# **ELKHORN SLOUGH** TECHNICAL REPORT SERIES 2010: 1

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# Assessment of the effects of nutrient loading in estuarine wetlands of the Elkhorn Slough watershed: a regional eutrophication report card

Brent Hughes, John Haskins, Kerstin Wasson

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# AUTHOR AFFLIATION

At the time the report was prepared, all three authors were employed by the Elkhorn Slough National Estuarine Research Reserve, 1700 Elkhorn Road, Watsonville, CA 95076. Email addresses for the authors can be obtained from the staff contact webpage of <u>www.elkhornslough.org</u>.

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

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#### **EXECUTIVE SUMMARY**

There are several consequences that arise from eutrophication that can affect the overall health of an ecosystem. Primary production is the key indicator in eutrophic estuaries, and it can facilitate microbial activity, cause hypoxic and anoxic conditions, and lead to an overall loss in biodiversity. However, symptoms of eutrophication within an estuary can be variable, especially if the estuary is hydrologically modified like Elkhorn Slough. Long-term dissolved nutrient water quality data combined with historical land-use data suggests that Elkhorn Slough and adjacent wetlands have been on the receiving end of intense nutrient loading during the second half of the 20<sup>th</sup> century and into the 21<sup>st</sup> century with signs that this pressure has been increasing. The goal of this study was to determine which areas in the Elkhorn Slough estuarine complex exhibit symptoms of the negative impacts of nutrient loading. We assessed eutrophication at 26 stations by rigorously quantifying various primary and secondary eutrophication indicators based on a method developed by NOAA for a national assessment of eutrophication status in United States estuaries. The Elkhorn Slough estuarine complex was determined to be highly eutrophic, with 7 of the 26 stations exhibiting hyper-eutrophic indications, 10 of 26 stations exhibited high eutrophic conditions, 8 of 26 stations exhibited moderate eutrophic conditions, and only one had low eutrophic conditions. Results indicated that Elkhorn Slough is highly impacted by nutrient loading. Sites that with greater tidal exchange have lower eutrophication expression compared to muted tidal sites. Rapid ecological improvements to impaired areas could be achieved by exposing to them to greater tidal exchange and decreased stagnation.

#### INTRODUCTION

Anthropogenic eutrophication, or an increase in the rate of supply of organic matter to an ecosystem due to human influences (Nixon 1995), is a phenomenon that has only recently received wide recognition and is of concern for coastal systems (Cloern 2001). The delivery of nutrients to surface waters is the biggest coastal pollution problem in the United States today (Nixon 1995, National Research Council 2000, Howarth et al. 2000, Smith and Schindler 2009), and is considered to be the cause of widespread eutrophication in coastal ecosystems (Bricker et al. 2007). Eutrophication can lead to increased hypoxia and anoxia events, the loss of foundation species and biodiversity, and even create biologic dead zones (Diaz and Rosenberg 2008, Vaquer-Sunyer and Duarte 2008, Fox et al. 2009, Turner et al. 2009). National assessments have determined that coastal eutrophication is on the rise, yet there is a general lack of data and understanding of eutrophication on the West Coast of the United States.

The focus of this investigation was one West Coast estuary, Elkhorn Slough in central California, which is located in a productive agricultural landscape. The water quality of Elkhorn Slough is under the influence of intense external human pressure due to nutrient inputs into the system. The Elkhorn Slough Foundation, Elkhorn Slough National Estuarine Research Reserve, and researchers from Moss Landing Marine Laboratories

developed a water quality monitoring program in 1988 to assess changes in nutrient inputs over the entire watershed. Results from this long-term monitoring program as well as other studies have demonstrated dramatic increases in dissolved nutrient concentrations over the last 30 years from freshwater sources (Nybakken et al. 1977, Caffrey et al. 1997, Caffrey et al. 2002, Johnson 2008). Dissolved nutrients also exceed the levels reported for normal estuarine waters and regulatory compliance. For example, nitrate coming into Elkhorn Slough from the Old Salinas River Channel are consistently  $> 1000 \mu$ mol, at times exceeding 3000  $\mu$ mol and concentrations in the main channel can exceed 300 µmol; whereas concentrations outside in Monterey Bay seldom reach 20 umol (Chapin 2004, http://www.mbari.org/lobo/loboviz.htm). These values far exceed the 70 µmol recommendation of the Central Coast Regional Water Quality Control Board (CCRWQCB) and United States National Estuarine Eutrophication Assessment (NEEA) (Carpenter et al. 1994, Bricker et al. 2003). Observations by Elkhorn Slough researchers over the last years have detected high phytoplankton concentrations, abundant and persistent macroalgal mats, and hypoxia events, presumably due to the high dissolved nutrient concentrations. Estimates of primary productivity in the Elkhorn Slough main channel exceed hyper eutrophic levels (Nixon 1995, Johnson 2008). Hypoxia events have been documented in the main channel and peripheral areas of Elkhorn Slough (Beck and Bruland 2000, Hughes 2009).

To date there has not been a rigorous attempt to determine whether nutrient loads translate into accepted indicators of eutrophication in Elkhorn Slough despite reports of high nutrient loads in Elkhorn Slough. The goal of this study was thus to provide an overall assessment or report card of the eutrophic condition at 26 monitoring stations (Figure 1, Tables 1 and 2) within the Elkhorn Slough watershed. These include sites along the Elkhorn Slough channel, as well as along the channels of Bennett Slough, Moro Cojo Slough, Tembladero Slough and the old Salinas river channel, all of which historically comprised the interconnected Elkhorn Slough estuarine complex. This study establishes a eutrophication baseline for Elkhorn Slough and informs management by indicating which areas are most threatened by nutrient loading. The survey design allows for reassessment every 5 years to detect changes in eutrophication indicators.



Figure 1. Water quality monitoring stations with eutrophication data (Table 2) interpolated in ArcView.

# SUMMARY OF METHODS

Methods for the Elkhorn Slough Eutrophication Report Card are modified from methods developed for NOAA's 1999 and 2007 NEEA, as well as detailed methods described by Bricker et al. 2003, and a eutrophication model described by Cloern (2001) (Figure 2). The method uses normalization techniques to transform highly variable data into scores, which are statistically comparable within and among estuaries. Statistical methods were modified to accommodate the known data sources and monitoring programs that exist in Elkhorn Slough. Many of these programs are described in detail in Appendices 1 and 2. Deviations from the method described in the NEEA and Bricker et al. 2003 were also necessary to address certain lacking information, such as toxic and nuisance algal blooms

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and the limited distribution of Submerged Aquatic Vegetation (SAV) (*Zostera marina*), which may be a secondary effect of eutrophication in Elkhorn Slough. There are also several parameters that have been included, but are not included in the NEEA and Bricker et al. (2003), yet have been considered to be important parameters from local experts. These parameters include ammonia as a human influenced pressure, and the secondary indicators: depth of sediment oxic layer and unionized ammonia (or free ammonia). Both sediment anoxia and unionized ammonia can be toxic to benthic assemblages, and unionized ammonia can be toxic to pelagic communities (US EPA 1999).



Figure 2. Coastal Eutrophication Model based on Cloern (2001) and Bricker et al. (2003).

Nutrient data used in the eutrophication report card were collected data from January 2004 to July 2009. Data of eutrophication indicators were collected during 2008-2009. All methods are described in detail in Appendix 1. Monthly water quailty grab samples were taken during ebb tide events to analyze the pressure (nutrients) as well as chl a concentrations at each site. The cover of floating algal mats was assessed monthly, and subtidal and intertidal cover was assessed during two summertime low tide events at select stations. Hypoxia was determined by collecting continuous data at 15-minute intervals over periods > 2 weeks using YSI data sondes at select stations. Hyperoxia data was collected during daytime monthly sampling events using YSI data sondes. Ammonia data from monthly grab samples as well as pH and temperature data from YSI data sondes collected simultaneously were used to calculate unionized ammonia. Sediment quality was determined from a one time assessment at select stations by taking sediment cores and measuring the sediment surface to anoxia layer depth. Scores were assigned to each parameter based on thresholds and when possible the frequency of the threshold (Bricker et al. 2003). Thresholds were based on literature values, with the exception of sediment the depth of the sediment oxic layer, which was determined based on the range of values within sites.

Eutrophic scores were averaged for primary indicators and secondary indicators for each site. Next, the average was taken between the primary and secondary indicators for an overall eutrophic score for each site. Eutrophication expression scores for each site were overlaid in ArcView, and spatially interpolated to determine spatial distribution of eutrophication in the Elkhorn Slough watershed.

# RESULTS

Scores of eutrophication expression ranged from low eutrophic to hyper eutrophic in the Elkhorn Slough complex. Of all sites only one received a low score, 31% received a moderate eutrophic score, most sites received a highly eutrophic score (38%), and 27% received hyper eutrophic scores (Table 2). Spatial analysis in ArcView determined that  $0.21 \text{ km}^2$  (1.5%) of the estuary is low eutrophic, 8.2 km<sup>2</sup> (57.1%) is moderately eutrophic, 4.19 km<sup>2</sup> (29.2%) is highly eutrophic, and 1.75 km<sup>2</sup> (12.2%) is hyper eutrophic (Figure 1).

Most sites received either a hyper (62%) or high (27%) external nutrient pressure (Table 2). This indicates that Elkhorn Slough is under intense pressure from external nutrient sources. Scores for each indicator (nitrate, phosphate, and ammonia) at all sites were generally hyper or high with a few sites having moderate nutrient pressure (Table 2, Appendix 2 Figures 1-3). Vierra Mouth, Reserve North Marsh, and Reserve Bridge were the only sites that received a moderate external pressure score. The lower score for Vierra Mouth is due to its proximity to the mouth and the large volume of Monterey Bay that enters the system and dilutes agricultural inputs. The Reserve sites also had a moderate score due to its adjacent lands being oak woodlands and devoid of intense agricultural use, also the Reserve sites are far from freshwater influences.

Primary indicators at the individual sites were highly variable and ranged from low to hyper-eutrophication. The average of primary indicators among the sites ranged from low (11%) to moderate (27% of sites) to high (31%) to hyper (31% of sites) (Table 2). Eutrophic expression of chl a ranged from low to hyper among sites, with 35% having a low eutrophic expression of chl a, 11% had a moderate expression, 19% had a high expression, and 35% of sites having a hyper eutrophic expression (Table 2, Appendix 2, Figure 4). The eutrophic expression of macroalgae ranged from low to hyper with 26% of sites having a low eutrophic expression of macroalgal cover, 8% had a high expression, and 66% had a hyper expression (Table 2, Appenix 2 Figure 5). The majority of algal coverage was from multiple species of the genus *Ulva*. The morphology (or species) of *Ulva* is dependent on various environmental variables, such as salinity and wave exposure. Ulva spp. found in protected habitats with a wide range of salinities tend to have a tubular morphology with hair-like peripheral branches, whereas less protected habitats with marine salinities tend to have broader blade morphology (Abbott and Hollenberg 1976). The tubular morphology of *Ulva* enables the thallus to float and form large floating mats characteristic of protected and tidally-muted areas (Appendix 1 Figure 2, Appendix 2 Figure 5).

Secondary indicators at the sites also were highly variable and ranged from low to hyper eutrophic expressions. The average of secondary indicators ranged from low (15% of sites) to hyper (42%), with 23% having moderate eutrophic expression and 20% having a high eutrophic expression (Table 2). Eutrophic expression of hypoxia ranged from low to hyper, with 44% of sites having a low eutrophic expression, 12% having a moderate eutrophic expression, and 44% had hyper expression of hypoxia (Table 2, Appendix 2 Figure 6). Expression of hyperoxia ranged from low (31%) to hyper (46%), and 23% had a high expression of hyperoxia (Table 2, Appendix 2 Figure 6). The sediment oxic layer ranged from low (40% of sites) to hyper (45%), with 5% of sites having a moderate expression and 10% had a high expression of hyperoxia (Table 2, Appendix 2 Figure 7). Expression of free ammonia ranged from low (one site) to moderate (11% of sites) to high (31% of sites) to hyper (54%) (Table 2, Appendix 2, Figure 8).

#### CONCLUSIONS

The wetlands of the Elkhorn Slough estuary are highly eutrophic based on the distribution of eutrophic expression scores (Figure 1, Table 2). Some sites were more impaired than others, and only one of the sites received a low eutrophication score. This was the first attempt to rigorously assess eutrophication in Elkhorn Slough using established and explicit criteria, and the results indicate that the ecosystem displays negative ecological responses to the intense pressure from anthropogenic nutrient loading. Results from this study indicate that eutrophication indicators expressed by Elkhorn Slough wetlands are equivalent to those at some of the most eutrophic estuaries in the United States.

Indicators of eutrophication in Elkhorn Slough have similar values to other highly eutrophic estuaries in the United States. Chlorophyll *a* concentrations at certain sites within the estuarine complex reach concentrations  $>200 \mu g/l$  during summer time blooms (Appendix 2 Figure 4). These values far exceed the hypereutrophic definition (>60  $\mu$ g/l) established by Bricker et al. (2003). Similar chlorophyll a concentrations have been observed in impaired estuaries on the Atlantic, such as Chesapeake Bay and Childs River (Harding Jr. and Perry 1997, Bricker et al. 2007). The Childs River is of particular importance because fish kills have been observed following phytoplankton blooms. Macroalgal blooms are a characteristic feature of Elkhorn Slough, and can completely cover intertidal mudflats of the estuary during the peak months of primary productivity in the summer. Intertidal, subtidal and floating algal mats have been observed to cover 90-100% of the surface at some sites (see Appendix 1 Figure 2 for example), regardless of flushing potential. Some sites have persistent intertidal and subtidal algal mats that are a permanent feature of the site, such as at Reserve Bridge (RBR) Whistlestop Lagoon (WL) (Appendix 2 Figure 5). Similar macroalgal blooms have been observed in other eutrophic estuaries, such as Waquoit Bay, MA (Bricker et al. 2007). Waquoit Bay provides an interesting comparison because highly eutrophic areas have been found to decrease benthic diversity and alter food webs, which are negative ecological effects (Fox et al. 2009).

One of the most biologically important indicators of eutrophication is low dissolved oxygen concentration. Hypoxia and anoxia events are widespread in Elkhorn Slough wetlands, but generally occur in areas behind water control structures, where residence time of the water is high and flushing of organic material is low. More than half of the stations, and half of the estuarine complex are behind water control structures, making hypoxia problematic in Elkhorn Slough (Figure 1, Appendix 2 Table 1). Even sites with full tidal exchange along the Elkhorn main channel can go hypoxic in the summertime (K. Johnson, from LOBO network), suggesting that oxygen dynamics even in these wellflushed areas have been impaired by anthropogenic nutrient loading. Hypoxia in West Coast estuarine waters has received little attention relative to East Coast estuaries. In the most recent NEEA, there was only one west coast estuary that had a high hypoxia event (Hood Canal), and Elkhorn Slough was listed as having low hypoxia (Bricker et al. 2007). Results from this report will change the perception of hypoxia in Elkhorn Slough, by incorporating many sites beyond the main channel that regularly go hypoxic. This report also demonstrates the usefulness of hyperoxia as an indirect measurement of hypoxia potential if only daytime measurements of dissolved oxygen are available. Hyperoxia in Elkhorn Slough and other eutrophic estuaries, such as Mondego Estuary, Portugal (Bricker et al. 2007) has been linked to high primary productivity, subsequently leading to daytime DO supersaturation and nighttime hypoxia.

Variation of eutrophic expression among sites gives insight as to how characterizing eutrophication of the entire estuary might lead to an under estimation or over estimation based on the spatial range of sites used in the assessment. Recent assessments aimed at comparing coastal systems on a national level have given Elkhorn Slough a moderate (Bricker et al. 2007) or fair (Office of National Marine Sanctuaries 2009) score. Both of these assessments focused on the main channel of Elkhorn Slough, and did not assess the state of the other wetlands that comprise the historical extent of interconnected estuarine wetlands. Our report is thus the first to examine the full spatial extent of estuarine habitats in the watershed, and to investigate variation within sites in the estuarine complex.

This study also provides some novel approaches of assessing eutrophication within an estuary. Few studies have addressed hyperoxia, sediment quality, and free ammonia as secondary indicators of eutrophication. The processes leading to these secondary indicators have been found to be a result of eutrophication. For example, hyperoxia is caused by super saturation of dissolved oxygen due to excessive amounts of primary production in the form of algal or phytoplankton blooms. Anoxic sediments are caused by deposition of organic material, which smothers bioturbators and facilitates anoxic tolerant organisms (i.e. *Capitella capitata*) and anaerobic bacteria (Pearson and Rosenberg 1978). Recent studies by Ritter et al. (2008) and Oliver et al. (2009) found that many of the sites (many were reported in this current study) with poor sediment quality also had decreased benthic invertebrate, fish, and shorebird abundances and diversity. Free ammonia production is of great importance and should be considered as a management objective in Elkhorn Slough because of the high concentrations of ammonium from either direct inputs into the system or from high densities of denitrifying bacteria. Free ammonia has been found to be toxic to fish and has been frequently

observed in Elkhorn Slough at levels far beyond the one established by the CCRWQCB (0.025 mg/l) (U.S. EPA 1999). Eutrophication tends to exacerbate this condition because high productivity leads to increases in pH and thus making the ammonium a proton donor and forming free ammonia (NH<sub>3</sub>).

Most sites within the Elkhorn Slough estuarine complex are under severe nutrient stress, yet not all of the sites have the same eutrophic signature. We are currently testing various hypotheses for these differences (Hughes, Haskins & Wasson, MS in prep.). Multiple factors appear to contribute to the propensity of sites to express indicators of eutrophication. The most important factor explaining differences between sites appears to be residence time, which is a function of tidal range at most sites. The sites that have moderate eutrophic scores are located in the main channel of Elkhorn Slough and have unrestricted tidal exchange (Appendix 1 Figure 1 & Table 1). There are three sites with restricted tidal exchange that have moderate eutrophication scores: Jetty Road, Whistlestop Lagoon, and Carneros Creek. The station at Jetty Road is located in a deep scour hole close to the mouth of the estuary and next to a series of large culverts that give this site a fairly substantial tidal range. Whistlestop Lagoon has a muted tidal range, yet is very deep and has a high volume to surface area ratio, which might assist in replenishing dissolved oxygen before hypoxia can occur. The site at Carneros Creek is characterized by unidirectional flow of freshwater, which, like daily tidal exchange, can prevent stagnation of water in this area. Thus, these results suggest that indicators of eutrophication can be decreased at Elkhorn Slough and other estuaries by increasing tidal range or otherwise decreasing residence time in managed wetlands behind water control structures.

Nutrient loading to the Elkhorn Slough estuary continues to pose a serious threat to its ecosystems, and nutrient concentrations in the lower estuary appear to be increasing over time (K. Johnson, unpubl. data; J. Haskins, unpubl. data). Coastal management strategies must address and reverse these trends to improve estuarine ecosystem health. But such a process is a long one, and while it is on-going, much more rapid improvements in ecological health can be achieved by simply improving management of water control structures at various parts of the estuary, to increase tidal exchange and reduce stagnation. A combined approach of nutrient management, water control structure management, and continued long-term monitoring in an adaptive management framework is essential for addressing the eutrophication problems characterized in this report, so that future "report cards" of eutrophication for the estuary can show improvement over time.

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Table 1. Water quality station information: Site Tag is a unique code established by the Central Coast Regional Water Quality Control Board, ID is the site acronym used in Figure 1 and the report card, Site Name is a descriptive name for the station, Waterbody indicates the slough region of the station, and Date Est. is the date when the first monthly water quality sample was taken.

Site Tag	ID	Site Name	Latitude	Longitude	Waterbody	Date Est.
306ELKAPC	APC	Elkhorn Slough at Azevedo Pond, Central	36.8439	-121.7513	Upper Slough	28-Mar-92
306ELKAPN	APN	Elkhorn Slough at Azevedo Pond, North	36.8471	-121.7545	Upper Slough	27-Mar-92
306ELKAPS	APS	Elkhorn Slough at Azevedo Pond, South	36.8423	-121.7469	Upper Slough	28-Mar-92
306BENEH1	BSE	Bennett Slough East of Highway 1	36.8215	-121.7834	Lower Slough	21-Sep-88
306BENWH1	BSW	Bennett Slough West of Highway 1	36.8209	-121.7909	Lower Slough	21-Sep-88
306CARBLR	CC	Carneros Creek at Blohm Road	36.8601	-121.7401	Upper Slough	23-Sep-89
306ELKHLE	HLE	Elkhorn Slough at Hudson's Landing East	36.8563	-121.7549	Upper Slough	01-Oct-89
306ELKHLW	HLW	Elkhorn Slough at Hudson's Landing West	36.8565	-121.755	Upper Slough	23-Sep-89
306BENJTR	JR	Bennett Slough at Jetty Road	36.8171	-121.7871	Lower Slough	21-Sep-88
306ELKKPD	KP	Elkhorn Slough at Kirby Park Dock	36.8398	-121.7437	Upper Slough	23-Sep-89
306MOREH1	MCS	Moro Cojo Slough East of Highway 1	36.7963	-121.7832	Lower Slough	21-Sep-88
309OSRMDW	MDW	Old Salinas River at Monterey Dunes Way	36.7719	-121.7897	Salinas River	14-Dec-91
306MORMLN	MLN	Moro Cojo Slough at Moss Landing Road, North	36.8	-121.7844	Lower Slough	05-Mar-91
306MORMLS	MLS	Moro Cojo Slough at Moss Landing Road, South	36.7997	-121.7847	Lower Slough	05-Mar-91
309OSRPRN	PRN	Old Salinas River at Potrero Road, North	36.7908	-121.7904	Salinas River	23-Sep-89
309OSRPRS	PRS	Old Salinas River at Potrero Road, South	36.7904	-121.7907	Salinas River	23-Sep-89
306ELKRBR	RBR	Elkhorn Slough at Reserve Bridge	36.8199	-121.7371	Reserve	23-Sep-89
306ELKRNM	RNM	Elkhorn Slough at Reserve, North Marsh	36.8364	-121.7323	Reserve	23-Sep-89
306ELKRSM	WL	Elkhorn Slough at Reserve, South Marsh (Whistle Stop Lagoon)	36.824	-121.74	Reserve	23-Sep-89
306MSLSKL	SKL	Moss Landing Harbor at Skipper's Landing	36.8106	-121.7864	Lower Slough	20-Sep-88
<b>305BENSTP</b>	SP	Bennett Slough at Struve Pond	36.8247	-121.7774	Lower Slough	21-Sep-88
309SLRBRG	SRB	Salinas River at the Highway 1/Railroad Bridge	36.7321	-121.7807	Salinas River	22-Sep-89
306ELKSTB	STB	Elkhorn Slough at Strawberry Rd	36.8296	-121.734	Reserve	28-Apr-98
309TEMPRS	TS	Tembladero Slough at Preston Street	36.7651	-121.7596	Salinas River	13-Jun-94
309TEMMOL	TS2	Tembladero Slough at Molera Rd	36.7722	-121.7876	Salinas River	07-Feb-06
N/A	VM	Vierra Mouth	36.8111	121.7792	Lower Slough	14-Mar-01

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Table 2. Eutrophication report card for 26 stations within the Elkhorn Slough estuarine complex. Site locations and full names corresponding to the abbreviations are provided in Table 1 and Figure 1. Appendix 1 explains the thresholds used for the categories for each parameter, and Appendix 2 describes how we obtained and analyzed data for each category. Eutrophication expression values are modified from Bricker et al. (2003).

Sites	Pressure				Primary I	ndicators	Seconda	ry Indicato	rs		LEVEL O	FEXPRESS	ION	
	(External 1	Nutrient Inpu	ts)											
(color denotes				Overall		Algal			Sediment	Free	Primary	Secondary	Overall	Eutrophic
eutrophic expression)	Nitrate	Phosphate	Ammonia	Avg.	Chl a	Cover	Hypoxia	Hyperoxia	oxic layer	Ammonia	average	average	average	Classification
HLW	1	1	0.75	0.92	0.75	1	0.5	0.75	0	0.75	0.88	0.50	0.69	High
KP	0.75	0.75	0.75	0.75	0.25	1	0.25	0.25	0	0.5	0.63	0.25	0.44	Moderate
MLN	1	1	0.75	0.92	0.25	0	N/A	0.25	0	0.75	0.13	0.33	0.23	Low
PRN	1	1	1	1.00	1	1	0.25	0.75	0	0.75	1.00	0.44	0.72	High
PRS	1	1	1	1.00	1	0	0.25	1	0	1	0.50	0.56	0.53	Moderate
RBR	0.75	0.5	0.75	0.67	0.25	1	0.25	0	0	0.5	0.63	0.19	0.41	Moderate
VM	0.5	0.5	0.5	0.50	0.25	1	0	0.25	0	0.25	0.63	0.13	0.38	Moderate
MDW	1	1	1	1.00	1	0	N/A	0.75	N/A	1	0.50	0.88	0.69	High
SKL	1	0.75	0.75	0.83	0	0	N/A	0.75	N/A	0.75	0.00	0.75	0.38	Moderate
TS	1	1	1	1.00	1	0	N/A	1	N/A	1	0.50	1.00	0.75	High
TS2	1	1	1	1.00	1	0	N/A	1	N/A	1	0.50	1.00	0.75	High
SRB	1	1	1	1.00	0.5	0	N/A	1	N/A	1	0.25	1.00	0.63	High
APN	0.5	0.75	1	0.75	0.25	1	1	1	0.75	0.75	0.63	0.88	0.75	High
BSW	0.5	1	0.5	0.67	0.25	1	1	0.75	0.75	0.75	0.63	0.81	0.72	High
HLE	1	1	1	1.00	0.75	1	0.5	1	0.5	1	0.88	0.75	0.81	Hyper
MCS	1	1	1	1.00	1	1	1	1	1	1	1.00	1.00	1.00	Hyper
MLS	1	1	1	1.00	0.75	1	1	1	1	1	0.88	1.00	0.94	Hyper
WL	0.5	0.5	0.5	0.50	0.25	1	0	0.25	1	0.75	0.63	0.50	0.56	Moderate
CC	1	1	1	1.00	1	0	N/A	0	N/A	1	0.50	0.50	0.50	Moderate
JR	0.75	0.75	0.5	0.67	0	1	N/A	0.25	0	0.5	0.50	0.25	0.38	Moderate
BSE	0.5	1	0.75	0.75	0.5	0.75	1	1	1	1	0.63	1.00	0.81	Hyper
RNM	0.25	0.5	0.5	0.42	0.5	1	1	0.25	1	0.75	0.75	0.75	0.75	High
SP	0.75	1	1	0.92	1	0.75	1	1	1	1	0.88	1.00	0.94	Hyper
APC	1	1	1	1.00	0.75	1	0	1	1	1	0.88	0.75	0.81	Hyper
APS	1	1	0.75	0.92	1	0	0	1	1	1	0.50	0.75	0.63	High
STB	0.5	0.5	1	0.67	0.75	1	1	0.75	1	1	0.88	0.94	0.91	Hyper

#### APPENDIX 1: MATERIALS AND METHODS

#### **Flushing Potential**

Sites were rated for flushing potential based on three categories: High, moderate, and low

(Appendix 1 Table 1). Tidal flushing is the capability of the site or estuary to flush out nutrient rich water or products of eutrophication and is based on several factors: tidal range, distance from the mouth, and freshwater inputs (persistent or periodic). Characterization of flushing potential for each site was based on empirical data and observations. The model proposed by Bricker et al. (2003) separates the flushing potential into tidal exchange and freshwater input.



For purposes of simplicity, tidal exchange and freshwater inputs were combined into one "flushing potential" category. Sites with a full tidal exchange (see Appendix 1 Figure 1 for a description of tidal exchange) or persistent freshwater input were considered to have a high flushing potential. Sites with a muted tidal exchange or a minimal tidal exchange but with a persistent input source were considered to have a moderate flushing potential. Sites with a minimal tidal exchange and no freshwater input were considered to have a low flushing potential. Sites behind water control structures in general have muted and minimal tidal exchanges.

# Pressure (External Nutrient Inputs)

Dissolved inorganic nutrients enter Elkhorn Slough from several different freshwater sources that include the Salinas River and Tembladero Slough (collectively the Old Salinas River Channel) to the south, Moro Cojo Slough to the southeast, Carneros Creek and Corn Cob Canyon Creek to the north, runoff from adjacent land areas (Figure 1). Elkhorn Slough also receives water from the nutrient rich Monterey Bay, especially during periods of upwelling during the late spring and early summer. The land surrounded by the freshwater systems is heavily influenced by agricultural practices and when the agricultural runoff gets into Elkhorn Slough it causes eutrophication. Water from the Old Salinas River Channel contributes the greatest volume of freshwater to the main channel of Elkhorn Slough, along with Moro Cojo Slough water; it is tidally pumped into the main channel of Elkhorn Slough. Carneros Creek and Corn Cob Canyon form the head of the estuary and flow directly into the estuary at a fraction of the input of the Old Salinas River Channel.

To determine the external pressure at each site grab samples were taken monthly at the 26 principal, long-term monitoring stations (Figure 1) from 2004-2009. Temperature,

dissolved oxygen, pH, salinity, and turbidity are taken at the time of sampling with YSI data sondes. Samples were filtered the same day they are collected. Samples were run at two different laboratories, Moss Landing Marine Labs (MLML) and Monterey County Consolidated Chemistry Lab (MCCCL).

#### 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 and Parsons, 1972). The MCCCL determined ammonia by using EPA 350.3 method (EPA 1993).

#### Nitrate + Nitrite as Nitrogen Analysis

MLML determines nitrate using a modified (Sakamoto et al, 1990) standard methods 4500 NO3 on an Alpkem flow injection autoanalyzer (Cleceri *et al.* 1998). MCCCL determines nitrate using EPA method 300.0 (EPA 1993). Some nitrate values were deleted due to being determined as outliers. Our method of determining outliers was to plot nitrate vs. salinity for each site. Since nitrate naturally decreases with increasing salinity concentrations values that exceeded the representative sites slope of nitrate to salinity concentration by greater than 2 standard deviations they were determined to be an outlier.

#### Orthophosphate as Phosphorous Analysis

MLML determines orthophosphate using a modified (Sakamoto *et al*, 1990) standard method 4500 PG on an Alpkem flow injection autoanalyzer (Cleceri et al. 1998). MCCCL determines orthophosphate using standard method 4500 P E (Cleceri *et al.* 1998).

#### **Primary Indicators**

Primary indicators generally refer to primary producers because they are the organisms that uptake the nutrient inputs. The definition of eutrophication is 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 and ephemeral green macroalgae were the primary indicators used in this study. Phytoplankton concentrations and macroalgal cover were not established as part of the monthly monitoring program prior to 2008. Therefore, this study included the monitoring of phytoplankton and macroalgal mats as part of the Elkhorn Slough eutrophication assessment.

#### Water Column Phytoplankton (Chlorophyll a) Assessments

Monthly water samples from the water quality monitoring stations were collected for the determination of laboratory measured chl *a* concentrations from August 2008 to July 2009. Water samples were filtered and extracted in 90% acetone, and run for chl *a* concentrations as detailed analysis in section 10200 H of *Standard Methods for the Examination of Water and Wastewater Analysis*. A modified single step method is used with a Turner Designs TD-700 fluorometer with 436 and 680 nm filters for these samples.

#### Macroalgal Assessments

At each of the water quality monitoring stations, by-eye estimates of percent cover of floating algal mats were made from August 2008 to July 2009. A visual survey was made at each of these same stations to determine the percent cover in relation to the surface area of the water body in question. Only algal cover visible at the water surface that can be seen within an oblique radius from the water's edge, closest to the sampling location were included in estimates. Algae had to be breaking the water surface, and benthic algae seen through the water column were excluded from coverage estimates. Algae growing on mudflats above the water line were not included. The total percent cover will be estimated for all species of algal pooled together that are observed under these conditions. Field percent cover sheets were used to calibrate by-eye estimates made with each monthly sampling. To increase accuracy percent cover was reported in 10% increments and the same observer was used throughout the study to increase precision (see Appendix 1 Figure 2 for an example).



Figure 2. 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 two-time assessment of floating, intertidal and subtidal macroalgal mats was performed in May and June 2009 at 17 of the 26 stations to take advantage of low daytime tides and the season of peak algal production. The stations were selected because they had marine salinities and comparable algal species (sites not included in this survey were generally tidal freshwater sites with little evidence of macroalgal cover). 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 by-eye estimates due to poor subtidal visibility. Fifteen points were randomly selected and sampled for the presence of green macroalgae to generate a percent coverage of the subtidal area.

#### **Secondary Indicators**

Secondary indicators of eutrophication are considered to be consequences that are not directly impacted by nutrient additions to a system (Cloern 2001). These consequences include hypoxia, decreases in submerged aquatic vegetation (i.e. eelgrass), reductions of sediment quality, changes in benthic community assemblages, loss of biodiversity and even dead zones. This study assessed three secondary indicators: hypoxia, sediment quality, and free ammonia production. Hypoxia is the product of eutrophication that causes the most concern because of its negative effects on populations, communities, and biodiversity (Vaquer-Sunyer and Duarte 2008, Diaz and Rosenberg 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: selfshading of the primary producer leading to respiration, organic deposition leading to microbial dissolved oxygen consumption, and night-time respiration of primary producers. The same process that leads to water column hypoxia can also cause sediment anoxia. Deposition and decay of algae can lead to the smothering and create anoxic conditions in the sediments. Sediment anoxia can cause losses in benthic community diversity and abundance, a process that 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 dissolved oxygen driven by eutrophication causes fluctuations to pH. High variation in pH coupled with high ammonia concentrations can lead to the production of unionized ammonia. Unionized ammonia production is of concern because it can be toxic to many fish species in Elkhorn Slough, such as the endangered steelhead trout (U.S. EPA 1999). The following equation describes eutrophication driven unionized ammonia production:

Photosynthesis/eutrophication  $\rightarrow (O_2+H_2O \rightarrow H^++HCO_3) \rightarrow (PH^+ \rightarrow NH_3 + H^+)$ 

#### Hypoxia Assessments

In addition to the single monthly day-time dissolved oxygen concentration measured as part of the monthly sampling, YSI Sondes were also deployed ~30 cm above the benthic surface and < 5m to the monthly water quality station for at least one lunar tidal cycle at selected sites (n=16) to obtain a more detailed understanding of dissolved oxygen concentrations over time. Stations were sampled around peak months of peak primary productivity from August 2008 to July 2009. Sampling was staggered due to the limited number of YSI data sondes. The resulting data were categorized into concentration-based groups representing oxic, hypoxic, or anoxic conditions. Further details of the methods used to account for drift over time and biofouling can be found in the protocols for the National Estuarine Research Reserve (NERR), system-wide monitoring program: http://cdmo.baruch.sc.edu/data\_dissemination.html#NERR%20Water%20Quality%20Dat a.

#### Hyperoxia Assessments

Hyperoxia data was also collected because it is a good indirect measurement of eutrophication and hypoxia potential (Bricker et al. 2007). Data was collected monthly using YSI data sondes to coincide with the monthly nutrient and chl *a* sampling.

#### Sediment Anoxia Assessments

A one-time assessment of sediment quality was done in May of 2009. Surveys were completed during low-tide at the same sites and time as the algal surveys. Benthic sediment cores (>50 cm) were taken at five 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 anoxia layer (Appendix 1 Figure 3). Five replicate measurements were taken within each core to capture variability within the core. Brown colored sediments indicated good sediment quality, whereas gray to black sediments indicated better sediment depths to anoxia layers indicated better sediment quality.



Figure 3. Sediment cores taken to measure sediment depth to anoxia layer.

#### Unionized Ammonia Assessments

Free ammonia was calculated using the ammonia concentration and simultaneously collected water quality parameters: pH and temperature (EPA 1999), using the following equation:

 $1/(1+10^{(pK-pH)}) * [Ammonia]$ , where pK has been 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

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Table 1. Summary of flushing potential at each site, along with the eutrophic condition of each site and nutrient pressure. Note that nutrient pressure does always cause high eutrophication because of the flushing potential of a system.

Sites	Flushing Potential	External
(color denotes	(Tidal Exchange and	<b>Nutrient Pressure</b>
eutrophic expression)	freshwater inputs)	
HLW	High	0.92
KP	High	0.75
MLN	High	0.92
PRN	High	1.00
PRS	High	1.00
RBR	High	0.67
VM	High	0.50
MDW	High	1.00
SKL	High	0.83
TS	High	1.00
TS2	High	1.00
SRB	High	1.00
APN	Moderate	0.75
BSW	Moderate	0.67
HLE	Moderate	1.00
MCS	Moderate	1.00
MLS	Moderate	1.00
WL	Moderate	0.50
CC	Moderate	1.00
JR	Moderate	0.67
BSE	Low	0.75
RNM	Low	0.42
SP	Low	0.92
APC	Low	1.00
APS	Low	0.92
STB	Low	0.67

Estuary expression value
0  to  0.3 = Low
0.3 to $0.6 =$ Moderate
0.6  to  0.8 = High
0.8  to  1.0 = Hyper

# APPENDIX 2: CRITERIA FOR EUTROPHIC ASSESSMENT

Statistical analyses of the eutrophic condition of water quality stations and Elkhorn Slough were based on the normalization techniques developed by Bricker et al. (2003). This method assigns values of eutrophic conditions or expression terms at all sites based on water quality and environmental data, as well as thresholds and frequency of occurrences. Pressure on the system in generally described as human influenced loads. However, due to the number of sites and limited hydrologic data, nutrient concentrations from 2005-2009 (instead of loads) were used to calculate pressure. The nutrient pressure on the system was based on values established by several sources: Central Coast Regional Water Quality Control Board, (1994), Bricker et al. (1999), and Bricker et al. (2003).

Thresholds for all parameters were modified to include a "hyper" category, this is due to most of the parameters in the estuary far exceeded (e.g. nitrate is at times two orders of magnitude greater) the high thresholds established by the Central Coast Regional Water Quality Control Board (1994), Bricker et al. (1999), and Bricker et al. (2003).

# Determination of Thresholds and Frequencies

Thresholds for nutrient, chl *a*, and hyperoxia data were determined by first taking the mean of the data throughout the study period, and comparing the result to reported thresholds (Appendix 2 Table 1). If the mean exceeded a certain threshold then it was determined to be periodic and assigned a eutrophication expression score (Appendix 2 Table 2). For example, the mean nitrate from HLW was 1.2 mg/L, therefore it was assigned a hyper threshold. If the threshold for the mean concentration fell below the hyper concentration, then it was next determined if was episodically hyper. This was determined by determining the threshold of the 90<sup>th</sup> percentile value, if the 90<sup>th</sup> percentile was above the hyper threshold then that parameter was determined to be episodically hyper. If the 90<sup>th</sup> percentile did fall within the hyper threshold range then the process moved down to the high threshold to determine if it met any of its criteria.

Frequency was not used in determining scores for algal cover, hypoxia, or sediment anoxia measurements. Hypoxia was based on continuous data sets and percentage of time hypoxic. Algal mat thresholds were determined by using monthly estimates of floating algal mats from 2008-09, as well as the two summer time surveys of intertidal and subtidal algal mats. Threshold standards were determined from a study from Nezlin et al. (2006), which examined relationships between ephemeral green macroalgal abundance and dissolved oxygen. The water surface, subtidal, and intertidal were treated as three unique zones, and if only one of the three habitats exceeded the threshold for macroalgae then the site was characterized by that threshold. There is a general lack of information describing threshold levels for depth to sediment anoxia layers, therefore frequency distributions were used to look for natural breaks in the data, and it can be assumed that

#### Individual Site Eutrophication Expression

The overall pressure at each site was determined by taking the average score of nitrate, phosphate and ammonia. Taking the average expression value among all parameters in the Primary Indicators and Secondary Indicators categories, and then averaging the overall scores of Primary and Secondary Indicators determined the overall expression of eutrophication at each site.

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Table 2. Pressure and eutrophication indicators along with their thresholds, associated score, and a description of sampling frequency. Threshold references: Chl *a*/nitrate/phosphate (Bricker et al. 2003), Ammonia (Caffrey et al. 1997 and Caffery 2002) from SF and Tomales Bays, Hypoxia EPA (2.3 mg/L), Hyperoxia (Bricker et al. 2007), Algal Cover (Nezlin et al. 2006), No definition for sed oxic layer, unionized ammonia (Central Coast Regional Water Quality Control Board Basin Plan 1994).

		Thresholds				
		Hyper	High	Mod	Low	Sampling Frequency
Parameters	Nitrate as N	> 1mg/L	0.5-1.0 mg/L	0.1-0.5 mg/L	<0.1 mg/L	Monthly grab samples from 2004-2009
	Phosphate as P	>0.5 mg/L	0.1-0.5 mg/L	0.01-0.1 mg/L	<0.01 mg/L	Monthly grab samples from 2004-2009
			0.14-0.42	0.01-0.14		
	Ammonia	>0.42 mg/L	mg/L	mg/L	<0.01 mg/L	Monthly grab samples from 2004-2009
	Chl a	>60 ug/L	20-60 ug/L	5-20 ug/L	<5 ug/L	Monthly grab samples 2008-2009
		>50% cover	20.50%	10.20%	<10%	Monthly for floating mats, 2 surveys of benthic
	Algal cover	> 50 /0 COVCI	20-3070	10-2070	<1070	mats
		Anoxic or Hypoxic	10-20% of	1.10% of time	0% of time	
	Hypoxia	>20% of time	time	1-10/0 01 time	070 01 time	%time hypoxic for >2 week periods
	Hyperoxia	>14 mg/L	12-14 mg/L	10-12 mg/L	<10 mg/L	Monthly sampling from 2007-2009
	Sed oxic layer	<1 cm	1-5 cm	5-10 cm	>10 cm	Summer 2009 survey
			0.01-0.025	0.005-0.01		
	Free Ammonia	>0.025 mg/L	mg/L	mg/L	<0.005 mg/L	Monthly grab samples from 2004-2009

Table 2. Logical decision process for determination of eutrophic condition, modified from Bricker et al. (2003).

Threshold	Frequency	Expression
Hyper	Periodic	1
Hyper	Episodic	1
High	Periodic	1
High	Episodic	0.75
Moderate	Periodic	0.5
Moderate	Episodic	0.25
Low	Periodic	0
Low	Episodic	0



#### **APPENDIX 2: DATA SUMMARY**

Figure 1. Mean nitrate as N collected monthly from 2004-2009 with error bars representing the 90<sup>th</sup> percentile. Dotted lines represent thresholds (Appendix 2 Tables 1-2). Note: Vertical axes of each graph are different scales.



Figure 2. Mean phosphate as P collected monthly from 2004-2009 with error bars representing the 90<sup>th</sup> percentile. Dotted lines represent thresholds (Appendix 2 Tables 1-2). Note: Vertical axes of each graph are different scales.



Figure 3. Mean ammonia as N collected monthly from 2004-2009 with error bars representing the 90<sup>th</sup> percentile. Dotted lines represent thresholds (Appendix 2 Tables 1-2). Note: Vertical axes of each graph are different scales.



Figure 4. Mean chl a concentrations collected monthly from July 2008 to August 2009 with error bars representing the 90<sup>th</sup> percentile. Dotted lines represent thresholds (Appendix 2 Tables 1-2).



Figure 5. Algal cover for three zones: water surface, intertidal and subtidal. Mean floating algal cover was collected monthly from August 2008 to July 2009. Subtidal and intertidal cover data was collected on two summer surveys in 2009. Dotted lines represent thresholds (Appendix 2 Tables 1-2). ND indicates no data, green 0 indicates no algae present.







Figure 7. Results from a 2009 survey characterizing sediment quality as a measurement of the depth of the sediment oxic layer down to the anoxic layer. Numbers in red indicate the depth of the layer, which was to small to represent graphically, and ND=no data collected. Dotted lines represent thresholds (Appendix 2 Tables 1-2).



Figure 8. Mean unionized ammonia collected monthly from 2004-09 with error bars representing the 90<sup>th</sup> percentile. Dotted lines represent thresholds (Appendix 2 Tables 1-2).