



Evaluating hyperspectral imaging of wetland vegetation as a tool for detecting estuarine nutrient enrichment

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ARTICLE INFO

Article history:

Received 1 March 2007

Received in revised form 15 April 2008

Accepted 3 May 2008

Keywords:

Wetland
Hyperspectral
Nitrogen
Water quality
HyMap

ABSTRACT

Nutrient enrichment and eutrophication are major concerns in many estuarine and wetland ecosystems, and the need is urgent for fast, efficient, and synoptic ways to detect and monitor nutrients in wetlands and other coastal systems across multiple spatial and temporal scales. We integrated three approaches in a multi-disciplinary evaluation of the potential for using hyperspectral imaging as a tool to assess nutrient enrichment and vegetation responses in tidal wetlands. For hyperspectral imaging to be an effective tool, spectral signatures must vary in ways correlated with water nutrient content either directly, or indirectly via such proxies as vegetation responses to elevated nitrogen. Working in Elkhorn Slough, central California, where intensive farming practices generate considerable runoff of fertilizers and pesticides, we looked first for long- and short-term trends among temporally ephemeral point data for nutrients and other water quality characters collected monthly at 18 water sampling stations since 1988. Second, we assessed responses of the dominant wetland plant, *Salicornia virginica* (common pickleweed) to two fertilizer regimes in 0.25 m² experimental plots, and measured changes in tissue composition (C, H, N), biomass, and spectral responses at leaf and at canopy scales. Third, we used HyMap hyperspectral imagery (126 bands; 15–19 nm spectral resolution; 2.5 m spatial resolution) for a synoptic assessment of the entire wetland ecosystem of Elkhorn Slough. We mapped monospecific *Salicornia* patches (~56–500 m²) on the ground adjacent to the 18 regular water sampling sites, and then located these patches in the hyperspectral imagery to correlate long-term responses of larger patches to water nutrient regimes. These were used as standards for correlating plant canopy spectral responses with nitrogen variation described by the water sampling program. There were consistent positive relationships between nitrogen levels and plant responses in both the field experiment and the landscape analyses. Two spectral indices, the Photochemical Reflectance Index (PRI) and Derivative Chlorophyll Index (DCI), were correlated significantly with water nutrients. We conclude that hyperspectral imagery can be used to detect nutrient enrichment across three spatial and at least two temporal scales, and suggest that more quantitative information could be extracted with further research and a greater understanding of physiological and physical mechanisms linking water chemistry, plant properties and spectral imaging characteristics.

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1. Introduction

Water quality in many estuaries, bays, and other coastal marine systems has declined progressively over the past century, often in response to increased runoff of nutrients from encroaching agricultural and urban areas (Vitousek et al., 1997a, Matson et al., 1997; Lotze et al., 2006). Intensive high-yield agriculture depends on addition of industrially produced nitrates (NO₃) and ammonium (NH₄). Their

global use increased sevenfold between 1960 and 1995 (Tilman et al., 2002), and an additional threefold increase is expected by 2050 (Tilman et al., 2001). The subsequent eutrophication of coastal and estuarine waters is intensifying (Nixon & Pilson, 1984; Cloern, 2001) with such symptoms as high concentrations of chlorophyll *a* (Beman et al., 2005), macro-algal and epiphytic blooms (Peckol & Rivers, 1996) and toxic algal blooms (Rabalais et al., 1996), all occurring with increasing frequency in coastal areas in much of the world. Nutrient enrichment of coastal and estuarine habitats often has far-reaching consequences with substantial economic and social costs (Turner et al., 1999) stemming from such events as fish-kills (Glasgow & Burkholder, 2000), restriction of shellfish aquaculture (Joint et al.,

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1997), degradation of seagrass beds (McGlathery, 2001), smothering of benthic organisms, and loss of biodiversity, ecological services, and other environmental damage (Vitousek et al., 1997a).

Traditional monitoring of water quality in estuarine and coastal systems relies on analyses of water samples collected periodically (e.g. weekly, monthly, or less frequently) at a few sites, with spatial coverage determined primarily by ease of access, which often excludes remote or otherwise inaccessible locations (e.g. private lands), and is sometimes characterized by discontinuous or irregular temporal coverage from opportunistic sampling (e.g. dependence on volunteer availability). This approach to monitoring water quality relies on instantaneous point samples in space and time, but the value of these data has limitations, including often poor correlations between nutrient concentrations measured in this way and levels of eutrophication measured, for example, as concentrations of chlorophyll *a*. In particular, low concentrations do not necessarily indicate the absence of eutrophication (Dettmann, 2001), especially when the intervals between samples are too large to capture ecologically important variation on other scales, such as daily to seasonal variation in nutrient inputs, water residence times, or unusual weather events (Bricker et al., 2003).

In California's coastal and estuarine habitats, nutrients usually arrive in pulses that vary spatially and temporally in frequency, duration and predictability (e.g. seasonal rainfall, or episodic influxes of wastewater). Although pulses of high nutrients produce transient peaks in the water column, nutrients also accumulate in soils and vegetation when loading rates are very high (Dettmann, 2001; Kamer et al., 2001). For Elkhorn Slough, a central Californian estuary, Chapin et al. (2004) concluded that adequate resolution of such processes as tides, diel cycling, and episodic runoff would require hourly *in situ* monitoring of water nutrient concentrations and physical conditions. While such a sampling program could capture temporal variation in great detail at a few sites, it would be expensive, labor intensive and impracticable for extended periods, and it would be impossible to gather data on spatial variation on analogous scales.

A second approach for detection and monitoring of nutrient runoff focuses on the consequences of nutrient enrichment by attempting to integrate these effects through time. The concentration of nutrients in the tissues of macro-algae or saltmarsh plants has been suggested as one such integrator of enrichment or eutrophication potential (Fong et al., 1998; Runcie et al., 2004; Boyle et al., 2004; Boyer & Fong, 2005; Cohen & Fong, 2006). Macro-algae and saltmarsh plants respond to seasonal and other variation by taking up nutrients differentially and using them partly for immediate growth; but they also store excess nutrients for future growth (Boyer et al., 2001; Kamer et al., 2001; Martins et al., 2001; Cohen & Fong, 2004). In estuaries surrounded by agricultural fields, the wetland vegetation can take up nutrients that would otherwise enter surface or ground waters, so the vegetation acts as a buffer zone, decreasing nutrient and silt loading from cultivated fields and pastures (Tilman et al., 2002). Unlike the instantaneous concentrations measured in water samples, the nutrient content of estuarine plant tissues integrates the nutrient regime over a period of time that is related to the duration of retention and delayed use of nutrients, including the effects of nutrient pulses likely to be missed by periodic water sampling (Cohen & Fong, 2006). Indeed, Valiela et al. (1997) indicate that in shallow estuaries with short residence times (like Elkhorn Slough), macro-algae and other persistent vegetation, rather than phytoplankton, are the most effective at intercepting nutrient pulses because phytoplankton tend to wash out of the system via tidal exchanges. Collectively, these studies suggest that estuarine vegetation has considerable potential as a practical bioindicator of temporal nutrient dynamics; but while tissue sampling for persistent vegetation like marsh plants may be effective when done less frequently (e.g. seasonally) than water sampling (e.g. weekly or monthly), the practical demands of extending such sampling and analysis of plant tissues over large areas to explore

spatial variation makes the task as laborious and time consuming as direct water sampling over large areas.

A third and increasingly common approach to environmental monitoring in estuarine systems uses airborne or satellite imaging to provide high spatial resolution, synoptic, and non-intrusive integration of spatial variation over large areas (Zhang et al., 1997; Phinn et al., 2000; Goetz, 2006). When high spectral resolution is also included, this approach may provide sensitive proxies for biophysical properties of wetland vegetation (Silvestri et al., 2002; Brando & Dekker, 2003; Li et al., 2005). Multispectral sensors, which have a small number (usually ≤ 10) of discontinuous, broad spectral bands, and relatively low spatial resolution (e.g. LANDSAT's 30 m pixels), have been used to delineate habitat boundaries and to partition ecosystems into functional units (Zhang et al., 1997; Harvey & Hill, 2001; Shuman & Ambrose, 2003), but they cannot adequately resolve either the fine-scale spatial heterogeneity and narrow ecotones common in most wetlands, or the subtle spectral shifts associated with physiological changes in plants. Airborne hyperspectral sensors, on the other hand, have many (≥ 100), narrow (2–15 nm), contiguous spectral bands; have high spatial resolution (≤ 3 m) when flown at low altitudes; and have great potential for mapping wetland vegetation and for correlating the spectral characteristics of plant species with environmental variables, including spatially variable nutrient uptake and storage. Therefore hyperspectral imaging should be useful for detecting water nutrient enrichment, provided that: 1) the vegetation is nutrient limited; 2) the vegetation's uptake of water column nutrients produces a spectral response; and 3) the spectral and spatial resolutions of the imagers are appropriate for detecting subtle spectral shifts within and between vegetation patches. Costs of acquisition usually limit the frequency of imaging (e.g. annual) and hence the temporal resolution of this approach, but when an image is acquired, it provides 100% spatial coverage for the watershed or coastal area of interest, and integrates past responses to nutrient variation over some period of time preceding the date of acquisition.

Each of these three approaches provides a different kind of data containing varying amounts of spatial and temporal information. Because they are acquired on different spatial and/or temporal scales, their results are not easily comparable, nor can they be readily extrapolated from one scale to another. In most cases, only one approach is used consistently in any one monitoring program.

In this paper, we integrate data from these three approaches to explore whether, and to what extent, hyperspectral imagery can be used to detect spatial variation in estuarine nutrient enrichment in Elkhorn Slough, a central Californian tidal wetland where fertilizer runoff from surrounding farmlands is a major nutrient source and a serious management concern. The three datasets comprising this study are: 1) a long-term *in situ* water monitoring program (monthly instantaneous point samples taken at 18 sites throughout the wetland); 2) a field fertilization experiment to determine nutrient and spectral responses of the dominant wetland plant, *S. virginica*, to nitrogen enrichment (using 40 small plots – 0.25 m² quadrats); and 3) an analysis of spatial variation of wetland vegetation spectra in high resolution, hyperspectral imagery covering the entire wetland, with correlated water quality and spectral variation (landscape scale). Collectively, these data allow us to explore the variation in nutrient enrichment across scales. The field fertilization experiment not only tested the first two assumptions underlying the use of hyperspectral imaging in detecting nutrient enrichment (nutrient limitation and spectral responses), but also allowed us to explore linkages, correlations, and extrapolations across the spatial (points, plots, landscape) and temporal (instantaneous measurements through cumulative effects) scales spanned by the three approaches.

We hypothesized that *S. virginica*, the dominant wetland plant, is nitrogen limited in Elkhorn Slough. Nutrient limitation has been studied extensively in California's salt marshes. For example, experimental applications of nutrients enhanced standing crop, above-

ground productivity and nitrogen content of *S. virginica* in a southern California marsh where, despite high nutrient levels in sediments, *S. virginica* responded strongly to applied N by increasing both biomass and tissue N concentration (Page, 1995; Boyer et al., 2001). Elkhorn Slough is also an estuary with high sediment nutrient levels, and data from a long-term water monitoring program have shown that instantaneous water nutrients vary greatly in space and time (Caffrey et al., 1997, 2007a). We conducted our field experiment to determine whether *S. virginica* is nutrient limited in Elkhorn Slough, and whether and how its reflectance spectra vary with nutrient levels. We then used the results from the experimental plots as an intermediate step between the water sampling and the image analysis. This allowed us to evaluate these assumptions at the landscape level, by using the high spectral and spatial resolution of the hyperspectral imagery spanning the entire wetland to locate areas of marsh vegetation with signatures of high nutrient levels.

Our spectral analyses addressed three scales of vegetation structure and complexity: leaf, canopy and landscape. Two levels (leaf and canopy) were measured by *in situ* spectroradiometry of experimental plots of *S. virginica*, while the third level (landscape) involved scaling up from the experimental plots to the sizes of monospecific *Salicornia* patches found throughout the estuary, by using image reflectance spectra to test extrapolations of the spectral relationships already found in the *in situ* experimental plots.

1.1. Hyperspectral measurement of plant nutrients

Measuring biochemical constituents of vegetation (e.g. chlorophyll, protein, water, lignin, etc.) with imaging spectrometry is a complex undertaking, since plant reflectance is strongly influenced by multiple, overlapping absorption features. This is particularly true for aquatic and wetland plants, where the signal from canopy water, often dominant in succulent vegetation, greatly confounds potential signals coming from other biochemical constituents. Absorption bands in the reflectance spectra of vegetation are generated largely by four main elements (hydrogen, carbon, oxygen, and nitrogen), and arise mainly from vibrations of C–O, O–H, C–H, and N–H bonds, their overtones, and many different combinations of these vibrations (Curran, 1989). Consequently, absorptions from different plant materials are usually similar and overlapping, and a single absorption band usually cannot be isolated and correlated directly with abundance of one plant constituent. This is in marked contrast to mineral reflectance spectra, which often have unique absorptions that can be isolated and related directly to that mineral's abundance in the substrate (e.g. Kruse et al., 1993; Clark, 1999). Nitrogen is primarily found in plant chlorophyll and proteins, and is usually reasonably well correlated with concentrations of chlorophyll *a*+chlorophyll *b* (Field & Mooney, 1986), which have fundamental absorptions centered at 460–480 nm and 650–670 nm. Measuring changes in a plant's chlorophyll content in response to nitrogen variation is therefore one way to assess nutrient enrichment with hyperspectral data. Nitrogen in plant proteins has additional absorptions in the Short Wavelength Infra-Red (SWIR), but they are often concealed by strong water absorptions in this region (Curran, 1989).

Most efforts to detect plant nutrients (primarily nitrogen) using hyperspectral data have been restricted to terrestrial vegetation, and many rely on reflectances from dried, ground plant material under controlled laboratory conditions (e.g. Kokaly & Clark, 1999; Kokaly, 2001; Curran et al., 2001). Few studies have attempted to extrapolate from laboratory and field data of single plants to the landscape level of airborne hyperspectral imagery. In one study aimed at deriving spatial estimates of canopy chemistry as a driver for a carbon balance model, Martin and Aber (1997) used NASA's Airborne Visible Infrared Spectrometer (AVIRIS) to relate hyperspectral image data in both the visible/Near Infrared (NIR) and SWIR regions to field measurements of canopy nitrogen and lignin concentrations in planted forest canopies.

Asner and Vitousek (2005) used imaging spectroscopy (AVIRIS) and photon transport modeling to measure leaf nitrogen concentrations and canopy water content in forest canopies in Hawaii. Their estimates of upper-canopy foliar N concentrations (derived from the imagery) were correlated strongly with ground-based measurements, and were used successfully to identify and map the geographic extent and biogeochemical impacts of two non-native species in this ecosystem. Using a similar methodology, Porder et al. (2005) used AVIRIS imagery to predict nutrient availability (primarily phosphorus, via area-integrated canopy P content) in an upland forest on the island of Kauai and illustrated the importance of scaling up from remote sensing analyses to the landscape level, especially when concerned with management and conservation of a particular area or ecosystem.

Other research efforts have focused on detection of indirect nutrient effects on plant reflectance spectra through changes in chlorophyll absorption and fluorescence. Perhaps the commonest technique for investigating spectral shifts in vegetation exposed to low nutrients or other adverse environmental conditions (i.e. environmental "stress") is the use of hyperspectral derivative ratios in the "red-edge" region of the spectrum, the boundary between the region of strong absorption of red light by chlorophyll (~680 nm), and the region of high, multiple scattering of radiation (~750 nm) in the leaf mesophyll (Lillesand & Kiefer, 2000). In derivative analyses, this region has several peaks that can indicate plant stress and leaf chlorosis before they become visible (Demetriades-Shah et al., 1990; Zarco-Tejada et al., 2000). The main peak used in derivative analyses of the red-edge reflectance is actually composed of two (or more) features corresponding to chlorophyll fluorescence maxima: a consistent peak around 700 nm, and a second around 725 nm (Zarco-Tejada et al., 2002, 2003). The magnitudes of these peaks change with different types of environmental stress, such as oil contamination of the soil (Jago & Curran, 1996), changes in temperature and humidity (Zarco-Tejada et al., 2003), soil-oxygen depletion, and subsequent changes in nutrient uptake from the soil (Smith et al., 2004).

Several studies demonstrate that changes in nutrient content and other environmental variables (natural gas, etc.) cause consistent, significant changes in the relative magnitudes of the 725 nm and the 700 nm peaks. Zarco-Tejada et al. (2002) reported that changes in the double peaks (~703 nm and 722 nm) of derivative reflectance spectra were correlated with increases in chlorophyll fluorescence and chlorophyll concentration. They used the ratio of derivative values at these wavelengths (725 nm/705 nm) to develop the Derivative Chlorophyll Index (DCI) which, they suggested, changes with pigment and chlorophyll fluorescence effects in the canopies of affected vegetation. Smith et al. (2004) subsequently examined changes in the DCI of vegetation exposed to high concentrations of natural gas in the soil. Lamb et al. (2002) experimented with nutrient enrichment in plants, and found that the 700 nm derivative peak was dominant in leaves with low nitrogen and low chlorophyll contents, but that the peak at ~725 nm became dominant in leaves with higher nitrogen and chlorophyll contents. The exact wavelength of each peak varies within a few nanometers among plant species, but the value of the double peak ratio invariably declines with increasingly adverse nutritional or other environmental conditions (Zarco-Tejada et al., 2002, 2003). Conversely, under increasingly beneficial conditions, such as higher nutrient availability, the value of the double peak ratio increases.

Another spectral index used to discriminate between nutrient levels in terrestrial as well as wetland vegetation is the Photochemical Reflectance Index (PRI; Gamon et al., 1997). The PRI is the ratio of narrow-band reflectances at 531 nm and 570 nm, and is an optical indicator of the efficiency of use of photosynthetically active radiation (PAR) across nutrient levels and plant species. The reflectance at 531 nm is a proxy for the xanthophyll cycle, a non-destructive mechanism that dissipates excessive radiation via pigment conversion (Gamon et al., 1992). The PRI is also closely related to a fluorescence-based indicator of the efficiency of use of light by the plant's photo system II (PSII). Gamon

et al. (1997) experimentally varied leaf nitrogen levels to demonstrate that nutrient-deficient leaves with lower photosynthetic rates have lower PRI values (reflecting a greater investment in photoprotection) than fertilized plants, and that this reduction holds true for annual, perennial deciduous, and perennial evergreen plants.

The major limitation of most previous studies of DCI and PRI, unlike the landscape-level studies of Martin and Aber (1997) and Asner and Vitousek (2005), is that the spectral measurements were carried out in laboratory environments on plants treated with fertilizers under controlled conditions, rather than using field or image spectral data. Tilley et al. (2003), on the other hand, measured the PRI and several other narrow spectral band reflectance indices of wetland vegetation (as opposed to drier terrestrial plants) under natural conditions in the field. Their goal was to detect changes in water column Total Ammonia ($TA = NH_3 + NH_4^+$) in a wetland ecosystem via hyperspectral reflectance of emergent estuarine vegetation, and they found that PRI was highly correlated with water column TA. The strongest correlation existed when the range of Total Phosphorus (TP) in the water was restricted, by excluding plants exposed to the highest and lowest TP. Restricting the range of salinities also improved the correlation, underscoring how spectral signals in natural ecosystems are affected by many other variables, including soil type, salinity, elevation and the concentrations of other nutrients. These results

suggest that hyperspectral data have the potential to detect estuarine nutrient enrichment, at least within a defined system, via a proxy based on vegetation responses to elevated nitrogen levels. While Tilley et al. (2003) obtained strong correlations using field spectrometry of wetland plants (rather than with more commonly studied terrestrial vegetation), they did not employ image data to scale the field spectrometry up to landscape scales.

Whether laboratory findings of spectral–chlorophyll–nitrate correlations actually can be scaled up to field and landscape scales is of prime interest to resource managers. Until results from *in situ* spectrometry can be scaled up to the landscape levels recorded by airborne or satellite hyperspectral imagers, many potential practical applications of hyperspectral remote sensing for management and conservation will remain only theoretical possibilities.

2. Materials and methods

2.1. Study system

2.1.1. Site

Elkhorn Slough (36.827° N, 121.758° W) is a shallow (2.5 m mean depth), meandering tidal estuarine embayment approximately 11.5 km long, that opens into Monterey Bay at Moss Landing, approximately

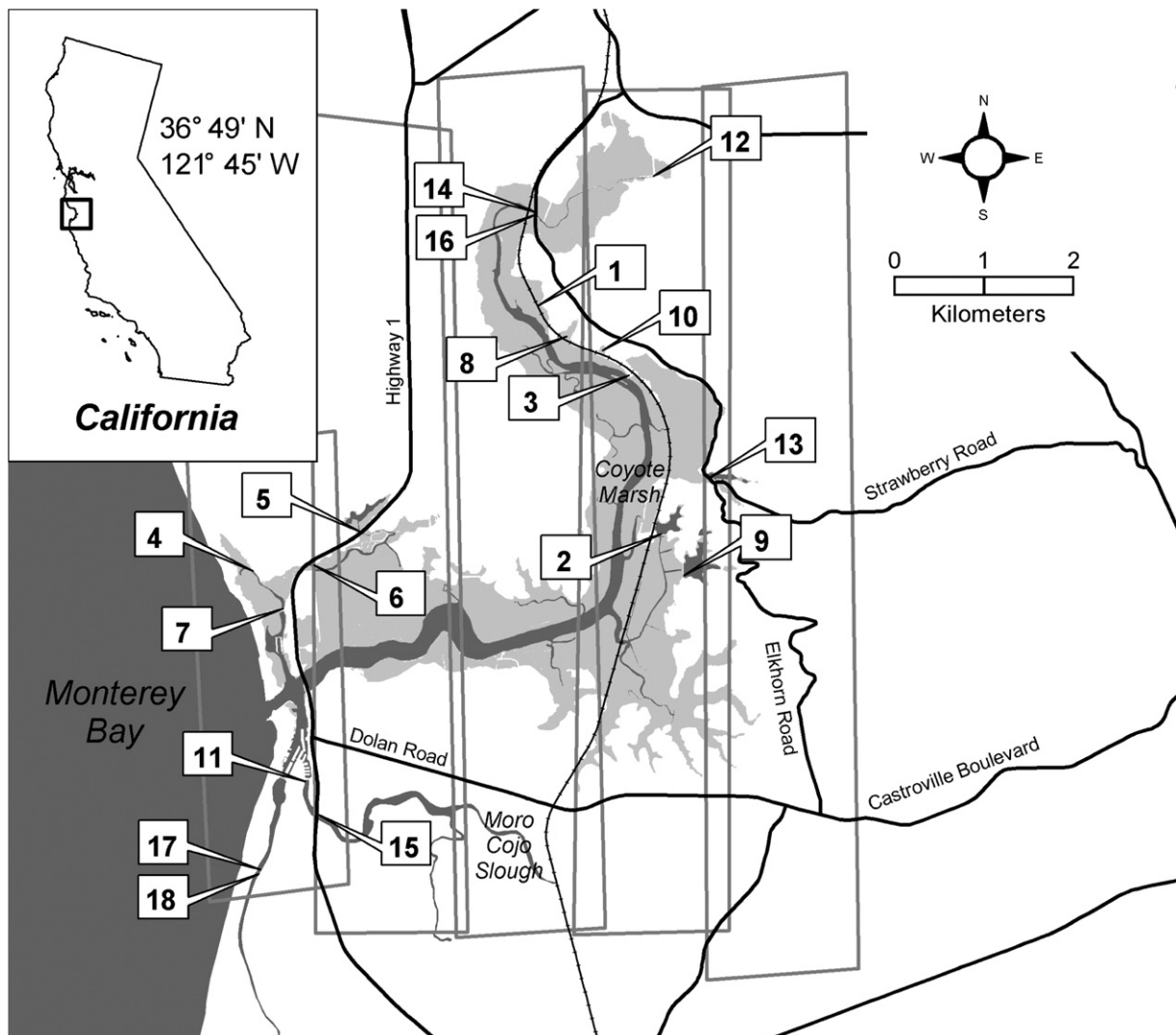


Fig. 1. Map of Elkhorn Slough, CA showing the main channel, wetland areas (shaded), the footprint of the HyMap imagery, and 18 water quality monitoring stations. Station numbers correspond to those listed in Table 1.

145 km south of San Francisco and 32 km north of Monterey (Figs. 1 and 2). Elkhorn Slough supports a high diversity of organisms and habitats and its waters, lying within the Monterey Bay National Marine Sanctuary, provide an important nursery area for commercial and recreational fish species. Saltmarsh habitat throughout its watershed occupies 1147 ha (Zimmerman & Caffrey, 2002), is brackish (25–35 psu), and is dominated by the halophytic angiosperm *S. virginica* L. (common pickleweed). The mean diurnal tide height is 1.7 m (Caffrey & Broenkow, 2002).

Several public agencies and private conservation groups have jurisdiction over Elkhorn Slough wetlands, including the National Oceanic and Atmospheric Administration which administers the Elkhorn Slough National Estuarine Research Reserve (ESNERR); the California Department of Fish and Game; and the Elkhorn Slough Foundation and The Nature Conservancy which, together, have purchased and protected over 3500 ac of watershed lands. These multiple designations and strategic land acquisitions, protecting approximately a quarter of the estuary's habitats, underscore the importance of Elkhorn Slough as a critical ecosystem.

Grazing, agriculture and other human activities over the past 150 years have greatly increased nutrient inputs to Elkhorn Slough (Caffrey, 2002; Caffrey et al., 2007a), while diking, draining and conversion to pasture have reduced both salt marsh area (Van Dyke & Wasson, 2005) and the ecological services, such as filtering and buffering, provided by the marsh. Agricultural fertilizers add 50–200 kg of nitrogen per hectare per year to the Elkhorn Slough watershed, but an estimated 50% of the added nitrogen remains unassimilated by crops, and instead is incorporated into soil organic matter or dissolved in ground or surface waters that drain into the tidal channels and marshes (Caffrey & Broenkow, 2002). Such non-point source pollution from farm chemicals (fertilizers and pesticides) is the primary cause of water quality degradation in Elkhorn Slough (Caffrey et al., 2002). Nutrient levels in some portions of Elkhorn Slough have increased 4-fold over the last 60 years, reflecting the regional shift to intensive agriculture (Caffrey et al., 1997; Caffrey et al., 2002). Most runoff occurs during the rainy season, October to March (Caffrey et al., 2007a), and varies considerably from year to year (Monterey County Water Resources Agency, unpublished data). There is little rain and thus limited leaching during the summer, so nitrate, ammonium and total inorganic nitrogen accumulate in the soil during this season (Los Huertos, 1999). Plants begin to assimilate the available soil nitrogen during the growing season (late winter and spring), but

with the first autumn rains, especially if heavy, some nitrate may be leached out before uptake.

2.1.2. *Salicornia virginica*

S. virginica L. is an emergent vascular plant species occurring at the lowest tide levels in saline coastal marshes of the western United States (Seliskar & Gallagher, 1983). It is a perennial, C₃ succulent, with a wide geographic range along the Pacific coast. It forms extensive, intertwining above-ground branches, about 0.5 m tall, each consisting of a woody stem surrounded by succulent leaves (Josselyn, 1983). It spreads rapidly by vegetative means, with low-lying stems producing new upright stems each spring. *S. virginica* is frequently bathed by higher tides, and can store excess salt within its tissues; in the autumn, as part of sloughing off salt-saturated tissues, it replaces most of its chlorophyll with anthocyanin pigments, which confer a characteristic red color to its leaves that contrast with the bright green color of spring and early summer (Josselyn, 1983).

2.2. Water quality

2.2.1. Water monitoring program

Concerns about elevated nitrogen concentrations and non-point source pollution have driven research and monitoring programs in Elkhorn Slough over several decades. The Elkhorn Slough National Estuarine Research Reserve (ESNERR) began a volunteer water quality monitoring program in 1988, measuring nutrients and several other environmental parameters monthly at up to 24 locations. Volunteers measure dissolved oxygen, temperature, turbidity, salinity, and pH *in situ* using a YSI 6600 sonde, then collect surface water samples that are filtered, iced and sent to the Monterey County Consolidated Chemistry Laboratory (MCCCL) for nutrient analyses through an arrangement between ESNERR and the Monterey County Water Resources Agency. Nitrate is analyzed using ion chromatography (Dionex model 80); inorganic phosphate is detected using the ascorbic acid method and a spectrophotometer; and ammonia is analyzed using an ion selective electrode. Samples are filtered for chloride prior to analysis to reduce interference.

2.2.2. Water quality data analyses

Data from the volunteer water quality monitoring program were provided by ESNERR after conducting standard quality assurance, and

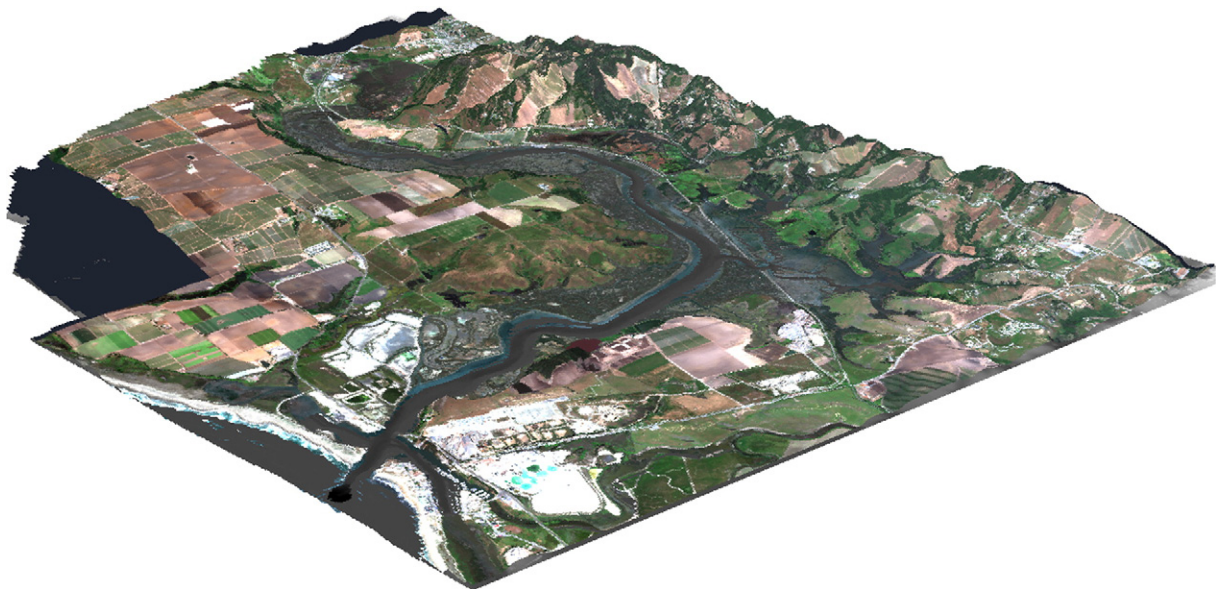


Fig. 2. 3-D HyMap hyperspectral image of Elkhorn Slough superimposed on a Digital Elevation Model (DEM) from LIDAR data to show the elevation gradient of the watershed, and the footprint of land use and human activities encroaching upon the estuarine embayment. (View from W-SW; vertical exaggeration 3×).

checking and compiling the data from volunteers and the MCCCL. We used data from all 24 monitoring stations for consideration of general patterns, then selected 18 stations (Fig. 1) for detailed analysis, based on their location within the boundaries of hyperspectral imaging in May 2000 (see below), and the presence of monospecific stands of *S. virginica* nearby. We chose nitrate, ammonium, phosphate, and salinity as the most relevant water quality data for this study. We calculated average values over several time spans, ranging from 1 to 10 years preceding image acquisition (May 2000) and for several seasonal subsets, including separating the productive (growth) season from the runoff season, and we then explored correlations between these nutrient values and various spectral indices in the experimental and imaging studies (see below).

We selected average values for the productive months (March through September) over the 5-years preceding image acquisition (March 1996–May 2000) for presentation in this paper, because that interval had the highest coefficient of determination (r^2) between nitrogen content and spectral indices (see below), and because perennial plants are known to accumulate nutrients throughout the productive season and store excess for use in subsequent years (Fong et al., 1998; Boyer et al., 2001). In addition, considering the longevity and expected N storage of the perennial *S. virginica*, five years is believed to be a reasonable time span for this plant to accumulate and store nitrogen in its tissues (J. Caffrey, pers. comm.). We summed nitrate and ammonium concentrations to give total water nitrogen (Table 1), and the mean values for total nitrogen and other environmental parameters from the 18 water monitoring stations were later used to ground truth the imagery, i.e. to establish correlations with image spectra.

2.3. Fertilization experiment and in situ spectroradiometry

2.3.1. Marsh fertilization and CHN analysis

We conducted a nutrient enrichment experiment at Coyote Marsh, on the Elkhorn Slough National Estuarine Research Reserve (Fig. 1). We established 40 quadrats (0.5×0.5 m) along a transect parallel to the shore through a monoculture of dense *S. virginica*. Most quadrats were spaced at 1.5 m intervals, but were further apart if vegetation was sparse, or if *Salicornia* was intermixed with other marsh plants (to ensure the plots were uniform and monospecific). Quadrats along the transect alternated between two treatments: 20 nitrogen-enriched alternated with 20 control (unmodified) quadrats. We applied 15 g of agricultural fertilizer-grade granular urea to the center of each

nitrogen enrichment quadrat, then gently patted the vegetation until the granules dispersed to the ground underneath. For the control treatment, we mimicked patting of the vegetation. We repeated these treatments every 10 days from 15 April to 15 August 2002.

We harvested the entire above-ground vegetation within a 15 cm×15 cm square at the center of each quadrat on August 18, 2002 using clippers, and immediately weighed the tissue as an estimate of biomass (wet weight). Before harvesting, we collected small tissue samples (~5 g) consisting of leaves from 3–4 different stems of 2–3 plants from every one of the 40 plots, with 3 replicated plant tissue samples from each plot. The tissue samples were stored overnight in Ziploc bags at 4–6 °C, then transferred to aluminum dishes and dried in an oven at 60 °C for 7 days. After cooling in a desiccator, the samples were pulverized using a “Wig-L-Bug®” mill (Pike Technologies). Carbon, hydrogen and nitrogen contents (CHN) were determined for each sample in a CHN Analyzer (Perkin-Elmer 2400 Series II) using 3 replicate subsamples (~3 mg each) from each sample. The CHN data were used to quantify differences in nitrogen concentration between fertilized and unfertilized plants.

2.3.2. Spectral response (leaf and canopy)

On August 17, 2002, before harvesting the biomass, we used a handheld spectroradiometer (Analytical Spectral Devices Inc. ASD FieldSpec® Pro FR) to measure both downwelling irradiance and upwelling radiance from *S. virginica* under clear skies near solar noon (~1315 PDT at this longitude). The ASD spectroradiometer has a 2150 nm spectral range from 350 nm in the ultraviolet (UV) to 2500 nm in the short wave infrared (SWIR), a 3–11 nm spectral resolution, and a 1.4–2 nm sampling interval. We measured the downwelling irradiance from a white standard ASD Spectralon® plate (LabSphere) before measuring each plant's radiance spectrum. The ASD software automatically calculated the plant's relative reflectance (%) by expressing the plant's radiance as a percentage of the downwelling irradiance. All ASD measurements used a 25° field-of-view (FOV) fore optic, and two readings were taken at different heights above each plant. The first reading measured reflectance only from leaves of *S. virginica* (leaf level) with the fore optic held 1 cm above the leaf surface at 45° to the ground, facing into the sun. The second reading measured reflectance from the canopy of each *S. virginica* quadrat (canopy level), and encompassed the variation in canopy characteristics of each plot viewed from above. The fore optic was held 1 m above the top of the *S. virginica* canopy at nadir (looking straight down, i.e. 90° from horizontal). The radius (R) of the area (A) sensed by the spectroradiometer at both leaf and canopy scales was

Table 1

Mean, standard deviations and range for nitrogen and phosphate concentrations in Elkhorn Slough waters at 18 water monitoring stations, arranged by increasing nitrogen levels

#	Station name	NO ₃ (±1 SD)	NO ₃ (Min–max)	NH ₃ (±1 SD)	NH ₃ (Min–max)	Total N (NO ₃ +NH ₃)	PO ₄ (±1 SD)	PO ₄ (Min–max)	Elevation (m)
1	Azevedo Pond, Upper	1.634 (1.540)	0.0–12	0.113 (0.045)	0.00–0.38	1.747	0.105 (0.030)	0.00–0.31	1.7
2	Reserve, South Marsh	1.908 (1.461)	0.0–14	0.101 (0.030)	0.00–0.21	2.009	0.126 (0.107)	0.00–0.99	1.3
3	Kirby Park	1.996 (1.838)	0.0–21	0.105 (0.042)	0.00–0.26	2.101	0.118 (0.042)	0.00–0.45	1.7
4	Bennett Slough, West	2.954 (2.332)	0.0–18	0.149 (0.049)	0.00–0.46	3.103	1.137 (0.892)	0.03–2.66	1.4
5	Struve Pond	3.389 (2.306)	0.0–18	0.394 (0.549)	0.00–3.76	3.783	0.517 (0.242)	0.07–2.20	3.0
6	Bennett Slough, East	3.247 (2.302)	0.0–19	0.157 (0.088)	0.00–0.84	3.404	0.203 (0.071)	0.08–0.62	1.6
7	Jetty Road	3.303 (2.975)	0.0–29	0.111 (0.032)	0.00–0.29	3.414	0.158 (0.047)	0.00–0.39	1.4
8	Azevedo Pond, Mid	4.252 (5.115)	0.0–31	0.356 (0.163)	0.00–1.42	4.608	0.252 (0.056)	0.00–0.47	1.6
9	Reserve Bridge	4.494 (4.234)	0.0–25	0.136 (0.035)	0.00–0.40	4.630	0.127 (0.072)	0.04–0.59	2.1
10	Azevedo Pond, Lower	4.891 (5.764)	0.0–39	0.248 (0.135)	0.00–1.24	5.139	0.766 (0.215)	0.11–2.00	5.1
11	Moss Landing Road South	7.636 (4.170)	0.0–33	0.309 (0.319)	0.00–3.03	7.945	0.348 (0.156)	0.00–1.48	0.3
12	Carneros Creek	8.971 (8.536)	0.0–51	1.492 (0.375)	0.00–3.91	10.463	0.510 (0.370)	0.00–3.24	1.1
13	Strawberry Road	14.718 (9.036)	0.0–54	0.201 (0.235)	0.04–1.73	14.919	0.152 (0.087)	0.00–0.59	0.8
14	Hudson's Landing West	15.816 (8.615)	0.0–70	0.161 (0.079)	0.00–0.54	15.977	0.190 (0.133)	0.00–1.61	1.0
15	Moro Cojo Slough	16.614 (8.530)	0.6–70	0.394 (0.256)	0.00–2.49	17.008	0.367 (0.227)	0.04–2.28	0.2
16	Hudson's Landing East	18.746 (10.274)	0.0–84	0.226 (0.159)	0.00–1.26	18.972	0.338 (0.228)	0.00–2.36	0.6
17	Potrero Road, North	35.710 (24.913)	3.0–123	0.185 (0.056)	0.00–0.520	35.895	0.349 (0.122)	0.00–1.08	1.2
18	Potrero Road, South	72.058 (31.179)	16.0–160	0.221 (0.092)	0.00–0.94	72.279	0.447 (0.196)	0.11–1.13	1.2

Each value is the average of productive months (March through September) of the years 1996–2000. All nutrient values are in mg/L, elevation is in meters (from 2004 LIDAR data). Station numbers correspond to numbers on Fig. 1.

calculated from the field of view (FOV) of the fore optic and the distance (D) from the target surface:

$$R = D \times \tan(\text{FOV}/2). \quad (1)$$

The area of surface sensed, calculated from the radius, was 0.1548 cm² at the leaf level (0.22 cm radius) and 1548 cm² at the canopy level (22.2 cm radius). The spectra were imported into ENVI® (ITT VIS Corp.) as four spectral libraries of 20 spectra each: fertilized leaf; fertilized canopy; control leaf; control canopy (Fig. 4). The bands corresponding to three regions of strong atmospheric water vapor absorption (924–986 nm, 1350–1428 nm, and 1756–1978 nm) were masked. We calculated the means and standard deviations (SD) of each library for the remaining bands using a standard IDL routine. We subsequently analyzed the spectral libraries using the methods outlined in Section 2.5 below.

2.4. Hyperspectral imaging

2.4.1. Image acquisition and calibration

HyMap hyperspectral imagery (440–2500 nm spectral range, 126 bands, 13–17 nm sampling interval, 15.5–19 nm bandwidths, 2.5–3.0 m spatial resolution; Cocks et al., 1998) was acquired over Elkhorn Slough on 3 May 2000, early in the dry season, by HyVista Corp. (Sydney, Australia). HyMap is a whisk-broom imager using diffraction gratings and four 32-element detector arrays. It has an FOV of 61.3°, and each swath is 512 pixels wide. Five flightlines covering Elkhorn Slough (Figs. 1 and 2) were flown at an average altitude of 1380 m, averaging 15–20% overlap between flightlines. The average elevation of the acquisition area was 16 m above sea level. Imaging occurred between 1326 PDT (start) and 1352 PDT (end) about 2 h after a high tide (1.9 m at 1125 PDT) and 4 h before a low tide (0.91 m at 1640 PDT). At this tide level in Elkhorn Slough, most of the *S. virginica* is emergent, but lower mud flats are still covered with water.

HyMap is a radiometrically stable sensor, partly due to an on-board stabilized lamp to monitor and correct for in-flight variation in radiometric sensitivity (Cocks et al., 1998); such variation is typically less than 1–2% over several hours (T. Cocks, pers. comm.). HyVista pre-processes the data from DN to radiance. We obtained the data in September 2000 as raw integer Radiances. We first converted each flightline to floating point Radiance (mW/cm²/nm/sr) using gain factor files provided by HyVista with the dataset. The data were subsequently converted to Reflectance as part of the atmospheric correction outlined below.

More than half of the spectral range of HyMap is affected by atmospheric gas absorptions, and we used the Fast Line-of-sight Atmospheric Analysis of Hyperspectral cubes (FLAASH®, ITT VIS Corp.) atmospheric correction algorithm to correct absorption and scattering effects. FLAASH is based on the atmospheric physics and spectroscopy described by the MODTRAN4 radiative transfer code (Andersen et al., 2000). We selected the following FLAASH parameters for the atmospheric correction: atmospheric model = Mid-Latitude Summer; aerosol profile = Maritime; atmospheric visibility = 65 km; CO₂ mixing ratio = 390 ppm; and aerosol scale height = 2 km. We also used the inverse of the radiance scale factors provided with the HyMap data. We atmospherically corrected each of the 5 flight lines separately, and subsequently georeferenced each flightline in ENVI using the Ground-Look-up Table (GLT) files provided by HyVista for each flightline. The HyMap imager sits in a fully gyro-stabilized mount to minimize instrument motion, but all aircraft motion is tracked with continuous DGPS and a time-coded Inertial Measurement Unit (IMU). This information is used to retrieve true ground position and is stored in a separate GLT (Cocks et al., 1998). Horizontal geospatial accuracy for relatively flat terrains such as the area encompassed by the Elkhorn Slough HyMap dataset is approximately 2–3 pixels (5–7 m). Once georeferenced, we mosaicked the 5 flightlines into a single image using

ENVI's map based mosaic function, which resulted in an average overlap of 15% between flightlines.

2.4.2. Extracting image spectra corresponding to *S. virginica*

Despite being the dominant saltmarsh species at Elkhorn Slough, *S. virginica* often co-occurs with other salt marsh plants, such as *Jau-meia carnosa* and *Frankenia salina*. To maximize accuracy of the analyses, we identified monotypic patches of *S. virginica* on the ground close to each water station, with the goal of comparing and analyzing image spectra of *Salicornia* from locations spanning a range of known water nitrate concentrations. We mapped these patches in May 2004 by walking around each patch with a Trimble GeoXT Explorer handheld GPS, to delineate GPS polygons of dense, homogeneous, monotypic stands of *Salicornia*. We carefully excluded any areas where *S. virginica* co-occurred with other salt marsh plant species. Suitable patches were obtained near 18 of the 24 water monitoring stations (Fig. 1). After collection, the GPS polygons were differentially corrected to ≤ 1 m accuracy using Trimble software and base data from the California State University Monterey Bay DGPS base station, then re-projected to match the imagery's projection model and datum. Image pixels within each polygon were then defined as 18 Regions of Interests (ROIs). These ROIs were used as image subsets, and a spectrum was extracted from every pixel within each of the 18 ROIs ($N=9\text{--}100$ pixels per ROI) to be used in the analyses at the "landscape level".

2.5. Spectral indices

The two spectral datasets acquired *in situ* (leaf and canopy scales) and the image spectral dataset (landscape scale) were analyzed using spectral indices identified in published literature as able to discriminate between plants with different nutrient levels by exploiting chlorophyll fluorescent properties of the plants. Spectral libraries at the leaf level were compared first. When statistically significant results were found, those indices were applied to spectral libraries at the canopy level; and significant results at the canopy level were then applied to the image spectra at the landscape level. Using these criteria, two spectral indices were chosen for the datasets and scales listed below:

- 1) Derivative Chlorophyll Index (DCI) (Zarco-Tejada et al., 2002):
(where R' = 1st derivative of reflectance)

$$(R'723 \text{ nm}) / (R'703 \text{ nm}) \quad (\text{ASD field spectra; leaf and canopy levels}) \quad (2)$$

$$(R'726 \text{ nm}) / (R'695 \text{ nm}) \quad (\text{HyMap spectra; landscape level}) \quad (3)$$

- 2) Photochemical Reflectance Index (PRI) (Gamon et al., 1997):

$$(R531 - R570) / (R531 + R570) \quad (\text{ASD field spectra; leaf and canopy levels}) \quad (4)$$

$$(R526 - R571) / (R526 + R571) \quad (\text{HyMap spectra; landscape level}). \quad (5)$$

The slight differences in the wavelengths used for *in situ* (Eqs. (2) and (4)) and image spectra (Eqs. (3) and (5)) indices were due to the coarser resolution of the HyMap spectral bands (13–17 nm) compared to the ASD spectral bands (3–11 nm).

3. Results

3.1. Water quality monitoring (point sampling)

Most of the long-term data were analyzed and published elsewhere (Caffrey et al., 1997, 2007a; Caffrey, 2002), so here we present only a summary of those data most relevant to our study and compare

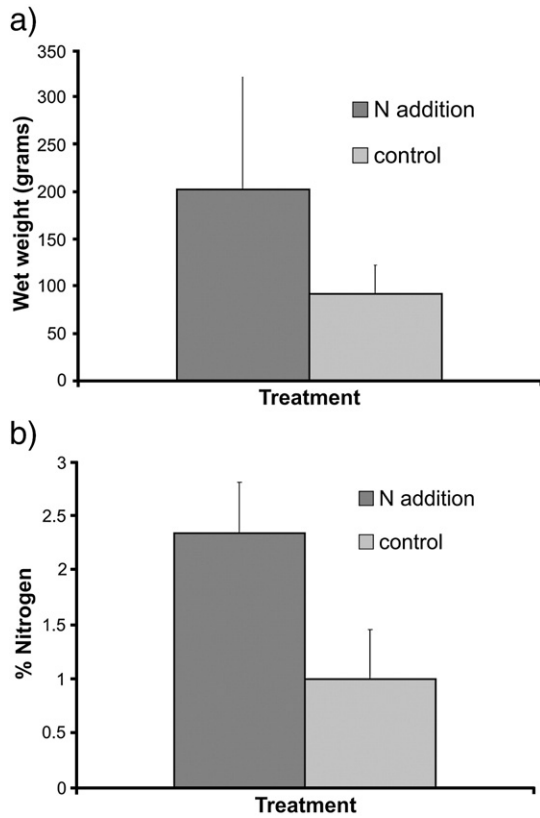


Fig. 3. Effects of nitrogen addition on *Salicornia virginica*: a) vegetation biomass (wet weight; mean \pm 1SD). b) Plant tissue nitrogen concentration (mean \pm 1SD).

it to longer-term trends. During the 12 years (1988–2000) of ESNERR’s monthly water monitoring data available at the time of image acquisition, the general patterns of spatial and temporal variation

within and among stations were consistent. Water nitrate concentrations in Elkhorn Slough were usually high compared to many other estuarine systems. Annual and interannual variations were also very high (Fig. 12.4 in Caffrey, 2002), reflecting the seasonal changes in nutrient inputs associated with precipitation and runoff patterns, including the recurrent droughts that have characterized the Californian climate since 1988.

Table 1 contains means and standard deviations of data from the seven-month growth period (March–September) for each year from 1996 to 2000. Long-term nutrient concentrations were lowest at or near the sites on the National Estuarine Research Reserve itself, and some distance from the most intensively farmed areas (e.g. South Marsh, Kirby Park); the highest values were at the sites in the lower old Salinas River channel, which drains into the lower reaches of Elkhorn Slough (i.e. Potrero Road North and South). These patterns are consistent with those at the 18 stations (Fig. 1) over the timeframe of this study. The general spatial and temporal patterns of nutrient enrichment in the present study are therefore consistent with the longer temporal and larger spatial trends in Elkhorn Slough reported by Caffrey et al. (1997, 2007a) and Caffrey (2002). At a few sites in our analyses, total water N concentrations exceeded the longer-term trends, but these increases were not significant (i.e. Lower Azevedo Pond, Carneros Creek, and both Hudson Landing stations; compare Table 1 with Caffrey et al., 1997). This increasing trend probably reflects intensification of agriculture in the ten years since Caffrey et al. (1997). Interannual variation remained high in the temporal subset of the present study (productive seasons between March 1996 and May 2000, Table 1), partly due to 1998, an anomalously wet El Niño year.

3.2. Field fertilization experiment (patch scale; temporal integration)

3.2.1. Vegetation quantity and quality

The addition of fertilizer in the field experiment at Coyote Marsh significantly increased *Salicornia* biomass; wet weight nearly doubled in the nitrogen addition plots (Fig. 3a; *t*-test, $P=0.007$). The subsequent CHN analyses of dried leaves showed that the nitrogen content of

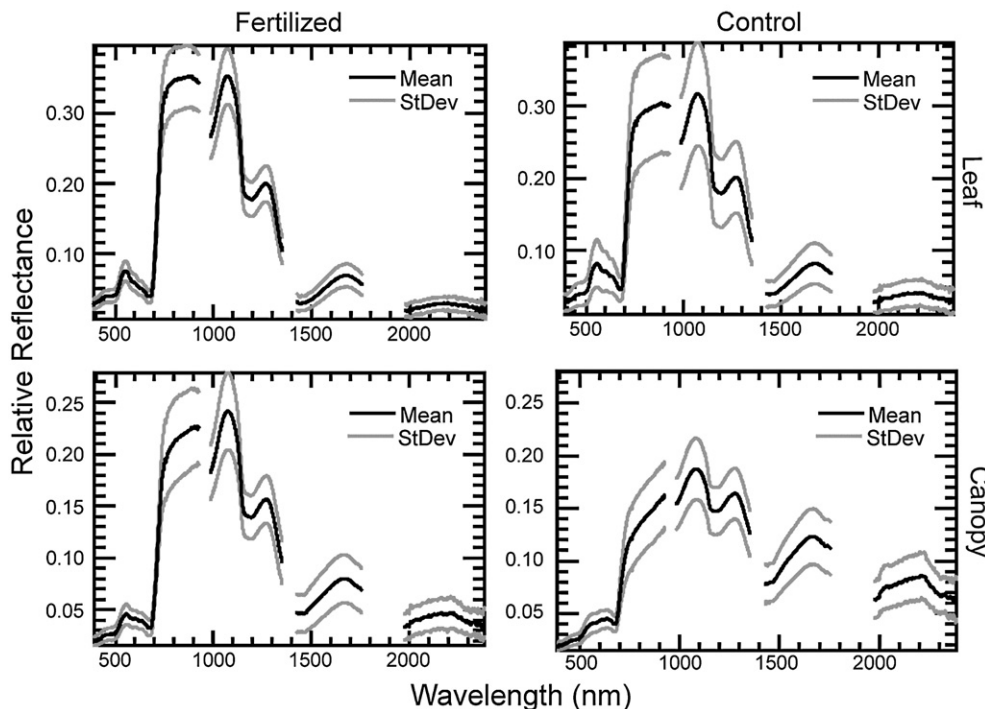


Fig. 4. Relative reflectances for spectral libraries acquired in the field (means \pm 1SD). Upper left: fertilized plants at leaf scale; lower left: fertilized plants at canopy scale; Upper right: unfertilized plants at leaf scale; and lower right: unfertilized plants at canopy scale. $N=20$ for each library.

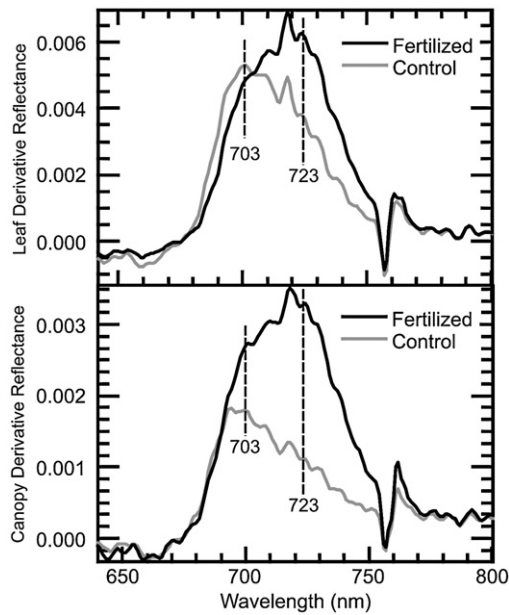


Fig. 5. First derivatives of mean reflectances at leaf (upper panel) and canopy (lower panel) levels for spectra of fertilized and unfertilized plants, showing changes in chlorophyll fluorescence in the 640–800 nm range. Vertical bars indicate wavelengths (703 and 723 nm) used by the Derivative Chlorophyll Index (DCI).

foliage in each of the 20 fertilized plots was significantly greater (~ 2.3 times) than in the 20 adjacent control plots (Fig. 3b; t -test of paired means, $P < 0.001$).

3.2.2. Spectral responses

The slope of the red-edge region was always steeper for fertilized plants, and their average NIR reflectance always higher compared to unfertilized plants (Fig. 4, left panels). Reflectances in the visible and NIR were considerably greater at the leaf level (Fig. 4, upper panels) than at the canopy level (Fig. 4, lower panels) where a greater shade

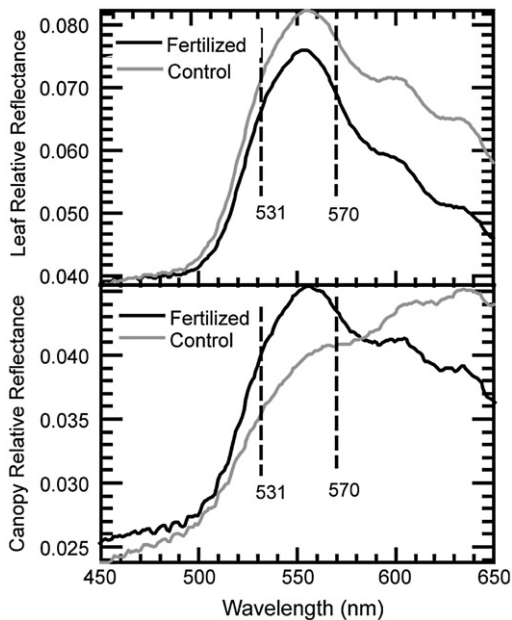


Fig. 6. Mean relative reflectance at leaf (upper panel) and canopy (lower panel) levels for spectra of fertilized and unfertilized plants, showing changes due to chlorophyll fluorescence in the 450–650 nm range. Vertical bars indicate wavelengths (531 and 570 nm) used by the Photochemical Reflectance Index–PRI.

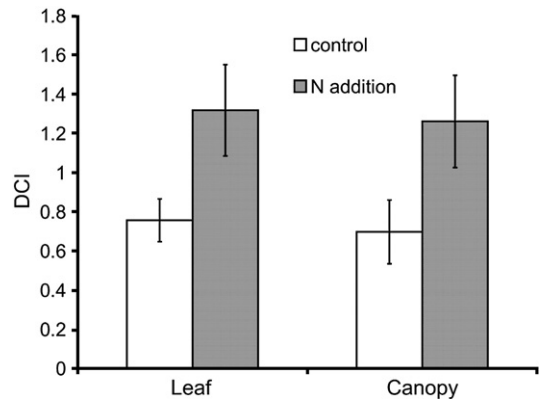


Fig. 7. Mean (± 1 SD) double peak ratios (703 nm/723 nm) of 1st derivatives of field reflectance spectra (Derivative Chlorophyll Index, DCI) of fertilized and unfertilized plots.

fraction made the spectra darker; this trend was reversed at longer wavelengths (SWIR) where the canopy spectra had higher reflectances, possibly due to contributions from the underlying soil. Canopy reflectances were also more variable (larger SD) in the SWIR region than leaf reflectances (Fig. 4). The first derivatives of reflectances in the red-edge region shifted towards longer wavelengths in fertilized plants at both leaf and canopy scales, and the magnitude of the 723 nm peak also increased relative to the 703 nm peak for fertilized plants (Fig. 5). Visually, vegetation in the fertilized plots appeared to be a brighter green than the relatively dull olive green of the unfertilized plots; and this was confirmed spectrally by a smaller contribution from yellow–red wavelengths (~ 570 –650 nm) in the fertilized plots than in the unfertilized plots at both leaf and canopy levels (Figs. 4 and 6). At both levels, reflectances from fertilized *S. virginica* were consistently lower in the 580–680 nm range than those from unfertilized plants (Figs. 4 and 6).

3.2.3. Spectral indices

Both spectral indices detected differences between treatments in the field fertilization experiment (Fig. 5). With the Derivative Chlorophyll Index (DCI), the DCIs (723 nm/703 nm) based on the first derivatives of field reflectance spectra from fertilized plants were significantly higher than those from unfertilized ones (Fig. 7; t -test, $P < 0.001$). This result was consistent at both leaf and canopy levels.

The Photochemical Reflectance Index (PRI) also detected treatment differences (Fig. 8), although the difference was smaller than for the DCI analyses. Values of PRI were significantly higher (less negative) for fertilized plants than for unfertilized plants, and the magnitude of this

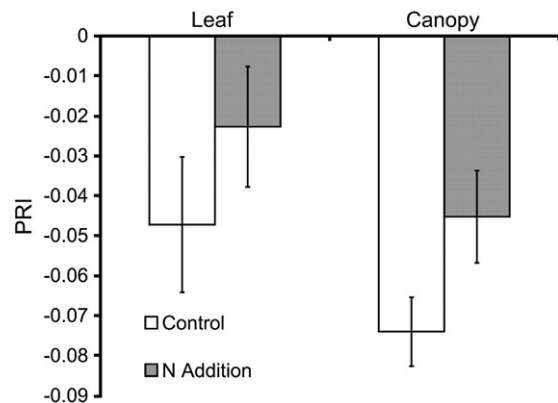


Fig. 8. Mean (± 1 SD) values of Photochemical Reflectance Index (PRI) calculated from field reflectance spectra of fertilized and unfertilized plots.

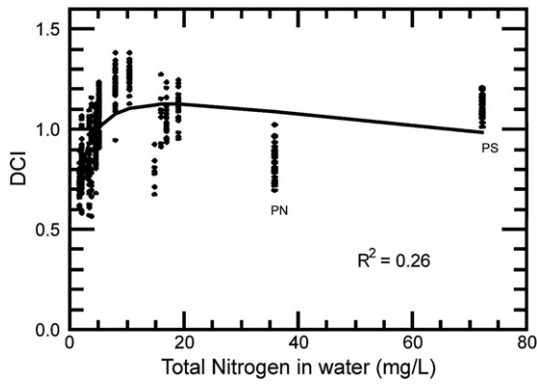


Fig. 9. Regression of Derivative Chlorophyll Index (DCI) from HyMap image spectra against mean total water Nitrogen (mean of productive seasons of 1996–2000) at 18 monitoring stations (logarithmic fit: $y=0.0959 \ln(x)+0.7975$). DCI values for image-extracted spectra from each 2.5×2.5 m pixel containing *Salicornia virginica*. PN = Potrero North station; PS = Potrero South station.

effect was similar at both leaf and canopy scales. These differences were significant at both leaf and canopy scales of spectral measurement (Fig. 8; *t*-test, $P < 0.001$).

3.3. Hyperspectral imagery (landscape scale; spatial and temporal integration)

Section 3.1 of these Results (above) described long-term (1988–2000) temporal and spatial variation in water quality at points throughout the Elkhorn Slough (Fig. 1) and then characterized 18 sites by the variation of the nutrient content of their waters during and between growing seasons (March–September) over five years (1996–2000; Table 1). Section 3.2 described responses of plant tissues (Fig. 3), reflectance spectra (Fig. 4), and two spectral indices (DCI and PRI; Figs. 5–8) of patches of *S. virginica* exposed to experimental additions of nitrogenous fertilizer in 2002. The current section uses spectra of *S. virginica* at each water-sampling site extracted from the image to link the point-scale water sampling (Fig. 1, Table 1) to the landscape-scale of Elkhorn Slough via analysis of the HyMap hyperspectral imagery.

We identified monospecific patches of *S. virginica* in the field near 18 of the water-sampling stations within the imaged area, mapped these patches on the ground as GPS polygons, superimposed the polygon coordinates onto the 2000 HyMap imagery to define Regions of Interest (ROI), and finally calculated the means and variances of two spectral

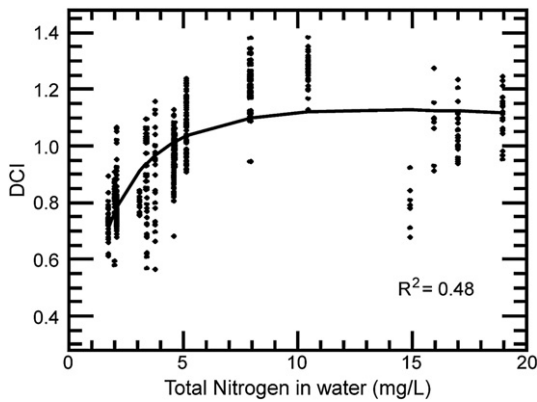


Fig. 10. Regression of Derivative Chlorophyll Index (DCI) from HyMap image spectra against mean total water Nitrogen (mean of productive seasons of 1996–2000) at 16 monitoring stations (logarithmic fit: $y=0.1849 \ln(x)+0.6813$). Potrero Rd. North and Potrero Rd. South omitted. DCI values for image-extracted spectra from each 2.5×2.5 m pixel containing *Salicornia virginica*.

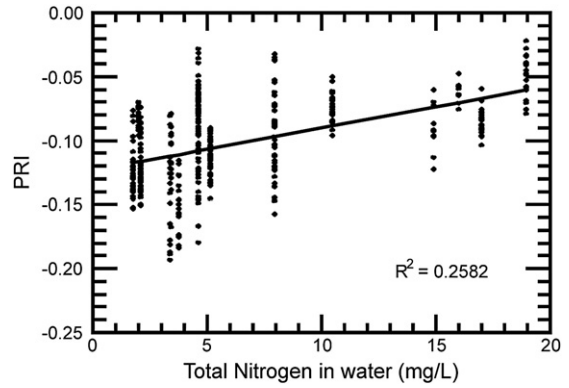


Fig. 11. Linear regression of Photochemical Reflectance Index (PRI) against mean total water Nitrogen (mean of 5 productive seasons, 1996–2000) at 15 monitoring stations. Bennett Slough West, Potrero Rd. North, and Potrero Rd. South stations were omitted.

indices (DCI and PRI) for all pixels within each ROI. There were significant correlations between average growing-season nutrient levels at the water quality stations and the spectral indices from adjacent *Salicornia* patches. When the DCI for first derivatives of double-peak ratio values for every pixel in the 18 ROIs were regressed against mean total water nitrogen over the growing season, there was a significant, more or less asymptotically increasing trend with increasing water nitrogen content (Fig. 9; $r^2=0.260$; $P < 0.01$; $N=18$ stations; 9–100 pixels per station). The apparent failure to asymptote completely appeared to be caused by declining DCIs at the highest N levels at two stations (Potrero North and Potrero South; see Fig. 1 for locations, Table 1 for nutrient data) where nitrogen concentrations were an order of magnitude higher (i.e. 35 and 72 mg/L; Fig. 9) than at low nitrogen stations. When the two Potrero stations were excluded from the analysis, the asymptotic fit improved considerably, and the r^2 almost doubled to 0.485 (Fig. 10; $P < 0.001$; $N=16$ stations). Figs. 9 and 10 show logarithmic fits.

Values of the second spectral index (PRI) also increased significantly with increasing water nitrogen content during the growing season (Fig. 11, $r^2=0.258$; $P < 0.001$), but only after three stations were excluded from the analysis: Potrero North and Potrero South were excluded for the reasons described above; Bennett Slough West was excluded because its phosphate concentrations were 50% higher (Table 1) and its PRI values were much lower than any other station. When these stations were included, the correlation was not significant (Fig. 12).

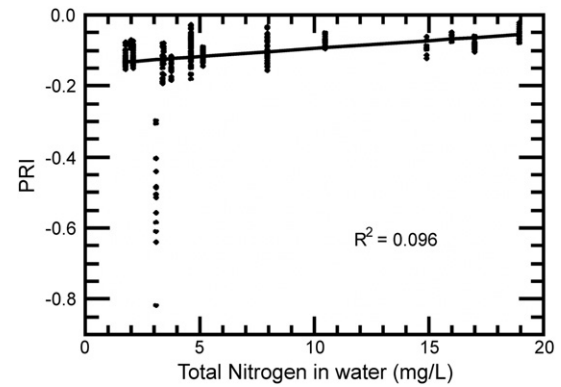


Fig. 12. Linear regression of Photochemical Reflectance Index (PRI) against mean total water Nitrogen (mean of 5 productive seasons, 1996–2000) at 16 monitoring stations, including Bennett Slough West. PRI values for image-extracted spectra from each 2.5×2.5 m pixel containing *Salicornia virginica*. Potrero Rd. North and Potrero Rd. South stations were omitted.

4. Discussion

Nutrient concentrations, especially nitrate, have been very high in the Elkhorn Slough watershed since the early 1990 s, even when compared to other eutrophic estuaries in California (e.g. Tomales Bay and San Francisco Bay) and elsewhere (Chesapeake Bay and Delaware Bay) (Caffrey et al., 1997; Caffrey, 2002). This is most likely due to the intensive agricultural production and limited drainage characterizing the Elkhorn Slough system. One of the greatest management challenges in Elkhorn Slough is quantifying effects of these inputs on ecosystem functioning, and designing possible remediation plans. Effects of non-point source pollution from agricultural runoff on estuarine biota and water quality are of particular concern (Caffrey, 2002).

Continuing water quality monitoring in Elkhorn Slough is essential, and new management practices and monitoring approaches are being evaluated by ESNERR and other agencies. To explore benefits of incorporating remotely-sensed data into new methods for effective monitoring, we examined indicators of nutrient enrichment in Elkhorn Slough in three different ways, concentrating on the most robust information that each contains about nutrients, while spanning several spatial and temporal scales. The major goal was to determine which, and how much, information is transferable across scales, at first qualitatively but ultimately quantitatively. This study was also one of the first explicit attempts to validate the use of hyperspectral imagery for detection of variation in estuarine nutrient enrichment, by using the spectral responses of the marsh vegetation as proxies for water nutrient concentration.

We first explored the patterns of nutrient concentrations in Elkhorn Slough waters, from published water quality and nutrient data for 24 sites at monthly intervals from 1988 to 2000, to gather information about mobile nutrients during their physical transport in the water column (Caffrey et al., 1997, 2007a; Caffrey, 2002). We used these data to evaluate patterns of variation in space and time, to describe temporal patterns of seasonal change, and especially, to identify sites with consistently high and consistently low nutrient concentrations and their correlations with marsh nutrient uptake and productivity.

Two main factors appear to control spatial variation in estuarine nutrient enrichment at Elkhorn Slough: 1) proximity to agricultural or other non-point source runoff; and 2) tidal exchange and water residence times within Elkhorn Slough (Caffrey, 2002). These trends are also apparent in the subset of the water quality data that we considered for this study. Sites nearest to the National Estuarine Research Reserve, and furthest from farmed areas, had the lowest nitrogen values (e.g. South Marsh, Kirby Park); while sites adjacent to agricultural fields, where they directly receive non-point source runoff, were consistently high (e.g. Potrero Road North and South; Table 1, Fig. 1); maximum N concentrations were comparable to those in effluent from sewage treatment plant (Caffrey et al., 1997). The longer residence time of water at the head of Elkhorn Slough than at its well-flushed mouth also explains some of the variation (Caffrey, 2002), however, as points in space and time, such water sampling data are unlikely to detect most short pulses or localized inputs of nutrients, and the ecosystem pathways, rates and ultimate fates (in groundwater, soil, sediments or organisms) of nutrients measured in water remain unknown.

Our second, experimental approach expanded the spatial scale to small patches (0.25 m² plots in an area about 200 m across) that allowed us to test the assumption that the dominant plant in Elkhorn Slough, *S. virginica*, is limited by nutrients (especially nitrogen), as has been shown for *Salicornia* in southern California (Page, 1995; Boyer et al., 2001). We determined that the spectral signature of *Salicornia* exposed to nutrient enrichment is distinct from that of unfertilized *Salicornia*, and is characterized by greater absorption between 555 nm and 680 nm (Fig. 4), a steeper red-edge

slope, and higher PRI and DCI values (Figs. 7 and 8). These patterns are all consistent with results from laboratory spectral measurements of dried plants and terrestrial vegetation, and they indicate that the chlorophyll content and photosynthetic activity of *Salicornia* increase with fertilization (Gamon et al., 1997; Kokaly & Clark, 1999; Kokaly, 2001; Zarco-Tejada et al., 2002; Mutanga et al., 2003). In both fertilized and unfertilized plots, lower PRI and DCI values at the canopy level than at the leaf level are probably due to greater shade fractions and increased mixing of non-photosynthetic surfaces in canopy level measurements.

Our third approach was based on analysis of high spatial resolution (2.5 m pixels) HyMap hyperspectral imagery of the entire wetland. After using a hand-held spectrometer to characterize spectral indicators of nutrient enrichment at two spatial scales (leaf and canopy levels) in the experimental plots, we then documented spectral variation in imagery of larger patches (landscape scales) in the wetland, by locating areas (56–500 m²) of monospecific *Salicornia* in close proximity to 18 of the water quality monitoring sites. We again found significant correlations between spectral signatures of *Salicornia* and nutrient levels at these sites. Although a direct causal relationship between water nitrate and adjacent *Salicornia* marshes cannot be established from these data alone, the consistency of the spectral responses of PRI and DCI across a range of water quality values suggests that these spectral indices may provide useful proxies for *Salicornia*'s physiological responses to nitrogen levels in waters inundating the plants.

Long-term nitrogen accumulation in plant tissues is likely to be part of *Salicornia*'s response to nutrient enrichment, since the plant's exposure to and opportunities for direct uptake of dissolved nitrogen are relatively unpredictable and ephemeral, given the short residence time of water in Elkhorn Slough (Caffrey et al., 2007a). When the marshes are flooded by incoming tides, nutrients are deposited or adsorbed on the plants and soils of the mudflats where *S. virginica* grows. Boyer and Fong (2005) suggested that transfer of water column nitrogen to salt marsh plants may be mediated by macro-algae through both leakage and tissue decomposition, since macro-algae commonly grow or are deposited among the bases of *S. virginica* and other marsh plants at lower tidal levels. When macro-algae co-occur with *Salicornia*, access to water column N derived from algal tissues could be as important a mechanism for wetland plants' nutrition as is direct uptake and assimilation of N inputs derived from upland runoff, the primary mechanism suggested by Boyer et al. (2001) and Caffrey et al. (2007b). In either case, plant nitrate uptake is a potentially large sink for nitrogen in estuarine systems. Chapin et al. (2004) calculated that perhaps 70% of nitrate lost from Elkhorn Slough is taken up by plants. Further work is needed to establish physiological and other mechanisms linking water nutrients, macroalgal metabolism, N-enrichment of *Salicornia* tissues, and spectral signatures of estuarine wetlands.

Results of DCI analyses of field spectra (Fig. 7) were consistent with those of Smith et al. (2004) and Lamb et al. (2002), who also found that ratios of the derivatives of reflectances at the two peak wavelengths increased in low nutrient plants. Similarly, regressions of DCI from image spectra against water nitrogen concentrations in Elkhorn Slough show that DCI increased with higher plant nutrient concentrations (Figs. 9 and 10). At the same time, two stations (Potrero North and Potrero South) with water nitrogen concentrations an order of magnitude higher than at the other stations, had lower DCI values than predicted by the regression. There was no observable response to water nitrogen higher than ~12 mg/L (Fig. 9) which may indicate either that a "saturation threshold" exists beyond which additional nitrogen is not used by *Salicornia*, or that some other environmental factor is now limiting plant performance. A third possibility is that high nitrogen values may be "stressful" to the plant, leading to lower DCI values for plants in exceptionally enriched waters. Other reflectance indices, such as NDVI, are known to have similar

asymptotic relationships with environmental gradients, especially at the canopy scale (Tilley et al., 2003).

The asymptotic relationship between the DCI spectral response and water nitrogen implies that the useful range of the regression for predictive purposes would be limited to concentrations ≤ 12 mg/L of total nitrogen, the value at which the DCI response starts leveling off (Figs. 9 and 10). During their analyses of long-term water quality in Elkhorn Slough, Caffrey et al. (1997) explored the occurrence of nitrate concentrations above an arbitrary threshold of 14 mg/L at each of the water quality monitoring stations over a 5-year span (1991–1996). Among the 18 sites considered in our study, only two stations exceeded 14 mg/L in more than 30% of samples from that analysis: 37% at Potrero North and 61% at Potrero South (Fig. 1). The other 16 stations exceeded this threshold only 0–8% of the time, with most stations exceeding it in $\leq 2\%$ of samples over the 5-year time span (Caffrey et al., 1997). This suggests that while the predictive power of the nitrogen enrichment correlation using the DCI approach seems to be limited to nitrate values ≤ 12 mg/L, this is the range most commonly experienced by *Salicornia* marshes in Elkhorn Slough, and this range spans conditions from unenriched to eutrophic.

Many factors may have contributed to greater variation in the DCI and (especially) PRI values from the HyMap images than in the ASD data from experimental plots, and hence to weaker correlations with nutrients at the landscape scale. These include, but are not limited to: the lower spectral resolution of the image spectra compared to the ASD spectra taken on the ground, which would reduce the detectability of narrow absorptions characterizing the DCI and PRI indices; greater mixing of substrates within pixels in the larger footprints of the HyMap versus the ASD; variation through time in water nutrient levels at individual water monitoring stations, not captured by the monthly data used for ground truthing the image spectra; and the unknown length of the period over which past environmental conditions continue to be expressed by the plants. Cumulatively, these factors probably contributed to the low coefficients of determinations of the PRI and DCI from image spectra. These factors also represent inherent limitations to using this approach as a management tool, unless they can be controlled or improved upon in future landscape level studies and applications.

For the VNIR range spanning photosynthetically active wavelengths, the ASD portable radiometer has 3–5 nm spectral resolution versus the 15.5 nm bands of HyMap. The HyMap spectra, acquired from an altitude of 1300 m, also have atmospheric distortions (partly addressed with the FLAASH program) that are not present in the ASD ground measurements. While HyMap's spatial resolution of 2.5 m pixels (from 1300 m) is better than many hyperspectral imagers, a 2.5 m pixel is still likely to include unwanted substrates, such as bare soil or dead plants, in addition to the targeted *Salicornia*. Cleaner relationships would be expected if the hyperspectral image had smaller pixels and/or narrower spectral bands (e.g. the Jet Propulsion Laboratory's AVIRIS sensor): in either case, pure *Salicornia* pixels and their subtle spectral absorptions could be detected more readily from airborne data. On the other hand, despite these potential practical limitations, the instantaneous airborne HyMap data from May 2000 are remarkably well correlated with the *in situ* water nitrogen concentrations averaged over five growing seasons (1996–2000) of *S. virginica*, and the coefficients of determination (Fig. 9–11) are realistic for many remote-sensing studies using image spectral data at landscape levels (e.g. Porder et al., 2005).

The present study appears to be the first in which either macro-algae or saltmarsh plants have been used as direct bioindicators of potential eutrophication in the field and at landscape scales. Most previous work on spectral responses to nutrient enrichment has been conducted primarily in the laboratory with terrestrial plants lacking the high water and salt contents that characterize *Salicornia* and other succulent salt marsh plants. The few previous studies exploring use of spectral indices as temporal integrators of estuarine nutrient enrich-

ment (Fong et al., 1998; Runcie et al., 2004; Boyle et al., 2004; Boyer & Fong, 2005; Cohen & Fong, 2006) were conducted either in experimental settings in the field under controlled conditions, or in laboratory microcosms that primarily used macro-algae.

Stronger statistical relationships between field and image data can also be expected with increasing understanding of the synergistic relationships of nitrogen with other factors affecting marsh vegetation. For example, effects of phosphorus (e.g. PO_4), fertilization of salt marsh plants have received little attention to date, because nitrogen is usually the limiting nutrient in marshes, but a few studies suggest that P fertilization can have significant effects on tissue P in *S. virginica* (Boyer et al., 2001). Phosphorus fertilization can also indirectly alter soil nitrate availability and its subsequent uptake by saltmarsh plants via indirect effects on denitrification and N-fixation (Caffrey et al., 2007b). In our analyses, the significance and predictive power of one index (PRI from image data) increased when the range of phosphate values was restricted (Fig. 11, compare to Fig. 12). Other researchers who have partitioned data by water phosphorus concentration, also report improved correlations of reflectance indices with water nitrogen variation (Tilley et al., 2003). However, the roles of phosphates on wetland plant reflectances remain largely unknown and are difficult to interpret. Furthermore, many previous studies show that reflectance spectra usually respond simultaneously to more than one environmental factor. Water nitrogen (Tilley et al., 2003), temperature and humidity (Zarco-Tejada et al., 2002), salinity, and soil-oxygen concentration (Smith et al., 2004) all tend to modify spectral signals in much the same way. While narrow band reflectance indices are certainly sensitive to plant responses from environmental stimuli, the responses usually lack unique features for identifying the particular environmental parameter causing the plant's response.

In laboratory studies, other hyperspectral methods have been proposed as ways to measure N content of plants, including the continuum-removed reflectance at SWIR wavelengths characteristics of nitrogen-containing amide bonds in plant proteins, and the normalized area calculated from the continuum-removed visible reflectance (Kokaly & Clark, 1999; Kokaly, 2001). The lower Signal-to-Noise Ratio (SNR) in the SWIR region of the HyMap and other imagers compared with laboratory instruments and the variability of the atmospheric column combine to make analyses using image spectra less sensitive. The airborne acquisition of Elkhorn Slough in May 2000 was flown with only a few hours notice that left insufficient lead time to acquire simultaneous ground truthing spectra with hand-held spectrometers, and thus to improve the atmospheric correction model and exploit the SWIR region using these additional methods. Although the science of removing optical atmospheric effects is developing rapidly, rigorous algorithms that work well without extensive ground calibration, especially in coastal and aquatic habitats, have yet to be developed.

Other uncertainties were associated with co-locating homogeneous *Salicornia* patches in the Elkhorn Slough imagery, partly because of the lag between image acquisition in May 2000 and the GPS mapping to define the boundaries of monotypic *Salicornia* patches in June 2004. We initially identified *Salicornia* patches directly from the HyMap imagery using standard classification routines (Jupiter et al., 2002), but concluded that the accuracy of the classifications of *Salicornia* cover was too low for purposes of the present study. While it is possible that the boundaries of some *Salicornia* patches mapped in the field may have changed since 2000, our continuing field observations at many sites suggest that most such changes occur slowly and probably were less than the pixel resolution (2.5 m) of the imagery.

The present results are encouraging indicators that water nitrogen regimes, physiological and spectral responses of plants, and remotely sensed hyperspectral imaging can be linked for assessing nutrient enrichment in wetlands. The spectral indices discussed here (PRI and DCI) clearly have some potential to serve as proxies for nutrient input, at least for qualitative mapping of relative nutrient variation and

gradients. We have correlated mean water column total nitrogen with the photosynthetic signatures of adjacent plants, but we recognize that this is not a direct spectral measurement of the concentration of nitrate in the water, and further work is needed to determine whether the steps between water concentrations and image spectra are sufficiently predictable to allow more quantitative estimates of water and plant nutrient levels from hyperspectral sensing.

Nutrient pollution and subsequent eutrophication continue to be among the most serious threats to estuarine ecosystems (Matson et al., 1997; Vitousek et al., 1997a; Kennish, 2002). In many wetlands, there are urgent needs for fast, reliable, and synoptic ways to detect, monitor and manage nutrient levels across entire systems (Cloern, 2001), and especially in ways that provide good correlations across several spatial and temporal scales. While our data are specific to the biogeochemical characteristics of Elkhorn Slough in a particular time interval, to the species *S. virginica*, and to the resolutions and SNRs of the HyMap and ASD instruments, we suggest that our approach could be extrapolated to other wetland systems should it prove to be robust in future studies of other estuarine systems. Hyperspectral imaging could then become a powerful tool for assessing and integrating the consequences of nutrient loading, especially over large areas, and where access is difficult.

Vitousek et al. (1997b) suggested that one way to slow the accelerating human alteration of natural ecosystems lies in applying interdisciplinary approaches that integrate methods spanning a range of biological, geological, atmospheric and marine sciences, to enhance understanding of interactions among the multiple components of anthropogenic global change. We propose that incorporating remote sensing into monitoring of estuarine nutrient enrichment follows the advice of Vitousek et al., because this technology is inherently interdisciplinary. By integrating knowledge from different sources and disciplines, our study provides one example of the advantages (and limitations) of such efforts, while also contributing to greater understanding of the consequences and ultimately, the mitigation of anthropogenic changes to coastal ecosystems. A remote sensing approach such as the one presented here should become increasingly relevant as nitrogen pollution of coastal waters becomes more widespread and severe as a direct consequence of the global expansion of industrialized agricultural practices (Beman et al., 2005). The fast pace at which hyperspectral remote sensing is evolving, and the wider availability and coverage offered by the advent of commercial hyperspectral satellite imaging should quickly broaden the scope and applicability of this technology.

Acknowledgments

We thank Sue Shaw (ESNERR volunteer) for collecting monthly water quality samples over more than two decades; Andrea Woolfolk (ESNERR) for help implementing the field fertilization experiment; Eric Van Dyke (ESNERR) for his GIS expertise; Brigitte Martini (HyVista Corp. and RRI) for useful discussions on HyMap and hyperspectral analysis; Laurel Fox (UCSC) for lab facilities for drying and grinding plant samples; William Pickles (LLNL/UCSC) for help securing the acquisition of HyMap imagery, and for spearheading hyperspectral imaging research at UCSC; John Ryan and Andy Fisher at MBARI for access to FLAASH software; NASA's Jet Propulsion Laboratory (JPL) for lending the ASD field spectrometer; and the staff of the Naval Postgraduate School Remote Sensing Center for their IDL programming, counsel and expertise. Finally, we thank Terry and Peter Cocks (HyVista) and Fred Kruse (Horizon GeoImaging) for including Elkhorn Slough in the 2000 HyMap Group Shoot. Financial support came primarily from a NOAA/CICEET grant (No. 01-453) to D.C. Potts, and a NASA Earth System Science Fellowship (NGT5-30415) to D. Siciliano.

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