from the Everglades. Creation of this model was only possible because long-term monitoring programs for water quality and seagrass were in place and provided the necessary data. Taylor and Rasheed (2011) were able to examine short and longer term impacts of an oil spill event on intertidal seagrass meadows by comparing potentially impacted areas with existing monitoring data and control meadows outside the oil spill area. The study demonstrated the value of long-term monitoring of critical habitats in high risk areas to effectively assess impacts (Taylor & Rasheed 2011). Neckles et al. (2012) discussed the importance of a multi-tiered approach to seagrass monitoring to meet conservation needs in which large scale mapping was supplemented with broader quadrat-based assessments of percent cover and canopy height and high resolution measurements of shoot density and biomass. This multi-tiered approach can accommodate conservation objectives at different spatial scales. Large scale mapping such as aerial photograph interpretation is useful to quantify resource extent and distribution over large regions. Transect- or quadrat-based monitoring of a limited number of characteristics can be used to quantify stressor/response relationships and produce estimates of the ecological condition of resources over broad areas (Neckles et al. 2012). To develop predictive models and diagnose causal relationships, a third level of monitoring that addresses a greater number of properties at fewer locations or index sites is needed (Neckles et al. 2012). Statistical and explanatory models built on higher resolution data can be used to interpret and predict resource patterns and condition at larger scales (Bricker and Ruggiero 1998).

Seagrass in Elkhorn Slough, CA

One of the most widespread seagrass species is eelgrass, *Zostera marina* L, which grows in shallow, protected waters, rooted in unconsolidated sediments. Plants can spread through vegetative rhizomatous expansion and sexual seed production. Under optimal conditions, eelgrass can form extensive monospecific meadows and can be a dominant organism in the estuary, functioning as an ecosystem engineer and providing many important ecosystem services (Hemminga & Duarte 2000; Moore & Short 2006; Bos et al. 2007). Because of the important ecosystem services eelgrass provides, *Zostera marina* has been selected as one of 6 key species to inform on-going restoration planning and adaptive management at Elkhorn Slough National Estuarine Research Reserve.

Eelgrass beds in Elkhorn Slough, CA provide nursery habitat for many fish species including California halibut, Pacific herring, northern anchovy, starry flounder, pipefish, several species of rockfish, surfperch, sticklebacks, and sole (Yoklavich et al. 2002, Brown 2006, Grant 2009), and support invertebrates such as nudibranchs, bivalves, gastropods, decapods and other crustaceans (Phillips 1984, Wasson et al. 2002, Grant 2009). Eelgrass beds on this coast have been shown to increase infaunal (Ferraro & Cole 2007, Hammerstrom, unpublished data) and epifaunal (Reed & Hovel 2006) invertebrate diversity in estuaries. Vertebrates including Brandt and sea otters use eelgrass beds as a food source and as habitat (Harvey & Connors 2002).

The earliest surveys of the Elkhorn Slough estuary indicated that eelgrass was plentiful in the lower slough in the 1930s (MacGinitie 1935). In 1947 a permanent harbor mouth was created in Moss Landing to accommodate commercial ship traffic (Browning et al. 1972). In the decades after the harbor mouth was created eelgrass largely disappeared from Bennett Slough and the main channel of Elkhorn Slough (Van Dyke & Wasson 2005). In the 1980s a shoal formed in the Seal Bend region of Elkhorn Slough and was colonized by eelgrass. The shoal expanded in 1995 when flooding in the Pajaro River added a tremendous amount of sediment into the slough, and eelgrass cover also expanded. Since that time eelgrass cover has continued to increase (Van Dyke, unpublished data).

Factors Affecting Abundance and Distribution of Eelgrass in Elkhorn Slough.

Many factors have influenced abundance and distribution of eelgrass in Elkhorn Slough. These factors were explored in an Elkhorn Slough Technical Report (Palacios 2010) and are summarized here. Tidal exchange is currently restricted in about one third of the estuarine habitat in Elkhorn Slough and no eelgrass is present in these modified areas, even in areas in which eelgrass was found historically. Water quality in the slough has decreased as a result of increased human land use. High concentrations of nutrients may have contributed to increased macroalgal abundance, which may in turn block light penetration or smother eelgrass shoots. Increased nutrient loading has also increased phytoplankton densities, which results in decreased water quality and clarity. There has been a net loss of eelgrass habitat due to erosion caused by construction and maintenance of the harbor mouth and possibly exacerbated by the reopening of Parsons slough to tidal flooding. There is active erosion of sediment on the channel-ward edges of the eelgrass bed at Seal Bend, which leaves exposed rhizomes extending out into the water column (Hammerstrom & Grant, pers. obs.).

The Elkhorn Slough estuary will continue to change over time, due to intentional management actions such as restoration projects, and due to unintentional consequences of agricultural nutrient loading or climate change. Monitoring the response of key estuarine species, including eelgrass, to these changes is a critical component of adaptive management of the estuary. For example, the Elkhorn Slough National Estuarine Research Reserve (ESNERR) recently completed construction of a water control structure at the entrance to the Parsons Slough complex to restore more historical hydrologic conditions. This sill will slightly decrease both the tidal currents and the tidal prism of the main channel of Elkhorn Slough. It is difficult to predict the effect these changes will have on eelgrass populations because multiple environmental variables including water temperature, velocity, and water quality may change as a result of modifications to current hydrologic conditions. Compounding this, very little is known about the eelgrass population in Elkhorn Slough. Researchers at the ESNERR have used aerial photography to describe the historical and current distribution of eelgrass (Van Dyke, unpublished data) and field surveys conducted in 2000 reported average shoot densities in the Seal Bend bed (Palacios & Zimmerman 2001). To our knowledge, however, no work has been completed which describes ecological characteristics of the eelgrass community. Given the strong likelihood of alteration in the habitat currently supporting eelgrass, it is imperative to establish a baseline of current conditions so that we can track changes to inform management decisions in the future. To date there have been three survey events: August 2010, January 2011, and August 2011.

The primary objectives of this vegetation monitoring were:
a) To characterize the eelgrass community in Elkhorn Slough in two areas with different water depth, proximity to Monterey Bay, current regime, and sediment composition, which span the range of conditions in which eelgrass currently grows within the slough.
b) To establish a baseline of eelgrass characteristics against which we can monitor future change due to changes in hydrographic conditions caused by human intervention and climate change.
c) To establish a long-term SAV monitoring program at Elkhorn Slough to advance efforts in the establishment of Elkhorn Slough as a NERR sentinel site for climate change.
d) To promote the use of long-term monitoring data to manage and protect Elkhorn Slough eelgrass beds as well as to encourage the use of the data for educational and stewardship purposes.

Methods

Site Description

Zostera marina forms monospecific beds in Elkhorn Slough. The Vierra site is approximately 900 m from Monterey Bay, while the Seal Bend site is approximately 2.2 km from Monterey Bay (Figure 1). Due to proximity to Monterey Bay and position in the channel, currents at Vierra are much stronger than those at Seal Bend (Breaker & Broenkow 2005). Because of the tidal exchange with Monterey Bay, both sites are commonly exposed to oceanic salinity of 33 – 35 ppt. There are seasonal pulses of freshwater that enter the slough and result in short-lived salinities of 10 to 20 ppt (data from Monterey Bay Aquarium Research Institute's Land/Ocean Biogeochemical Observatory). These pulses tend to occur during the rainy season, from November to March each year. Water temperatures range from 10 to 22 ^{o}C, with an average temperature of approximately 13.5 ^{o}C (data from Monterey Bay Aquarium Research Institute's Land/Ocean Biogeochemical Observatory). Nutrient loads in Elkhorn Slough are high year-round, resulting in highly eutrophic wetlands (Hughes et al. 2010). Agricultural runoff enters from the Elkhorn Slough and Lower Salinas Valley watersheds, adding nitrate and phosphate to the slough system (Hughes et al. 2010).

In the Seal Bend site, eelgrass forms patchy beds interspersed with bare areas of up to 10 m across in water depths of -0.5 to -2.0 m below MLLW (Hammerstrom & Grant, pers. obs.). Occurring on the fringes of the eelgrass in shallow water adjacent to the shoreward edge of the bed are large expanses of *Ulva* sp. The *Ulva* occurs in two basic forms: one form consists of large blades that may be layered upon each other, while the other form consists of aggregations of fragmented and senescent blades. In both cases the sediment underlying the *Ulva* is usually black, fine-grained, and anoxic, smelling strongly of hydrogen sulfide. Small patches of *Ulva* may also be found interspersed in the eelgrass beds with the *Zostera* shoots growing up through the *Ulva*. Occasional small aggregations of red algae from the family Gracilariaceae may also be found intermixed with eelgrass. Sediment at Seal Bend is fine-grained and high in organic content (Hammerstrom, pers. obs.).

At the Vierra site, eelgrass is found in depths of -0.5 to -3 m below MLLW. Eelgrass is more sparse but evenly distributed with fewer bare patches than occur at the Seal Bend site. In shallow water near the shore small patches (< 0.5 m across) of fleshy red Gracilariaceae and *Sarcodiotheca gaudichaudii* sometimes occur. *Ulva* sp. may also occur but in much smaller aggregations than are found at Seal Bend. Sediment is coarser, with lots of shell fragments and little organic detritus (Hammerstrom & Grant, pers. obs.).

Monitoring Protocol

Submerged aquatic vegetation monitoring was conducted following the NERRS SWMP Bio-Monitoring protocol (Moore 2009). The NERRS SWMP protocol is for non-destructive annual sampling of percent cover, shoot density, and canopy height for each species present in each sampling plot along established transects. Our original intention was to structure the monitoring according to the SeagrassNet protocol because it samples for biomass and sediment grain size in addition to the metrics mentioned above and we thought that data collected in the SeagrassNet protocol would fulfill both monitoring programs. However, we were directed to use the NERRS protocol for our sampling design to allow direct comparisons with other NERRs and encouraged to supplement the SWMP protocol with SeagrassNet sampling. Once we began work we realized we did not have the time or resources to sample both protocols so we concentrated our efforts on the NERRS protocol.

We collected data in addition to that called for by the NERRS protocol. Our intent was to create a comprehensive baseline of life history and community data against which future change could be measured. To capture the range of conditions in which eelgrass exists in Elkhorn Slough, we chose two sites for monitoring (Figure 1). In the final design, five transects were established at each site with multiple plots along each transect. Transects were oriented perpendicular to the edge of the bed and endpoints at the deeper, channel-ward edge were determined by examining aerial photographs of the beds. In order to achieve good spatial coverage, transects were spaced approximately 100-150 m apart at Seal Bend (Figure 2) and 50-75 m apart at Vierra (Figure 3).

At Seal Bend individual sampling plots were spaced at 10 m intervals while at Vierra plots were spaced at 16.5 m intervals. Transects begin at the edge of the eelgrass bed closest to the central channel in Elkhorn Slough and extend up into the intertidal at an elevation approximately equal to MLLW. Transect lengths range from 90 to 190 m. Earlier sampling designs were comprised of fewer transects and plots (Appendix 1). The final design was adopted to ensure adequate macroalgal sampling and transects can easily be extended into the marsh should the emergent vegetation be sampled in the future. In order to maintain consistency, plot locations from the earlier sampling design were included in the final design.

During our first survey we used differential GPS to navigate to each plot and marked all plots with buoys. Once at the plot, a diver inserted a PVC pole to mark the location for future surveys. We quickly discovered that marking individual plots required multiple short dives that used up bottom time inefficiently. Additionally, our permanent transect poles were disturbed by sea otters and seals and difficult to relocate in low visibility water. In subsequent surveys we therefore used a differential GPS to locate transect end and midpoints for each survey and temporarily marked the end points with tall PVC poles. We then ran transect tapes along the length of each transect and used the tapes to swim along the bottom from plot to plot while using SCUBA, referring back to our datasheets to ensure that proper distance intervals were travelled between plots. While swimming transects we often came across previously unlocated PVC poles, so we are confident that our methods enable us to resample fairly precisely. While possibly not resampling the exact same location each time, if we were careful during transect tape deployment we could get within a meter of the original plot as located with differential GPS.

At each permanent data plot, we collected data using a 50 cm x 50 cm quadrat placed 1 m to the east of the transect tape. If eelgrass was present in the plot, the leaves were carefully lifted so that the quadrat could be worked down to the surface of the sediment. Time and water depth were recorded from a diving watch and a SCUBA depth gauge. We visually estimated percent cover of SAV within the quadrat for each macrophyte species present. We then counted all eelgrass shoots present within the quadrat. Canopy height was measured to the nearest 0.5 cm using a fabric measuring tape by gathering up all the leaves and extending the longest leaf next to the tape. The number of flowering shoots in the quadrat was recorded. Notes were made on the presence of grazing and grazers and the presence of *Labyrinthula zosterae*, eelgrass wasting disease. In several instances visibility was so poor that percent cover of eelgrass was not estimated. In those cases, "NA" was entered into the dataset for percent cover, although quantitative values are still given for shoot density as these counts were done by feel. At the transects' channel-ward endpoints we attached a new transect tape and swam parallel to the transect out into the channel to the farthest extent of eelgrass and noted that distance on the datasheet. We also measured and noted distance from selected midpoints of the two outermost transects for each site to the perpendicular edge of the eelgrass bed. These measurements will allow us to track bed expansion and contraction over time.

For each survey and site, we randomly chose to destructively sample either even or odd plots. Once the determination was made by coin toss, a second diver was assigned the task of sampling either all even or all odd plots for eelgrass and macroalgal biomass. Biomass was collected approximately 1 m to the west of the transect tape so that destructive sampling did not occur within the data plot where counts were made. We sampled eelgrass biomass by haphazardly collecting one terminal adult vegetative shoot and its associated belowground rhizome for each plot (SeagrassNet 2002). Macroalgal biomass samples were collected by placing a 25 cm x 25 cm quadrat approximately 1 m west of the transect tape and placing all macroalgae present in the quadrat into a fine mesh bag. Macroalgae and eelgrass were rinsed in fresh water and frozen. Prior to analysis samples were thawed. We standardized the eelgrass samples by processing 7 cm of rhizome material contiguous to the terminal shoot. Samples were separated into leaves, stems, and roots and rhizomes following the SeagrassNet protocol, dried to a consistent weight in a dehydrator, and weighed to the nearest 0.0001g.

To characterize sediment grain size in each seagrass bed we collected one or two 4 cm diameter sediment cores per transect during each survey. In addition, we collected one or two 10 cm diameter sediment cores per transect to estimate eelgrass seed bank density. Each seed bank core was sieved over a 0.5 mm screen and all residue was stored in a plastic bag. Sediment samples and residues were held at 3^{o}C until processing was completed. Seed core residues were examined under a dissecting scope at 4 -10 x and all seeds were counted. Particle size analyses were carried out with a Beckman-Coulter LS 13 320 laser particle size analyzer with an aqueous module equipped with a pump and a built-in ultrasound unit. This module analyzes very small (~1 g) amounts of sediments and the measured size distributions ranges from 0.04 µm to 2 mm. Measurements of such a wide particle size range are possible because the particle sizer equipped with the aqueous module combines conventional laser beam diffraction with polarized intensity differential scatter (PIDS), which measures particles between 0.4 and 0.04 µm (Beckman Coulter Inc. 2003). A total of 32 grain size samples were analyzed and 17 seed bank cores were processed.

Data on macroalgal cover were not collected during the August 2010 and January 2011 surveys. After feedback from other NERRs we incorporated macroalgal sampling into our protocol. For the third survey we collected percent cover data on all macroalgal species present in our sampling plots. In many cases species level identifications required the use of a microscope and the presence of reproductive structures. We therefore identified macroalgae to the lowest possible taxon while in the field.

All surveys were conducted using SCUBA. At each plot we recorded time as well as water depth in feet using SCUBA gauges. Water depths were then converted to metric units and corrected for tidal height relative to MLLW using tide tables for the closest available tide station. These corrected depths are presented as elevations in cm in the dataset. We have found, however, that these elevation data are unreliable. Because the water surface was rarely completely calm, water depths so shallow, and our SCUBA depth gauge measurements so coarse, there was a lot of error associated with the elevation values. For presentation purposes we extracted elevation data along each transect from the bathymetry recently completed by the Seafloor Mapping Laboratory at CSUMB. While these data are more reliable than the data we generated using SCUBA depth gauges, communications with staff at the Seafloor Mapping Lab revealed that the vertical resolution of the data is approximately ± 10 cm and that the presence of eelgrass may have caused some regions of bathymetry to read as higher in elevation than they actually are. Bathymetric data must therefore be interpreted with caution.

We also attempted to measure relative differences in light intensity within and between sites using HOBO Pendant® Loggers (Onset Computer Corp) but the loggers were dug up and disturbed by otters and seals present at the Seal Bend field site. We observed this disturbance while we were sampling and also found pits where our loggers had previously been deployed. Due to the expense of replacing data loggers and the loss of data when loggers could not be relocated we abandoned this data collection effort after the first survey.

Data Analysis

These surveys were primarily conducted to establish a baseline dataset of eelgrass community metrics in Elkhorn Slough. Data were first graphed to examine basic relationships of percent cover and shoot count as they varied along transects within a site. Prior to analysis all data were examined for normality and variance homogeneity; if necessary, square-root transformations were applied to make data meet assumptions. All analyses were conducted using the SAS software package (SAS 9.3).

We utilized 2 way ANOVA to describe the effect of site (a proxy for the various differences between sites mentioned above) and survey date (August 2010, January 2011, August 2011) on shoot density, percent cover of eelgrass, canopy height, above to belowground biomass ratio, aboveground biomass, belowground biomass, and total biomass of eelgrass shoots collected during our destructive sampling. Tukey honestly least significant difference test was used to conduct all post-hoc comparisons.

Results

Monitoring Protocol

Our protocol changed over time as we received feedback from NERRS staff and as we tested proposed methods in the field. Unlike many NERR settings the eelgrass beds in Elkhorn Slough are primarily subtidal, requiring that monitoring work be performed using SCUBA. Even in the low intertidal stretches of transects the sediment is often so soft that working on SCUBA is much easier than trying to walk from plot to plot. At the Seal Bend site in particular, low visibility meant that we started out doing a lot of bounce dives, diving to examine a plot but then having to return to the surface to swim to the next plot to collect data. The time for surface swimming, bounce dives and navigation to individual plots with a GPS was prohibitive and inefficient. Recognizing this, we reduced the number of plots in our second survey by removing two transects at Seal Bend. Feedback from Ken Moore prompted us to add a fourth transect of 5 plots at the Vierra site. Concern about capturing variability and presence of macroalgae drove the extension of transects at both sites into shallower water. Thus the first and second surveys differ from the third in transect and plot number (Appendix 1).

At both sites, and in fact throughout Elkhorn Slough the deepest extent of the eelgrass is difficult to delineate using aerial photography because of water depth and poor visibility. However, we determined transect endpoint positions by examining aerial photos with the intention of measuring extent of eelgrass beyond each transect endpoint using a transect tape, following the methods outlined in the SeagrassNet protocol. In this way we thought we would be able to track bed expansion and contraction. In hindsight, another alternative is to extend transects further into deeper water. While our measurements enable us to detect presence or absence of eelgrass at the edges of the bed, our data don't reflect anything about the condition of the edges of the bed (except in our field notes, but this is not quantitative). In future surveys a hybrid of the methods would be best: use of a transect tape to measure extent and quantitative data collection at the terminus.

Data Analysis

In general vegetative shoot density was higher at Seal Bend than Vierra and higher in winter than in summer (Table 1). Zeros in the dataset were infrequent and tended to occur at the shoreward edges of the beds. Flowering shoot density was highly variable and tended to be lower in winter than summer. Of 175 quadrats total, only 49 contained flowering shoots, anywhere from 1 to 6 in the 0.25 m^2 area, and only 2 times were flowering shoots noted during the January 2011 survey.

Seed density was almost four times higher in Seal Bend sediments than in Vierra sediments (Table 1). In addition, the seed bank was less patchy in Seal Bend, with 87% of seed cores containing seeds versus only 66% of Vierra cores containing seeds. Seed density cores were collected as time permitted rather than systematically so all data were averaged by site regardless of date collected.

Both grazing and wasting disease were present at each site (Table 2). It appears that both were more widespread at Seal Bend than at Vierra, with grazing almost twice as frequent and wasting disease (*Labyrinthula zosterae)* three or more times as frequent. Wasting disease presence was generally limited to infections around grazing scars and did not appear to be causing whole leaves to blacken and die as has been reported elsewhere. Although we do not have quantitative data on which grazers were most active, our field observations are that the isopod *Idotea resecata* is the primary grazer at Seal Bend, while at Vierra we found mostly the gastropod *Alia carinata*, although the later may be more of a scavenger than a true grazer. We saw very few, if any *Idotea* at Vierra and no *Alia* at Seal Bend.

Our most extensive survey was in August 2011 and those data are presented in Figures 4 and 5. Two other pieces of data other than eelgrass and macroalgal cover are presented: 1) bathymetry data from CSUMB's Seafloor Mapping Laboratory latest maps of Elkhorn Slough (black line that changes with distance from shore); and 2) furthest measured extent of eelgrass from the transect channel end point (green vertical line). For reference there is also a horizontal dashed line delineating MLLW. Immediately apparent is the lack of overlap in presence of eelgrass and macroalgae, possibly due to niche partitioning.

In over 100 plots examined, eelgrass and macroalgae co-occurred only 3 times, once at Seal Bend (transect D) and twice at Vierra (transects C and E). We observed this throughout our surveys, that macroalgae occurred in bare patches in the eelgrass beds or at the fringes of the beds, rather than co-occurring within a plot of eelgrass. There appears to be a relationship of macroalgal presence and elevation, with macroalgae occurring at Seal Bend at higher elevations (mostly *Ulva* sp.) and macroalgae occurring at Vierra at lower elevations (mostly Gracilariaceae).

Two-way ANOVA was conducted testing the effect of site (Seal Bend vs. Vierra) and date (August 2010, January 2011, August 2011) on shoot density, percent cover of eelgrass, canopy height, and above to below ground biomass ratio (Figure 6). Results for shoot density and biomass ratio are presented in Table 3. Shoot density was significantly different among sites, but not survey date. In contrast, above to belowground biomass ratio was not significantly different among surveys or sites (overall model $p = 0.43$). Analyses for percent cover and canopy height, presented in the bottom panel of Table 3, both found significant interactions between survey date and site ($p = 0.0014$ and $p = 0.0041$, respectively).

In order to determine patterns in percent cover and canopy height, both data sets were examined using one-way ANOVA to test effects of survey date within each site individually. Those results are presented in Table 4. Percent cover was significantly affected by survey date at both Seal Bend and Vierra ($p < 0.0001$ and $p = 0.006$, respectively). Pairwise comparisons revealed that at Seal Bend percent cover was significantly lower during the January 2011 survey than either of the two summer surveys, which were not different from each other. Similarly at Vierra, percent cover in the January 2011 survey was significantly lower than cover in the August 2011 survey, but all other pairwise comparisons were not significantly different. Canopy height was also significantly different among surveys. At Seal Bend canopy height in August 2011 was significantly greater than canopy height in January 2011 or August 2010, which were not different from each other. At Vierra, summer canopy heights were significantly greater than January 2011 canopy height, but not different from each other.

Aboveground biomass was significantly affected by both site and survey (Table 5, Figure 7). On a per-shoot basis, Seal Bend shoots were significantly more robust than Vierra shoots. Aboveground biomass was significantly greater in the summer surveys than the winter survey, but summer values were not different from each other. The same patterns held true for total biomass, probably driven by the fact that proportionally, aboveground biomass was about an order of magnitude greater than belowground biomass (Table 5, Figure 6). There were no differences in belowground biomass among survey dates ($p = 0.1755$, Table 5), but Seal Bend shoots had significantly more belowground biomass than Vierra shoots ($p = 0.0026$, Table 5).

We observed marked differences in sediment grain characteristics between the two sites. Deployment of ground anchors and collection of biomass samples was much more difficult at Vierra than at Seal Bend. At first glance our grain size analysis results do not appear to support our field observations. Although mean percent sand was slightly lower in Vierra sediment than Seal Bend sediment (Figure 7, top panel), this result is due to three outlier samples with very small particle sizes. If we exclude those outliers the sand fraction at Vierra increases 61%. This grain size variability is reflected in the comparison between mean and median grain size (Figure 7, bottom panel). Mean and median grain size were very similar at Seal Bend but less similar at Vierra due to the more poorly sorted sediments occurring at Vierra. We did not measure sediment organic content but it is probably greater at Seal Bend where grain size is smaller and the percent of small-sized fractions is greater (and even more so when Vierra outliers are removed: 11% clay and 39% silt at Vierra versus 14% clay and 57% silt at Seal Band).

Discussion

Data collected during three eelgrass surveys allowed us to provide a thorough characterization of eelgrass habitat in Elkhorn Slough. In addition to shoot density, percent cover, and canopy height, macrophyte parameters required by the NERRS monitoring protocol, we also measured flowering shoot density, above and belowground biomass, seed bank density of eelgrass and sediment grain size. We also noted presence of grazing and wasting disease in each plot. Prior to these surveys, our knowledge of Elkhorn Slough eelgrass was limited to annual interpretation of location and size of beds delineated via aerial photography and a single survey of vegetative shoot density carried out in 2000. Remote sensing has allowed us to track changes in eelgrass abundance and distribution over broad spatial scales but information gleaned through aerial photography cannot tell us about stressor/response relationships or give us estimates of the ecological condition of eelgrass habitat. For higher resolution information of ecosystem properties and for diagnosing causal relationships we need a ground-based approach (Short et al. 2006, Neckles et al. 2012).

Intensive monitoring is needed to understand the mechanisms and causes of change detected at larger scales. In some cases intensive monitoring may highlight changes in habitat quality before these changes can be detected over greater spatial scales. In a series of case studies, Short et al. (2006) determined that quarterly monitoring over 2-4 years revealed declines in cover, biomass, and density, even though areal coverage remained the same at several sites. Hands-on monitoring enabled researchers to explain declines due to reasons such as waterfowl foraging, eutrophication, development impacts, and a shift to shallower water (Short et al. 2006). In one case managers were able to respond with waterfowl population control policies designed to minimize impacts. In another, researchers are now testing various hypotheses to explain change related to a depth shift, possibly an indirect effect of successful marine protected area management (Short et al. 2006). This early detection of change is essential if managers are to make policy changes to halt and reverse degradation of valuable habitat before coastal ecosystems experience irreparable loss. Conversely, intensive monitoring may inform management that no action is needed even when declines in resources are observed. For example, declines in seagrass abundance were linked to an oil spill in Australia (Taylor & Rasheed 2011). In this study examination of long-term monitoring data demonstrated that an observed decrease in biomass was likely due to natural interannual variation, and subsequent recovery of biomass to pre-spill levels was observed in both control and impacted sites without intervention or mitigation (Taylor & Rasheed 2011).

We observed resource partitioning between seagrasses and macroalgae at both sites. At Seal Bend almost all of the macroalgae was *Ulva* sp. and it was usually found in higher elevations, between the eelgrass bed and the intertidal mudflat (Figure 4). This pattern was more pronounced on the western side of the bed, in transects A, B, and C. On the eastern side of the bed, there was some *Ulva* in the middle and deeper ends of transects but the apparent lack of co-occurrence of algae and eelgrass persisted. In contrast, we found no *Ulva* at Vierra, but instead a fleshy red in the family Gracilariaceae dominated the macroalgal assemblage. The algae were again most prevalent on the shallow edge of the eelgrass bed although not necessarily at higher elevations, as was the case in Seal Bend. The shoreline is erosional on the south bank of Elkhorn Slough where the Vierra bed occurs and there is very little mudflat or intertidal zone. If the slightly higher elevation in the eelgrass transects from 40 to 130 m from shore is not an artifact of the bathymetry data, it may be that the red algae are occurring in slightly deeper water depths, occupying an area with light levels too low to support eelgrass. We did not test the reasons for the distribution of macroalgae but presumably it is driven by a combination of light, desiccation and hydrodynamics. At both sites it appears that in optimal habitat, eelgrass is able to outcompete macroalgae, at least at the levels of macroalgal biomass we observed.

Seagrass above to belowground biomass (AG/BG) ratios are highly variable and range anywhere between about 0.15 and 7 (Fourqurean & Zieman 1991, Hemminga et al. 1994, Dunton 1996, Hemminga 1998). In general, though, ratios tend to be around 1, meaning there is a balanced distribution of biomass between above and belowground components (Duarte & Chiscano 1999). Belowground tissues, in addition to anchoring seagrasses, also serve a resource storage function. But roots and rhizomes are heterotrophic, thus they depend exclusively on aboveground leaves for energy and oxygen (Zimmerman & Alberte 1996). Light attenuation is often the primary factor regulating seagrass productivity and biomass distribution (Mateo et al. 2006) and high ratios of above to belowground biomass can be indicative of light regimes that are less than optimal. Less light reaching the eelgrass means less oxygen for belowground tissue respiration, resulting in the build-up of sulfides and ammonium, toxic to seagrasses at high concentrations (Mateo et al. 2006). In a related species, *Zostera noltii*, belowground production was increased in plants with high AG/BG ratios and maximum growth at low light was recorded in plants with low AG/BG ratios (Olivé et al. 2007). Furthermore, *Z. noltii* in factorial experiments responded to low light with high AG/BG ratios and only allocated more production to belowground roots and rhizomes at high current velocity when light was saturating (de los Santos et al. 2010). Plants at both Elkhorn Slough sites exhibit a high AG/BG ratio which appears to be consistent over time, indicating that pressures acting on the eelgrass are also fairly consistent over time rather than seasonal. At the Vierra site we sometimes observed exposed rhizomes within the bed, while at the Seal Bend site there is a permanent erosional feature at the southern edge of the bed. If water quality declines caused a further decrease in light attenuation, eelgrass might respond by allocating even more productivity to aboveground photosynthetic tissues. Yet the proportion of belowground tissue is already low, meaning that anchoring and carbohydrate storage capacities would be further reduced and the beds' ability to respond to stochastic events such as storms might be compromised.

In general, eelgrass appears to be growing in shallower water/higher elevations at Seal Bend when compared to Vierra (Figure 4, 5). Most eelgrass is found at or slightly below MLLW at Seal Bend, while at Vierra most eelgrass occurs at -1 to -2 m below MLLW. Our field observations support this and suggest that two factors may be contributing. The first is light. Visibility is almost always better at the Vierra bed than at the Seal Bend bed, likely due to the closer proximity to Monterey Bay and the clear water delivered to Elkhorn Slough with each incoming tide. Sediment grain size is larger at Vierra and therefore sediment is less likely to be resuspended and cause poor visibility. The second factor contributing to eelgrass bed elevation differences is erosion. At Vierra, there is very little substrate at MLLW as this is where the water depth changes most dramatically due to the erosion on the shoreline. At Seal Bend eelgrass grows right to and over the erosional face of the channel. There is a sharp-drop off at the edge of the bed in about -1 m below MLLW, with rhizomes extending out into the water column where the sediment has been eroded out from under the root-rhizome matrix. This erosional interface was present at each survey we conducted and appears to be a permanent feature. The extent of eelgrass at Seal Bend (vertical green line) appears to correspond with the erosional face of the channel (black horizontal line) in Figure 4, suggesting that perhaps eelgrass would extend further into the channel if the bathymetry changed more gradually.

Eelgrass shoot density in Elkhorn Slough ranges from a mean of 74.2 shoots m^{-2} at Vierra in August 2011 to a mean of 188.9 shoots m^{-2} at Seal Bend in January 2011. These values are similar to the mean shoot density measured in April 2000 of 110 shoots m^{-2} (Palacios, unpublished data) and much higher than the mean of 56.2 shoots m^{-2} found in a small eelgrass bed in Moss Landing Harbor in 2009. In contrast, eelgrass shoot density in San Francisco Bay ranged from 5 to 80 shoots m^{-2} in different beds throughout the central and south bays in 2006-2008 (Boyer & Wyllie-Echeverria 2010). In Morro Bay eelgrass shoot densities ranged from 33.2 to 82.5 shoots m^{-2} in 2007, but values have been steadily declining since then and eelgrass cover was essentially zero in 2011(Kitajima & Gillespie 2007, A. Gillespie, pers. comm.). Eelgrass beds in other west coast locations also show quite a bit of variability. Thom et al. (2003) reported values ranging from less than 10 to over 100 shoots m^{-2} at six sites in Willapa Bay, WA and from 40 to 200 shoots m^{-2} at four sites in Coos Bay, OR. All these sources have reported remarkable variability from year to year, bed to bed and even from transect to transect within a bed, variability which is reflected in Elkhorn Slough as well. It will take several more years of data to establish if shoot density in Elkhorn Slough is increasing, declining, or remaining stable over time.

Flowering densities ranged from 1 to 6 per 0.25 m^2 quadrat, or 4 to 24 m^{-2} where they occurred, although flowers were patchily distributed. Flowering shoot density was higher in August 2010 and August 2011 than in January 2011. In fact, flowers were present in 25 to 71% of quadrats in the summer surveys and only a single flowering shoot was found at each site in January 2011. This is not surprising since flowering tends to be highest in the summer months (Thom 1990). Our flowering shoot densities were very similar to densities in San Francisco Bay (Boyer & Wyllie-Echeverria 2010) and Willapa Bay, WA and Coos Bay, OR (Thom et al. 2003).

It has long been assumed that vegetative reproduction is the primary means by which perennial seagrass beds are maintained, yet research on genetics and seedling recruitment have demonstrated that sexual reproduction may play a larger role than previously thought in bed persistence (Alberte et al. 1994, Muñiz-Salazar et al. 2006). Although it is difficult to track seedling recruitment in extant eelgrass beds, Boyer and Wyllie-Echeverria (2010) suggested that perennial beds in San Francisco might experience substantial recruitment from seed because of the similar levels of flowering density and genetic variability they observed in annual and perennial beds. Given that there is evidence of high genetic diversity in Elkhorn Slough eelgrass beds (Alberte et al. 1994) and similar rates of flowering compared to eelgrass beds in San Francisco Bay, beds in Elkhorn Slough may also be undergoing considerable recruitment from seed set. Our seed core data show that eelgrass seeds are being incorporated into the sediment seed bank in densities of approximately 100 to 400 seeds m^{-2}. In one of the only published reports of eelgrass seed banks, densities in *Zostera marina* beds in central Japan ranged from 5-21 seeds m^{-2} from a perennial bed to 200-1200 seeds m^{-2} in an annual bed (Morita et al. 2007), values roughly similar to those we observed in Elkhorn Slough. Although long distance dispersal of seeds is possible through the transport of flowering shoots on currents (Harwell & Orth 2002), experiments with broadcasting of seed demonstrate that seeds settle rapidly, dispersing only a few meters before being incorporated into the sediment (Orth et al. 1994). This may in part explain why we have rarely observed new patches and beds of eelgrass forming in the slough. Seeds tend to recruit closely to parent plants and flowering shoots entrained in the extreme tidal currents of Elkhorn Slough are likely to be rapidly exported into less favorable habitats.

Spatial cover of eelgrass was highest in summer surveys and lowest in the winter survey. This pattern is typical of temperate eelgrass beds and has also been found in Oregon (Rumrill & Sowers 2008) and New Jersey (Kennish et al. 2008). Unlike the Elkhorn Slough beds, the shoot densities in South Slough, OR beds followed a similar pattern to percent cover, with lowest shoot densities occurring in winter months (Rumrill & Sowers 2008). Thom et al. (2003) did not examine seasonal patterns of density but did note an increase in density and flowering that corresponded with a shift to warmer winters and cooler summers associated with the transition from El Niño to La Niña oceanic conditions during the study period. Because many of the closest California eelgrass beds are surveyed annually, we don't know how shoot densities changed with seasons.

In general, certain seasonal patterns are expected in temperate eelgrass beds. Percent cover, shoot density, canopy height, and biomass are all generally greater in summer months and lesser in winter months (Olesen & Sand-Jensen 1994, Hauxwell et al. 2006, Kennish et al. 2008, Rumrill & Sowers 2008). Our results followed the same pattern with the exception of lower shoot counts in the summer surveys and higher shoot counts in the winter survey. One possible explanation for this is self-thinning, or density-dependent mortality, whereby as the plants grow in size and biomass the population begins to drop shoots. This is contrary to supposition by Olesen & Sand-Jensen (1994), who predicted seasonal biomass variability to occur predominantly due to changes in shoot size. Of course we don't know how much of the observed pattern in our shoot densities is an actual pattern and how much is simply background variability. In December 2011 Brent Hughes recorded shoot density along the middle transect at each bed. Density remained about the same at Seal Bend (means $\pm$ standard errors: 126.3 $\pm$ 11.9 shoots m^{-2} in December 2011 versus 127.4 $\pm$ 14.4 shoots m^{-2} in August 2011) but increased at Vierra (142.0 $\pm$ 11.7 in December 2011 versus 74.2 $\pm$ 9.2 shoots m^{-2} in August 2011). Thus it is possible this is a real pattern, but only further surveys will enable us to distinguish potential patterns in seasonal shoot densities from variability inherent in the system.

Field-based monitoring data are essential to improve management of eelgrass habitat. As shown by Short et al. (2006), field-based monitoring of declining eelgrass canopy height and shoot density enabled researchers and managers to adopt a policy of waterfowl population control to mitigate effects of herbivory before declines were lethal. In another study, Taylor & Rasheed (2011) were able to demonstrate that short-term declines in aboveground biomass were due to natural seasonal variability rather than an affect of a local oil spill. Eight months post-spill, biomass and shoot density increased to be within historical ranges without action by managers (Taylor & Rasheed 2011). In both of these instances, analysis of detailed monitoring parameters enabled researchers to describe, explain, and track changes in seagrass habitat, a process that would have been impossible had they relied solely on mapping from aerial photography. In Elkhorn Slough we have demonstrated that although eelgrass beds have increased in size in recent years, biomass partitioning indicates that light limitation is negatively impacting plants. Variability in vegetative and flowering shoot density and percent cover may be seasonal but more surveys are necessary before we will know if eelgrass populations are stable or changing.

Recommendations for future monitoring

Our primary objectives in these surveys of submerged vegetation were to characterize the eelgrass community in Elkhorn Slough, to establish a baseline of conditions against which we can measure future changes, to establish a long-term monitoring program at Elkhorn Slough, and to promote the use of long-term monitoring data to manage and protect Elkhorn Slough eelgrass beds. Our data have demonstrated a great deal of variability in the basic population and community characteristics of Elkhorn Slough eelgrass beds. More surveys, preferably seasonal surveys, are necessary to distinguish natural seasonal and annual variability from changes that might occur due to altered hydrodynamics, nutrient regimes, sea level rise or climate change (see discussion of oil spill impacts above). Examples from the literature have demonstrated that field-based monitoring enables researchers to establish causal relationships between stressors and eelgrass response variables and to create predictive models that can be used to inform management decisions. In the case of Elkhorn Slough eelgrass beds, above to belowground biomass ratios are very high, meaning that eelgrass is allocating more energy into production of photosynthetic tissue, a result of light limitation. If water quality and concomitant water clarity decline, eelgrass may not produce enough belowground rhizome material to withstand currents in Elkhorn Slough. Conversely, increased light availability could lead to a shift in biomass partitioning, an indication that ecosystem health is improving.

One piece of information that is still missing is the integration of remote sensing with field vegetation monitoring. Aerial photography can tell us something about the extent of eelgrass beds in Elkhorn Slough but without ground-truthing we don't know if current interpretation is correct, especially given that we know the beds are fringed with macroalgae. The same is true of other remote sensing methods such as multibeam bathymetry. Field vegetation monitoring can give us an idea of what is occurring at a selected group of sites. Ground-truthing would enable us to tie these two monitoring data sets together. The delay in georectifying and interpreting aerial photography has caused difficulty in ground-truthing to date. Perhaps working with additional partners (e.g., California State University's Seafloor Mapping Lab) to utilize sidescan sonar and quicker turnaround of sonar data interpretation would enable us to conduct ground-truthing surveys with a shorter lag time. Once an initial ground-truthing effort is completed, our ability to compare and integrate remote sensing and field vegetation monitoring efforts would be strengthened.

We have suggestions for additional work, were funds to become available. We suspect that several factors contributed to observed differences between the beds, including light, sediment organic and nutrient content, and eelgrass productivity. We attempted to measure relative light levels at various places in both beds but were unsuccessful in relocating most of our dataloggers. The loggers, while relatively inexpensive, also measure light in the visible spectrum rather than the photosynthetically active spectrum utilized in research and models to make predictions about restoration potential of proposed habitats. A series of light surveys measuring photosynthetically active radiation (PAR) would enable us to better characterize the range of habitat eelgrass occupies in Elkhorn Slough. In their 2010 report, Boyer and Wyllie-Echeverria mentioned limiting sediment organics to < 4% when choosing restoration sites. We do not currently know what the sediment organic content is in the eelgrass beds in Elkhorn Slough but with minimal effort we could gather these data and use them to inform future work. Sediment pore water nutrient concentrations might indicate whether or not the allocation of most biomass to aboveground tissues is a result of light attenuation and the subsequent nutrient enrichment that occurs. Finally, eelgrass productivity measures would be instrumental in fully characterizing the beds in Elkhorn Slough. An evaluation of growth dynamics and energetics of eelgrass, especially changes in biomass and productivity, is crucial to understanding the contribution of eelgrass to higher trophic levels in the slough.

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Table 1. Mean density and standard deviation of *Zostera marina* vegetative shoots, flowering shoots, and seeds. Seed cores were not collected systematically in time or space and so all data were averaged regardless of survey and sampling location within site.

	Seal Bend			Vierra		
	Aug 2010	Jan 2011	Aug 2011	Aug 2010	Jan 2011	Aug 2011
Vegetative Shoots	155.0 ± 19.0	188.9 ± 16.0	127.4 ± 14.4	97.6 ± 9.9	126.0 ± 10.3	74.2 ± 9.2
Flowering Shoots	1.4 ± 0.5	0.1 ± 0.1	2.8 ± 0.7	0.5 ± 0.4	0.2 ± 0.2	5.1 ± 1.1
Seeds		432.9 ± 130.4			113.2 ± 33.2	

Table 2. Percentage of *Zostera marina* plots in which we saw evidence of grazing and *Labyrinthula zosterae*, eelgrass wasting disease. We did not look for wasting disease in the August 2010 survey.

	Seal Bend			Vierra		
	Aug 2010	Jan 2011	Aug 2011	Aug 2010	Jan 2011	Aug 2011
Grazing	37 %	13 %	8 %	20 %	10 %	0 %
Wasting Disease	ND	43 %	93 %	ND	15 %	9 %

Table 3. Two-way ANOVA results testing the effect of site (Seal Bend vs. Vierra) and Survey Date (July 2010, January 2011, July 2011) on above to belowground (AG/BG) biomass ratio and shoot density (top panel) and percent cover and canopy height (bottom panel).

	Shoot Density				AG/BG Biomass Ratio			
	DF	MS	F	p-value	DF	MS	F	p-value
Model	5	74580	14.64	<0.0001	5	3.67	0.99	0.43
Site	1	360065	70.66	<0.0001	1	2.29	0.62	0.43
Survey Date	2	2025	0.40	0.67	2	7.63	2.06	0.13
Site x Date	2	6173	1.21	0.30	2	0.40	0.11	0.90

	Percent Cover				Canopy Height			
	DF	MS	F	p-value	DF	MS	F	p-value
Model	5	9730	29.6	<0.0001	5	0.55	22.0	<0.0001
Site	1	21470	65.3	<0.0001	1	0.06	2.36	0.13
Survey Date	2	11344	34.5	<0.0001	2	1.21	48.2	<0.0001
Site x Date	2	2247	6.83	0.0014	2	0.14	5.68	0.0041

Table 4. One-way ANOVA results testing the effect of survey date on percent cover and canopy height, analyzing sites separately because of a significant site x survey date interaction in the two-way ANOVAs.

	Seal Bend				Vierra			
	DF	MS	F	p-value	DF	MS	F	p-value
% Cover	2	11044	30.3	<0.0001	2	1524	5.57	0.006
Canopy Ht	2	0.65	22.7	<0.0001	2	0.70	37.1	<0.0001

Table 5. Results of two-way ANOVAs testing the effects of site (Seal Bend vs. Vierra) and survey date (August 2010, January 2011, August 2011) on shoot biomass.

	Aboveground Biomass				Belowground Biomass				Total Biomass			
	DF	MS	F	p-value	DF	MS	F	p-value	DF	MS	F	p-value
Model	5	3.01	7.09	<0.0001	5	0.14	3.49	0.0064	5	4.34	7.61	<0.0001
Site	1	8.12	19.12	<0.0001	1	0.38	9.61	0.0026	1	12.05	21.14	<0.0001
Survey Date	2	2.78	6.55	0.0023	2	0.07	1.78	0.1755	2	3.58	6.28	0.0029
Site x Date	2	0.68	1.61	0.2051	2	0.09	2.15	0.1231	2	1.24	2.19	0.1182

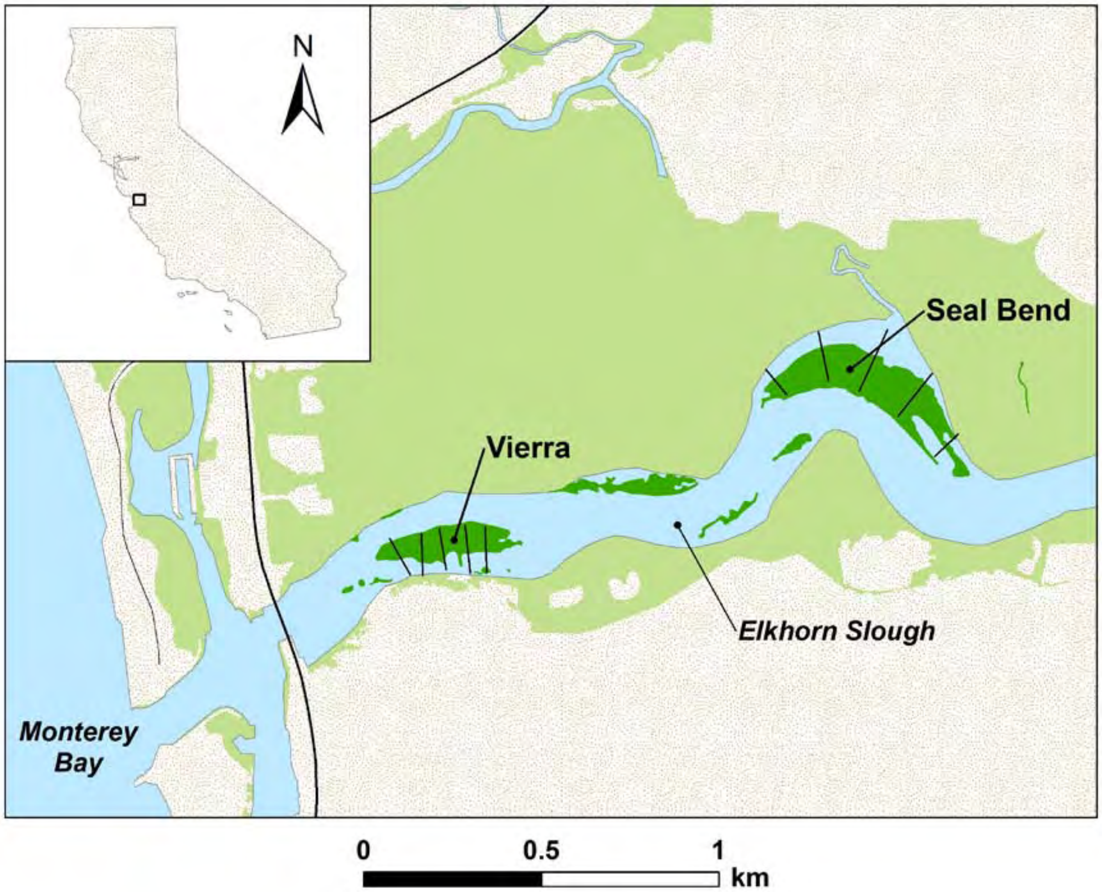


Figure 1. Map showing location of submerged aquatic vegetation transects established at two sites, Seal Bend and Vierra, in Elkhorn Slough. The eelgrass polygons (dark green) are taken from Eric Van Dyke's interpretation of aerial photographs from 2007-2009.

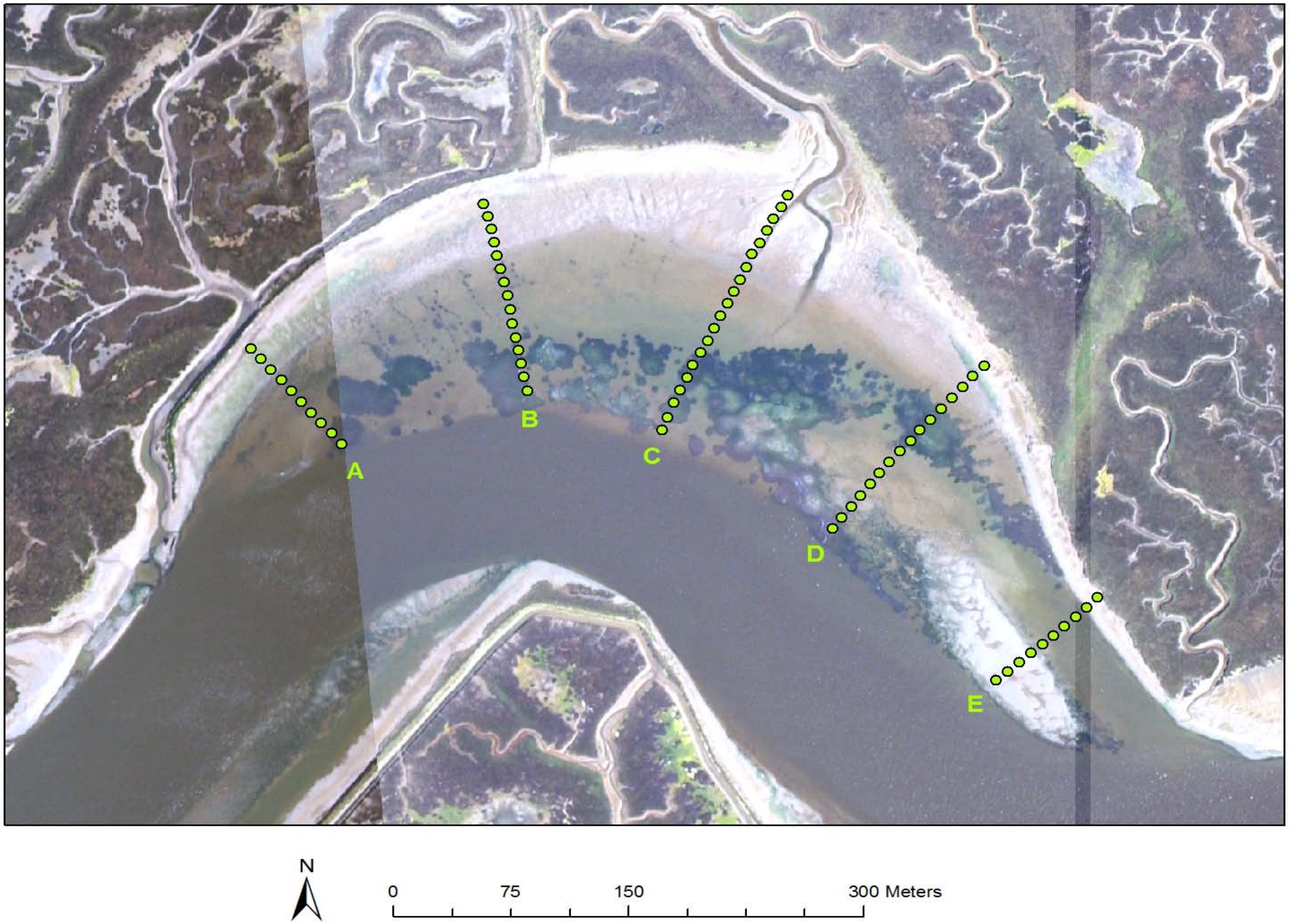


Figure 2. Seal Bend sampling site with permanent sampling plots marked along each of the five established transects.

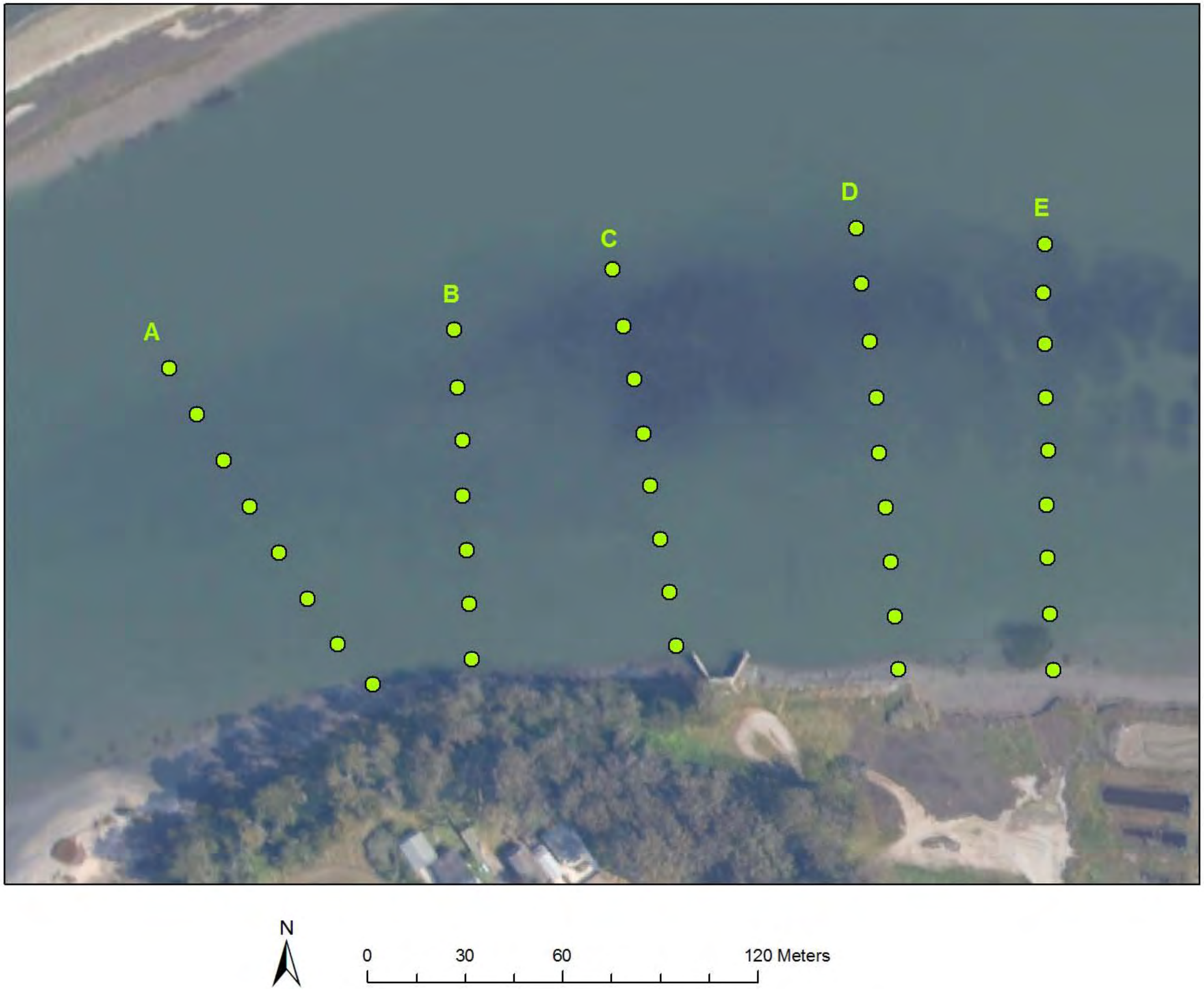


Figure 3. Vierra sampling site with permanent sampling plots marked along each of five established transects.

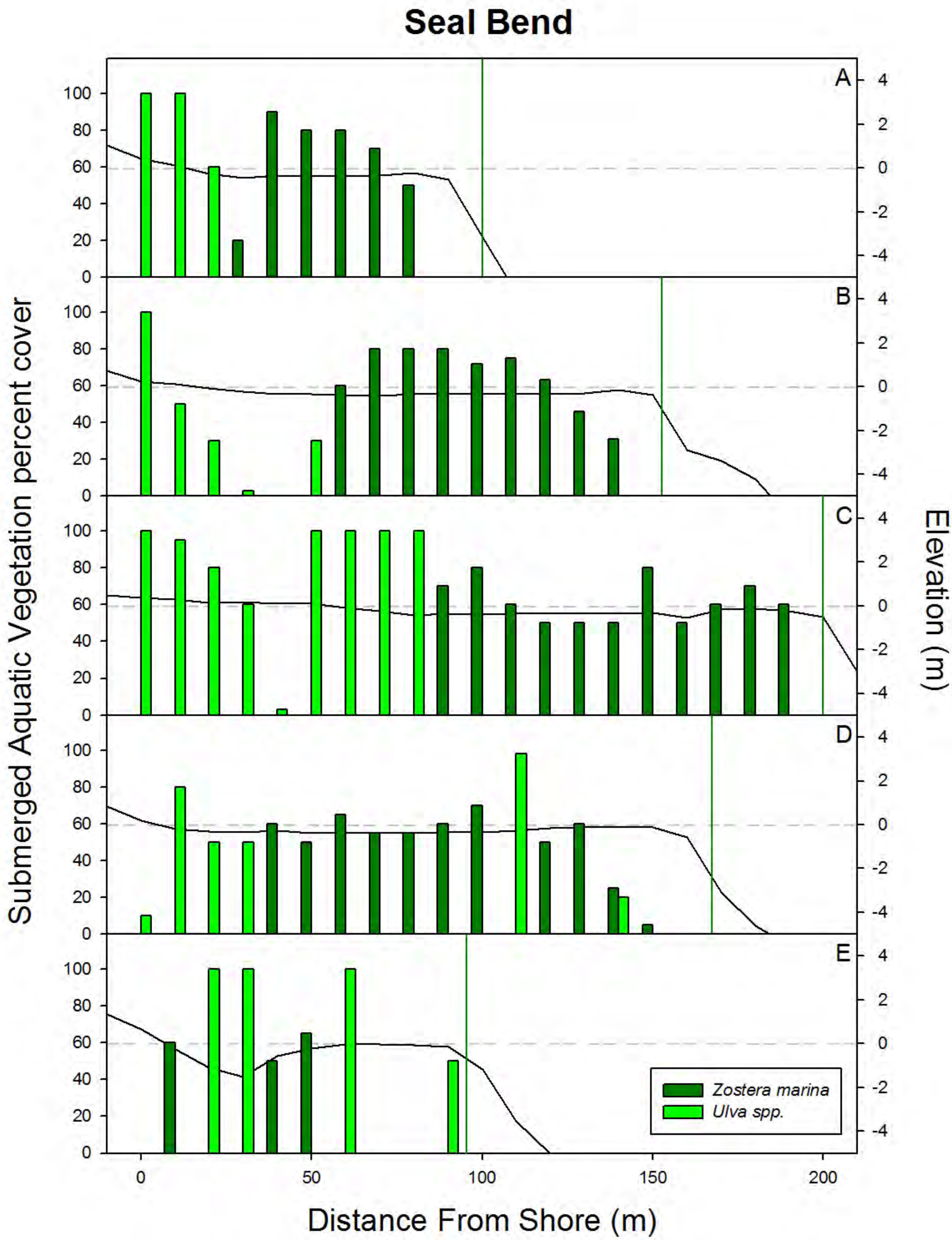


Figure 4. Percent cover of eelgrass, *Zostera marina*, and *Ulva* sp(p). in each sampling plot along the 5 permanent transects at Seal Bend. Data are from August 2011. Also depicted are the furthest extent of

eelgrass along each transect (green vertical line), MLLW (grey horizontal dashed line), and transect bathymetry (black horizontal line). Vertical exaggeration of bathymetry is approximately 6x.

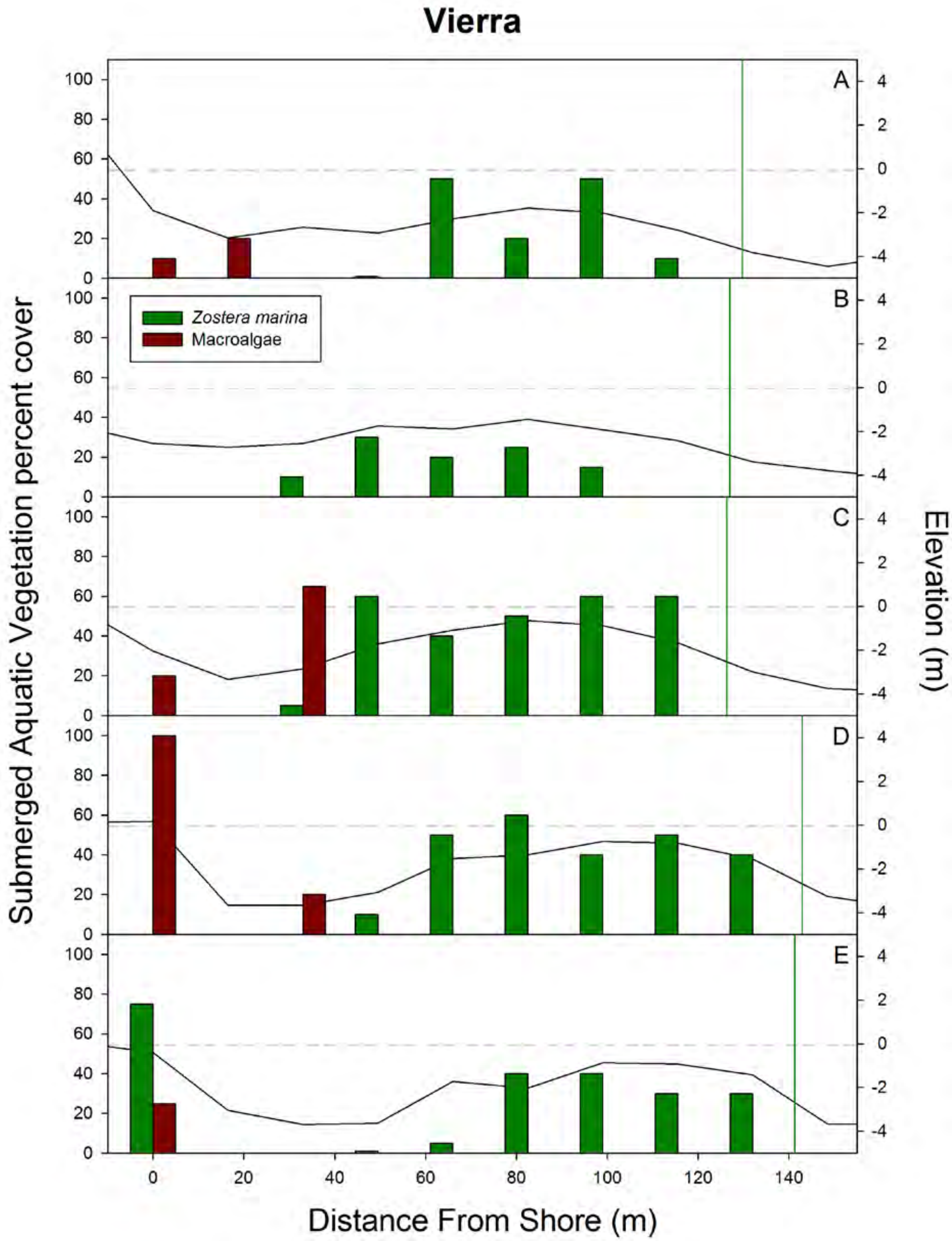


Figure 5. Percent cover of eelgrass, *Zostera marina*, and macroalgae, primarily Gracilariaceae, in each sampling plot along the 5 permanent transects at Vierra. Data are from August 2011. Also depicted are the furthest extent of eelgrass along each transect (green vertical line), MLLW (grey horizontal

dashed line), and transect bathymetry (black horizontal line). Vertical exaggeration of bathymetry is approximately 5x.

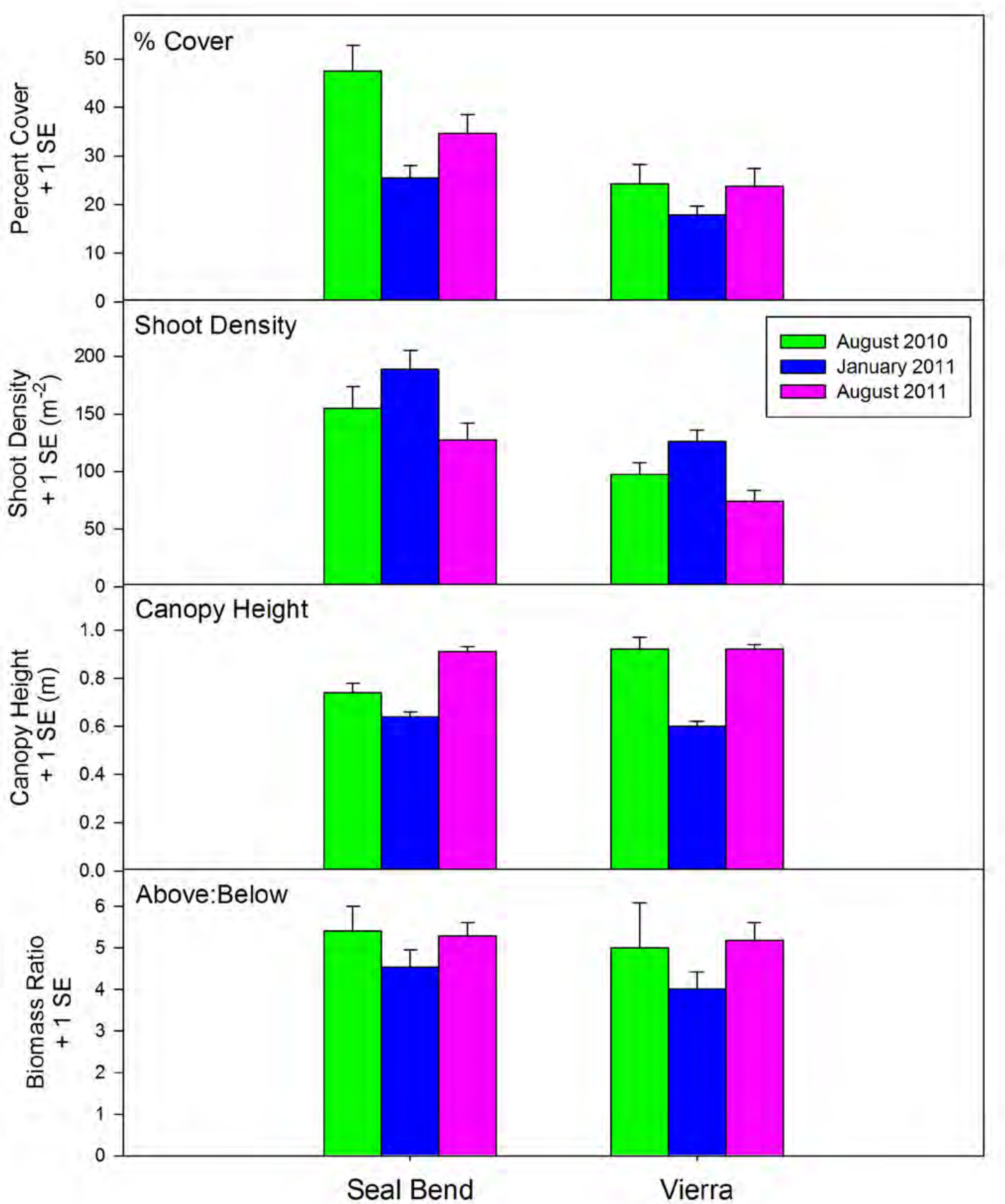


Figure 6. Means and standard errors of *Zostera marina* percent cover, vegetative shoot density,

canopy height, and above to belowground biomass ratio by site and survey, using individual sampling plots as replicates.

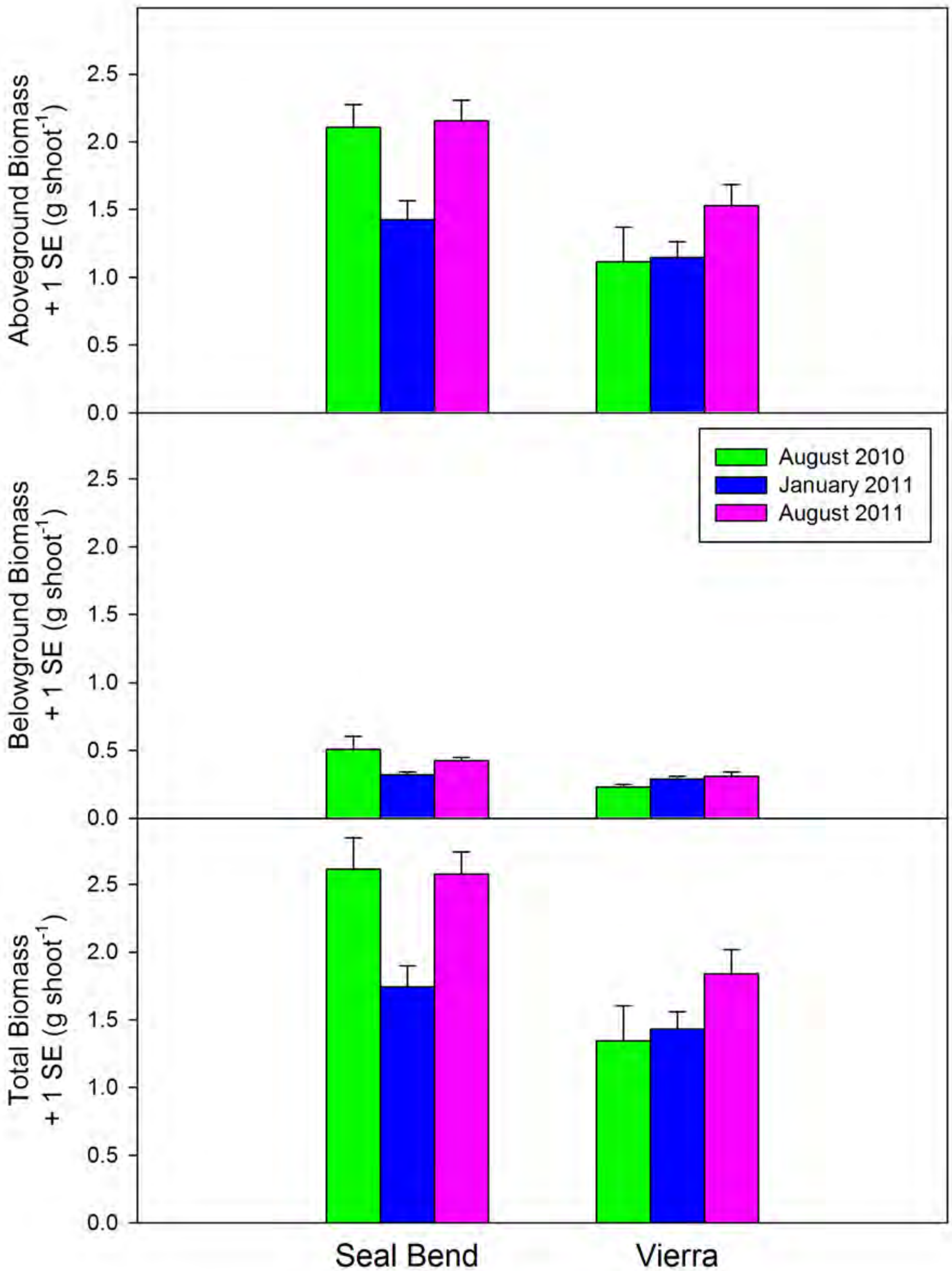
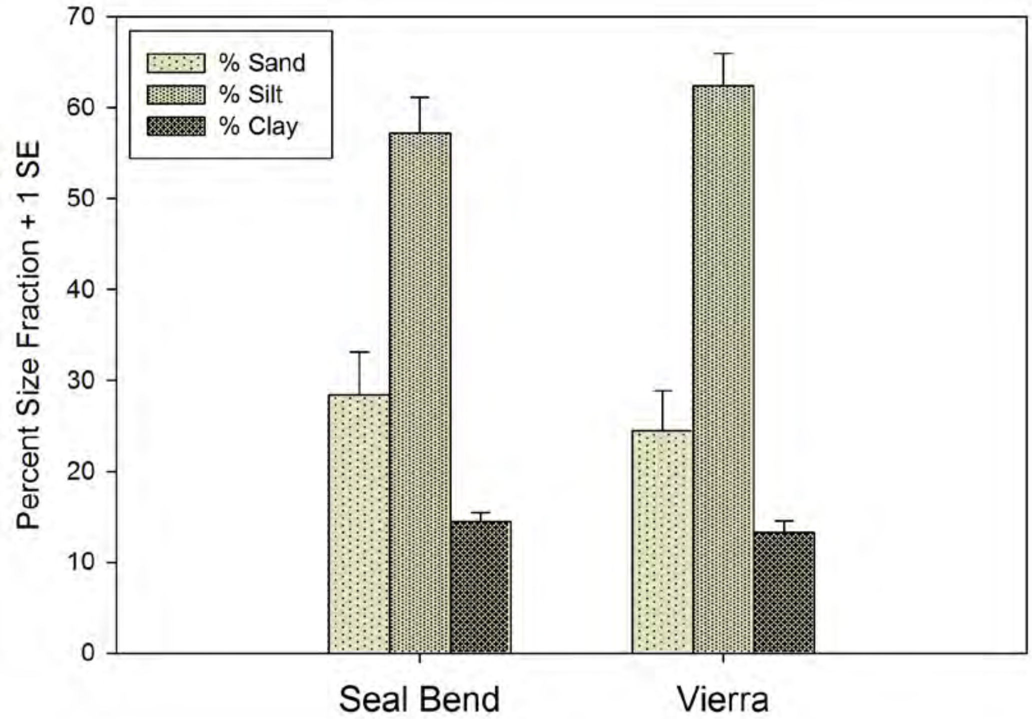


Figure 7. Means and standard errors of shoot biomass values for each site and survey.



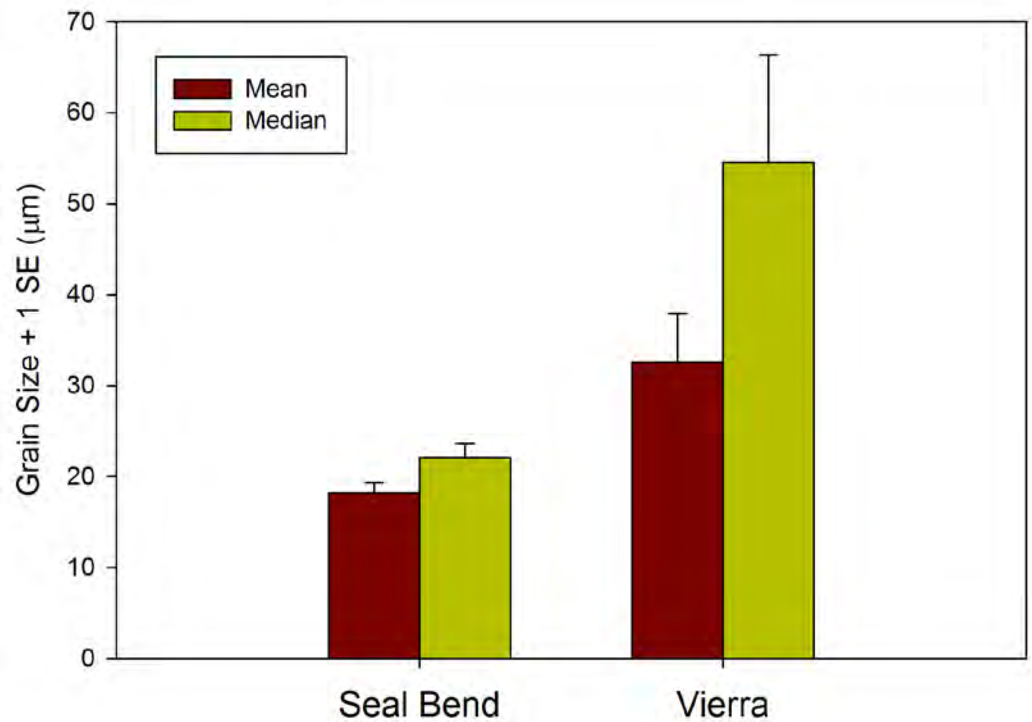
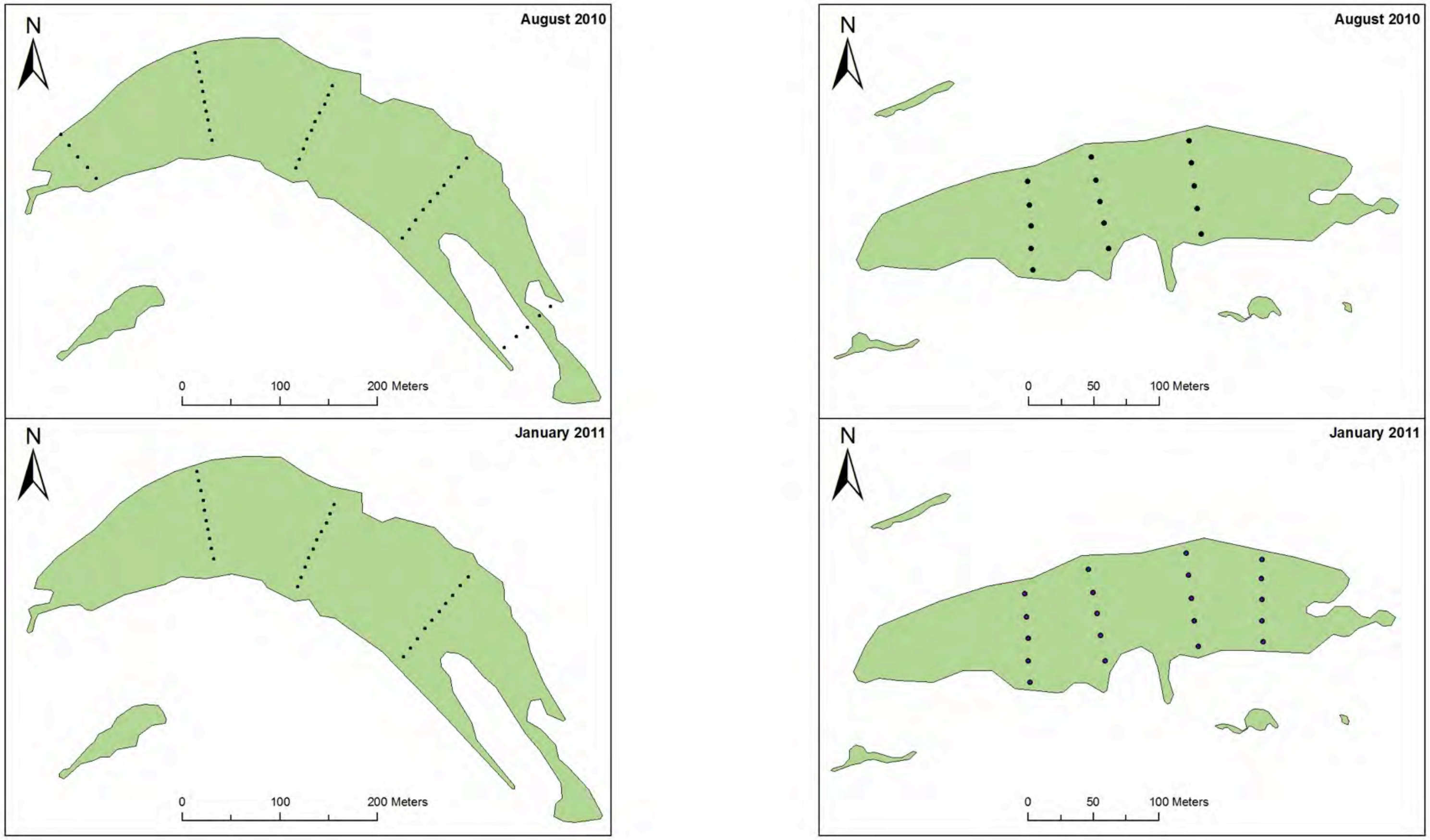


Figure 8. Percent sand, silt, and clay (top panel) and mean and median sediment grain size (bottom panel) and standard errors for each site, regardless of collection date.



Appendix 1. Plot and transect locations for August 2010 and January 2011 sampling designs. The Seal Bend site is in the left panel and the Vierra site is in the right panel. The seagrass polygons are taken from Eric Van Dyke's interpretation of aerial photographs from 2007-2009.