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Research Paper

# Characterization of cDOM in the Elkhorn Slough estuary using EEM spectroscopy and its potential for macrophyte monitoring

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ABSTRACT

The Elkhorn Slough estuary is an upwelling-influenced system located in Monterey Bay, California. It is of great ecological importance, as it provides essential habitat to over 750 species, and is one of the largest estuaries in California. The sources and distribution of chromophoric dissolved organic matter (cDOM) in Elkhorn Slough are strongly influenced by geochemical processes linked to the transition between sea and continent, and to anthropogenic activity from adjacent lands. In 2016, water samples were collected from across the Elkhorn Slough estuary to assess the sources and spatial dynamics of cDOM and its components throughout the system. Using EEM spectroscopy, seven fluorophores (four humic-like and three protein-like), and three distinctive cDOM regions were identified through a combination of traditional peak analysis, Principal Components Analysis (PCA) and interpretation of different fluorescence indices. The majority of the sites in the lower slough, receiving water from Monterey Bay and the Old Salinas River watershed, presented a balanced mix of humic-like and protein-like materials. Sites in the proximity of eelgrass beds had distinctive cDOM signals with predominant protein-like components of autochthonous origin. Water from the upper slough, subject to high nutrient input and excessive macroalgal development, was mostly composed of humic-like matter linked to recent biological activity and macrophyte development. Distribution of cDOM along the main channel of the Elkhorn Slough estuary was influenced by hydrological processes, allochthonous input, and macrophyte distribution. The large contribution and high specificity of macrophyte-derived chromophores identified in this study suggest that EEM spectroscopy could be an effective tool to monitor macrophytes in locations where environmental conditions are unfavorable.

## 1. Introduction

Dissolved organic matter (DOM) in the ocean is linked to primary production, and plays a key role in shaping aquatic ecosystems (Aluwihare and Repeta, 1999). The variability of DOM in natural waters is often traced via optical measurements, since its optical properties are defined by its chemical nature (Jaffé et al., 2008). Fluorescence of a water sample is related to its concentration, and higher intensity of exciting radiation results in more fluorescence and higher emission intensity (Mayerhöfer and Popp, 2019). Colored dissolved organic matter (cDOM) refers to the optically active fraction of DOM, that is commonly characterized using fluorescence spectroscopy. Fluorescent compounds can be identified using excitation-emission matrices (EEMs), resulting from repeated emission scans over a range of excitation wavelengths or vice-versa (Coble, 1996). EEM spectroscopy is fast and allows for detection of small-scale variation in DOM composition. It is used to obtain information on the number, nature, and abundance of the fluorophores present in a sample. Recent studies have used EEM spectroscopy to understand physical and biological processes taking place in aquatic systems (e.g., Huguet et al., 2010; Murphy et al., 2008; Stedmon et al., 2003).

Measuring fluorescence in cDOM can be helpful to discern the sources, circulation, and mixing of different water masses (Stedmon et al., 2003). Transition ecosystems that connect continental and oceanic environments receive water from multiple sources that include marine cDOM, terrestrially-derived cDOM, bacterial degradation, and autochthonous production of cDOM (Wang et al., 2014). In coastal systems, degradation of land and aquatic plants is a major source of DOM (Hansell and Carlson, 2014). Production of cDOM can occur via extracellular release, senescence, predatory grazing, or viral lysis (Bertilsson and Jones, 2003). Additionally, wastewater discharges might also influence cDOM distribution. In estuaries, a combination of interacting physical, chemical, and microbial processes result in cDOM cycling (Murphy et al., 2008).

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In shallow productive environments, macrophytes are known to generate protein-like cDOM and supply an important amount of autochthonous DOM (Lapierre and Frenette, 2009). The relative contribution of algae and aquatic plants as sources of cDOM in estuaries varies depending on algal input and geophysical characteristics of each individual system (Bertilsson and Jones, 2003). Seagrasses and macroalgae are thought to be significant sources of cDOM in areas where there is little freshwater input and terrestrial runoff (Clark et al., 2016). In coastal waters, there are two predominant fluorescence signals: humic-like fluorescence, occurring at 420-450 nm emission wavelengths from excitation at 230–260 and 320-350 nm, and protein- or amino acid-like fluorescence observed at an emission of 300–305 and 340-350 nm emission wavelengths from excitation at 220 and 275 nm (Coble, 1996). The main fluorophores identified via EEM spectroscopy in coastal environments are shown in Table 1.

The purpose of this study was the characterizations of cDOM throughout the Elkhorn Slough estuary in California. This was achieved by evaluating the relative contributions and spatial distributions of the different cDOM components to identify their sources throughout the estuary. This study also assessed the effectiveness of EEM spectroscopy to estimate macrophyte abundance and distribution in eutrophic estuarine environments, something which has not been previously addressed.

## 2. Methods

### 2.1. Study location

The study took place in the Elkhorn Slough estuary, located in the center of Monterey Bay, California (Fig. 1). The main channel of Elkhorn Slough is approximately 10 km long, 100 m wide and 3 m deep on average, with depth being 8 m at the mouth and decreasing landward (Broenkow and Breaker, 2005). Two distinctive climatic seasons can be appreciated: a dry season (March to October) and a wet season (November to February), with high seasonal variability in physical, chemical, and biological composition of the estuary (Smith, 1973). Regardless of season, two distinctive water types are found in the main channel, based on chemical properties: offshore water, and upper slough water (Smith, 1973). Although it is well established that the residence time for lower slough waters is short, the degree to which waters from the upper slough mix with waters from the lower slough is unknown (Broenkow and Breaker, 2005). Adjacent lands are primarily used for agricultural purposes, resulting in waters with high nutrient concentrations being transferred to the slough (Smith, 1973). As a result, Elkhorn Slough is nutrient enriched, with high autochthonous biological activity and anthropogenic influence, and algal blooms that are frequent in the Spring. The dominant macrophytes in the Elkhorn Slough estuary are short-lived opportunistic macroalgae of the *Ulva* genus, and eelgrass (*Zostera marina*). Their distribution along the main channel can be attributed to a combination of factors such as bathymetry, tidal inundation, nutrient runoff and herbivory (Grant, 2009; Schaadt, 2005; Vila Duplá, 2020). There are two large *Z. marina* beds in the lower slough, located approximately 1–2 km from the mouth of the estuary: one halfway between Vierra Mouth and Seal Bend, and one off the Northern shore of Seal Bend. These beds are found in depths ranging from 0.5 to 2 m below mean lower low water (MLLW) (Hammerstrom and Grant, 2012), and shoot density that can be as high as 350 shoots/$m^2$ in the month of November (Vila Duplá, 2020).

In this study, surface water samples were collected from ten different locations throughout the main channel of Elkhorn Slough (Fig. 1). The sites chosen were located at the mouth of the estuary on either side of the Highway Bridge (HB 1–2), on the eelgrass beds near Seal Bend (SB 3–4), South of Rubis Creek (RC 5–6), along the Elkhorn Slough National Estuarine Research Reserve (RR 7–8), and among decomposing algal mats near Kirby Park (KP 9–10). These locations represent the variability and gradients in freshwater input, nutrient runoff, and macrophyte abundance along the estuary. Letter codes were given based on predictions of pairs of sites whose water masses had similar sources due to the hydrographic, chemical and biological conditions of their locations.

**Table 1**
Designation and description of the major fluorescent components in coastal waters (Cheng et al., 2015; Coble, 1996). This notation will be used throughout the paper.

| Peak | $Ex_{max}$ (nm) | $Em_{max}$ (nm) | Description |
|---|---|---|---|
| A | 260 | 380–460 | UV fulvic acid[a] |
| B | 270–280 | 300–310 | Tyrosine-like, autochthonous origin[a] |
| T | 270–280 | 320–350 | Tryptophan-like, autochthonous origin[a] |
| M | 300–320 | 380–420 | Humic-like, marine origin[a] |
| C | 330–370 | 420–480 | Visible-band fulvic acid[a,b] |
| R | 230 | 355–375 | Tryptophan-like, associated with eelgrass[b] |
| a | 230–250 | 400–450 | Humic-like, terrestrial origin (exported from agricultural catchments)[b] |
| N | 280–300 | 365–380 | Biologically-derived, primary production[b] |

[a] Coble (1996).
[b] Cheng et al. (2015).

### 2.2. Data collection and processing

Samples from the ten chosen sites were collected during low tide on November 10th 2016, and stored in ice until they reached the laboratory. Unconcentrated samples were filtered through 0.7 μm Whatman glass fiber filters (GF/F) into acid-cleaned Falcon tubes. Filter choice was based on the characteristics of the study system; particles within the 0.2–0.7 μm range might have a significant impact in highly productive environments, but they have little influence on cDOM composition in aquatic systems with terrestrial DOM input like the Elkhorn Slough estuary (Massicotte et al., 2017). All samples were refrigerated and processed within a week of being collected. Data on water quality across all sites on the sampling date can be found in Appendix A.

A SPEX Fluoromax-3 spectrofluorometer (© Jobin Yvone, Inc., USA) operated in ratio mode (S/R) was used to obtain fluorescence readings. Before analyzing samples, monochromators and spectrometers were calibrated when necessary and reference scans were run with milli-Q water and quinine sulfate to standardize data output. Water samples contained in 1 cm quartz cuvettes were run across an excitation range of 230-500 nm and an emission range of 300-600 nm. The increments were of 5 nm on the excitation axis and 3 nm on the emission axis, for a total of 55 emission scans per matrix and measurements of fluorescence intensity at 55,550 Ex/Em wavelength pairs. EEM scans were run unmasked, as Raman and Rayleigh scatter corrections were performed after acquiring the data using MATLAB software (© 2016 MathWorks, Natick, MA).

A MATLAB Graphical User Interface (GUI) developed by The Alliance of Coastal Technologies (ACT) (Johengen et al., 2012) was used to process and plot EEM data; to obtain quantitative readings of fluorescence intensity, to adjust fluorescence readings based on several corrections, and to create contour plots. In MATLAB, four different types of corrections were made to fluorescence readings (Fig. 2), and reflected in the contour plots:

1. Raman Area: data normalization by dividing the area under the Raman curve.
2. Subtract Blank: reduction of the intensity of the scatter peaks by subtracting the EEM run on Milli-Q water.
3. Quinine Sulfate: conversion of all data to Quinine Sulfate Units (QSE) using the slope of a standard curve relating fluorescent signal intensity at 450 nm and quinine sulfate concentration, following recommendations by Johengen et al., 2012.

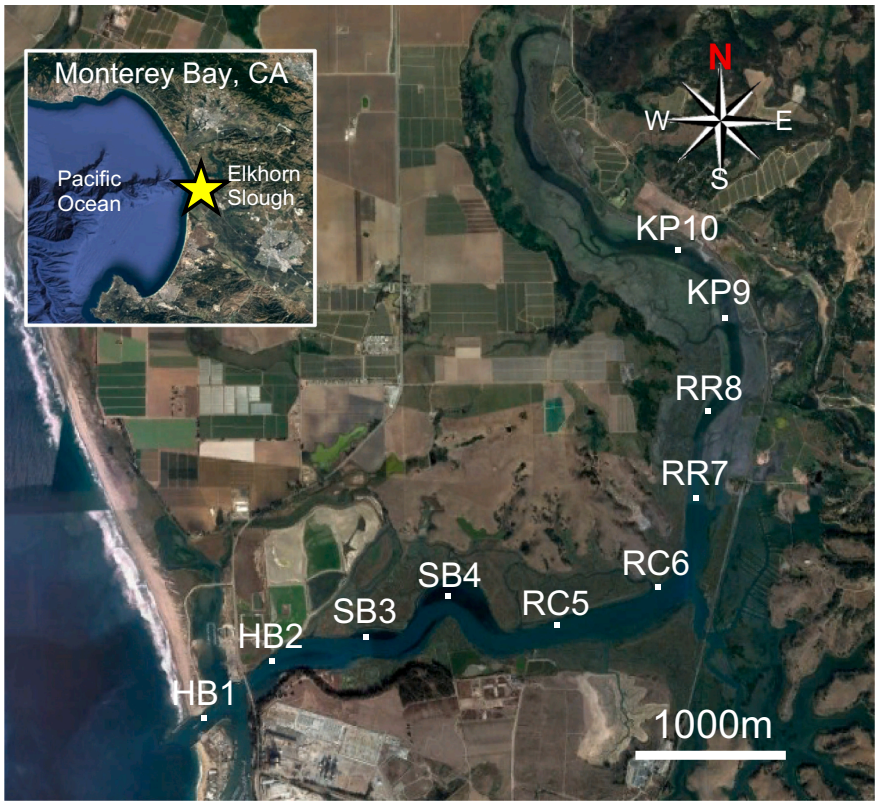


**Fig. 1.** Study location and sampling sites. Location of Elkhorn Slough on a map of Monterey Bay (top left) and collection sites in the main channel of Elkhorn Slough. Letter codes indicate nearby landmarks to each pair of collection sites: HB=Highway Bridge, SB=Seal Bend, RC = Rubis Creek, RR = Research Reserve, KP=Kirby Park, and numbers (1−10) indicate proximity to the mouth of the estuary. Images from Google Earth Pro 7.3.3.7699 (September 15, 2018). Monterey Bay, California. 36.8259°N 121.7569°W, Eye alt. 70 km (Monterey Bay map) & 8.27 km (Elkhorn Slough map).

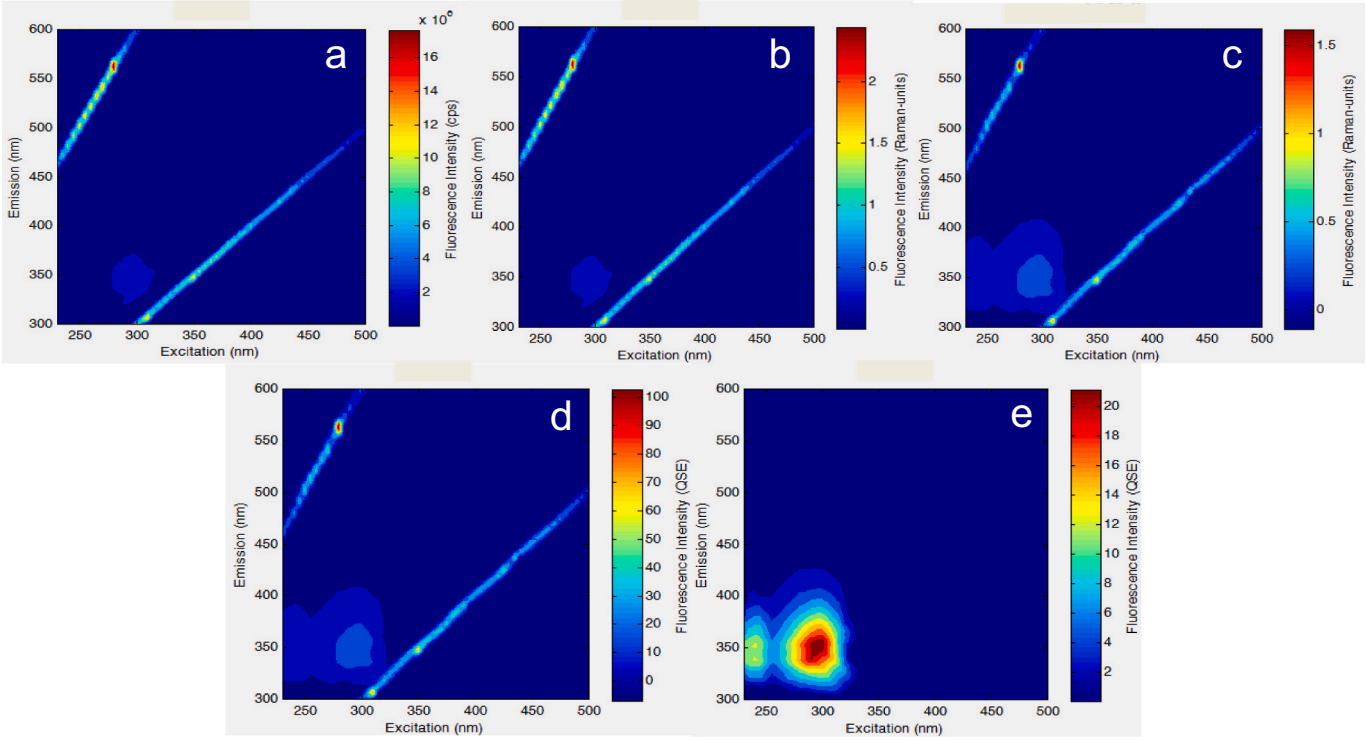


**Fig. 2.** The four types of MATLAB corrections performed by ACT's GUI reflected in one of the samples' contour plot. a) Uncorrected sample. b) Raman Area. c) Subtract Black. d) Quinine Sulfate. e) Scatter Removal.

4. Scatter Removal: removal of Raman and Rayleigh scatter peaks following Zepp et al. (2004) method.

Additionally, MATLAB R2020a (Mathworks, Natick, MA) was used to conduct a PARAFAC analysis using the drEEM toolbox. The optimal number of PARAFAC-derived components was determined after an

outlier analysis and model validation following Murphy et al.'s (2008) recommendations.

### 2.3. Data interpretation

In this study, an initial characterization of cDOM was conducted based on Coble's (1996) traditional analysis of EEM data: 'peak-picking' from contour plots, identifying the positions of wavelength-independent fluorescence maxima ($Ex_{max}/Em_{max}$), and measuring fluorescence intensity at $Ex_{max}/Em_{max}$ for each location. After identifying the main cDOM components in the system, their relationship to each other was assessed to check for potential common sources. Their relative contribution to total fluorescence was also compared by calculating peak ratios at each site, and the ten sampling sites sorted and grouped according to their cDOM properties. Additionally, fluorescence index (FIX), humification index (HIX), and biological index (BIX) values were calculated and interpreted following McKnight et al. (2001), Zsolnay et al. (1999) and Huguet et al. (2009), respectively, using the following formulas:

$$FIX = \frac{I_{370/450}}{I_{370/500}}$$

$$\mathrm{HIX} = \frac{\sum \mathrm{I}_{435\rightarrow480}}{\sum \mathrm{I}_{300\rightarrow345}}$$

$$BIX = \frac{I_{310/380}}{I_{310/430}}$$

In the equations, $I$ indicates fluorescence intensity at each specific excitation/emission wavelength pair or range. Finally, cDOM sources were identified at each location by contrasting results of the different analyses.

### 2.4. Statistical analysis

JMP Pro 13.0 software (© 2016 SAS Institute Inc.) was used for statistical analysis. To evaluate differences in mean total fluorescence and intensity of the main identified fluorophores by the 'peak-picking' method among the groups of sites, one-way ANOVA was used when the distributions of the data were normal and Kruskal-Wallis test when they were not normal, as inferred from normal quantile plots and Goodness-of-Fit Shapiro-Wilk test. These were followed by Tukey's or Wilcoxon rank sum tests, respectively, to discern which specific groups were different from each other. To evaluate the relationships among the different fluorophores identified in the study, significance values of pairwise correlation tests, previously adjusted using the Holm-Bonferroni method, were examined. To compare fluorescence indices across groups of sites, one-way ANOVA and Tukey's test were used where significant differences were detected. To better characterize the different cDOM fractions present in all sampling locations throughout the Elkhorn Slough estuary, Principal Components Analysis (PCA) paired with k-means cluster analysis were used. PCA was performed via the correlation matrix and using the Kaiser criterion to select the appropriate number of factors, which included those with eigenvalues greater than 1.

## 3. Results

### 3.1. Traditional EEM analysis

EEM contour plots and corrected fluorescence readings obtained using MATLAB indicated similar fluorescent signals among pairs of sites in close proximity and with predicted similar water sources (same letter code). In this study, seven fluorescent peaks were identified from contour plots of 10 EEMs from water samples from the Elkhorn Slough estuary (Fig. 3). Of the fluorophores identified, four were associated with humic-like cDOM components (A, C, M, a) and three were associated with protein-like components (N, R, T). This initial analysis resulted in the sorting of all 10 EEMs into three groups of similar cDOM composition. Group 1 included samples from Highway Bridge (HB1, HB2) and Ruby's Creek (RC5, RC6), which had a balanced mixture of humic-like and tryptophan-like fluorophores. Group 2 included samples from Seal Bend (SB3, SB4), which presented predominant tryptophan-like fluorophores that were exclusive to these sites, and the overall highest fluorescence intensity. Finally, Group 3 included samples from the Elkhorn Slough National Estuarine Research Reserve (RR7, RR8) and Kirby Park (KP9, KP10), which had predominant humic-like fluorophores and a tryptophan-like fluorophore that was exclusive to these sites.

A significant difference in total fluorescence intensity was observed among the three groups of sites, with mean fluorescence being more than six times lower in Group 1 than Group 3. Fluorescence intensity of peaks associated with humic-like cDOM: peak A, peak C, peak M and peak a, were significantly higher in Group 3 than in Group 1. There was no trace of humic-like cDOM in SB3, and peak a was not present in either of the sites in Group 2. Fluorescence intensity of signals associated with protein-like cDOM: peak T and peak R, was higher in Group 1 than in Group 2 and Group 3, respectively. Peak R was not present in any of the sites in Group 3, and peak N was present almost exclusively in Group 2. Statistical analysis is shown in Table 2.

There were strong positive correlations among humic-like peaks A, C, M, and a ($r > 0.90$). Similarly, there was a strong positive correlation between protein-like peaks N and R ($r = 0.97$) and a moderate positive correlation between peaks T and N ($r = 0.82$). The contribution of each of the seven fluorophores identified to total fluorescence intensity is shown in Fig. 4.

### 3.2. Principal components and PARAFAC

There were two main principal components (PCs) in the model, and together they explained approximately 98% of the variation in the data: PC1 explained 62.4% and PC2 explained 35.5% of the variation (Fig. 5). The four humic-like fluorophores (A, C, M, and a) loaded on both PC1 and PC2, and the three protein-like fluorophores (N, R, and T) loaded on PC2 only, as determined by the loading matrix and loading plot (Fig. 5a). The three groups into which the ten sampling sites were previously sorted based on the results of traditional analysis appeared as separate clusters in the biplot; however, the k-means cluster analysis found two main clusters: one including the sites near Kirby Park (KP9–10) and one including the remaining eight sites (Fig. 5b).

Based on PARAFAC analysis, five independent cDOM components were identified: three humic-like and two protein-like. The five-component model explained 99.2% of the variability in the dataset. Table 3 shows the characteristics of each PARAFAC component.

### 3.3. Fluorescence, Humification, and biological indices

There were significant differences in fluorescence index (ANOVA, $F_{2,9} = 22.81$, $p = 0.009$) and humification index (ANOVA, $F_{2,9} = 8.21$, $p = 0.0146$) among the groups of sites sorted by cDOM characteristics (Fig. 6). Group 3 had a mean FIX value of 2.6, significantly higher than the 1.5 and 1.4 mean values of Group 1 (Tukey's test, $p = 0.0014$) and Group 2 (Tukey's test, $p = 0.0028$), respectively. Group 2 had a mean HIX value of 1.6, significantly lower than the 2.2 and 2.1 mean values of Group 1 (Tukey's test, $p = 0.0124$) and Group 3 (Tukey's test, $p = 0.0409$), respectively. The biological index did not differ among the groups of sites (ANOVA, $F_{2,9} = 0.7894$, $p = 0.7849$), with constant BIX values of $1.6 \pm 0.2$ throughout the estuary.

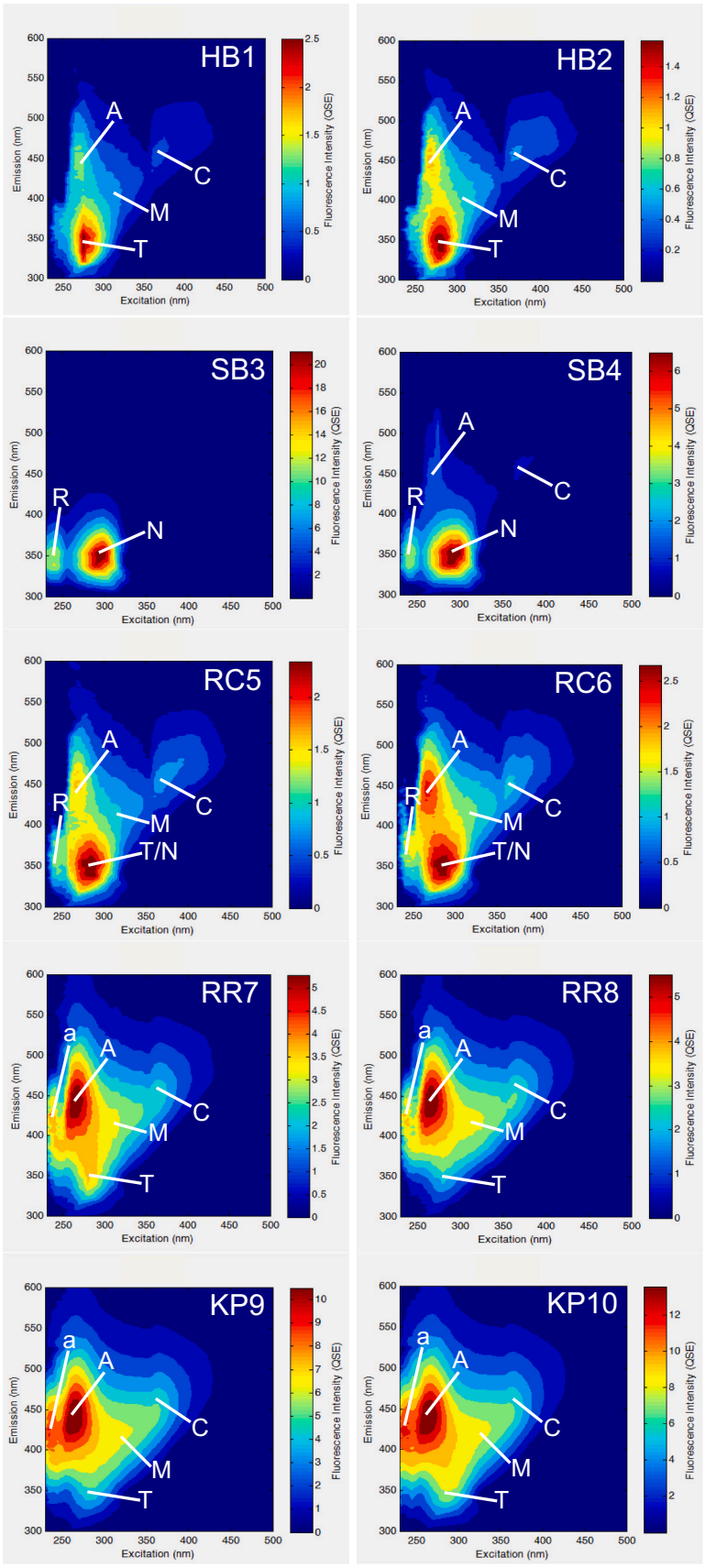


**Fig. 3.** EEM contour plots showing fluorescent signals in water samples from Elkhorn Slough. Fluorescence intensity is expressed in Quinine Sulfate Units (QSE) and fluorescence peaks are labelled with their corresponding letters following the traditional classification in Table 1.

**Table 2**
Statistical analysis of differences in total and peak fluorescence intensity among the three groups of cDOM sites defined across Elkhorn Slough. Non-significant differences are not shown in the table.

| Fluorescence Intensity | Groups compared | Test | Statistic | p |
|---|---|---|---|---|
| Total | 1–2-3 | Kruskal-Wallis | $\chi^2 = 6.54$ | 0.0379 |
| Peak A | 1–2-3 | Kruskal-Wallis | $\chi^2 = 6.63$ | 0.0364 |
| | 1–3 | Wilcoxon rank sum | Z = 2.16 | 0.0304 |
| Peak C | 1–2-3 | Kruskal-Wallis | $\chi^2 = 7.85$ | 0.0197 |
| | 1–3 | Wilcoxon rank sum | Z = 2.16 | 0.0304 |
| Peak M | 1–2-3 | Kruskal-Wallis | $\chi^2 = 7.28$ | 0.0262 |
| | 1–3 | Wilcoxon rank sum | Z = 2.16 | 0.0304 |
| Peak a | 1–2-3 | Kruskal-Wallis | $\chi^2 = 7.32$ | 0.0258 |
| | 1–3 | Wilcoxon rank sum | Z = 2.18 | 0.0294 |
| Peak T | 1–2-3 | One-Way ANOVA | $F_{2,7} = 6.47$ | 0.0257 |
| | 1–2,1–3 | Tukey's test | – | 0.0222 |
| Peak R | 1–2-3 | Kruskal-Wallis | Z = 8.36 | 0.0153 |
| | 1–2,1–3 | Wilcoxon rank sum | Z = -2.31 | 0.0211 |

## 4. Discussion

This was the first study to apply EEM spectroscopy for characterization of cDOM in the Elkhorn Slough estuary. Elkhorn Slough is a unique region to study cDOM dynamics due to its high diversity of fluorescent signals, which covers nearly the entire known variability in cDOM properties within a fairly small area. In this study, chemical properties of slough water were predicted to be highly influenced by nutrient runoff from anthropogenic activities, and macrophyte distribution, in addition to hydrological and geomorphological characteristics of the estuary. Overall, relative abundance of humic-like and protein-like fluorophores across Elkhorn Slough vary throughout the year, with the latter being lowest during the fall (Jaffé et al., 2008). These seasonal trends suggest that protein-like components are primarily controlled by primary productivity, while humic-like components are more influenced by hydrological processes (Maie et al., 2006). While protein-like fluorescence overwhelms humic-like fluorescence in estuaries impacted by industrial activities and where autochthonous production eclipses terrestrialy-derived signals (Osburn and Stedmon, 2011), humic-like fluorescence predominates in eutrophic estuaries with little tidal influence. A more comprehensive study of Elkhorn Slough with monthly sampling would be very clarifying and would help to characterize temporal variability of DOM and capture the effects of rainfall, upwelling, and other seasonal factors.

Significant differences in total fluorescence intensity throughout Elkhorn Slough were likely due to precipitation, tidal excursion, and terrestrial input in the upper slough. Bai et al. (2017) compared fluorescence intensities of an inland sea with multiple freshwater inputs and pollution sources, and long residence times to those of a semi-enclosed sea with low water residence times, finding that fluorescence was significantly higher in the first. In fact, presence of the humic components identified in this study and higher fluorescence values are correlated with lower salinity (Bai et al., 2017; Kowalczuk et al., 2013). Overall, my results are coherent with previous studies that described two different regions in Elkhorn Slough's main channel based on distinct physical (Breaker et al., 2008), chemical (McLaughlin et al., 2006; Novak, 2011), and biological (Smith, 2009) characteristics: the upper slough and the lower slough. However, through EEM analysis, a section within the lower slough comprising the two sites on Seal Bend (SB3–4) that presented unique cDOM signals was found, likely due to the presence of large eelgrass beds. Despite the fact that seagrasses and macroalgae release DOM consisting mainly of labile, low-weight compounds (Sgroi et al., 2017), their fluorescence properties are unlike one another. Three distinct regions were defined to represent the cDOM variability throughout Elkhorn Slough, hence the ten sampling sites were sorted into three groups.

With the exception of Seal Bend, DOM in the lower slough region (Group 1) was a mixture of autochthonous protein-like components and humic materials, including fulvic acid and other components of terrestrial and anthropogenic origin. All seven fluorophores identified at the study location were present in the lower slough; humic-like signals

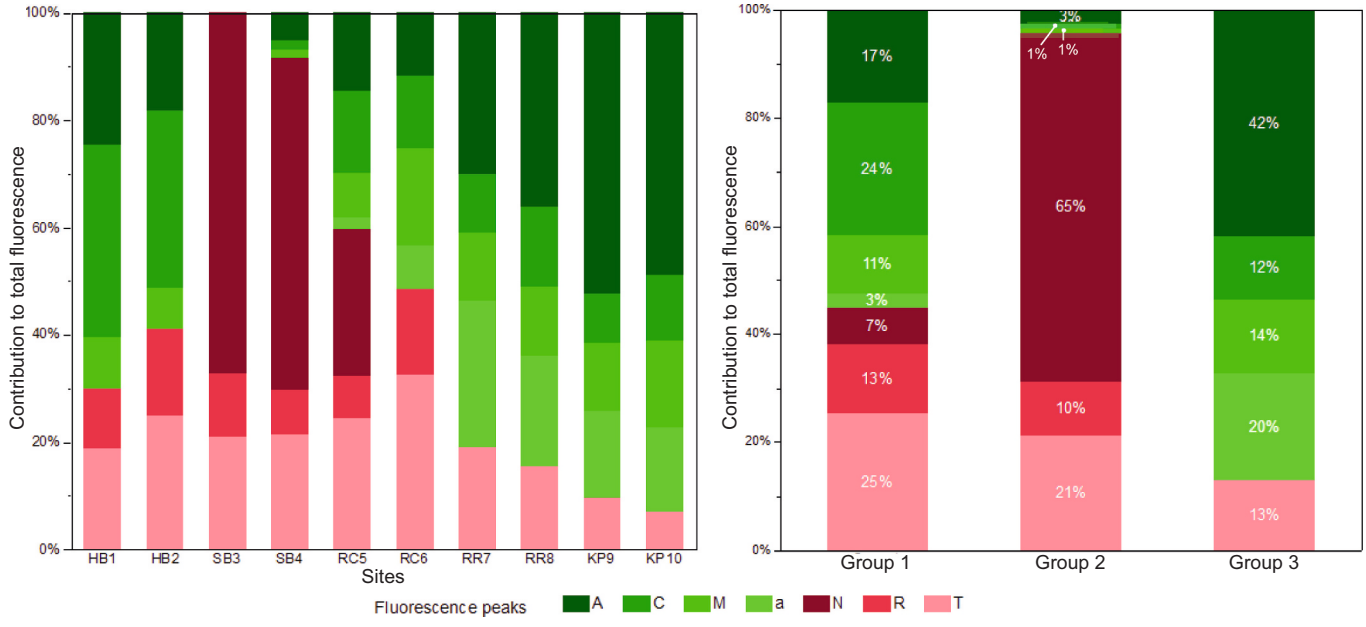


**Fig. 4.** Percent contribution of the seven fluorescence peaks identified to the total EEM fluorescence intensity. a) Contribution of each fluorophore at each of the ten sampling sites (HB1-KP10). b) Mean contribution of each fluorophore within each group of sites. Fluorophores associated with humic-like cDOM (A, C, M, a) are represented in green tones, and fluorophores associated with protein-like cDOM (N, R, T) are represented in red tones. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

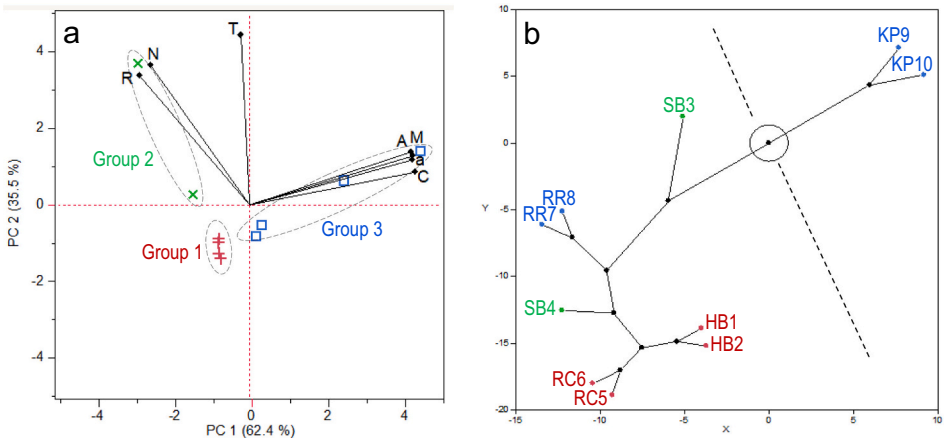


**Fig. 5.** Results of PCA using the seven identified fluorophores and k-means cluster analysis. a) Biplot showing loadings on a two PCs model and scores for the 10 samples throughout Elkhorn Slough. b) Constellation plot showing two main clusters as determined by the k-means method.

**Table 3**
Characteristics of the five PARAFAC-derived fluorescent components: $Ex_{max}$, $Em_{max}$, traditional peak designation (Coble, 1996; Cheng et al., 2015), and other studies that previously identified these components. Secondary excitation and emission maxima are indicated in brackets. For descriptions of each component, refer to Table 1.

| Component | $Ex_{max}$ | $Em_{max}$ | Peak | Previous studies |
|---|---|---|---|---|
| C1 | 280 | 340 | T or N | Coble (1996); Stedmon and Markager (2005); Yamashita and Jaffé (2008); Santín et al. (2009); Cheng et al. (2015); Mangalgiri et al. (2017) |
| C2 | 260 | 445 | A | Coble (1996); Stedmon and Markager (2005); Yamashita and Jaffé (2008); Santín et al. (2009); Mangalgiri et al. (2017) |
| C3 | 230 | 425 (350) | R or a | Murphy et al. (2008); Yao et al. (2011); Yao et al. (2011); Cawley et al. (2012); Cheng et al. (2015); Xie et al. (2018) |
| C4 | 370 (270) | 455 | C | Coble (1996), Stedmon et al. (2003); Stedmon and Markager (2005); Murphy et al. (2008); Yamashita and Jaffé (2008); Shutova et al. (2014); Mangalgiri et al. (2017); Xie et al. (2018) |
| C5 | 305 | 380 (405) | M | Coble (1996); Stedmon and Markager (2005); Yamashita and Jaffé (2008); Santín et al. (2009) |

contributed 55% of the total fluorescence, and protein-like signals accounted for the remaining 45%. Parlanti et al. (2000) reported high variability in the EEM spectra of seawater, concluding that marine DOM contains various types of protein-like and humic-like chromophores. It is well established that the residence time for lower slough waters is very short (Broenkow and Breaker, 2005), and that this section of the estuary is subject to constant horizontal mixing. Interestingly, the strongest cDOM signal in Group 1 (T), which accounted for 25% of its total fluorescence, is associated with protein tryptophan-like sources, likely of autochthonous origin. This was the only signal present in all samples along the estuary, slightly more prominent in the upper slough than in the lower slough. The second strongest was a visible-band fulvic acid signal (C) that is associated with terrestrial humic-like matter of anthropogenic or agricultural origin, and which contributed 24% of the total fluorescence. The main source of humic material in this region is likely fertilizer-rich waters from the lower Salinas Valley and Tembladero watersheds that enter the Moss Landing Harbor at the mouth of the estuary via the old Salinas River channel (Hughes et al., 2011).

The DOM characteristics of Seal Bend (Group 2) were quite different

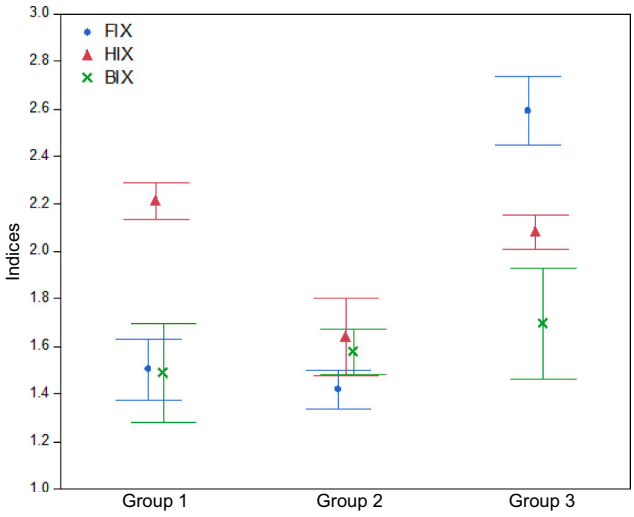


**Fig. 6.** Comparison of fluorescence index (FIX), humification index (HIX), and biological index (BIX) among the three groups of sites. Error bars indicate variability among sites within each group.

from those of the remaining regions of Elkhorn Slough, since it was primarily composed of protein-like substances (96% contribution to total fluorescence), and humic-like contributions (4%) were very minimal. At Seal Bend, there was a predominant biologically derived component (N) associated with high primary production that contributed 65% of the total fluorescence at the two sites. Two tryptophan-like components (R and T) were also present at this location, contributing 10% and 21%, respectively, to the total fluorescence. The high correlation between these protein-like components suggests that they have a common source and very similar biogeochemical properties. Signals N and R have been identified in various studies conducted in seagrass ecosystems, concluding that seagrasses can be a significant source of DOM in coastal systems (Stabenau and Zika, 2004; Cheng et al., 2015). Despite the predominant protein-like fluorescent signals at the Group 2 sites, there was no trace of tyrosine-like molecules, a finding coincident with Cheng et al.'s (2015) characterization of seagrass cDOM. The presence of eelgrass at Seal Bend leads to the definition of an additional cDOM region with unique properties that are different from those of the traditional sections of the slough based on hydrogeochemical properties. It seems like macrophyte diversity is responsible for the high variability in cDOM composition in the Elkhorn Slough system.

In contrast with Seal Bend, the upper slough (Group 3) was primarily

composed of humic-like cDOM, which contributed 87% of the total fluorescence. The most prominent signals were of allochthonous origin; a terrestrial humic-like component associated with recent material (A), and UV fulvic acid associated with agricultural catchments (a), which contributed 42% and 20% to the total fluorescence in that region, respectively. Another important contribution was that of a microbial humic-like cDOM signal (M), thought to come from wastewater or agricultural catchments. With the exception of signal C, the remaining humic-like fluorophores presented higher fluorescence intensities in the upper slough than the lower slough. The strong correlation among the four humic signals is indicative of a possible common origin and similar chemical composition. The lands surrounding Elkhorn Slough are mainly used for agricultural purposes, and irrigation waters with high nutrient concentrations enter the slough seasonally (Broenkow and Breaker, 2005). The high proportion of humic-like components is likely a result of rain-induced discharge of terrestrial material, since sampling took place at the beginning of the wet season and there had been a rainfall event (20 mm) only two weeks before (Moss Landing Marine Laboratory Weather Station). Moreover, nutrient levels in Elkhorn Slough are among the highest for United States estuaries (Caffrey et al., 2002); something that is much more evident in the upper slough than the lower slough, since tides are responsible for mixing and removing ¾ of the mean high-water volume daily (Smith, 1973).

The fluorescence index (FIX), humification index (HIX) and biological index (BIX) are commonly used to discern the sources of cDOM. Higher FIX values translate into higher autochthonous to allochthonous ratios and overall higher proportions of fulvic substances (McKnight et al., 2001). FIX values of 1.4 or less, such as the mean value of the sites on Seal Bend (Group 2), indicate terrestrially derived cDOM. On the other hand, sites in Group 1 had a FIX value between 1.4 and 1.9, suggesting its cDOM components are derived from both terrigenous and biogenic matter. Xie et al. (2018) reported similar FIX values of 1.4 in an anthropogenically perturbed estuary in Southern China; however, there are not much data available for upwelling influenced eutrophic estuaries. Sites in Group 3 had an average FIX value much higher than 1.9, an indication of biologically derived cDOM. The four sites in Group 3 (RR7–8, KP9–10) are in the upper slough, a region that is minimally influenced by tidal action due to the estuary's morphology (Breaker et al., 2008). This area is particularly propitious for excessive macroalgal development due to terrestrial and anthropogenic input from adjacent agricultural land that drains directly into the estuary (Novak, 2011). Even though sampling took place in November, thick mats of the macroalga *Ulva* spp. were observed in the upper slough. In coastal systems, autochtonous DOM is essentially derived from algae, and algal release of DOM is often regulated by nutrient deficiency and light intensity (Bertilsson and Jones, 2003).

Conversely, the humification index (HIX) is the ratio of allochthonous to autochthonous substances (Zsolnay et al., 1999). Values higher than 10 are associated with high humification degree, while values lower than 4 are associated with low humification degree. As expected, Group 2 presented the lowest HIX values that were significantly different from those of Group 1 and Group 3, since DOM from the seagrass ecosystems is known to have strong autochthonous contributions and a poor humification degree (Cheng et al., 2015). However, the humification degree was quite low (<2.5) throughout the entire estuary, suggesting that composition of DOM is strongly linked to biological activity. Group 3's low mean HIX values despite the predominance of humic-like components might be due to the fact that some of the fluorescence values used to calculate the HIX ratio fall in the Raman scatter band, and had to be estimated. Scatter corrections, though essential to discern patterns and identify predominant fluorescence signals qualitatively, can lead to inaccuracies when calculating indices. Given that Elkhorn Slough is a highly eutrophic system, and that this study was conducted during the wet season, HIX values were expected to be much higher, such as those reported in similar ecosystems (e.g. Hu et al., 2017; Huguet et al., 2009; Zhao et al., 2017).

The biological index (BIX) is an indicator of autotrophic productivity, and high BIX values (>1) indicate the presence of organic matter freshly released into the water (Huguet et al., 2009). Studies focused on characterization of cDOM in natural ecosystems rarely include BIX values, notably missing for United States West Coast estuaries. All three groups of sites throughout Elkhorn Slough had high BIX values, which suggests that autochnthonous DOM of macrophyte origin is a key contributor to the total pool of organic matter in the Elkhorn Slough estuary. However, the relative contributions of algal-derived and seagrass-derived DOM, through processes associated with their development and decay, vary among the different regions. In this study, the specificity of fluorescence signals linked to degrading seagrass detritus in Group 2, and to excessive macroalgal development in Group 3, suggest that there is a strong link between dominant macrophytes and cDOM properties in coastal systems. Macrophytes are known to generate DOM via release of photosynthate or release of dissolved and particular contituents due to aging (Sgroi et al., 2017). However, cDOM properties differ among macrophyte types. For example, eelgrass-derived DOM is very distinctive and quite different from mangrove or algal-derived DOM (Cawley et al., 2014; Cheng et al., 2015; Ferrari and Mingazzini, 1995).

Macrophytes are likely the most important supply of authochtonous DOM in shallow, productive systems like Elkhorn Slough, since they produce approximately eight times more than phytoplankton on an areal basis (Wetzel, 1983). EEM spectroscopy offers potential as a fast, non-destructive approach to monitor macrophyte abundance and distribution where conditions are unfavorable for application of other techniques. Multiple studies have compared different methods of submersed macrophyte sampling, such as plot-based surveys, hydroacoustic techniques, and satellite remote sensing (Johnson and Newman, 2011; Nelson et al., 2006; Radomski and Holbrook, 2015), concluding that factors like water depth, reflectance, stratification, cloud cover, and species buoyancy decrease the accuracy and precision of methods that require the least effort. Dierssen et al. (2019) evaluated the effectiveness of remote sensing to detect eelgrass in Elkhorn Slough, and found that detection of the deeper beds via the combination of pseudo true-color imagery and broadband satellite imagery was not reliable over time due to the dark sediment, turbid water, and other environmental conditions. Even though traditional field sampling techniques yield the highest accuracy (Wetzel and Likens, 2000), large scale macrophyte surveys are very costly and are rarely able to capture biomass, cover and density of macrophytes across entire aquatic systems (Nelson et al., 2006). Future studies should address whether EEM spectroscopy could be used as a complementary tool to other non-intrusive methods to increase macrophyte detection effectiveness, i.e., the highest precision with the least effort, in other coastal systems. .

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A

Table A1. Water quality data on the day and time of sampling from monitoring stations throughout Elkhorn Slough. All values are expressed in S.I. units. Data from NOAA National Estuarine Research Reserve System (NERRS).

| Sites[a] | Station | Temperature | Sp. Conductivity | Salinity | DO % | DO (mg/L) | Level[b] | pH | Turbidity |
|---|---|---|---|---|---|---|---|---|---|
| HB | Vierra Mouth | 15.2 | 49.18 | 32.2 | 96.2 | 7.9 | 1.62 | 8.1 | 5 |
| RC | South Marsh | 16.5 | 49.50 | 32.5 | 77.1 | 6.2 | 1.75 | 7.9 | 5 |
| RR | North Marsh | 16.9 | 46.30 | 30.1 | 49.2 | 4.1 | 0.84 | 7.4 | 25 |
| KP | Acevedo Pond | 17.6 | 48.20 | 31.5 | 71.1 | 5.5 | 1.78 | 7.9 | 3 |

[a] There wasn't available water quality data for sites SB3–4 as there wasn't a monitoring station at Seal Bend.
[b] Tidal datum based on sea level, using NAVD88 as reference.

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