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# Identifying factors that influence expression of eutrophication in a central California estuary

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ABSTRACT: Coastal eutrophication models have proposed that various environmental conditions can serve as filters mediating the effects of nutrient loading on coastal ecosystems. Variation in such filters due to natural or anthropogenic causes can potentially lead to varied responses in overall eutrophication expression as well as in individual eutrophication indicators. In this study, we sought to identify factors that affect eutrophication expression at contrasting sites within one nutrient-loaded estuary in central California. We developed and applied a eutrophication expression index to 18 sites in the Elkhorn Slough estuary and then used principal components analysis of environmental drivers (nutrients) and filters to determine how they relate to overall eutrophication expression as well as to individual eutrophication indicators. We also examined the relationship between one key filter, tidal range, and eutrophication indicators. Elkhorn Slough was determined to be a moderately eutrophic estuary, with individual sites varying from being low to hypereutrophic. Eutrophication expression was explained mostly by tidal range, depth, temperature, salinity, distance to estuary mouth, and turbidity, but not by nutrient concentrations. Tidal range in particular correlated strongly with most eutrophication indicators. Sites with artificially dampened tidal range through water control structures were more eutrophic than sites with full tidal exchange. Results from this study emphasize the importance of filters in mediating the negative ecological effects of eutrophication. Coastal managers can decrease eutrophication expression at a local scale by managing for filters (e.g. increasing tidal exchange to managed wetlands), complementing efforts to reduce eutrophication at a regional scale by decreasing nutrient loading.

KEY WORDS: Eutrophication · Elkhorn Slough · Hypoxia · Environmental filters · Tidal range · *Ulva* · Chl *a*



## INTRODUCTION

Over the last 70 yr, the addition of nitrogen to the earth's surface has doubled, mainly due to the production of industrial nitrogen for fertilizer (Vitousek et al. 1997, Gruber & Galloway 2008, Schlesinger 2009). This has caused eutrophication via sustained delivery of anthropogenic nutrients to surface waters. Defined as an increase in the rate of organic matter additions to an ecosystem, eutrophication is one of the biggest coastal pollution problems the world faces today (Nixon 1995, Howarth et al. 2000, National Research Council 2000, Smith & Schindler 2009). Eutrophication can lead to algal blooms, hypoxia events, decreases in biodiversity, and even dead zones, all of which can fundamentally change an ecosystem and its ecologic function (Cloern 2001, Diaz 2001, Diaz & Rosenberg 2008).

The earliest studies of eutrophication focused on lakes, where nutrient additions often trigger direct ecological effects (Vollenweider 1976), such as increases in primary productivity and changes in

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ecosystem metabolism. Contemporary models of coastal eutrophication have been modified from the earlier freshwater models to include both direct responses or primary indicators (i.e. changes in algal growth and phytoplankton production) and indirect responses or secondary indicators (i.e. changes in dissolved oxygen and sediment biogeochemistry) with increased nutrient additions (Cloern 2001).

Coastal eutrophication models also accommodate more varied response levels by eutrophication indicators to nutrient loading. In lakes, the magnitude of the response is typically correlated with the magnitude of nutrient additions. In coastal systems, the response does not always correlate directly with the magnitude of nutrient additions. This is due to physical characteristics of the coastal environment that can mitigate or filter the effects of nutrients on primary indicators, or the effects of primary on secondary indicators (Fig. 1) (Cloern 2001). The importance of eutrophication filters can be illustrated with a comparison of eutrophication indicators in different estuaries with similar nutrient inputs. For example, San Francisco and Chesapeake Bays have similar nutrient inputs, yet productivity in San Francisco Bay is much less than that in Chesapeake Bay due to numerous differences in filters, such as residence time, depth, stratification, and tidal amplitude (Cloern 2001). The main physical filter in San Francisco Bay is a high tidal amplitude that leads to higher turbidity decreasing light availability for primary producers, whereas Chesapeake Bay has a lower tidal amplitude that leads to greater water column light penetration and higher primary productivity.

Studies identifying factors that affect eutrophication expression are rare, but vital for coastal management (McGlathery et al. 2007). Many earlier studies examining variation in drivers and filters of eutrophication either compared differences between estuaries or used time series analysis to make inferences (e.g. Monbet 1992, Rabalais et al. 1996, Sandén & Håkansson 1996, Harding & Perry 1997, Allen et al. 1998, Zimmerman & Canuel 2000, Cloern 2001); however, confounding factors such as geomorphology (for regional comparisons) and weather (for time series) decrease the rigor of these inferences about the relationship between eutrophication drivers, filters, and expression. Examining spatial variation within an estuary is perhaps the most powerful approach for identifying key filters because of fewer problems with confounding variables, and because more replication is possible. This approach has been implemented in only a few estuaries on the Atlantic coast of North America (see Boynton et al. 1996, Kemp et al. 2005), and never on the Pacific coast.

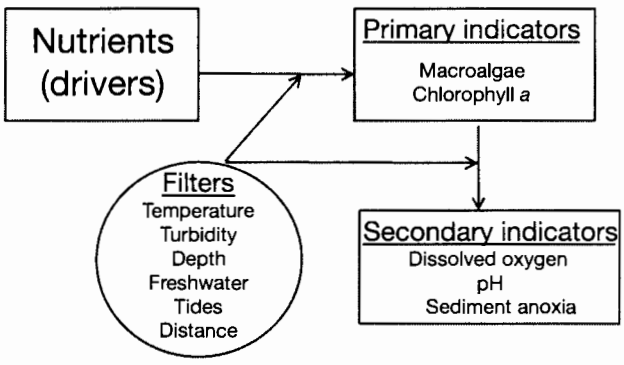


Fig. 1. Coastal eutrophication model and hypothesis. Modified from Cloern (2001)

In this study, we examined the spatial variability of eutrophication expression within an estuary on the central California coast with high nutrient loads and investigated the role of filters in mediating eutrophication expression. We systematically investigated 18 sites to assess the elements of Cloern's (2001) model (Fig. 1): drivers, filters, primary and secondary indicators. We developed and implemented a eutrophication expression index (EEI) to obtain a single value for the expression of eutrophication indicators at each site, and characterized spatial patterns of this index across the entire estuarine complex encompassing the 18 sites. We used principal components analysis (PCA) to determine whether nutrients and/or filters correlated with the EEI or with individual eutrophication indicators. Finally, we examined one key filter, tidal range, in greater detail using linear regression to elucidate the relationship between tidal range and individual eutrophication indicators.

## MATERIALS AND METHODS

### Study system

The study location was Elkhorn Slough, which is a small California estuary located in Monterey Bay (see Fig. 2). The estuary is comprised of multiple interconnected channels, including Elkhorn Slough proper, Bennett Slough, Moro Cojo Slough, and the old Salinas River channel; however, the entire estuarine complex is generally referred to as the Elkhorn Slough estuary because the Elkhorn Slough channel is the largest and the only channel that is not obstructed at its mouth by a water control structure. The estuary is heavily influenced by surrounding agricultural practices as well as tidally driven processes leading to nu-

trient loading on variable temporal and spatial scales. Elkhorn Slough has some of the highest levels of dissolved nutrients among US estuaries (Caffrey et al. 1997, 2002, Fry et al. 2003) (Table 1a). Long-term data suggest that nutrient levels have increased over the last 70 yr (Caffrey 2002). Biomass of phytoplankton and macroalgae is also high (Table 1b). Some Elkhorn Slough habitats also suffer from chronic periods of nighttime hypoxia and anoxia, and daytime hyperoxia (Beck et al. 2001, Caffrey et al. 2010), along with a high occurrence of sulfate reducing bacteria, and high fluctuations in pH due to high productivity. The Elkhorn Slough estuary has been hydrologically altered by dikes, culverts, and tide gates (see Fig. 2), which has created artificial dampening of the tidal range upstream of water control structures. High nutrient concentrations and high primary productivity combined with high intra-estuary tidal range variation create an ideal system to test the coastal eutrophication model proposed by Cloern (2001).

Within the estuary, we selected 18 sites that are highly variable in a number of physical factors, such as distance from the mouth, tidal restriction, depth, temperature, turbidity, and freshwater influence (see Fig. 2; Tables S1 & S2 in Supplement 1 at www.int-res.com/articles/suppl/m439p031_supp.pdf). We selected 6 sites with full tidal range (~2.7 m maximum daily tidal range) and 12 with an artificially restricted tidal range (0.05– 1.6 m maximum daily tidal range).

Table 1. Summary statistics of (a) drivers (nutrient concentrations), (b) primary indicators, and (c) secondary eutrophication indicators in Elkhorn Slough during the study period of July 2008 to June 2009. Algal biomass was collected from the intertidal at only 10 sites; all the remaining values are based on averaging of data from all 18 sites (see Fig. 2). aRPD: apparent redox potential discontinuity layer. Methods for obtaining these values are described in Supplement 1 at www.int-res.com/articles/suppl/m439p031_supp.pdf

| | Mean | Median | SD | Max. | Min. |
|---|---|---|---|---|---|
| **(a) Drivers** | | | | | |
| Nitrate (mg N $l^{-1}$) | 1.74 | 0.09 | 6.11 | 56.40 | 0.00 |
| Ammonia (mg N $l^{-1}$) | 0.10 | 0.06 | 0.16 | 1.56 | 0.00 |
| Phosphate (mg P $l^{-1}$) | 0.17 | 0.05 | 0.33 | 2.35 | 0.01 |
| **(b) Primary indicators** | | | | | |
| Subtidal algal cover (%) | 34 | 35 | 32 | 90 | 0 |
| Intertidal algal cover (%) | 36 | 33 | 31 | 90 | 0 |
| Floating algal cover (%) | 9 | 0 | 20 | 100 | 0 |
| Fresh algal biomass (g $m^{-2}$) | 658.4 | 148.6 | 920.0 | 2244.4 | 0.0 |
| Dry algal biomass (g $m^{-2}$) | 103.7 | 20.0 | 128.0 | 330.5 | 0.0 |
| Chl *a* (µg $l^{-1}$) | 12.67 | 4.41 | 26.07 | 231.89 | 0.00 |
| **(c) Secondary indicators** | | | | | |
| Time hypoxic (%)[a] | 16.4 | 6.1 | 23.2 | 73.1 | 0.0 |
| Daytime dissolved $O_2$ (mg $l^{-1}$) | 8.7 | 8.2 | 3.7 | 19.4 | 0.0 |
| Daytime pH | 8.2 | 8.2 | 0.4 | 9.3 | 7.2 |
| aRPD (cm) | 11.1 | 1.5 | 15.7 | 50.0 | 0.0 |

[a]Hypoxia defined as $O_2$ < 2.3 mg $l^{-1}$

## Data collection

We sampled eutrophication drivers, filters, and primary and secondary indicators of eutrophication at the 18 sites in Elkhorn Slough with the goal of assessing spatial patterns of eutrophication and determining whether drivers or filters correlate with them. Data were collected at varying frequencies because some parameters, e.g. nutrients and water quality variables, were part of a 20 yr water monitoring program with a monthly sampling frequency, while others (algal cover, hypoxia, and sediment quality) were sampled only in targeted surveys for this study of eutrophication. For each site, we used a single value to characterize each driver, filter and indicator. How this single value was obtained differed by variable, and is summarized below and explained in detail in Supplement 1 at www.int-res.com/articles/suppl/m439p031_supp.pdf, which also specifies and justifies the variables used for the various analyses.

### Drivers

Dissolved inorganic nutrients enter Elkhorn Slough from several different freshwater sources that include the Salinas River and Tembladero Slough (which both flow into the Old Salinas River channel) to the south, Moro Cojo Slough to the southeast, Carneros Creek to the north, as well as runoff from adjacent land areas (Fig. 2) (Caffrey et al. 2007). Elkhorn Slough also receives water from the nutrient rich Monterey Bay, especially during periods of upwelling in late spring and early summer (Chapin et al. 2004). Water from the Old Salinas River channel contributes the greatest load of nutrients to the main channel of Elkhorn Slough; this nutrient rich water is tidally pumped up the main channel of Elkhorn Slough (Jannasch et al. 2008). Carneros Creek forms the head of the estuary and flows directly into the estuary, supplying a fraction of the nutrient input of the Old Salinas River channel.

To evaluate drivers of eutrophication, we collected surface water samples monthly at each site on ebbing tides during July 2008 to June 2009, and analyzed them for concentrations

of nitrate as N, ammonia as N, and phosphate as P. We calculated the annual mean of these monthly measurements to obtain a single measurement for each nutrient per site.

### Filters

Various physical parameters that have been identified as potential eutrophication filters were measured at each site: turbidity, temperature, salinity, depth, distance to estuary mouth, and tidal range. Turbidity, temperature, and salinity measurements were taken with data sondes (YSI) during monthly collection of nutrients from July 2008 to June 2009. To obtain a single value to characterize the filter at each site, we used mean annual turbidity, the 90th percentile of temperature (because warm temperatures are important for hypoxia), and the negative of the 10th percentile of salinity (to emphasize the role of freshwater input).

We assessed the channel depth at each site during one low tide survey in May 2009, and calculated the distance of water channels from sampling sites to the estuary mouth by measuring distances on georeferenced aerial imagery in ArcGIS. To assess tidal range, a YSI sonde with a pressure transducer was deployed at each site for 2 to 4 wk during the prime hypoxia months in the summer and early fall of July 2008 to June 2009. The sonde sampled water depth every 15 min, and we used these measurements to calculate the maximum daily tidal range during this period.

### Primary indicators

In order to assess primary indicators of eutrophication, we sampled chl *a*, and floating, subtidal, and intertidal algal mats at each site. We measured chl *a* concentrations at each site from July 2008 to June 2009 during the same monthly grab sampling used to assess nutrients. We used the annual mean of these monthly values to obtain a single value to characterize each site. The percent cover of floating algal mats was visually estimated monthly during the same time as water sampling, and the annual maximum of these monthly values was calculated and used to characterize each site. Subtidal and intertidal benthic algal cover was assessed once on an extreme low tide during May 2009 in order to capture one of the peak months of primary productivity in Elkhorn Slough.

### Secondary indicators

We collected data on secondary indicators of eutrophication, which included hypoxia, daytime dissolved oxygen (DO) variation, hyperoxia, sediment quality, pH, and free ammonia. Hypoxia was determined in July 2008 to June 2009 during the same 2 to 4 wk deployments of YSI data sondes as were used for assessing tidal range, with dissolved oxygen being measured every 15 min during this deployment. The percent of the deployment time with values of DO below the EPA's low criterion of 2.3 mg $l^{-1}$ (US EPA 2000) was used as the single value to characterize hypoxia at each site. Daytime DO variation, hyperoxia, and pH data were collected at the same time as water sampling during daytime monthly sampling events (July 2008 to June 2009) using YSI data sondes. For DO variation, the annual mean of monthly values of DO variation from 100% saturation was used to characterize each site. For hyperoxia and pH, the respective 90th percentiles of monthly DO and pH measurements were used. Free ammonia concentration was calculated from the same monthly grab samples used for analysis of nutrient drivers. To accurately assess average free ammonia concentration at each site, we averaged data for a longer period (from 2004–2009) because this variable displayed greater temporal variation than the other variables. Sediment quality was assessed once in the summer of 2009 by measuring depth to the apparent redox potential discontinuity (aRPD) layer. To determine the aRPD depth, a caliper was used to measure the distance between the sediment surface and the transition from brown oxic surface sediments to black reduced sediments below, with smaller values being associated with sediment anoxia or hypoxia and poor quality. This was a semi-quantitative approach to determine the depth of oxygen penetration in sediments due to faunal mediated particle and porewater mixing. As the aRPD has been found to correlate well with the actual RPD depth, porewater DO, and sediment redox potential (Rosenberg et al. 2001, Diaz & Tefry 2006), visual assessments of aRPD depth have been used as a proxy for sediment quality in benthic habitat assessments (Karlsson et al. 2010, Shumchenia & King 2010).

## Statistical analysis

### Eutrophication expression index

We assessed the overall eutrophication condition of each of the 18 sites using a synthetic EEI modified

from Bricker et al. (2003). The method uses normalization techniques to transform highly variable data into a eutrophication index, which is statistically comparable within and among estuaries. Statistical methods were modified to accommodate the known data sources and monitoring programs that exist in Elkhorn Slough. The data used and analyses conducted are described in detail in Supplements 1 & 2 at www.int-res.com/articles/suppl/m439p031_supp.pdf.

Eutrophication values were generated for 8 eutrophication indicators (subtidal, intertidal, and floating algal cover, chl *a*, hypoxia, hyperoxia, aRPD, and free ammonia) based on established thresholds. Next, the values for primary indicators and secondary indicators were averaged for each site. Then the average between the primary and secondary indicators was calculated and used for an overall EEI for each site. We used these values to map the spatial extent of eutrophication status with areas being delineated by the highest high water level (Fig. 2), and further utilized these site-specific values to estimate an overall eutrophication score for the estuary. For areas behind water control structures, we assumed spatial homogeneity, and used a site-specific value for the full area of tidal restriction. For contiguous areas that experience full tidal exchange, we interpolated values between sites as a simple function of distance. To produce an estuary-wide score, we weighted eutrophication scores by both area ($A$) and volume ($V$) to determine any differences between area-based vs. volume based scores (Supplement 2).

### Principal components analyses

To determine whether drivers or filters were related to the EEI and eutrophication indicators, we used PCA. Separate PCAs were performed for the driver, filter and indicator data, where each site was treated as a replicate. A single value per site was used for each driver, filter, and indicator, as described above. We used 3 drivers (nitrate, phosphate, ammonia), 6 filters (temperature, turbidity, depth, salinity, tidal range, and distance to mouth), 3 primary indicators (chl *a*, subtidal algal cover, and floating algal cover), and 3 secondary indicators (DO variation, aRPD, and pH). Each variable was tested for skewness and homogeneity of variance and log transformation was used if one of the assumptions was violated (Underwood 1997). The PCA was run using SPSS statistical software (v. 17) by developing a correlation matrix using a varimax rotation method. Principal components (PC) with eigenvalues $<0.9$ were discarded because they did not explain more than the original variables (Clarke & Warwick 2001). Variables associated with each component with an absolute correlation (PC weights) value $<0.40$ were also discarded. This was a conservative PC weight based on previous reported values (Graham 2003, Hughes 2010) and reduced the complexity of interpreting an excessive number of variables.

The scores for the first 2 PCs for each site were plotted and coded by their EEI to visualize the influence of drivers and filters on eutrophication expression. PC weights were used to label each axis to help characterize the filters and drivers of eutrophication.

### Determination of key eutrophication drivers and filters

In addition to the above analyses identifying key correlates of the EEI, we conducted additional analyses to determine which potential drivers and filters had the strongest relationship with eutrophication indicators. To do this, we used principal component regression (PCR) because it provides a robust analysis when there is a high degree of multicollinearity, such as in the driver and filter variables (Graham 2003). The principal component scores were the predictor variable used to examine the combined relationships of significant variables of the principal component and the response variable (eutrophication indicators). The same PC scores of the driver and filter variables described above were used for the PCR. However, if a predictor variable (i.e. drivers or filters) had an absolute correlation value $>0.4$ for one or more PCs, then it was removed from the analysis to avoid complications of interpreting PC modes, and was used as a stand-alone variable in the multiple regression. PCA was again run without the stand-alone variable. A PCA was run on eutrophication indicators to help identify the importance of filters and drivers of eutrophication, using a similar data set as that for the EEI, with some modifications to avoid multicollinearity of certain variables (see Supplement 1).

After we determined the treatment of the various PCs, we ran the PC regression using a stepwise multiple regression to determine the key predictor PCs of eutrophication using SPSS statistical software (v. 17). A multiple regression was run using the driver and filter PCs as the predictor against each significant indicator PC. Running correlation analysis of predictor PCs (drivers and filters) against indicator PCs

enabled identification of key drivers, filters, and indicators of eutrophication in Elkhorn Slough. Partial correlation coefficients (PCC) were used to determine positive or negative relationships while holding other variables constant. All alpha levels were set at 0.05.

### Linear regressions of tidal range vs. eutrophication indicators

One of the key filters apparent from the spatial analysis and from the PC analysis was tidal range, which is artificially restricted at many of the study sites. To more closely examine the specific relationship between tidal range and eutrophication indicators, we conducted simple linear regressions between tidal range and 6 eutrophication indicators (chl *a*, subtidal algal cover, floating algal cover, DO variation, aRPD, and pH). A Bonferroni correction was applied to avoid Type I errors by taking the original alpha and dividing it by the number of independent tests to generate a more conservative alpha.

To better understand the relationship between DO variation (measured in our monthly water quality sampling) and hypoxia, we also conducted a linear regression between DO variation and percent time each site went hypoxic. This helped us to determine if DO variation was a useful indicator of hypoxia. The relationship between tidal range and hypoxia was graphed to examine the relationship between these variables that are of high coastal management interest.

## RESULTS

### Eutrophication expression: estuary-wide mean and spatial variation between sites

#### Eutrophication expression index

Eutrophication indices varied spatially across the 18 sites, from low to hypereutrophic (Fig. 2, Tables S2 & S4). The main channel of Elkhorn Slough was moderately eutrophic near the mouth and middle, and increased to highly eutrophic near the head. The overall spatially averaged EEI value for the Elkhorn Slough estuarine complex was 0.539 and 0.450 for area-based and volume-based assessments, respectively, making it a moderately eutrophic estuary (Table S3c).

#### Drivers

Nutrient concentrations in Elkhorn Slough were very high compared to reported eutrophication thresholds (Table 1a). Mean nitrate and phosphate exceeded high thresholds, and mean ammonia reached moderate to high thresholds based on values reported by Caffrey et al. (1997), Caffrey (2002), and Bricker et al. (2003). However, nutrient concentrations were highly variable among sites (Table S2); 33% of the sites had means exceeding the high nitrogen threshold (>1.0 mg N $l^{-1}$), and 39% had means that exceeded the high phosphorous threshold (>0.1 mg P $l^{-1}$) established by Bricker et al. (2003). Nutrients were highest at sites that were closest to freshwater inputs (i.e. Old Salinas River channel and Carneros Creek) (Table S2), and were at times 50 and 20× greater than the high threshold proposed by Bricker et al. (2003) for nitrogen and phosphorous, respectively.

#### Primary indicators

Mean algal cover, biomass, and chl *a* in all sites in Elkhorn Slough were moderate (Table 1b); however, at least one primary indicator was high at all sites (Table S2). Although there was high cover of algae at many sites, only a few species of green macroalgae comprised the algal assemblages in Elkhorn Slough.

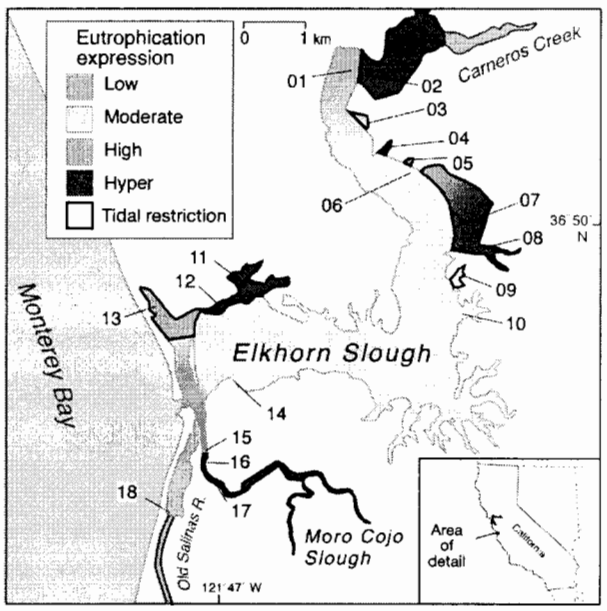


Fig. 2. Monitoring stations in Elkhorn Slough with spatially interpolated eutrophication indices. See Table S1 in Supplement 1 at www.int-res.com/articles/suppl/m439p031_supp.pdf for site descriptions

Table 2. Principal component weights for eutrophication (a) drivers, (b) filters and (c) indicators. Percent variance explained among variables are in parentheses. aRPD: apparent redox potential discontinuity layer; DO: dissolved oxygen. Significant weights >0.40 are highlighted in **bold**

| | Principal component | |
|---|---|---|
| **(a) Drivers** | 1 (63.5%) | 2 (30.0%) |
| Nitrate[a] | **0.863** | 0.389 |
| Ammonia[a] | **0.962** | −0.032 |
| Phosphate[a] | 0.114 | **0.984** |
| **(b) Filters** | 1 (43.1%) [41.9%] | 2 (28.1%) [33.6%] |
| Tidal range[b] | **−0.532** | **−0.548** |
| Subtidal depth[a] | **−0.691 [−0.714]** | −0.333 [−0.326] |
| Temperature | 0.227 [0.252] | **0.936 [0.926]** |
| Freshwater | **0.853 [0.860]** | 0.009 [−0.008] |
| Turbidity | **0.769 [0.795]** | −0.148 [−0.137] |
| Distance to mouth | −0.246 [−0.184] | **0.853 [0.901]** |
| **(c) Indicators** | 1 (49.6%) | 2 (25.4%) |
| Chl *a*[a] | 0.354 | **0.845** |
| Floating algal cover | **0.733** | 0.000 |
| Subtidal algal cover | 0.250 | **−0.863** |
| Daytime pH | **0.740** | **0.584** |
| aRPD | **−0.941** | −0.016 |
| Daytime DO variance | **0.732** | 0.062 |

[a]Variable log transformed prior to analysis
[b]Removed as stand-alone variable for multiple regression; data in square brackets are the PC weights after tidal range was removed

Floating algal mats were dominated primarily by *Ulva intestinalis*, but also included *Rhizoclonium riparium* and *Chaetomorpha* sp. Subtidal zones were dominated by *U. lactuca, U. expansa*, and *U. lobata*. The intertidal zone was dominated by *U. lactuca, U. intestinalis, R. riparium, Chaetomorpha* sp., and *Gracilariopsis andersonii*.

### Secondary indicators

There was also significant expression of secondary indicators of eutrophication in the estuary (Table 1c). This includes periods of hypoxia, anoxia, high pH, and anoxic sediments. There was also high variability in hypoxia, daytime DO, pH, and sediment quality among sites (Table S2).

### Correlation between EEI and indicators

Nutrient levels were not strongly correlated with the EEI of the sites; the plot of PC1 vs. PC2 Drivers displayed no separation in eutrophication expression among the sites despite their great variation in nutrient concentrations (Fig. 3a, Table 2a). While the PC analysis did not show clustering of sites characterized by nutrients alone into the eutrophication categories, it is noteworthy that hypereutrophic sites were never found near the origin of the axes, suggesting some role of nutrients at sites with a hypereutrophic status.

Eutrophication filters were strongly correlated with the EEI. The plot of PC1 vs. PC2 Filters shows clear separation of sites by their EEI (Fig. 3b). All 6 filters had significant PC weights in the PCA analysis (Table 2b), so these filters all appear to contribute to variation in eutrophication patterns (Fig. 3b, Table 2b). Sites with greater tidal range, depth, salinity, and lower turbidity (PC1) generally had lower eutrophication expressions, whereas sites with greater freshwater inputs, turbidity, and lower depths and tidal ranges had higher eutrophication expressions. This gradient among the sites segregates marine from estuarine sites. The PC2 axis indicated that sites with lower eutrophication had lower temperatures and were closer to the mouth of the

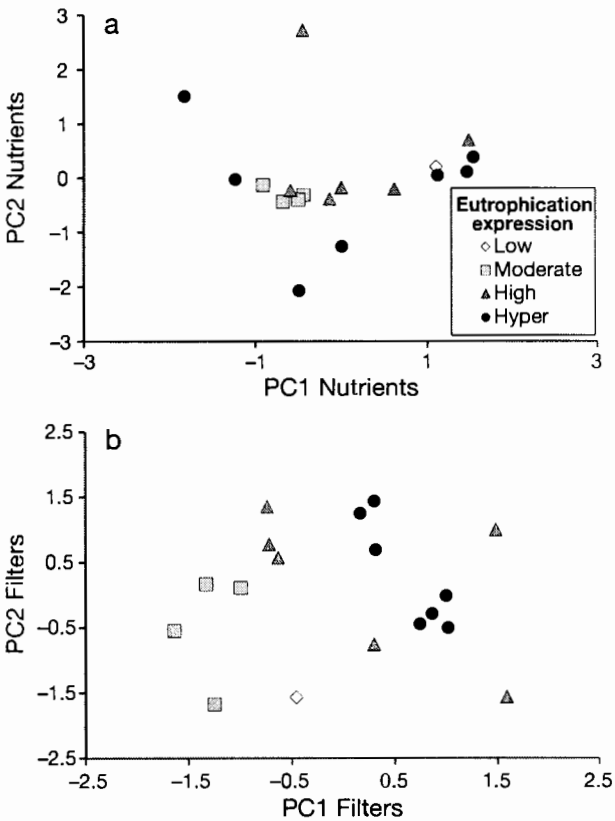


Fig. 3. Principal components analysis (PCA) of (a) nutrients and (b) filters. Points represent PC scores for each site (N = 18), with eutrophication expression index being indicated by markers. See Table 2a,b for description of significant PC weights

estuary, and sites further from the mouth with higher temperatures had higher eutrophication indices.

Eutrophication filters were also correlated with the eutrophication indicators. To run the analysis, we first removed tidal range from the PC Filters as a stand-alone variable because it loaded significantly on both axes (Table 2b). Next, we ran a multiple regression with tidal range as the dependent variable and PC1 and PC2 as the independent variables to ensure that there was no collinearity between the PCs and tidal range ($F_{1,17}$ = 2.951, $R^2$ = 0.156, p = 0.105). We ran the multiple regression with PC1 and PC2 Filters, tidal range as a stand-alone variable, PC1 Drivers (nitrate and ammonia) and phosphate as a stand-alone variable, since phosphate was the only variable with a significant weight for PC2 (Table 2a). The multiple regression resulted in a significant negative relationship of PC1 Indicators with tidal range (Fig. 4) and a significant positive relationship of PC2 Filters with PC1 Indicators (Table 3). Tidal range correlated positively with aRPD and negatively with floating algal cover, daytime DO variance, and daytime pH. In contrast, PC2 Filters (temperature and distance to mouth) were negatively correlated with aRPD and positively correlated with floating algal cover, daytime DO variance, and daytime pH. Lastly, PC2 Indicators had a significant positive correlation with PC1 Filters (Table 3). More specifically, higher freshwater inputs and turbidity were correlated with greater chl *a* concentrations and daytime pH, whereas greater subtidal depth was positively correlated with greater subtidal algal cover (Table 2b,c).

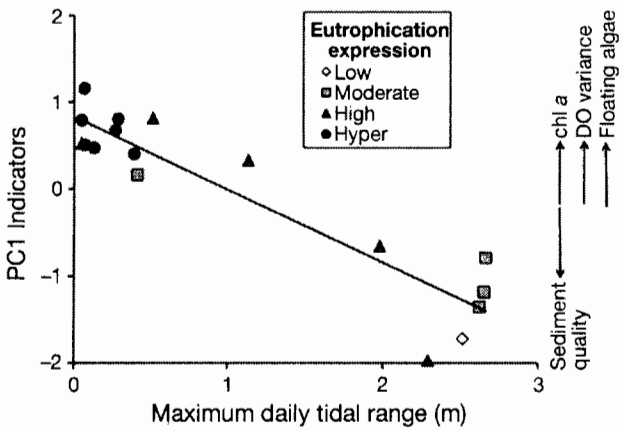


Fig. 4. Multiple regression analysis of principal component (PC) scores for tidal range vs. PC1. Variables with significant PC weights are indicated on each axis. Variables included in the stepwise regression were PC1 Drivers, phosphate, PC1 Filters, PC2 Filters, and tidal range (see Table 3 for statistical results)

Table 3. Stepwise multiple regression of eutrophication indicators versus principal components (PCs) of eutrophication drivers and filters (N = 18). PCC: partial correlation coefficients, used to determine relationships while holding other variables constant. The independent variables were PC1 Drivers, phosphate, PC1 Filters, PC2 Filters, and tidal range

| | Entered variables | df | $R^2$ | *F* | p | PCC |
|---|---|---|---|---|---|---|
| **PC1 indicators** | | | | | | |
| Step 1 | Tidal range | 1 | 0.768 | 52.923 | <0.0005 | −0.876 |
| Step 2 | PC2 filters | 2 | 0.823 | 34.804 | <0.0005 | 0.234 |
| **PC2 indicators** | | | | | | |
| Step 1 | PC1 filters | 1 | 0.417 | 11.458 | 0.004 | 0.646 |

Nutrients were never entered into the stepwise multiple regression models as significant explanatory variables along with filter variables, indicating that filters explained the most variation in eutrophication. However, nutrients had a weak significant relationship with eutrophication indicators when analyzed without filter variables. The PC2 Indicators had a significant negative relationship with phosphate ($F_{1,17}$ = 4.689, $R^2$ = 0.227, p = 0.046, PCC = 0.476). Sites with high phosphate were associated with high chl *a* concentrations and pH, but low subtidal algal cover (Table 2a,c).

### Relationship between tidal range and eutrophication indicators

Tidal range had the most significant correlation with the eutrophication indicators (Figs. 2 & 3, Table 3), indicating that it is the strongest contributor to the separation of sites by filters. Therefore, a reduced model (simple linear regression) was used to more closely examine pairwise relationships between tidal range and individual indicators. Tidal range was correlated with all of the eutrophication indicators except for subtidal algal cover (Table 4). Tidal range was negatively correlated with chl *a*, floating algal cover, daytime pH, and daytime DO variation; and was positively correlated with aRPD.

Since hypoxia is a primary concern for overall ecosystem health, we investigated the relationship between DO variation (used in the previous analyses) and percent time hypoxic at sites where DO was measured continuously (every 15 min) during 2008–09. There was a significant relationship between DO variation and percent time hypoxic (Fig. 5a).

Table 4. Simple linear regression analysis of individual eutrophication indicators (chl *a*, floating algal cover, subtidal algal cover, daytime pH, apparent redox potential discontinuity [aRPD], and daytime dissolved oxygen [DO] variance) vs. tidal range. All alphas were set at 0.05, while conservative measurements of alpha were set at 0.01 using a Bonferroni correction. *Significant relationship ($\alpha = 0.05$). **Highly significant Bonferroni correction ($\alpha = 0.01$)

| Predictor variable | Response variable | df | $R^2$ | *F* | ß | p |
|---|---|---|---|---|---|---|
| Tidal range | chl *a*[a] | 1 | 0.292 | 6.584 | −0.459 | 0.021* |
| | Floating algal cover | 1 | 0.445 | 12.838 | −23.555 | 0.002** |
| | Subtidal algal cover | 1 | 0.004 | 0.059 | −1.172 | 0.811 |
| | Daytime pH | 1 | 0.470 | 14.212 | −0.197 | 0.002** |
| | DO variation | 1 | 0.269 | 5.882 | −8.793 | 0.027* |
| | aRPD | 1 | 0.714 | 39.853 | 11.571 | <0.0005** |

[a]Variable log transformed prior to analysis

Next, we examined the relationship between tidal range and percent time hypoxic at all of our sites, which resulted in a significant negative relationship (Fig. 5b). The role of tidal range was also readily visualized by examining the geographic patterns of expression of eutrophication and tidal restriction (Fig. 2). In particular, all hypereutrophic areas were those that were behind water control structures.

## DISCUSSION

### Importance of scale in determining eutrophication expression

Overall, the estuarine wetlands of Elkhorn Slough exhibit levels of eutrophication equal to or exceeding those of other estuaries that are considered eutrophic in the United States, e.g. San Francisco Bay, Newport Bay, Chesapeake Bay, and Tampa Bay (Scavia & Bricker 2006). Elkhorn Slough wetlands exceed thresholds for nutrient concentrations, algal cover and biomass, chl *a*, hypoxia and anoxia, sediment quality, and free ammonia production. The overall eutrophication score for the entire estuary, based on spatial averaging, was moderate. However, eutrophication expression within the estuary was highly variable (low to hypereutrophic). For instance, some sites are hypoxic a large percentage of time while others never become hypoxic. Eutrophication assessments at the single estuary scale could be improved by capturing both spatial and temporal scales of variation in eutrophication indicators, providing a range of observed values as well as averages. Although estuary-scale scores are useful for large-scale geographic characterizations, results from this study within a relatively small estuary with high variation among sites show that a single score is probably not very useful at a local scale. Also, managers should be cautious in using spatial analysis to determine overall eutrophication expression because volume-based estimates can yield a different expression than area-based estimates. In the case of Elkhorn Slough, the area-based score was higher than the volume-based score because many of the restricted areas had low volume, yet had extensive intertidal area.

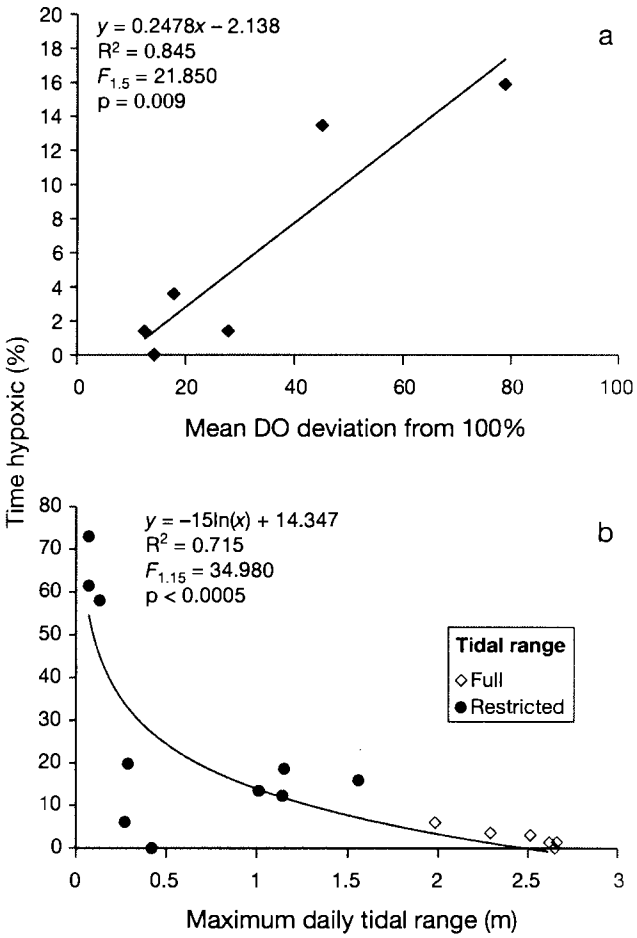


Fig. 5. Linear regression showing the relationship between (a) hypoxia vs. DO deviation, and (b) percent time hypoxic vs. tidal range. Tidal range was measured during 2 to 4 wk deployments and does not necessarily reflect the maximum tidal range for the year, which is similar for all fully tidal sites. Sites in (b) were labeled according to tidal range category: full (no water control structure, ◇) or restricted (behind water control structure, ●)

### Importance of filters in mediating eutrophication expression

The dramatic variation in eutrophication expression between sites enabled us to conduct powerful analyses that examined key correlates of eutrophication expression, and could be a useful approach for other estuaries. Taken together, the filters we measured explained site variation in eutrophication expression, as summarized by the strong correlation (Table 3) between filters and eutrophication indicators. The powerful analytical approach allowed us to examine groupings of co-varying drivers, filters, and indicators, as well as individual ones that were dominant, and to examine complex collinearity. Physicochemical filters explained much of the variation in overall eutrophication expression among sites, as well as variations in levels of individual indicators among sites. Also, different filters were important for different indicators; tidal range explained variation in patterns of floating algal mats, DO deviation, hypoxia, pH, aRPD, and chl *a*; temperature and distance to mouth explained variation in floating algal mats, DO deviation, pH, and aRPD; and depth, freshwater, and turbidity explained variation in pH, subtidal algal cover, and chl *a*. Other studies have tested filters of eutrophication (Monbet 1992, Boynton et al. 1996, Kemp et al. 2005), but our study is the first powerful test within a single estuarine system of the model proposed by Cloern (2001), and our results strongly support this model.

Filters not only explained variation in eutrophication expression, but actually did so better than did drivers. Sites with the highest nutrient concentrations were not the ones with the highest eutrophication scores, or with significant positive correlations with individual indicators such as algal cover or hypoxia. Of all the eutrophication indicators, only chl *a* and daytime pH had a significant positive relationship with nutrients (phosphate), but this is probably a weak correlation because only 25% of the variation in the PC2 Indicators axis was explained by its variables (chl *a*, subtidal algae, and aRPD) (Table 2c). It should be noted that nutrient loads at each site were not measured and could have shown a correlation with eutrophication expression, but we consider this unlikely based on our knowledge of these sites. This finding contrasts with the results of studies in other estuaries where nutrient concentrations have been shown to correlate well with eutrophication symptoms, e.g. Maryland coastal bays (Boynton et al. 1996) and Chesapeake Bay (Kemp et al. 2005). It is possible that such correlations only occur over lower ranges in nutrient concentrations; Elkhorn Slough has much higher nutrient concentrations than these other estuaries, with a mean nitrogen concentration of 1.7 mg $l^{-1}$ and a maximum of 56.4 mg $l^{-1}$. In very nutrient-loaded systems, nutrient concentrations may not be as good predictors of eutrophication as are various filters.

### Tidal range as a key filter of eutrophication expression

Tidal range has been identified in other studies as an important variable affecting estuarine eutrophication (Monbet 1992, Cloern 2001, Martinetto et al. 2010), and our results from Elkhorn Slough found tidal range to be the single most important filter of eutrophication expression, both in the multivariate and univariate analyses. Decreases in tidal range correlated significantly with increased DO variation, increased chl *a*, increased daytime pH, decreased sediment quality, and increased cover of floating algal mats. In our study, variation in tidal range was the result of artificial water control structures, but the results are also relevant to natural variation in tidal range (i.e. differences between micro- and macrotidal estuaries). Other studies of natural variation in tidal range between estuaries (Young et al. 1997, Edgar et al. 2000) revealed significant water quality and biological community differences attributed to tidal range.

There are numerous mechanisms by which tidal range may affect eutrophication expression at Elkhorn Slough and other estuaries. A filter such as tidal range can affect both how nutrients stimulate primary production or how such production can lead to secondary indicators of eutrophication; our modification of Cloern's conceptual model indicates both of these potential roles for filters (Fig. 1). Greater tidal amplitude increases tidal mixing, which leads to decreases in stratification and residence time, and increases the transport of primary producers and nutrients out of the system (Nixon et al. 1996, Cloern 2001, Uncles et al. 2002). Martinetto et al. (2010) found that increases in tidal range can filter the effects of eutrophication on secondary indicators (hypoxia and benthic invertebrate abundance); despite high nutrient inputs and algal growth, secondary indicators of eutrophication were absent in areas of high tidal exchange. Increases in residence time can lead to higher temperatures and phytoplankton blooms (Largier et al. 1997, Valiela et al. 1997). Temperature directly affects the photosynthetic and respiration rates of algae, especially in opportunistic species like

*Ulva lactuca* and *U. linza* (Kanwisher 1966, Lüning 1990). The increase in metabolic activity may lead to greater fluctuations in secondary indicators, such as pH and DO, as noted in this study at sites with lower tidal ranges and higher temperatures.

Coastal managers can influence tidal range in wetlands behind water control structures. Most water control structures at Elkhorn Slough and other estuaries around the world were constructed to allow farming in floodplains and prevent inundation of farmed fields with salt water (Caffrey & Broenkow 2002, Williams & Orr 2002). For Elkhorn Slough, there are 2 aspects of agricultural development that have negative consequences for the estuarine ecosystem: nutrient run-off and artificial tidal dampening; these work in concert to influence eutrophication expression. The most eutrophic sites are the ones behind water control structures. Negative ecological effects of water control structures, both direct and indirect, include loss of diversity or decreases in abundances of ecologically important species (Sanzone & McElroy 1998, Ritter et al. 2008). However, the results from this study indicate that even moderate increases in tidal exchange through water control structures can mediate the worst eutrophication problems, and thus potentially enhance biological communities. For example, increasing the tidal range of most restricted sites in Elkhorn Slough to ~1 m could dramatically reduce hypoxia (Fig. 5b). Although increases in tidal range offer managers potential for more local control, reductions of nutrient inputs at the watershed scale must be the ultimate solution to eutrophication problems in the long run.

## Filter analyses to examine variation within estuaries: future studies

This study used a rigorous approach to identify filters mediating eutrophic expression. The success of this approach came from using 18 different sites within an estuary, which gave us statistical power to investigate correlations between eutrophication and filters. This approach is readily transferrable to other systems, if a design using multiple sites is implemented, with measurement of primary and secondary indicators and factors that may serve as potential filters. The multivariate approach could also be applied to larger scales (e.g. regional, national, and global), or could be used to examine even finer scales within single estuaries.

There is great value for researchers to monitor filters and indicators (not just nutrients) for characterizing and predicting eutrophic conditions in coastal environments. This approach does not necessarily require a large investment of resources. In this study, we were able to use a combination of monthly sampling, one-time rapid assessment surveys, and 2 wk hypoxia deployments to obtain very robust statistical relationships. This type of effort is within the capabilities of many coastal monitoring organizations and highlights the importance of spatial coverage across varying sites. Physico-chemical features were emphasized, using easily measurable parameters such as depth, salinity, and temperature. Although this study did not examine the actual mechanisms by which these parameters may affect eutrophication (i.e. underlying biogeochemical processes), it identified key parameters that correlate with eutrophication expression. These parameters have value in informing management strategies because managers can regulate freshwater or tidal inputs that affect flushing, whereas they do not directly manage biogeochemical processes.

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# Identifying factors that influence expression of eutrophication in a central California estuary

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**Supplement 1.** Data sources: field sampling, laboratory methods, and analyses

The foundation dataset for this study came from an ongoing 20 yr water quality monitoring project being conducted at the 18 sites. We used the monthly water quality data from 2008–09 that were a part of this long-term program, and supplemented this with short-term assessment of additional indicators and filters during the same period. Below we summarize the field and laboratory methods for each element as well as the statistical methods applied to the data both for the eutrophication expression index (EEI), principal components analyses (PCA), and linear regression. Some variables used for the PCA and linear regression analyses differed from those used in the EEI because the EEI included variables that are commonly used for coastal management targets with defined thresholds, and some of these variables could not be used for parametric statistics testing the coastal eutrophication model for several reasons. Also, the EEI model is more robust with more indicator variables, even if they do not apply to some sites (S. Bricker pers. comm.).

**Nutrient analysis.** To determine the nutrient concentrations at each site, grab samples were taken monthly at 18 monitoring sites (Fig. 2, Table S1) from July 2008 to June 2009. Samples were GFF filtered the same day they were collected and analyzed within 24 h. Samples were run at 2 different laboratories, the Moss Landing Marine Labs (MLML) and the Monterey County Consolidated Chemistry Lab (MCCCL); regular cross lab comparisons ensured high correlations between results for the 2 labs. For statistical analyses, the mean annual values (i.e. mg N or P $l^{-1}$) from samples collected during the 12 mo sampling period for nitrate, ammonia, and phosphate were used. All values were reported as the concentration of nitrogen (nitrate and ammonia) or phosphorous (phosphate). These data were used only for the PC and regression analyses, and not for the EEI.

***Ammonia as nitrogen analysis:*** The determination of ammonia in sea-water was conducted at MLML using a modified method as described in Standard Methods 4500-NH3 (Strickland & Parsons 1972). The MCCCL determined ammonia by using EPA 350.3 method (US EPA 1993).

***Nitrate as nitrogen analysis:*** MLML determined nitrate using the modified (Sakamoto et al. 1990) standard methods 4500 NO3 on a flow injection autoanalyzer (Alpkem; Clesceri et al. 1998). MCCCL determined nitrate using EPA method 300.0 (US EPA 1993).

***Orthophosphate as phosphorus analysis:*** MLML determined orthophosphate using the modified (Sakamoto et al. 1990) standard method 4500 PG on a flow injection autoanalyzer (Alpkem; Clesceri et al. 1998). MCCCL determined orthophosphate using standard method 4500 P E (Clesceri et al. 1998).

**Filters.** Eutrophication filters were measured at each site and included turbidity, temperature, salinity, depth, distance to estuary mouth, and tidal range. Turbidity, temperature, and salinity measurements were sampled using data sondes (YSI) during monthly collection of nutrients from July 2008 to June 2009, by taking surface water measurements near the water collection site. Depth was determined by taking the average of 5 thalweg

measurements during spring low tide at each site and calibrating them to the mean tide level for fully tidal sites by adjusting for tidal height at the time of measurement. The distance to the estuary mouth was determined using Google Earth's path ruler measurement tool by following the channel contours to each site. Lastly, tidal range was measured for each site between July 2008 to June 2009 during 2 to 4 wk deployments of YSI data sondes that sampled depth every 15 min. The maximum daily tidal range observed during this 2 to 4 wk period was used as the value for this filter. For fully tidal sites, this value was sometimes lower than the maximum daily tidal range observed over a whole year (~3 m for all fully tidal sites during the most extreme tides of the year). For 3 sites with restricted tidal exchange that receive significant freshwater inputs (2, 16, 17), the measured water level changes were likely largely due to freshwater backing up in the wetland during high tide when 1-way tide gates were closed by water pressure; however, we had no way of estimating the contribution of freshwater or tidal water to the water level fluctuations and thus considered them all as 'tidal range' for simplicity. For this small subset of our sites, 'range in water levels' would be a more accurate descriptor than 'tidal range'. Filter data were used only for the PC and regression analyses, and not for the EEI.

**Primary indicators.** Primary indicators generally refer to primary producers because they are the organisms that take up nutrients. Eutrophication is defined as the increase in the rate of primary production (Nixon 1995). Cultural eutrophication is an increase in the rate of primary productivity due to anthropogenic inputs. Water column phytoplankton, ephemeral green macroalgae, and one species of red alga were the primary indicators used in this study. We assessed proxies for the biomass of these algal components and did not directly assess rate of primary productivity.

***Water column phytoplankton (chlorophyll a) assessments:*** Monthly water samples from the 18 sites were collected for the determination of laboratory measured chl *a* concentrations from July 2008 to June 2009. Water samples were filtered then extracted in 8 ml of 90% acetone, and run for the determination of chl *a* concentrations after 24 h as detailed in the analysis in section 10200 H of Clesceri et al. (1998). For these samples, a modified single step method using a fluorometer (Turner Designs TD-700) with 436 and 680 nm filters was employed(Welschmeyer 1994). The mean annual chl *a* concentration for each site during the study period was used in all statistical analyses (EEI, PCA, and linear regression).

***Macroalgal assessments:*** At each of the monitoring sites, visual estimates of percent cover of floating algal mats were made monthly at the same time as water sample collection from July 2008 to June 2009. The percent of the water surface that was covered by algae was assessed within a ~50 m radius of the water sampling site. Floating algae as well as benthic algae breaking the water surface were included; benthic algae seen through the water column were excluded from percent cover estimates because water clarity differed greatly among sites. Algae growing on mudflats above the water line were not included because the area of the intertidal varied greatly among sites. All algal species were pooled to estimate the total percent cover. Photos of ground-truthed percent cover were used to calibrate visual estimates made at each monthly sampling. To increase accuracy, estimates of percent cover were reported in 10% increments and the same observer was used throughout the study to increase precision (see Fig. S1 for an example). The maximum percent cover of floating algal mats for each site over the course of the study period was used for all statistical analyses (EEI, PCA and linear regression).

**Fig. S1.** Floating *Ulva intestinalis* mat at Moss Landing Road South with ~80% cover. Solid red line indicates the oblique plane, and cover beyond this point was not sampled

A one-time assessment of subtidal and intertidal macroalgal mats was performed in May 2009 to take advantage of low daytime tides and the season of peak algal production. Floating and intertidal algal mats were surveyed using the same techniques described above. Subtidal algal mats were sampled using random point contact (RPC) within the same survey area used for floating algal mats. The RPC method was used instead of visual estimates due to poor subtidal visibility. Twenty points were randomly selected and sampled for the presence of green macroalgae to generate a percent cover of the subtidal area. The percent cover of subtidal algal mats from each site sampling was used for both statistical analyses. Intertidal algal cover was not used in PCA or linear regression, but was used for the EEI for sites with a tidal range >1. This was because the intertidal zone was very narrow and not comparable at sites with a restricted tidal range, but was useful for a general characterization of algal mats in Elkhorn Slough (Table 1) and for determining the algal condition for the EEI at sites with a tidal range >1 m.

**Secondary indicators.** Secondary indicators of eutrophication are considered to be parameters that are indirectly influenced by nutrient additions to a system (Cloern 2001). These parameters, or consequences, include hypoxia, decreases in submerged aquatic vegetation (i.e. eelgrass), reductions in sediment quality, changes in benthic community assemblages, loss of biodiversity and even dead zones. This study assessed 3 secondary indicators: dissolved oxygen (DO), sediment quality, and pH (pH was also used to calculate free ammonia production for developing the EEI). Hypoxia is the product of eutrophication that causes the most concern because of its negative effects on populations, communities, and biodiversity (Diaz & Rosenberg 2008, Vaquer-Sunyer & Duarte 2008, Fox et al. 2009, Turner et al. 2009). Hypoxia occurs when increased nutrient levels cause increased primary productivity which can cause hypoxia due to several different processes: self-shading of the primary producer leading to net respiration, organic deposition leading to increased microbial DO consumption, and increased night-time respiration of primary producers. The same processes lead to water column hypoxia and sediment anoxia. Deposition and decay of algae can lead to smothering and create anoxic conditions in the sediments. Poor sediment quality (i.e. sediment anoxia) can cause losses in benthic community diversity and decrease the abundance of ecologically important species. This process has been observed in Elkhorn Slough (Oliver et al. 2009). Reductions in sediment habitat quality can limit the distribution of important trophic prey items, such as clams and worms. Fluctuations in DO driven by eutrophication cause fluctuations in pH. High variation in pH coupled with high ammonia concentrations can lead to the production of un-ionized ammonia (free ammonia), which is of concern because it can be toxic to many fish species in Elkhorn Slough, such as the endangered steelhead trout (US EPA 1999). The following equation describes eutrophication driven un-ionized ammonia production:

$$\text{Photosynthesis/eutrophication} \rightarrow \Uparrow O_2 + H_2O \rightarrow H^+ + HCO_3^- \rightarrow \Uparrow pH + NH_4^+ \rightarrow NH_3 + H^+$$

***DO variation:*** Data were collected monthly using YSI data sondes to coincide with the monthly nutrient and chl *a* sampling. DO variation was calculated by subtracting the measured DO concentration in % saturation from 100%. The mean DO variation measured monthly at each site over the study period was used in the PC and regression analyses because it was more statistically robust and directly comparable among sites without violating assumptions of PCA and linear regression in comparison to hypoxia and hyperoxia measurements. The percent of time a site experienced hypoxia and hyperoxia was determined using continuous measurements. However, due to the limited number of sondes, we could not deploy at all of the sites at the same time; thus, we did not use these data for parametric tests to avoid violating the assumptions of independence.

***Hyperoxia:*** Hyperoxia data were also collected because they are a good indirect measurement of eutrophication and hypoxia potential (Bricker et al. 2007). Data were collected monthly using YSI data sondes to coincide with the monthly nutrient and chl *a* sampling. Hyperoxia data were similar to those used to assess DO variation, but were instead generated using the 90th percentile DO at each site over the 12 mo sampling period. Hyperoxia data were used for the EEI but not for the PCA and regression analyses.

***Hypoxia assessments:*** In addition to the single monthly daytime DO concentration measured as part of the monthly sampling, YSI sondes were also deployed ~30 cm above the benthic surface and <5 m from the monthly water quality site for at least one lunar tidal cycle (2 to 4 wk) to obtain a more detailed understanding of DO concentrations over time. Sites were sampled around peak months of peak primary productivity from July 2008 to June 2009. Sampling was staggered due to the limited number of YSI data sondes. These sonde deployments were the same ones as were used to calculate maximum tidal range at these sites, as briefly described above under filters. The resulting data were categorized into concentration-based groups representing oxic, hypoxic, or anoxic conditions (Table S3a). Further details of the methods used to account for drift over time and biofouling can be found in the protocols of the National Estuarine Research Reserve (NERR), system-wide monitoring program: http://cdmo.baruch.sc.edu/data_dissemination.html#NERR%20Water%20Quality%20Data. Hypoxia data were compared to DO variation data to test the assumption that they were correlated, and these 2 parameters were also used to generate the EEI, and for simple linear regression analyses. For each site, the percent of time the DO fell below 2.3 mg $l^{-1}$ (US EPA 2000) was used to indicate the degree of hypoxia. Hypoxia data were used for the EEI in preference to DO variation data because hypoxia has defined thresholds, which were essential in generating the EEI. For Fig. 5, Sites 4 and 5 were excluded because they were considered as outliers due to their being seasonal ponds, which may affect their biogeochemistry and DO.

***Sediment quality assessments:*** A one-time assessment of sediment quality was done in May 2009. Surveys were completed during low tide at the same water monitoring sites and time as the algal surveys. Benthic sediment cores (>50 cm) were taken at 5 random locations in the same subtidal zone where algal surveys were done. Cores were moved to shore and split apart to measure the depth of the sediment surface to the black anoxic layer (Fig. S2). Five replicate measurements were taken within each core to capture variability within the core. Brown colored sediments indicate good sediment quality, whereas dark gray to black sediments indicate sediment anoxia and sulfate reduction. This layer is generally considered to be the product of high organic deposition and of poor habitat quality for all benthic infauna (except for anaerobic bacteria) due to the anoxic environment. A greater apparent redox potential discontinuity (aRPD) layer indicates better sediment quality. The mean aRPD (cm) taken from the 5 replicates in each core at each site was used for the EEI, PC, and regression analyses.

**Fig. S2.** Sediment cores taken to measure the aRPD

***pH:*** Like DO, pH is driven by primary productivity and is therefore a good indicator of eutrophication. High pH values are indicative of eutrophic areas. We collected daytime pH using YSI data sondes and used the high range of pH values from each site to assess eutrophication. The high pH (90th percentile) was calculated using data collected monthly at each site during the day over the course of the study period. The 90th percentile of pH was used for the PC and regression analyses but not for the EEI, where free ammonia was instead used since it has defined thresholds essential for the EEI.

***Un-ionized (free) ammonia assessments:*** Free ammonia was calculated using the ammonia concentration and simultaneously collected water quality parameters from 2004–2009: pH and temperature (US EPA 1999), using the following equation: 1 (1+10^(pK–pH)$^{-1}$ × [Ammonia], where pK was described by Emerson et al. (1975) with the following equation: pK = 0.09018 + 2729.2 (273.2 + T)$^{-1}$, where T is temperature in degrees Celsius.

Free ammonia was only used in the calculation of the EEI but not for the PCA or regression because ammonia as a secondary indicator is not independent of ammonia as a driver variable.

**Table S1.** Description of monitoring sites in Fig. 2. Sites designated as having full tidal range had no water control structures, while restricted tidal range sites were behind water control structures that artificially restricted tidal range

| ID | Site name | Latitude | Longitude | Tidal range |
|---|---|---|---|---|
| 1 | Hudson Landing | 36.8565 | –121.7550 | Full |
| 2 | Porter Marsh | 36.8563 | –121.7549 | Restricted |
| 3 | Azevedo Pond North | 36.8471 | –121.7545 | Restricted |
| 4 | Azevedo Pond Central | 36.8439 | –121.7513 | Restricted |
| 5 | Azevedo Pond South | 36.8423 | –121.7469 | Restricted |
| 6 | Kirby Park | 36.8398 | –121.7437 | Full |
| 7 | Reserve North Marsh | 36.8364 | –121.7323 | Restricted |
| 8 | Strawberry Pond | 36.8296 | –121.7340 | Restricted |
| 9 | Whistlestop Lagoon | 36.8240 | –121.7400 | Restricted |
| 10 | Reserve Bridge | 36.8199 | –121.7371 | Full |
| 11 | Struve Pond | 36.8247 | –121.7774 | Restricted |
| 12 | Bennett Slough East | 36.8215 | –121.7834 | Restricted |
| 13 | Bennett Slough West | 36.8209 | –121.7909 | Restricted |
| 14 | Vierra | 36.8111 | 121.7792 | Full |
| 15 | Moss Landing Harbor at Moss Landing Road, North | 36.8000 | –121.7844 | Full |
| 16 | Moro Cojo Slough at Moss Landing Road, South | 36.7997 | –121.7847 | Restricted |
| 17 | Moro Cojo Slough East of Highway 1 | 36.7963 | –121.7832 | Restricted |
| 18 | Old Salinas River channel at Potrero Road, North | 36.7908 | –121.7904 | Full |

**Table S2.** Summary statistics partly used to generate the eutrophication expression index (EEI), as well as the complete data set used to generate the multivariate (principal components analysis PCA and PC multiple regression) and univariate parametric statistics (simple linear regression). Sites are listed from hyper to low eutrophication expression. See Tables S3a–c & S4 for determination of EEI and scores. Rest.: restricted, Mod.: moderate *Indicators used in the EEI model, but not in the PCA model

| | Site # | 2 | 4 | 8 | 11 | 12 | 16 | 17 | 1 | 3 |
|---|---|---|---|---|---|---|---|---|---|---|
| | Tidal range classification | Rest. | Rest. | Rest. | Rest. | Rest. | Rest. | Rest. | Full | Rest. |
| | Eutrophication expression index | Hyper | Hyper | Hyper | Hyper | Hyper | Hyper | Hyper | High | High |
| Drivers | Mean nitrate (mg $l^{-1}$) | 2.655 | 0.003 | 0.040 | 0.208 | 0.046 | 1.140 | 5.026 | 1.156 | 0.057 |
| | Mean phosphate (mg $l^{-1}$) | 0.197 | 0.136 | 0.015 | 0.035 | 0.038 | 0.355 | 0.353 | 0.112 | 0.059 |
| | Mean ammonia (mg $l^{-1}$) | 0.154 | 0.008 | 0.173 | 0.131 | 0.015 | 0.374 | 0.202 | 0.105 | 0.046 |
| Filters | Max daily tidal range (m) | 0.27 | 0.05 | 0.29 | 0.07 | 0.07 | 0.13 | 1.15 | 1.98 | 1.56 |
| | Mean low tide depth (m) | 0.34 | 0.10 | 0.06 | 0.25 | 0.21 | 0.15 | 0.06 | 0.36 | 0.09 |
| | Distance from mouth (km) | 10.15 | 8.04 | 7.52 | 3.59 | 1.96 | 1.42 | 1.89 | 10.07 | 8.14 |
| | 90th% temperature (°C) | 22.8 | 20.6 | 24.3 | 20.2 | 20.0 | 19.8 | 21.2 | 21.3 | 24.1 |
| | 10th% salinity | 9.3 | 21.4 | 23.1 | 16.4 | 16.5 | 6.8 | 12.7 | 18.6 | 31.3 |
| | Mean turbidity (NTU) | 14.6 | 24.7 | 17.7 | 37.8 | 25.9 | 12.7 | 12.0 | 8.2 | 6.2 |
| 1° Indicators | Summer % subtidal algae | 30 | 80 | 0 | 30 | 40 | 0 | 0 | 50 | 75 |
| | *Summer % intertidal algae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 65 | 10 |
| | Maximum % floating algae | 40 | 80 | 90 | 50 | 100 | 100 | 50 | 0 | 30 |
| | Mean chl *a* (μg $l^{-1}$) | 15.0 | 10.4 | 17.1 | 31.0 | 9.0 | 17.2 | 27.2 | 9.6 | 2.4 |
| 2° Indicators | aRPD (cm) | 6.4 | 0.2 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 | 16.8 | 4.8 |
| | 90th% pH | 8.95 | 8.58 | 8.69 | 8.95 | 8.88 | 8.56 | 9.06 | 8.29 | 8.63 |
| | Mean DO variation | 53.9 | 27.8 | 34.8 | 25.2 | 64.8 | 31.1 | 26.7 | 22.2 | 78.9 |
| | *% Time hypoxic | 6.1 | 0 | 19.9 | 61.5 | 73.1 | 58.1 | 18.6 | 6.0 | 15.92 |
| | *90th% free $NH_4$ (mg $l^{-1}$) | 0.042 | 0.017 | 0.049 | 0.060 | 0.023 | 0.045 | 0.054 | 0.022 | 0.018 |

Table S2. (continued)

| | Site # | 5 | 7 | 13 | 18 | 6 | 9 | 10 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|
| | Tidal range classification | Rest. | Rest. | Rest. | Full | Full | Rest. | Full | Full | Full |
| | Eutrophic expression index | High | High | High | High | Mod. | Mod. | Mod. | Mod. | Low |
| Drivers | Mean nitrate (mg $l^{-1}$) | 0.031 | 0.171 | 0.131 | 15.754 | 0.120 | 0.077 | 0.152 | 0.101 | 4.075 |
| | Mean phosphate (mg $l^{-1}$) | 0.941 | 0.065 | 0.095 | 0.345 | 0.055 | 0.043 | 0.045 | 0.040 | 0.202 |
| | Mean ammonia (mg $l^{-1}$) | 0.015 | 0.070 | 0.087 | 0.098 | 0.046 | 0.023 | 0.040 | 0.036 | 0.116 |
| Filters | Max daily tidal range (m) | 0.05 | 1.01 | 1.14 | 2.29 | 2.66 | 0.42 | 3.26 | 3.1 | 2.51 |
| | Mean low tide depth (m) | 0.08 | 0.20 | 0.12 | 0.17 | 0.28 | 2.07 | 1.80 | 0.74 | 0.26 |
| | Distance from mouth (km) | 7.60 | 6.61 | 1.87 | 2.44 | 7.11 | 5.56 | 5.43 | 1.40 | 1.37 |
| | 90th% temperature (°C) | 24.2 | 20.2 | 18.1 | 17.8 | 20.1 | 20.2 | 18.9 | 15.5 | 16.9 |
| | 10th% salinity | 11.6 | 31.3 | 24.7 | 10.4 | 28.5 | 32.2 | 31.8 | 32.3 | 21.4 |
| | Mean turbidity (NTU) | 51.2 | 8.0 | 20.5 | 65.7 | 9.3 | 7.2 | 8.4 | 5.6 | 9.4 |
| 1° Indicators | Summer % subtidal algae | 0 | 40 | 70 | 0 | 40 | 90 | 0 | 65 | 0 |
| | *Summer % intertidal algae | 0 | 0 | 35 | 30 | 48 | 0 | 90 | 10 | 0 |
| | Maximum % floating algae | 0 | 90 | 80 | 0 | 0 | 10 | 0 | 0 | 0 |
| | Mean chl *a* (μg $l^{-1}$) | 58.1 | 6.3 | 2.2 | 6.5 | 4.0 | 2.1 | 4.7 | 4.6 | 2.9 |
| 2° Indicators | aRPD (cm) | 0.4 | 0.0 | 2.6 | 50.0 | 23.8 | 0.0 | 29.0 | 34.6 | 30.6 |
| | 90th% pH | 9.01 | 8.54 | 8.20 | 8.10 | 8.35 | 8.37 | 8.41 | 8.20 | 8.02 |
| | Mean DO variation | 61.3 | 45.1 | 25.7 | 17.9 | 27.9 | 28.4 | 12.5 | 14.2 | 9.0 |
| | *% Time hypoxic | 0 | 13.48 | 12.3 | 3.6 | 1.43 | 0 | 1.41 | 0.02 | 3 |
| | *90th% free $NH_4$ (mg $l^{-1}$) | 0.052 | 0.017 | 0.011 | 0.007 | 0.005 | 0.004 | 0.010 | 0.010 | 0.011 |

*Indicators used in the EEI model, but not in the PCA model

**Supplement 2.** Calculation of the eutrophication expression, index

A single eutrophication index was calculated to synthesize the overall eutrophication status of each of the 18 sites. Calculation of this index was based on the normalization techniques developed by Bricker et al. (2003). The method involves converting continuous data to categorical assessments for numerous indicators, and then averaging these to yield a composite score. This method assigns values of eutrophic conditions or expression terms at all sites based on water quality and environmental data, and thresholds and frequency of occurrences of values from each site. Thresholds for all parameters were modified to include a 'hyper' eutrophication category for conditions significantly exceeding the 'high' category defined by Bricker et al. (2003); this is because most of the parameters in the estuary far exceeded the high thresholds established by Carpenter et al. (1994) and Bricker et al. (2003) at various sites in Elkhorn Slough.

Deviations from the method described by Bricker et al. (2003) were also necessary to address certain missing information, such as toxic and nuisance algal blooms and the limited distribution of submerged aquatic vegetation (SAV) (i.e. *Zostera marina*), which may be a secondary effect of eutrophication in Elkhorn Slough. There are also several parameters that are not included in Bricker et al. (2003) but have been included in this index because they are considered to be important parameters in Elkhorn Slough. These parameters include the secondary indicators: hyperoxia, aRPD and free ammonia. Both sediment anoxia and un-ionized ammonia can be toxic to benthic assemblages, while un-ionized ammonia can be toxic to pelagic communities (US EPA 1999).

**Determination of thresholds and frequencies.** Numeric scores were assigned to each parameter based on thresholds, and when possible, to the frequency with which the threshold is exceeded (Table S3a,b) (Bricker et al. 2003). Thresholds were based on literature values, with the exception of the aRPD, which was determined based on the range of values within sites.

Rather than simply averaging continuous data and then determining whether average values for a site fell within particular thresholds, we used a more sophisticated calculation method for chl *a*, hyperoxia, and free ammonia, which included information on the frequency of monthly deployments that exceeded particular values. Categorical assessments for these variables were thus made by combining data on frequency of events as well as on mean values and 90th percentiles.

Frequency was not used in determining values for algal cover, or sediment quality because insufficient independent samples were available. Hypoxia values were based on continuous data sets and percent time a given site was hypoxic. Algal mat thresholds were determined by using monthly estimates of floating algal mats from 2008–09, as well as the summertime surveys of intertidal and subtidal algal mats. The maximum intertidal, subtidal, or floating algal cover values were used in combination, so if a site had high intertidal cover but low subtidal and floating cover, then it was still determined that the algal cover for this site was high. Threshold standards were determined from a study by Nezlin et al. (2006), which examined relationships between ephemeral green macroalgal abundance and DO. There is a general lack of information describing threshold levels for aRPD; therefore, frequency distributions were used to look for natural breaks in the data, and sites with a high aRPD were assumed to have good sediment quality. Values for these parameters were assigned with the following scoring scheme: hyper = 1.0, high = 0.75, moderate = 0.5, and low = 0.0.

**Eutrophication expression index.** The overall eutrophication expression index for each site was determined by averaging values of all parameters in the primary indicators and secondary indicators categories, and then averaging the overall values of primary and secondary indicators (Table S3c). The final expression value was assigned a eutrophication classification of either low, moderate, high, or hyper based on the scale of Bricker et al. (2003) (Table S4).

To produce an estuary-wide score, we weighted eutrophication scores by both area ($A$) and volume ($V$) to determine any differences between area-based vs. volume based scores. Volume assessments were based on the mean tide level water volume ($V_{MTL}$) for individual spatially discrete areas. Volume at mean tide level was calculated using a 1 m grid size digital elevation model of Elkhorn Slough, which was produced using a combination of bare-earth lidar and bathymetric surveys completed in April 2005 on Airborne 1 (El Segundo, CA) for the Remote Sensing Center at the Naval Postgraduate School (Monterey, CA), with elevation error estimates ranging from 10–30 cm. In some locations, the digital elevation model data quality was poor due to

the difficulties of surveying extremely shallow subtidal depths using lidar or traditional bathymetric survey techniques. In these locations, field data was used to estimate water volume by mapping water area and multiplying by average depth. We used the following equations to develop an overall eutrophication score:

$$\text{Area-based EEI}_{\text{estuary}} = [\sum (A \times EEI)_{score\ x–n}] \times [\sum (A)_{score\ x–n}]^{-1}$$

$$\text{Volume-based: EEI}_{\text{estuary}} = [\sum (V_{MTL} \times EEI)_{score\ x–n}] \times [\sum (V_{MTL})_{score\ x–n}]^{-1}$$

The resultant expression was assigned an overall estuary eutrophication expression category using Table S3c, which was modified from a similar table in Bricker et al. (2003).

**Table S3a.** Eutrophication indicators assessed as categorical variables with defined thresholds among categories

| Eutrophication indicators Numeric score | Eutrophication categories and distinguishing thresholds | | | | |
|---|---|---|---|---|---|
| | Hyper 1 | High 0.75 | Mod 0.5 | Low 0 | Source |
| Chl *a* | >60 μg $l^{-1}$ | 20–60 μg $l^{-1}$ | 5–20 μg $l^{-1}$ | <5 μg $l^{-1}$ | Bricker et al. 2003 |
| Algal cover | >50% cover | 20–50% | 10–20% | <10% | Nezlin et al. 2006 |
| Hypoxia | >20% of time | 10–20% of time | 1–10% of time | 0% of time | Bricker et al. 2007 |
| Hyperoxia | >14 mg $l^{-1}$ | 12–14 mg $l^{-1}$ | 10–12 mg $l^{-1}$ | <10 mg $l^{-1}$ | Nezlin et al. 2006 |
| aRPD | <1 cm | 1–5 cm | 5–10 cm | >10 cm | No reference |
| Free ammonia | >0.025 mg $l^{-1}$ | 0.01–0.025 mg $l^{-1}$ | 0.005–0.01 mg $l^{-1}$ | <0.005 mg $l^{-1}$ | Carpenter et al. 1994 |

**Table S3b.** Decision matrix for determination of eutrophic condition for chl *a*, hyperoxia, and free ammonia, modified from Bricker et al. (2003). (Values for algal cover, hypoxia, and aRPD were established using only thresholds from Table S3a and not the following table.) If the mean value for a given indicator exceeded the threshold for a given threshold, then it was determined to be chronic, but if the mean value did not exceed a given threshold but exceeded the 90th percentile, did then it was determined to be episodic

| Threshold category | Frequency | Expression score |
|---|---|---|
| Hyper | Chronic | 1 |
| Hyper | Episodic | 1 |
| High | Chronic | 1 |
| High | Episodic | 0.75 |
| Moderate | Chronic | 0.5 |
| Moderate | Episodic | 0.25 |
| Low | Chronic | 0 |

**Table S3c.** Development of overall eutrophication ex-pression index (EEI) for each site by averaging primary and secondary indicator expression. These overall scores for each site are reported in the last 2 columns of Table S4

| Expression index | Average score |
|---|---|
| Hyper | >0.8 |
| High | 0.6–0.795 |
| Moderate | 0.3–0.595 |
| Low | <0.295 |

**Table S4.** Eutrophication expression index (EEI) for each site, as well as the eutrophication scores for primary and secondary indicators used to calculate the EEI. Scores for the primary and secondary indicators were calculated as described in Table S3a,b; the overall average in the second to the last column was converted to a categorical assessment in the final column as described in Table S3c

| | Primary indicators | | Secondary indicators | | | | Level of expression | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Site ID | Chl *a* | Algal cover | Hypoxia | Hyperoxia | aRPD | Free ammonia | Primary average | Secondary average | Overall average | Eutrophication classification |
| 1 | 1 | 1 | 0.5 | 0.75 | 0 | 1 | 1.000 | 0.563 | 0.781 | High |
| 2 | 0.75 | 1 | 0.5 | 1 | 0.5 | 1 | 0.875 | 0.750 | 0.813 | Hyper |
| 3 | 0.25 | 1 | 1 | 1 | 0.75 | 1 | 0.625 | 0.938 | 0.781 | High |
| 4 | 0.75 | 1 | 0 | 1 | 1 | 1 | 0.875 | 0.750 | 0.813 | Hyper |
| 5 | 1 | 0 | 0 | 1 | 1 | 1 | 0.500 | 0.750 | 0.625 | High |
| 6 | 0.25 | 1 | 0.25 | 0.25 | 0 | 1 | 0.625 | 0.375 | 0.500 | Moderate |
| 7 | 0.5 | 1 | 1 | 0.25 | 1 | 1 | 0.750 | 0.813 | 0.781 | High |
| 8 | 1 | 1 | 1 | 0 | 1 | 1 | 1.000 | 0.750 | 0.875 | Hyper |
| 9 | 0.25 | 1 | 0 | 0.25 | 1 | 0.75 | 0.625 | 0.500 | 0.563 | Moderate |
| 10 | 0 | 1 | 0.25 | 0.25 | 0 | 0.75 | 0.500 | 0.313 | 0.406 | Moderate |
| 11 | 1 | 0.75 | 1 | 1 | 1 | 1 | 0.875 | 1.000 | 0.938 | Hyper |
| 12 | 0.5 | 0.75 | 1 | 1 | 1 | 1 | 0.625 | 1.000 | 0.813 | Hyper |
| 13 | 0.25 | 1 | 1 | 0.75 | 0.75 | 1 | 0.625 | 0.875 | 0.750 | High |
| 14 | 0 | 1 | 0 | 0 | 0 | 0.75 | 0.500 | 0.188 | 0.344 | Moderate |
| 15 | 0.25 | 0 | 0 | 0.25 | 0 | 0.75 | 0.125 | 0.250 | 0.188 | Low |
| 16 | 1 | 1 | 1 | 1 | 1 | 1 | 1.000 | 1.000 | 1.000 | Hyper |
| 17 | 1 | 1 | 1 | 1 | 1 | 1 | 1.000 | 1.000 | 1.000 | Hyper |
| 18 | 1 | 0.75 | 0.25 | 0.75 | 0 | 1 | 0.875 | 0.500 | 0.688 | High |

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