

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).

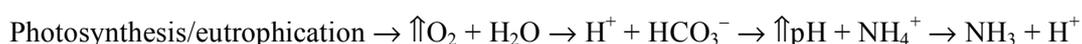
Macroalgal assessments: At each of the monitoring sites, visual estimates of percent cover of floating algal mats were made monthly at the same time as water sample collection from July 2008 to June 2009. The percent of the water surface that was covered by algae was assessed within a ~50 m radius of 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:



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: $1 / (1 + 10^{(pK - pH)}) \times [\text{Ammonia}]$, where pK was described by Emerson et al. (1975) with the following equation: $pK = 0.09018 + 2729.2 / (273.2 + T)$, 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.	Full	Rest.						
	Eutrophication expression index	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 <i>a</i> (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 <i>a</i> (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:

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

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

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				Source
	Hyper 1	High 0.75	Mod 0.5	Low 0	
Chl <i>a</i>	>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 expression 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

Site ID	Primary indicators		Secondary indicators				Level of expression			Eutrophication classification
	Chl <i>a</i>	Algal cover	Hypoxia	Hyperoxia	aRPD	Free ammonia	Primary average	Secondary average	Overall average	
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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