

**THE IMPACTS OF NUTRIENT LOADING AND ENVIRONMENTAL CONDITIONS ON THE FISH ASSEMBLAGE
AND AVAILABLE NURSERY HABITAT IN ELKHORN SLOUGH**

A report prepared for the Nature Conservancy and the Elkhorn Slough Foundation

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EXECUTIVE SUMMARY

Changes in nearshore fish assemblages are influenced by diverse factors and elucidating the important drivers of these changes can be challenging. Some factors that can contribute to changes in nearshore fish assemblages include environmental conditions, fishing pressure, and available habitat, such as nursery grounds. In this study we examined the changes in a fish assemblage from a coastal estuary over time and correlated those changes with changes in physical and chemical parameters. Elkhorn Slough is an estuary located in the heart of Monterey Bay, CA, and is the largest estuary on the central coast of California between San Francisco and Morro Bay. It is an important nursery habitat for a variety of marine and estuarine species in the Monterey Bay region, including species of flatfish that are primarily present as juveniles in the slough prior to migrating out to Monterey Bay where adult populations are targeted as commercial and recreational fisheries. In addition, the predominance of agricultural land use in the watershed makes Elkhorn Slough an ideal site for investigating the impacts of nutrient loading and environmental conditions on the fish assemblage. We used a 40-year data set of estuarine fish abundance from otter trawls and beach seine surveys, combined with water quality, and climatic indices to determine the important factors driving variability in the estuarine fish assemblage.

Elkhorn Slough's estuarine habitats have changed considerably in the last century, and these changes have caused dramatic shifts throughout the biological communities of the slough, including the fish assemblage. Of the original extensive estuarine network, 50% is now behind water control structures which greatly limit tidal exchange and have converted many formerly favorable habitats to unfavorable conditions. The seaward portion of the main channel of Elkhorn Slough deepened dramatically, and tidal creeks widened and deepened, as a result of the construction of the Moss Landing harbor at the mouth of the slough in 1947, which triggered significant erosion and the export of large amounts of soft sediments. Nutrient-loading to the entire estuarine complex also increased dramatically over the past 50 years. Run-off that flows directly into the estuary from the adjacent 2.69 million acre agricultural landscape of the Salinas Valley is the primary driver of eutrophication in the estuary.

We investigated the indirect effects of nutrients and associated eutrophication had on the estuarine fish assemblage by examining variation in hypoxic conditions, a known correlate of eutrophication and a condition that many fish are known to avoid. To identify periods of hypoxia in the estuary we developed an index of dissolved oxygen anomaly (DOA). The DOA detected hypoxic conditions as early as the 1970s when nutrient concentrations started increasing beyond the baseline levels of Monterey Bay upwelling and historical conditions. Since the 1970s nutrient concentrations in the estuary have increased exponentially, yet the DOA conditions have remained stable. Hypoxic conditions in the estuary were correlated with high salinities and temperatures, which occurred during El Niño relaxation events.

Since the 1970s the fish assemblage declined in total species richness and abundance. This change in diversity was primarily driven by declines in several surfperch species and near disappearance of starry flounder; as well as the complete disappearance of 13 rare species, as well as white croaker, blue rockfish, kelp greenling, striped bass, kelp pipefish, and barred surfperch, species which were all common in the 1970s. In the last two decades (1990s-2000s) hypoxic periods in the estuary, as identified by the DOA, had lower species diversity and abundances than normoxic periods. The declines in species diversity during more recent hypoxic periods were driven by declines in several species of flatfish, as well as complete loss of 12 rare species, and the following commonly caught species: sand sole,

rubberlip surfperch, bocaccio, longjaw mudsucker, onspot fringehead, grey smoothhound, and brown smoothhound. The combined effects of hypoxia and habitat change resulted in the loss of 59% of fish species in the estuary, at least 28% of declines in fish diversity were attributed to eutrophication and hypoxia alone.

In addition to our community level analysis, we specifically examined the three most abundant and economically important species of flatfish (California halibut, English sole, and speckled sanddab) in the estuary by modeling their predicted probability of occurrence using raw DO concentrations. We determined that all three flatfish species in the estuary were very sensitive to hypoxic conditions. Modeling of the DOA and spatial modeling of flatfish probabilities indicated that El Niño induces favorable conditions in the estuary for flatfish by decreasing hypoxic conditions in two ways: 1) relaxation of upwelling intensity leading to the inability of the deep sea DO minimum layer to reach the lower estuary, and 2) increased flushing through rain events in the mid and upper estuary.

Our results demonstrate that estuarine fish assemblages are sensitive to changes in habitat conditions. Specifically, increases in hypoxia combined with change to physical habitats can decrease the functional role of the estuary in supporting fish, which can lead to losses in the overall biodiversity of the system. Flatfish in general are very sensitive to hypoxic conditions as demonstrated by the overall lower presence during hypoxic periods, as well as declined presence in areas where eutrophication and the effects of nutrient loading are intensified. Flatfish in Elkhorn Slough were almost completely absent when dissolved concentrations were < 4 mg/L, and declines were detected in even moderate hypoxic conditions (4-8 mg/L). Therefore, certain species of flatfish (California halibut, English sole, and speckled sanddab) should be considered as candidate indicator species for assessing the effects of nutrient loading in coastal systems. Future research should investigate the relationship between the juvenile flatfish assemblage in the estuary and the offshore adult populations to determine the effect of estuarine habitat condition on offshore flatfish assemblages, as well as testing the generality of our model in other estuaries.

INTRODUCTION

Nearshore fish assemblages are shaped by diverse factors, such as recruitment variation, oceanographic forces, available habitat, and environmental conditions. The interaction of these multiple effects on economically important fish assemblages can make it difficult to elucidate their relative importance. Due to increases in the human populations along the world's coasts in the last century there have been additional stressors placed on nearshore fish populations. Hypoxia along with over-fishing are considered to be the two major stressors on fish assemblages (Jackson et al. 2001). Hypoxia has been increasing exponentially in the world's coastal ecosystems in the last century, and has been indirectly caused by nutrient loading through a process called eutrophication (Vaquer-Sunyer and Duarte 2008). Eutrophication and hypoxia can lead to negative consequence for fish assemblages, through loss of essential fish habitat, a decrease in growth and reproductive capacity, and even fish kills (Diaz and Rosenberg 2008; Vaquer-Sunyer and Duarte 2008; Carlisle and Starr 2009).

Hypoxia, defined as the reduction in normal or optimal dissolved oxygen concentrations, is a sublethal stressor that has a variety of biochemical and physiological effects on fish (Rabalais et al. 2002; Wu 2002; Lefrancois and Claireaux 2003; Vaquer-Sunyer and Duarte 2008). When challenged with low oxygen levels, fish generally use several strategies to survive. One of the first strategies that fish use to maintain delivery of oxygen to body tissues is to increase rate of flow of water across the gills, through increased buccal pumping or swimming speed (Randall 1982; Carlson and Parsons 2001) or increasing gill diffusion (Randall 1970; Randall 1982; Wu and Woo 1995). Fish may also increase the capacity of their blood to transport oxygen by increasing red blood cell concentration and oxygen binding capacity of hemoglobin (Wood and Johansen 1972; Randall 1982). Another strategy is to reduce oxygen demand by reducing activity levels and metabolic rate to conserve energy (Storey 1988; Schurmann and Steffensen 1994; Hochachka 1997) and/or switching to anaerobic metabolic pathways (Dunn and Hochachka 1986; Hochachka 1997). The energetic costs incurred by these physiological responses to hypoxia and the concurrent reduction in feeding that often accompanies them (Thetmeyer et al. 1999) can be dramatic and reduce individual fitness through reducing rates of growth and reproduction and increasing mortality (Diez and Davenport 1990; Petersen and Pihl 1995; Keckeis et al. 1996; Chabot and Dutil 1999).

Many species actively avoid habitats with low dissolved oxygen concentrations (Pihl 1991; Wannamaker and Rice 2000; Breitburg 2002), and as a result dissolved oxygen levels play an important role in determining the distribution and patterns of habitat use of marine fishes in both coastal (Breitburg 2002) and pelagic ecosystems (Prince et al. 2010). Hypoxia reduces the quality and quantity of habitats (Breitburg et al. 2009), and in extreme cases low dissolved oxygen levels can lead to fish kills or the formation of large "dead zones" (Rabalais et al. 2002; Service 2004). Upon encountering hypoxic conditions, fish typically exhibit avoidance behavior and move to habitats with more suitable conditions (Breitburg 2002). This change in habitat availability may result in an increased use of suboptimal habitats or habitat compression. In certain circumstances, habitat compression may increase susceptibility to fishing pressure (Pihl 1991; Wannamaker and Rice 2000) as population densities increase in remaining suitable habitat (Coutant 1985; Eby and Crowder 2002; Brandt and Mason 2003; Craig and Crowder 2005; Aalbers et al. 2010).

Shifts in the distribution of fish in response to hypoxia can have broader population and ecosystem level ramifications (Wu 2002; Breitburg et al. 2009). As species move to avoid hypoxic conditions, food web

structure and function in both hypoxic and nearby normoxic habitats are affected (Pihl 1994; Breitburg 2002; Baird et al. 2004; Craig and Crowder 2005). In hypoxic habitats, species diversity, richness, and biomass decreases as organisms die or leave affected areas (Dauer 1993; Pihl 1994; Diaz and Rosenberg 1995; Petersen and Pihl 1995; Wu 2002) and there is a concomitant shift in trophic interactions as well (Breitburg et al. 1994). Not only the structure of food webs change as species distributions shift, but shifts in competitive and predator-prey dynamics alters food web function (Breitburg 2002; Domenici et al. 2007). As a result, the broad scale ecological consequences of hypoxia can be dramatic and it may take as long as several years for community structure and function to return to normal following hypoxic events (Josefson and Widbom 1988; Diaz and Rosenberg 1995). However, it is important to remember that the impact of hypoxia is mediated by species specific physiological limitations as well as life stage (Breitburg 2002), so not all species or even life stages are affected in the same way or to the same extent, with some tolerant species doing well in hypoxic conditions and more sensitive species being eliminated (Dauer 1993).

Elkhorn Slough, an estuary located in Monterey Bay on the central coast of California, has been greatly affected by anthropogenic activity over the last century. One of these activities has been intense nutrient loading from the surrounding agricultural landscape, which has caused widespread eutrophication and hypoxia (Beck and Bruland 2000; Caffrey 2002; Hughes et al. 2011) (Figure 1). An earlier study by Elkhorn Slough National Estuarine Research Reserve (ESNERR) staff documented that biological communities in Elkhorn Slough, including fish, are strongly impacted by artificial tidal restriction, which degrades water quality (Ritter et al. 2008). Ritter et al. (2008) documented that two estuarine flatfish (Speckled sanddab and California halibut) are significantly more abundant in habitats with full tidal exchange than in habitats with partially muted tidal exchange, suggesting that these species were sensitive to water quality. As these two commercially important species, as well as many others, use the estuary as a nursery (Brown 2006), decreased water quality in the estuary may have important ramifications for productivity of the estuary as a nursery habitat. To date there has yet to be a study in the region that describes the effect of anthropogenic nutrients on the nursery function of Elkhorn Slough, and its support for juvenile flatfish.

The purpose of this study was to provide *The Nature Conservancy (TNC)* with information and reports that can be used to inform their strategies for engaging stakeholders that affect and are affected by estuarine ecosystem conditions. In particular, we elucidate how nutrient loading affects fish, and more specifically flatfish, in Elkhorn Slough, and how improvement of water quality and concomitant improvement of nursery habitat could potentially improve off-shore commercial fisheries of flatfish (Figure 2). We hypothesize that variable eutrophic conditions, more specifically hypoxia, will negatively impact the fish assemblage, especially flatfish, of Elkhorn Slough depending on the severity of eutrophication. Flatfish are a functional group of interest due to their potential sensitivity to bottom-water hypoxia, and the importance of Elkhorn Slough for their nursery grounds (Brown 2006). We hypothesized that the expression of nutrient loading (i.e. hypoxia) is variable in space and time and dependent upon environmental conditions (i.e. El Niño Southern Oscillation [ENSO], Upwelling, Pacific Decadal Oscillation [PDO], and North Pacific Gyre Oscillation [NPGO]), and that hypoxic years in the estuary could result in declines in offshore adult flatfish populations.

Due to the diversity of data sets available, we used a multifactorial approach to investigate the relative importance of several factors that influence fish assemblages in Elkhorn Slough. These data sources

include long-term data sets on estuarine fish communities, water quality, estuarine ecosystem dynamics, and oceanographic data (all of which are described below).

METHODS

Treatment of data

To determine if varying water quality and habitat conditions affect the fish assemblage in Elkhorn Slough we first assembled various data sets from long-term monitoring programs from the Monterey Bay Region (see the Appendix 1 for detailed descriptions of various data sets).

Treatment of fish data

We used a long-term fish monitoring dataset (1970-2010) that used a combination of otter trawls (N = 626) and beach seines (N = 318) to sample the fish assemblage at various sites within Elkhorn Slough (see Appendix 1 for detailed description) (Figure 1). The fish monitoring dataset captured a high degree of temporal and spatial variability, which made it ideal to test the effect of varying water quality on the fish assemblage. We identified the sites that were most consistently sampled through the entire time series, and used those in the analysis. Since water quality sampling stations and fish monitoring stations differed, we grouped fish monitoring stations based on proximity to the nearest water quality station.

Otter trawl locations were broken into various regions within the slough based on known physical and biological characteristics (Figure 1). The nearest water quality station was used to match up water quality data with otter trawl data. Beach seines had spatially defined sampling locations and were assigned to the nearest water quality station. Each fish sampling event (otter trawls and beach seines) were located < 1 km and within < 30 days to the nearest water quality sampling event. It was assumed that the monthly water quality sample was a good indicator for the overall water quality condition for the fish sample.

We transformed all fish density data to presence/absence due to the difficulty and lack of specific information on net size and trawl time for each trawl and beach seine. We then pooled the presence/absence data for each date at each site (N = 170 otter trawls, N = 85 beach seines). We did this to avoid violating assumptions of independence and weighting dates (which were sample replicates in the analysis) where multiple samples (i.e. trawls) were often collected. Instead we noted the number of trawls taken on each date and used this as a variable in the logistic regression analysis. Thus we assumed that sampling date (> 1 month between replicates) and sampling station (>1 km apart) were independent samples.

Treatment of Water Quality Data

We used several data sets that span from 1970-present as well as samples collected in 1928 that serve as background data. These data were collected monthly at various stations around the estuary (Figure 1) (Appendix 1). The parameters we used for analyses in this study were Dissolved Oxygen (DO) (mg/L), Nitrate (mg/L), Temperature (°C), and salinity (ppt), as these were factors known to affect fish presence in estuarine environments. Other parameters collected such as pH and turbidity were not used in the analysis to reduce the artifacts of multicollinearity in the regression analysis. Also, preliminary analyses determined that these other parameters were not important correlates with variation in the fish

assemblages. We used the raw monthly values for DO, temperature, salinity, and nitrate for correlations with fish presence and absence.

We also developed a cross-validation technique by calculating a dissolved oxygen anomaly (DOA) to identify hypoxic periods in the slough that could be correlated with the fish assemblage. The DOA was calculated using the entire ESNERR water quality record (1988-2011) at stations that were sampled within the fish sampling range ($N = 6$) by calculating Z-scores: $(\text{Global Mean} - \text{Raw DO [mg/L]}) / \text{Global SD}$. The average monthly value among all the sampling sites was used for a single monthly value that represented the DO condition for the estuary for that month. We used the DOA, which is simply a standardized variance value, because we wanted to assess the slough-wide DO condition for monthly sampling and not individual sites to address high site and temporal variability (Hughes et al. 2011). DO variation is also an excellent indicator for eutrophication and hypoxic conditions (which generally occur during nighttime or early morning hours when sampling does not occur). The use of the DOA also allowed us to assess the overall eutrophic condition in the entire slough, and the potential threat to the entire fish assemblage.

Treatment of oceanography data

We used El Niño Southern Oscillation (ENSO), Pacific Decadal Oscillation (PDO), North Pacific Gyre Oscillation (NPGO) and local Monterey Bay Upwelling indices, to investigate the relative effects of large scale climate variation on the Elkhorn Slough water quality and fish assemblage. These indices are reported as mean monthly values, so we matched the month of each fish and water quality sample to the corresponding ENSO, PDO, NPGO, and Upwelling indices, and used those in the statistical analyses.

Analytical Methods

Bottom-up threats to the Elkhorn Slough ecosystem

We first characterized the primary threats to Elkhorn Slough, nutrients and changes to the tidal volume exchange (changed due to erosion and restoration activities), by constructing a time series (1928-2011) based on the water quality data described above. For nitrate, all samples were collected in the lower part of the estuary near the mouth. The three-month running average of the monthly mean among lower slough sites was plotted and used in a simple linear regression analysis to determine if nitrate increased over time. We used the three-month running average to smooth results of nitrate that could have been driven by anomalous monthly patterns. We coupled this with the five-year running average of agricultural fertilizer sales (in tons of N) from Monterey County (California Department of Food Agriculture & Central Coast Regional Water Quality Control Board), as this is the primary source of nutrients in the system (Hughes et al. 2011). We plotted the DOA using monthly mean of all study sites averaged in two year increments to make surveys from 1970-71 and 1974-76 comparable to the recent ESNERR data (1988-2011). Lastly, we plotted tidal prism data from Broenkow and Breaker (2005) to indicate erosional rates in the estuary.

Key correlates of the fish assemblage

We used non-metric Multidimensional Scaling (nMDS) to assess the key environmental correlates of the multivariate fish assemblage for both otter trawls and beach seines. We calculated a similarity matrix between samples with the presence-absence fish assemblage data using the Bray-Curtis technique. We

then divided this into hypoxic and normoxic regimes based on the DOA value associated with each fish sampling effort. The 1970s were also used as a reference period to compare to the hypoxic and normoxic regimes. These three different regimes were compared using the ANOSIM function in Primer (v. 6). Next, we used the SIMPER function in Primer to determine which species were contributing to differences in the three different regimes. The SIMPER function uses the Bray-Curtis similarity matrix to calculate the dissimilarity between groups, and the species that are contributing most to the dissimilarity.

We also calculated a dissimilarity matrix on the associated environmental parameters (DOA, ENSO, Upwelling, PDO, NPGO) using Euclidean distances. The environmental parameters were normalized prior to the matrix calculation. Next, we used the RELATE function in Primer (v. 6) to determine the overall relationship between the fish assemblage and environmental matrices. We then used the BEST function in Primer to determine the environmental parameters that correlate with variation within the fish community structure.

To determine differences in diversity and abundance during the three regimes (1970s, and Hypoxic and Normoxic for 1989-2010) (fixed factor, 3 levels) and season (fixed factor, 4 levels) we compared the mean daily species richness and mean abundance for otter trawls using a full-factorial two-way Analysis of Variance (ANOVA) using SPSS (Statistical Software V. 20). Dependent variables were checked for homogeneity of variances (Levene's Test) and normality (Shapiro-Wilk Test). Data that violated these assumptions were square-root transformed (Underwood 1997). We used a Least Squares Difference (LSD) multiple comparisons test for ANOVAs resulting in significant differences of the independent variables.

Key correlates with select individual fish species

To explore potential effects of hypoxia on flatfish species we examined differences in presence/absence data of the three most abundant and commercially important flatfish species (English sole, speckled sanddab, California halibut) found in the estuary. To determine the key correlates with individual flatfish species we used logistic regression with the GLM package in R (v. 2.15). Logistic regression is derived from the Generalized Linear Model family, and differs from linear regression because it relaxes assumptions on normality of response variables by using binary (i.e. presence/absence) data instead of continuous data. It then models the probability of presence/absence of a species using a linear function of the predictor variables. The predictor variables used were DO (mg/L), nitrate (mg/L), temperature (°C), salinity (ppt), and monthly index values for ENSO, Upwelling, PDO, NPGO, as well as daily sampling effort. We did not include sampling location or seasonal effects in the analysis because flatfish were generally caught at all times of the year throughout the primary sampling stations (Figure 1) in Elkhorn Slough. The same process was replicated using beach seine data.

We determined the key correlates using the Akaike Information Criterion (AIC), which selects the best model by incorporating all variables, similar to a stepwise multiple regression. Once the significant correlates were identified, we applied a reduced logistic regression by correlating the presence/absence data to the significant individual environmental predictors to determine the direction of the correlation. Last, we applied the DO concentration (mg/L) as the only predictor variable to determine general patterns in the relationship between DO and flatfish presence. All alphas were set at 0.10 to reduce Type II errors that fail to reject the null hypothesis.

To determine the spatial extent of flatfish habitat in Elkhorn Slough we modeled the probability of flatfish presence (using logistic regression curves) as a function of hypoxia extent in the estuary. To determine the level of hypoxia we calculated the 10th percentile of DO for each water quality station using the monthly ESNERR (1989-2011) water quality dataset. Dissolved oxygen data were organized and formatted for use within ArcGIS Basic 10.0 (ESRI 2010) using MS Excel 2007. Each sampling site had a column with site name, X coordinate, Y coordinate, sampling month, day, and year, and measured absolute DO value. All data from 1988 – 2011 were then categorized by sampling site and separated into individual worksheets containing data for that specific site. Data were also categorized into worksheets by sites containing data from periods categorized as with or without El Niño Southern Oscillation (ENSO) events, e.g.; El Niño, La Niña, or “La Nada” (periods with no significant ENSO event). For each site the tenth percentile of DO was calculated. The tenth percentile values for each site were imported into ArcGIS and shapefiles created. The shapefiles consisted of “All Years”, “El Niño Years”, “La Niña Years”, and “La Nada Years”.

ArcGIS with the Spatial Analyst extension was used to interpolate DO values among sites within Elkhorn Slough. The interpolation method used was Spline with Barriers, which attempts to fit a surface among all values while minimizing the amount of curvature while respecting breaks and discontinuities imposed on the surface. Spline interpolation is considered the optimal method for representing smoothly varying data such as temperature (Childs 2004) and therefore should adequately estimate DO gradients throughout Elkhorn Slough. Four interpolated grids (rasters) for DO values were created for each data set. These rasters were exported as text XYZ files with X and Y being the spatial coordinates and Z being the interpolated DO values. In MS Excel the DO values for each grid cell were used to calculate the probability of the presence of the three fish species of interest based on the predicted probabilities from logistic regressions. The new XYZ files were then imported back into ArcGIS and converted into rasters of fish probability for each species and each data period.

Next, we validated our temporally robust logistic regression model by using a spatially robust sampling effort of flatfish in Elkhorn Slough. A 2005 survey by Ritter et al. (2008) thoroughly sampled the Elkhorn Slough fish assemblage using beach seines at 16 stations strategically located (< 500 m) to the nearest water quality monitoring station (Figure 1). Each station was sampled in the spring and again in the summer. We used each sampling date at each station as a replicate in a logistic regression analysis. The logistic regression analysis was run using presence/absence for flatfish species as the dependent variable and the 10th percentile DO calculated for the entire ESNERR dataset (1989-2011). We combined all flatfish species (speckled sanddab, California halibut, starry flounder, fantail sole, and California tonguefish) into one functional group because of low replication among the individual species during the survey period.

Drivers of dissolved oxygen

Nutrient loading, eutrophication, and their side-effects are often masked by physical processes such as freshwater inputs, water temperature, storms, or channel mixing to name a few (Cloern 2001). This is especially true for Elkhorn Slough where tidal range and channel depth act as filters to negative responses of nutrient loading at sites within the estuary (Hughes et al. 2011). For this study we set out to determine the main physical drivers for the expression of hypoxia in the estuary since we hypothesized that hypoxia rather than nutrient loading has the stronger influence on the fish assemblage.

First, we explored the correlates of variation of DO using Principal Components Analysis (PCA). We used the raw monthly values for DO, temperature, and salinity for each water quality site that

was in proximity of the fish monitoring program in Elkhorn Slough for the entire ESNERR water quality record (1988-2011). The PCA was run using SPSS statistical software (v. 20) by developing a correlation matrix using an unrotated method. We used scores from the first PC because it explained the most variation within the data, and we averaged the PC scores among all sites to generate a single value for each month. The mean monthly PC was plotted along a time series axis to explore patterns in the water quality data.

Next, we further explored the key correlates of hypoxia by using continuous water quality monitoring stations within the estuary. This also served as cross-validation technique for the DOA, but used more temporally explicit continuously collected data (every 15 minutes) rather than spatially explicit data. We used ESNERR's South Marsh (Figure 1, #10) and Vierra Mouth (Figure 1, #14) water quality monitoring stations, which have been sampling since 1995 and 2001, respectively with YSI (Yellow Springs Instruments) data sondes (Figure 1) (Appendix 1). The Vierra Mouth site is closer to the mouth of the estuary and therefore more influenced by oceanographic processes, whereas the South Marsh site is located half-way up the estuary where residence times are higher and is more representative of mid to upper estuarine sites. We characterized hypoxia at each site by calculating the 10th percentile of DO for an entire calendar year, and then used step-wise multiple regression to determine the key correlates by using the mean annual values for temperature, salinity, Monterey Bay upwelling, ENSO, and PDO.

RESULTS

Bottom-up threats to the Elkhorn Slough ecosystem

Elkhorn Slough is an estuary under tremendous pressure from impaired water quality and hydrological change. Fertilizer sales in the surrounding watershed increased 30-fold in the period from 1928-2009 (Figure 3). This has resulted in downstream water quality impairment in Elkhorn Slough. Nitrate concentrations near the mouth of the estuary, the area closest to the main freshwater source (the lower Salinas Valley), increased exponentially from 1928-2011 ($F_{1,278} = 378.9$, $P < 0.0005$, $R^2 = 0.577$) (Figure 3). There was a 1000 fold increase in nitrate from 1928-2011, and 50-fold increases from 1928-1970 and 1970-2011, respectively. The two-year DOA average revealed that Elkhorn Slough was more hypoxic in the 1970s relative to the 1988-2011 mean ($\mu = 8.62 \text{ mg/l} \pm 3.55 \text{ SD}$) (Figure 4). Hypoxia was highly variable from 1988-2011, and only one period (1992-1994) was more severely hypoxic than the 1970s. At the same time nutrient concentrations were increasing there was an increase in the tidal prism the estuary (Figure 3). Since the opening of the Moss Landing harbor mouth in 1947 the tidal prism increased to 3 times the original size. This increase in the tidal prism was both due to the erosion of the main channel, tidal creeks, and marsh and to restoration of tidal exchange to a major wetland area (Parsons complex) accounting for ~25% of the tidal volume of the estuary (Broenkow and Breaker 2005, Van Dyke and Wasson 2005).

Key correlates of the fish assemblage

There were strong differences in the fish community assemblage among the three regimes of the 1970s, and hypoxic and normoxic regimes of the 1990s-2000s for otter trawls ($\text{Global } R = 0.39$, $P = 0.0001$) (Figure 4a) and beach seines ($\text{Global } R = 0.19$, $P = 0.0001$) (Figure 4b). The 1970s fish assemblage was significantly different than both the 1990s-2000s hypoxic (Otter trawls ANOSIM *Pairwise Test Statistic*

= 0.551, $P = 0.0001$; Beach seines ANOSIM *Pairwise Test Statistic* = 0.408, $P = 0.0001$) and normoxic regimes (Otter trawls ANOSIM *Pairwise Test Statistic* = 0.389, $P = 0.0001$; Beach seines ANOSIM *Pairwise Test Statistic* = 0.299, $P = 0.003$). There were also significant differences between hypoxic and normoxic regimes of the 1990s-2000s for both otter trawls (ANOSIM *Pairwise Test Statistic* = 0.098, $P = 0.025$) and beach seines (ANOSIM *Pairwise Test Statistic* = 0.099, $P = 0.0004$). Species that contributed to general differences between the 1970s and 1990s-2000s regimes for otter trawls and beach seines included the starry flounder, white surfperch, blacksurfperch, cabezon, and northern anchovies, which had a greater presence in the 1970s (Tables 1a-b, 2a-b). For otter trawls there was a greater presence of English sole, California halibut, and speckled sanddab in the 1990s-2000s (Tables 1a-b). Differences between the hypoxic and normoxic periods of the 1990s-2000s for otter trawls were attributed to greater presence of nearly every species during normoxic regimes (Table 1c) and in general flatfish had lower presence in both otter trawls (Table 1c) and beach seines (Table 2c) during the hypoxic regimes.

There was a strong relationship between the fish assemblage and their associated environmental/climatic conditions for otter trawl data (DOA, ENSO, PDO, NPGO, and Upwelling) (RELATE $Rho = 0.22$, $P = 0.0001$). The factors contributing most to the community composition were the DOA, PDO, and Upwelling (BEST *Correlation* = 0.230). The DOA was the only factor to appear as a significant contributor to variation in the community assemblage for all reported permutations, further indicating that it is an important factor influencing the Elkhorn Slough fish assemblage. However, there was not a significant relationship between the beach seine fish community assemblage and any environmental parameter (RELATE $Rho = -0.021$, $P = 0.66$)

Fish diversity declined in Elkhorn Slough from the 1970s to 1990s-2000s, and more recently hypoxic periods had significant declines in fish diversity for trawl surveys compared to normoxic periods. The two-way ANOVA resulted in a significant effect of regime and season, but no regime*season effect on the square-root transformed fish species richness for trawl surveys (Table 3a) (Figure 5a). The LSD test revealed significantly lower species richness for the hypoxic regime of the 1990s-2000s compared to both the normoxic regime of the 1990s-2000s ($P = 0.013$) and the 1970s ($P = 0.002$). However, there was no significant difference between species richness for the 1970s and 1990s-2000s normoxic regime ($P = 0.387$). Only the winter was significantly different than other seasons and was significantly lower in fish diversity among all the regimes than summer ($P = 0.001$) and fall ($P = 0.002$), all other seasons were not significantly different from each other.

There was also a significant decline in fish diversity for beach seine data (square-root transformed) since the 1970s compared to hypoxic (LSD $P < 0.0005$) and normoxic (LSD $P = 0.002$) regimes of the 1990s-2000s, yet no significant difference between the hypoxic and normoxic regimes of the 1990s-2000s (LSD $P = 0.485$) (Table 3b) (Figure 5b). For beach seines there was a significant regime and regime*season effect, however, no season effect (Table 3b).

The species associated with diversity loss were explored by examining presence/absence data from otter trawls and beach seines combined. A total of 98 fish species were caught in otter trawls and beach seines during the 1970-2010 study period (see Appendix 2 for rank abundance species list). Of the total species 51 were considered to be rare ($< 1\%$ presence in all surveys). Between the 1970s and the 1990s-2000s there was a total loss of six species that were common ($> 1\%$ presence in all surveys) in the 1970s surveys: white croaker, blue rockfish, kelp greenling, striped bass, kelp pipefish, and barred surfperch.

However, there were no new species observed for commonly caught species during the 1990s-2000s since the 1970s. Furthermore, there was a total loss of 26 rare species (< 1% presence) from the 1970s to 1990s-2000s, whereas there were only 13 rare species gained, resulting in a net loss of 19 species (common + rare), or 19.5% of the total species pool. During the more recent 1990s-2000s period, there were seven common species that were only caught during normoxic periods (sand sole, rubberlip surfperch, bocaccio, longjaw mudsucker, onspot fringehead, grey smoothhound, and brown smoothhound), whereas only Pacific sardines were exclusively caught during hypoxic periods. There were an additional 16 rare species that were only caught during normoxic periods compared to 4 that were caught only in hypoxic periods. This all totals in a net loss of 18 species during hypoxic v. normoxic periods, or 27.7% of the entire species pool during the 1990s-2000s. The combined effects of hypoxia and other factors (erosion, fishing, climate) on species diversity loss ($1 - [(Total\ species\ pool - 1990s-2000s\ Hypoxic\ species\ richness) * (Total\ species\ pool)^{-1}]$) resulted in a 58.8% decline in fish species richness in the last four decades.

Fish abundance declined from the 1970s to 1990s-2000s, and more recently hypoxic periods had significant decreases in fish abundance for both trawl and seine surveys relative to normoxic periods. The two-way ANOVA on the square-root transformed mean abundance of fish caught in trawls determined a significant regime, season, and regime*season effect (Table 4a) (Figure 6a). The LSD test determined that the 1990s-2000s hypoxic regime had significantly lower trawl fish abundances than both the 1990s-2000s normoxic regime ($P < 0.0005$) and the 1970s ($P < 0.0005$). The 1990s-2000s normoxic regime was marginally lower in fish abundance than the 1970s ($P = 0.098$). Only the winter was significantly different than other seasons and was significantly lower in fish abundance among all the regimes than spring ($P = 0.057$), summer ($P < 0.0005$) and fall ($P = 0.003$), all other seasons were not significantly different from each other.

There was also a significantly lower fish abundance caught in beach seines (square-root transformed fish abundance) of the 1990s-2000s hypoxic regime compared to the 1970s (LSD $P = 0.002$) and the 1990s-2000s normoxic regime (LSD $P = 0.03$), yet no significant differences between the 1970s and the 1990s-2000s normoxic regime (LSD $P = 0.181$) (Table 4b) (Figure 6b). Only the summer was significantly different than other seasons, and was significantly greater for all seasons (LSD $P < 0.0005$, all seasons).

Key correlates with individual flatfish species

Elkhorn Slough is an important nursery for flatfish in the Monterey Bay region given that is the largest estuary in the region and it contributes disproportionately to offshore populations relative to other habitats in the area (Brown 2006). For the 1970-2010 study period the majority of the three target flatfish species caught in otter trawls were of juvenile size: California halibut (98.0%), English sole (95.3%), and speckled sanddab (69.1%). The high proportion of juveniles emphasizes the nursery function of the estuary.

Hypoxic conditions had negative effects on flatfish species in Elkhorn Slough. For the three target species (California halibut, English sole, and speckled sanddabs) there were significant declines in predicted presence as a function of decreasing dissolved oxygen (Table 5) (Figure 7). The positive correlation with DO and flatfish presence were consistent for both otter trawls and beach seine surveys for English sole and speckled sanddab, but were only significant for otter trawl surveys of California halibut. Temperature was also consistently included in the best-fit models using AIC, indicating that it is

also an important factor affecting flatfish. However, the correlation between species differed with both English sole and speckled sanddab increasing with decreased temperatures, and California halibut increasing with increased temperatures. Climatic and oceanographic conditions were also significant predictors in the logistic regression models, however, there were few consistent patterns among the species in both otter trawl and beach seine datasets, suggesting that local habitat conditions are more important factors influencing flatfish species in the estuary. Otter trawl surveys indicated that California halibut and speckled sanddab were positively correlated with increases in El Niño conditions. On the other hand, English sole were strongly correlated with increases in local upwelling, and this pattern was consistent for both otter trawls and beach seines.

Spatial modeling of flatfish presence and hypoxia determined the spatial extent of flatfish habitat quality in the estuary. The lower section of the main channel of Elkhorn Slough was the highest quality habitat for flatfish (Figure 8). Flatfish habitat quality generally worsened outside of the lower main channel of the estuary. Peripheral areas of the estuary where tides were restricted by water control structures were the poorest habitat for flatfish in the estuary. Spatial patterns of the predicted probability of flatfish presence were consistent with previous spatial modeling efforts (Figure 1) (Hughes et al. 2011). This indicated that eutrophication and the consequences of nutrient loading had negative effects on flatfish.

The logistic regression model as well as the spatial modeling of predicted probabilities was validated using the 2005 spatially explicit survey of 16 stations (Figure 1) around Elkhorn Slough (Table 6) (Figure 9). The logistic regression analysis of flatfish presence indicated that the probability of flatfish occurrence decreases with increased hypoxia. Sites that were historically hypoxic were devoid of flatfish during the 2005 surveys. The threshold for absence was ~ 4 mg/L DO (Figure 9), and this value was consistent to the lower threshold from the logistic regressions at the historical fish sampling locations (Figure 7). These results indicate that flatfish are sensitive to hypoxic conditions and that thresholds for complete absence are 4 mg/L DO, and declines in presence occur between 4 and 8 mg/L DO.

Drivers of the DOA

Along with the high spatial variability in hypoxic conditions (Hughes et al. 2011), there was also high temporal variation in hypoxia as revealed by the DOA. Analysis of the intra-annual variation among the sites in the estuary revealed the estuary generally was hypoxic during the summer months of June–October with DO conditions improving in the winter and spring (Figure 10).

There was high inter-annual variability in the DOA, which suggested external drivers of hypoxia in the estuary (Figure 11a). The periods from 1988–1994, 2000–2001, 2005–2006, and 2010–2001 were hypoxic regimes in the estuary followed by normoxic regimes from 1995–1999, 2002–2004, and 2007–2009. Three variables, DO concentration, temperature, and salinity, were highly correlated in space and time based on PCA (Table 7) (Figure 11a). PC1 explained both intra- and inter-annual variation in DO patterns in the estuary. Like the seasonal patterns shown in the DOA (Figure 10), PC1 explained similar seasonal patterns with increased hypoxia in the summer months that were highly correlated with increased temperature and salinity, followed by relaxation of hypoxia to normoxic conditions in the winter that were correlated lower temperature and salinity.

The PCA of key correlates of hypoxia in the estuary was validated at one of the continuous water quality monitoring stations. At the South Marsh station (Figure 1, #10) in the central portion of the estuary mean annual salinity was the key correlate of the annual lower limit of DO according to the stepwise

regression analysis (Table 8a) (Figure 11b). There was a negative correlation between the mean annual salinity and 10th percentile DO, indicating that increased freshwater inputs improve hypoxic conditions. However, at the Vierra Mouth station (Figure 1, #14) in the lower part of the estuary the lower DO limit was driven primarily by Monterey Bay upwelling conditions (Table 8b) (Figure 11c). Increased upwelling events were highly correlated with increases in hypoxia.

The results of the DOA resulted in positive effects of low salinity and temperature as well as decreases in the upwelling index on the hypoxic condition of the estuary. Climatic conditions along the eastern Pacific are driven by ENSO patterns, which in turn affect patterns of precipitation and wind-driven processes, such as upwelling. Increases in El Niño intensity results in increased local precipitation and can relax upwelling intensity (Friederich et al. 2002; Chavez 1996). The increase frequency and intensity in El Niño could benefit Elkhorn Slough's hypoxic condition and thus flatfish in two ways: 1) increased precipitation (as indicated by decreases in salinity) increases the flushing of the estuary which has been shown to decrease eutrophication and hypoxia in the estuary (Hughes et al. 2011), and 2) relaxation of local upwelling that brings hypoxic water from the deep sea over the continental shelf during intense upwelling years (Booth et al. 2012).

Given the evidence for salinity driven (Figure 11b) and upwelling driven (Figure 11c) hypoxia, we explored the potential indirect effects of ENSO conditions on DO and flatfish presence in the estuary. Modeled DO and probability of flatfish presence determined that increased El Niño intensity improved DO conditions and increased the proportion of suitable habitat for flatfish in Elkhorn Slough. During El Niño conditions the overall conditions of DO in the main channel of Elkhorn Slough had notably improved conditions which translated into improved probability of presence for all three target flatfish species (California halibut, English sole, and speckled sanddab) (Figure 12). During La Niña conditions DO generally decreased the habitat suitability for flatfish, and were of intermediate condition during La Niña periods. The spatial modeling results thus supported the indirect effects of ENSO on the fish habitat condition as a function of DO.

DISCUSSION

Ecosystem threats and the loss of biodiversity

Nearshore and estuarine ecosystems are currently under threat from multiple stressors, which include fishing pressure, climate change, and pollution (Jackson et al. 2001; Harley et al. 2006; and Nixon 1995). Increases in these pressures have caused shifts to marine communities because of perturbations that alter food-webs, fitness, and ultimately biodiversity (Fox et al. 2009; Vaquer-Sunyer and Duarte 2008; Worm & Lotze 2006). In this study we used an estuarine fish assemblage to assess the effects of anthropogenic nutrient pollution as the stressor. Long-term nutrient and dissolved oxygen data determined that Elkhorn Slough has received increasing nutrient inputs during the 40-year time series and that it had demonstrated symptoms of high nutrient loading and hypoxia starting in the early 1970s. Nitrate concentrations in the estuary started exceeding values expected from local upwelling (~0.14 – 0.28 mg/L) (Pennington and Chavez 2000) as early as the early 1970s. The consequences of this eutrophication include reductions in the diversity of the fish assemblage through significant declines in species richness and abundance.

Dissolved oxygen concentrations in Elkhorn Slough are symptomatic of a “hyperventilating estuary” (Beck and Bruland 2001). The productivity is high throughout the estuary, however that does not always result in the secondary symptoms of hypoxia (Hughes et al. 2010). Ironically, certain areas of the estuary benefit from the artificially increased tidal prism (due to engineering at the mouth) because of increased flushing potential of the estuary and thus enhancement of the overall water quality. Yet, the increased tidal prism has eroded important shallow-water habitat, such as marsh, and deepened the main channel and tidal creeks (Van Dyke and Wasson 2005). This combined with decades of increasing nutrient loading and sustained eutrophication should be considered two of the main drivers of the loss in fish diversity in the system, and therefore support previous studies documenting diversity losses from anthropogenic stressors.

During the two decades between 1990-2010 there were major declines in the diversity of the fish assemblage that link closely to hypoxic periods. During the hypoxic periods there were declines in 78% of species caught in otter trawls, yet there were no notable differences between hypoxia and normoxia for fish assemblages sampled by beach seines. When compared to the 1970s there were declines in fish presence during hypoxic years for 83% and 76% of species caught in otter trawls and beach seines, respectively. Yet when compared to the 1970s the fish presence during the 1990s-2000s normoxic periods declines were less severe than 1990s-2000s hypoxic periods because only 61% and 38% of species caught in otter trawls and beach seines, respectively. This result was reflected in the fish species richness among the three regimes using otter trawl data, where richness during the 1990s-2000s hypoxic period declined compared to the 1970s and the 1990s-2000s normoxic period. Analysis of species richness between the 1970s and normoxic 1990s-2000s showed no significant differences. Yet, there were significant differences in fish species richness between the 1970s and both normoxic and hypoxic periods of the 1990s-2000s in beach seine surveys, but no significant differences between the more recent normoxic and hypoxic periods.

Differences observed between otter trawl surveys and beach seines were likely due to differences in tidal height, where otter trawls sample more subtidal channel habitat and beach seines sample more intertidal mudflat habitat. Differences in the species assemblage as well as diversity between the more recent hypoxic and normoxic periods was likely due to decreases in bottom water DO in subtidal channels leading to net losses in available habitat to fish, as sampled by otter trawls. These fish may take refuge in shallower water where increased mixing occurs and thus less hypoxia. This conclusion is supported by the lack of significant differences in fish diversity in beach seines during the most recent decades. However, declines in fish diversity, as sampled by beach seine, when comparing the more recent decades to the 1970s indicated that there has been an overall loss in shallow habitat quality in the estuary. This may result in declines in diversity in shallow water habitats important for fish who use shallow water as a refuge from hypoxia.

There were also major declines in the abundance of fish from the 1970s to the 1990s-2000s as well as declines during the 1990s-2000s hypoxic periods when compare to normoxic conditions. This result was consistent with patterns in species richness. Unlike species richness, declines in abundances occurred in both otter trawl and beach seine data sets. This result indicates that since the 1970s there has been significant loss of fish diversity due to habitat loss, and the consequences of habitat loss are amplified during hypoxic periods in the estuary.

Losses in fish species richness and abundance may be due to a combination of avoidance behavior from hypoxia as well as loss of habitat for important prey items. Barry et al. (1996) demonstrated that certain fish species in Elkhorn Slough feed exclusively on specific invertebrate functional groups (i.e. benthic infauna or epifaunal crustaceans, infaunal worms, mollusks, or plankton). Therefore, losses in the function of shallow-water habitats due to eutrophication and hypoxia could deter certain fish species from using those areas. Out of the 18 species studied by Barry et al. (1996) from 1976-1980, five have severely declined or have gone locally extinct from the estuary (barred surfperch, black surfperch, starry flounder, white surfperch, northern anchovy) since the 1970s. This indicated that these species were negatively affected by loss of habitat in the estuary because they feed exclusively on certain invertebrate groups and since the 1970s was a hypoxic period in the estuary they were not as sensitive to this stress. However, other species listed in the Barry et al. (1996) study have prey or are themselves more sensitive to hypoxia because their populations declined significantly during hypoxic periods. These hypoxic sensitive species include speckled sanddab, English sole, sand sole, staghorn sculpins and longjaw mudsucker. Determining the specific mechanism (physical tolerances v. variation in prey abundance) for hypoxia effects on fish is difficult because benthic invertebrate diversity and abundances have been found to decline in more eutrophic parts of the estuary (Oliver et al. 2009).

Flatfish as indicator species

Flatfish in Elkhorn Slough as sampled in this study were sensitive to small changes in DO, and this was supported by multiple lines of evidence (e.g. logistic regression of the long-term fish and water quality data sets, as well as spatially explicit monitoring of sites around the estuary with varying water quality). These patterns of negative effects of hypoxia on flatfish are consistent with results from other studies showing the same negative trends (Eby and Crowder 2002; Stierhoff et al. 2006; Switzer et al. 2009). These results emphasize the utility of flatfish as ecosystem indicators. More specifically, flatfish as a group can be used as indicators of eutrophication and nutrient loading in coastal ecosystems.

In Elkhorn Slough the three target species used in this study had general trends of reduced presence in areas and periods of increased hypoxia. Spatial modeling determined that areas that had DO concentrations < 4 mg/L resulted in complete absence of flatfish as a functional group. In shallow water habitats English sole and speckled sanddab both had $< 10\%$ probability of presence. In Elkhorn Slough areas with a DO of < 4 mg/L were generally poor conditions for flatfish, and even small decreases below normoxia (4-8 mg/L) had markedly lower flatfish presence. Sites that frequently go hypoxic are numerous in Elkhorn Slough and are severely eutrophic. Hughes et al. (2011) determined that about 1/3 of the estuary is highly eutrophic, and results from this study indicate that those areas are generally devoid of flatfish. Spatial modeling of DO and flatfish presence determined that these peripheral areas are consistently hypoxic, and when combined with hypoxic years in the main channel could cause severe consequences for offshore adult populations in the following years due to significant declines in suitable nursery habitat. This reasoning is supported by a previous study by Brown (2006) who showed that Elkhorn Slough as an important nursery habitat for local populations of commercially important flatfish. More specifically, ~50% of locally caught English sole caught in central California were found to use the estuary as a nursery (Brown 2006).

Filters for coastal eutrophication and hypoxia

A decade ago, Cloern (2001) proposed the concept that environmental filters can mask the effects of nutrient loading in coastal areas. There have been exponential increases in nutrient concentrations in Elkhorn Slough in the last century. Nitrate concentrations at the mouth were consistently greater than local upwelling conditions and nitrate concentrations from the early 20th century. Recent decades consistently had concentrations that were on average 20-60 times greater than Monterey Bay upwelling. Results from this study as well as others (see Caffrey 2002) indicate that Elkhorn Slough is one of the most nutrient loaded estuaries in the world. Ironically, this intense nutrient loading has not caused increases in the severity of hypoxia since the 1970s. In fact, eutrophication as a function of hypoxia was worse in the 1970s. The estuary has benefited from an increased tidal prism in the estuary and increased precipitation in the last few decades that have increased the flushing of the main channel of the estuary. However, peripheral areas that have artificially reduced tidal ranges from levees and culverts, are experiencing hypoxia and anoxia that has persisted for several decades, which accounts for 1/3 of the estuarine area.

The expression of nutrient loading through eutrophication and hypoxia is controlled by intra and inter-annual environmental factors. Hypoxia generally increases in the summer months when temperature and productivity peaks. June is the one of the months when macroalgal production peaks in the estuary (B. Hughes, unpublished data). The peak in productivity can drive increased respiration rates that lead to increased hypoxia. June also coincides with local upwelling conditions in the estuary, which can have a two-fold effect on hypoxia through shoaling of the deep-water DO minimum layer that spills over the continental shelf (Novak et al. 2012) and into the estuary, upwelling also increases the fog layer, which can suppress productivity and lead to net increases in ecosystem respiration rates (Caffrey et al. 2010).

Eutrophication combined with environmental conditions have synergistic effects on local fish assemblages. On interannual scales we found that ENSO conditions drive a high degree of variability in DO and flatfish presence through indirect measures. First, increases in El Niño intensity is generally correlated with increased precipitation along the central coast of California (Friederich et al. 2002). This increase in rainfall increases flushing activity and decreases hypoxia, as evident by the negative correlation between annual lows in DO and mean annual salinity. Second, increased El Niño intensity suppresses upwelling (Chavez 1996) and therefore reduces the shoaling of the deep DO minimum layer over the continental shelf and into the estuary, resulting in better DO conditions in the lower part of the estuary. The effects of El Niño was supported by logistic regression correlations with increased California halibut and speckled sanddab presence with increased El Niño conditions. These results suggest that environmental conditions have important effects on the eutrophic condition of temperate north Pacific estuaries and should be considered when assessing the consequences of nutrient loading.

CONCLUSIONS AND RECOMMENDATIONS

We have established a framework that used estuarine fish communities as indicators for nutrient loading in northeast Pacific estuaries. In this study we took advantage of many long-term monitoring programs that have existed for decades in Elkhorn Slough. We conclude that:

- 1) *The estuary itself has been chronically nutrient loaded for over four decades, and because of this has allowed us to investigate the impacts of nutrient loading in coastal environments.*
- 2) *Fish species richness and abundance, particularly flatfish abundance, was a sensitive indicator of hypoxia. Hypoxia negatively affects fish assemblages, including commercially valuable flatfish, both temporally (in more hypoxic years) and spatially (in more hypoxic places).*
- 3) *El Niño conditions decreases the negative effects of nutrient loading, specifically hypoxia, through increased flushing during rainy years and suppression of upwelling that lowers the depth of the deep sea DO minimum layer.*
- 4) *Due to the strong environmental mediators on hypoxia there isn't a direct, immediate relationship between nutrient concentrations and negative impacts to beneficial uses (high nutrient years are better for fish due to freshwater flushing).*

Although it has been shown that returning an ecosystem to historic conditions after decades of eutrophication is nearly impossible (Duarte et al. 2008), we still encourage local managers to work on reducing the inputs to a system that has been under intense stress for over four decades. Improved land management has already resulted in improved water quality in small parts of the estuary (Gee et al. 2010). Indeed, our results suggest that recovery from the negative impacts of hypoxia may be fairly rapid, as demonstrated by the short-term increases in fish richness and abundance documented in years of lower hypoxia. Furthermore, sites with high hypoxia due to limited exchange would likely benefit almost immediately in their function as fish habitat if tidal range were increased to 1 m (about 50% of natural tidal exchange).

Determining consequences from indirect perturbations like nutrient loading is often difficult and needs multiple years of monitoring to accurately determine causation. Therefore, we recommend that managers and ecologists concerned about the state of an ecosystem establish long-term monitoring programs that are designed to answer questions on the consequences of natural and anthropogenic change. Lastly, given the economic fragility of funding for environmental monitoring, estuarine ecologists and managers can use fish monitoring as relatively inexpensive means to assessing ecosystem health. Comparative analysis of fish diversity and abundance combined with the presence of flatfish species outlined in this study are useful indicators of estuarine condition. We recommend that future studies should focus on the connectivity of estuarine and offshore fish populations to determine the indirect effect of nursery conditions on the offshore adult populations.

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Table 1. SIMPER analysis of presence/absence data for species contributing the greatest dissimilarity in **otter trawl** surveys between the 1970s to the (a) hypoxic and (b) normoxic regimes of the 1990s-2000s, and the (c) hypoxic and normoxic regimes of the 1990s-2000s. Bold numbers indicate the regime with greater presence.

(a)

Groups 1970s & Hypoxic

Average dissimilarity = 73.21

<i>Species</i>	<i>1970s %Presence</i>	<i>Hypoxic %Presence</i>	<i>Dissimilarity</i>	<i>Diss/SD</i>	<i>Contrib%</i>	<i>Cum.%</i>
Starry Flounder	0.94	0.11	8.17	1.59	11.15	11.15
White Surfperch	0.76	0.22	6.01	1.14	8.21	19.36
Black Surfperch	0.72	0.33	5.34	1.05	7.30	26.66
Staghorn Sculpin	0.71	0.41	5.27	0.91	7.20	33.86
Shiner Surfperch	0.78	0.44	5.23	0.97	7.14	41.00
Speckled Sanddab	0.49	0.74	4.92	0.87	6.72	47.73
English Sole	0.33	0.44	4.41	0.84	6.02	53.74
California Halibut	0.02	0.41	3.88	0.74	5.30	59.04
Cabazon	0.33	0.26	3.41	0.74	4.66	63.70
Pile Surfperch	0.35	0.22	3.30	0.79	4.51	68.21
Walleye Surfperch	0.30	0.04	2.49	0.63	3.39	71.60
Bat Ray	0.22	0.11	2.38	0.57	3.25	74.85
Plainfin Midshipman	0.23	0.04	2.08	0.55	2.83	77.69
Bay Pipefish	0.21	0.00	1.94	0.48	2.64	80.33
Pacific Herring	0.17	0.04	1.80	0.47	2.45	82.78
Dwarf Perch	0.15	0.15	1.72	0.57	2.34	85.13
Rockfish Brown	0.21	0.04	1.68	0.51	2.30	87.42
Northern Anchovy	0.16	0.00	1.52	0.40	2.08	89.50
Topsmelt	0.15	0.04	1.50	0.44	2.05	91.55

(b)

Groups 1970s & Normoxic

Average dissimilarity = 66.94

<i>Species</i>	<i>1970s %Presence</i>	<i>Normoxic %Presence</i>	<i>Dissimilarity</i>	<i>Diss/SD</i>	<i>Contrib%</i>	<i>Cum.%</i>
Starry Flounder	0.94	0.16	6.62	1.52	9.89	9.89
White Surfperch	0.76	0.25	5.08	1.14	7.58	17.47
Black Surfperch	0.72	0.25	4.76	1.11	7.11	24.58
Speckled Sanddab	0.49	0.81	4.24	0.91	6.34	30.92
English Sole	0.33	0.58	3.99	0.98	5.96	36.87
Shiner Surfperch	0.78	0.58	3.98	0.84	5.95	42.82
Staghorn Sculpin	0.71	0.72	3.57	0.74	5.33	48.15
Cabazon	0.33	0.25	3.12	0.75	4.67	52.82
Pile Surfperch	0.35	0.18	2.83	0.77	4.23	57.05
Walleye Surfperch	0.30	0.12	2.56	0.68	3.82	60.87
Plainfin Midshipman	0.23	0.19	2.38	0.67	3.55	64.42
Northern Anchovy	0.16	0.23	2.35	0.62	3.51	67.93
Pacific Herring	0.17	0.18	2.24	0.60	3.34	71.27

Table 1(b) continued.

Goby Arrow	0.15	0.19	2.20	0.59	3.29	74.56
Bat Ray	0.22	0.14	2.19	0.60	3.27	77.83
Pipefish	0.00	0.32	2.16	0.63	3.23	81.05
Brown Rockfish	0.21	0.12	1.96	0.59	2.92	83.98
Bay Pipefish	0.21	0.04	1.89	0.50	2.83	86.80
California Halibut	0.02	0.25	1.83	0.54	2.73	89.53
California Tonguefish	0.02	0.26	1.78	0.59	2.67	92.20

(c)

*Groups Hypoxic & Normoxic**Average dissimilarity = 67.80*

<i>Species</i>	<i>Hypoxic %Presence</i>	<i>Normoxic %Presence</i>	<i>Dissimilarity</i>	<i>Diss/SD</i>	<i>Contrib%</i>	<i>Cum.%</i>
Staghorn Sculpin	0.41	0.72	6.40	0.84	9.44	9.44
English Sole	0.44	0.58	5.82	0.80	8.59	18.03
Shiner Surfperch	0.44	0.58	5.46	0.85	8.06	26.09
California Halibut	0.41	0.25	5.25	0.71	7.74	33.83
Speckled Sanddab	0.74	0.81	4.97	0.60	7.32	41.15
Black Surfperch	0.33	0.25	3.83	0.75	5.64	46.80
Cabezon	0.26	0.25	3.60	0.67	5.32	52.11
Pipefish	0.15	0.32	3.41	0.67	5.03	57.15
California Tonguefish	0.19	0.26	2.99	0.69	4.42	61.56
White Surfperch	0.22	0.25	2.96	0.70	4.37	65.93
Pile Surfperch	0.22	0.18	2.57	0.65	3.8	69.73
Bat Ray	0.11	0.14	2.57	0.42	3.79	73.52
Northern Anchovy	0.00	0.23	2.04	0.50	3.01	76.52
Starry Flounder	0.11	0.16	2.03	0.51	2.99	79.51
Pacific Herring	0.04	0.18	2.00	0.45	2.95	82.46
Goby Arrow	0.00	0.19	1.88	0.45	2.77	85.23
Plainfin Midshipman	0.04	0.19	1.80	0.50	2.65	87.89
Walleye Surfperch	0.04	0.12	1.57	0.37	2.31	90.20

Table 2. SIMPER analysis showing the species contributing the greatest dissimilarity in **beach seine** surveys between the 1970s to the (a) hypoxic and (b) normoxic regimes of the 1990s-2000s, and the (c) hypoxic and normoxic regimes of the 1990s-2000s. Bold numbers indicate the regime with greater presence.

(a)

Groups 1970s & Hypoxic

Average dissimilarity = 64.73

<i>Species</i>	<i>1970s %Presence</i>	<i>Hypoxic %Presence</i>	<i>Dissimilarity</i>	<i>Diss/SD</i>	<i>Contrib%</i>	<i>Cum.%</i>
Starry Flounder	0.89	0.05	8.50	1.88	13.12	13.12
Black Surfperch	0.67	0.11	6.23	1.18	9.63	22.75
White Surfperch	0.67	0.11	5.85	1.19	9.04	31.79
Topsmelt	0.56	0.92	5.16	0.86	7.97	39.77
Goby Arrow	0.22	0.53	4.96	0.97	7.66	47.42
Northern Anchovy	0.44	0.21	4.78	0.87	7.39	54.82
Shiner Surfperch	0.67	0.61	4.73	0.88	7.30	62.11
Staghorn Sculpin	0.78	0.71	3.99	0.74	6.17	68.28
Bat Ray	0.22	0.26	3.48	0.71	5.38	73.66
Cabezon	0.33	0.21	3.42	0.80	5.29	78.95
Speckled Sanddab	0.33	0.16	3.23	0.77	4.99	83.94
Pacific Herring	0.22	0.05	2.55	0.54	3.94	87.88
Dwarf Perch	0.22	0.11	2.09	0.61	3.23	91.11

(b)

Groups 1970s & Normoxic

Average dissimilarity = 66.97

<i>Species</i>	<i>1970s %Presence</i>	<i>Normoxic %Presence</i>	<i>Dissimilarity</i>	<i>Diss/SD</i>	<i>Contrib%</i>	<i>Cum.%</i>
Starry Flounder	0.89	0.12	8.27	1.66	12.35	12.35
Black Surfperch	0.67	0.06	6.54	1.22	9.77	22.12
White Surfperch	0.67	0.06	6.12	1.22	9.13	31.25
Shiner Surfperch	0.67	0.32	5.55	1.05	8.28	39.53
Topsmelt	0.56	0.71	5.14	0.90	7.67	47.20
Northern Anchovy	0.44	0.09	4.89	0.86	7.31	54.51
Speckled Sanddab	0.33	0.44	4.46	0.92	6.65	61.16
Arrow Goby	0.22	0.38	4.22	0.82	6.31	67.47
Cabezon	0.33	0.15	3.29	0.76	4.91	72.38
Staghorn Sculpin	0.78	0.85	3.28	0.62	4.90	77.28
English Sole	0.22	0.26	3.19	0.74	4.76	82.04
Pacific Herring	0.22	0.12	2.98	0.60	4.45	86.49
Bat Ray	0.22	0.06	2.65	0.54	3.95	90.44

Table 2 (c) SIMPER output.

*Groups Hypoxic & Normoxic**Average dissimilarity = 56.17*

<i>Species</i>	<i>Hypoxic %Presence</i>	<i>Normoxic %Presence</i>	<i>Dissimilarity</i>	<i>Diss/SD</i>	<i>Contrib%</i>	<i>Cum.%</i>
Shiner Surfperch	0.61	0.32	6.93	0.96	12.34	12.34
Goby Arrow	0.53	0.38	6.76	0.91	12.03	24.37
Staghorn Sculpin	0.71	0.85	5.47	0.68	9.73	34.11
Speckled Sanddab	0.16	0.44	5.23	0.87	9.31	43.42
Topsmelt	0.92	0.71	4.77	0.61	8.50	51.92
Bat Ray	0.26	0.06	3.60	0.58	6.40	58.32
Northern Anchovy	0.21	0.09	3.13	0.56	5.57	63.89
Cabazon	0.21	0.15	3.07	0.61	5.47	69.37
English Sole	0.00	0.26	3.00	0.57	5.34	74.71
Threespine Stickleback	0.11	0.15	2.90	0.50	5.16	79.87
Slendernosed pipefish	0.08	0.15	2.13	0.47	3.79	83.66
Pacific Herring	0.05	0.12	1.78	0.41	3.16	86.82
California Halibut	0.13	0.03	1.73	0.39	3.08	89.90
Starry Flounder	0.05	0.12	1.71	0.41	3.04	92.94

Table 3. Results from a two-way ANOVA testing for the effects of season and regime on fish species diversity (square-root mean species richness/day) for (a) otter trawls and (b) beach seines. Significant values are in bold.

(a) Otter trawls

<i>Source</i>	<i>Type III Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F</i>	<i>P</i>
<i>Corrected Model</i>	23.550	11	2.141	4.89	0.000
<i>Regime</i>	4.428	2	2.214	5.057	0.007
<i>Season</i>	6.338	3	2.113	4.825	0.003
<i>Regime * Season</i>	4.173	6	0.695	1.588	0.154
<i>Error</i>	69.177	158	0.438		
<i>Total</i>	819.544	170			
<i>Corrected Total</i>	92.727	169			

(b) Beach seines

<i>Source</i>	<i>Type III Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F</i>	<i>P</i>
<i>Corrected Model</i>	23.245	10	2.324	5.207	0.000
<i>Regime</i>	5.918	2	2.959	6.628	0.002
<i>Season</i>	2.6	3	0.867	1.942	0.13
<i>Regime * Season</i>	9.751	5	1.95	4.368	0.002
<i>Error</i>	33.035	74	0.446		
<i>Total</i>	357.5	85			
<i>Corrected Total</i>	56.28	84			

Table 4. Results from two-way a ANOVA testing for the effects of season and regime on mean fish abundance (square-root mean abundance/day) for (a) otter trawls and (b) beach seines. Significant values are in bold.

(a) Otter trawls

<i>Source</i>	<i>Type III Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F</i>	<i>P</i>
<i>Corrected Model</i>	1505.633	11	136.876	6.891	0.000
<i>Regime</i>	496.173	2	248.086	12.49	0.000
<i>Season</i>	298.341	3	99.447	5.007	0.002
<i>Regime * Season</i>	264.094	6	44.016	2.216	0.044
<i>Error</i>	3138.401	158	19.863		
<i>Total</i>	13573.324	170			
<i>Corrected Total</i>	4644.034	169			

(b) Beach seines

<i>Source</i>	<i>Type III Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F</i>	<i>P</i>
<i>Corrected Model</i>	2248.951	10	224.895	7.152	0.000
<i>Regime</i>	347.468	2	173.734	5.525	0.006
<i>Season</i>	1069.367	3	356.456	11.335	0.000
<i>Regime * Season</i>	1476.695	5	295.339	9.392	0.000
<i>Error</i>	2327.039	74	31.446		
<i>Total</i>	9569.416	85			
<i>Corrected Total</i>	4575.99	84			

Table 5. Logistic regression results testing the effects of DO, temperature, salinity, nitrate, ENSO, PDO, NPGO, local upwelling, and daily sampling effort on presence/absence data for (a) California halibut, (b) English sole, and (c) speckled sanddab using both otter trawl and beach seine data. First, the best fitting model was determined using the lowest AIC value and we reported the best fitted model using multiple logistic regression. Last, the model was reduced down to using only DO as the predictor to test for generality of DO effects. Significant values are in bold.

(a) California halibut

Otter Trawls:

Best-fit Model				
<i>Source</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>P</i>
<i>Temperature</i>	0.136	0.075	1.811	0.0702
<i>DO</i>	0.200	0.107	1.867	0.0620
<i>ENSO</i>	0.604	0.260	2.328	0.0199
<i>AIC</i> = 142.02				

DO Model				
<i>Source</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>P</i>
<i>DO</i>	0.246	0.105	2.345	0.019

Beach Seines:

Best-fit Model				
<i>Source</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>P</i>
<i>Temperature</i>	1.071	0.474	2.261	0.0238
<i>NPGO</i>	-2.470	1.098	-2.248	0.0245
<i>Upwelling</i>	-0.053	0.026	-1.991	0.0464
<i>AIC</i> = 31.42				

DO Model				
<i>Source</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>P</i>
<i>DO</i>	-0.007	0.237	-0.028	0.978

Table 5. (continued) Logistic regression analysis.

(b) English sole

Otter trawls:

Best-fit Model				
<i>Source</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>P</i>
DO	0.183	0.093	1.983	0.0473
Upwelling	0.0178	0.004	4.705	0.0000003
<i>AIC</i> = 201.12				

DO Model				
<i>Source</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>P</i>
DO	0.256	0.087	-0.028	0.00326

Beach Seines:

Best-fit Model				
<i>Source</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>P</i>
Temperature	-0.595	0.215	-2.763	0.00573
Upwelling	0.020	0.007	2.647	0.00812
<i>AIC</i> = 99.90				

DO Model				
<i>Source</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>P</i>
DO	0.409	0.215	1.906	0.0566

Table 5. (continued) Logistic regression analysis.

(c) Speckled sanddab

Otter trawls:

Best-fit Model				
<i>Source</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z</i>	<i>P</i>
<i>Temperature</i>	-0.152	0.064	-2.392	0.0168
<i>DO</i>	0.209	0.094	2.203	0.0276
<i>ENSO</i>	0.489	0.221	2.215	0.0268
<i>AIC</i> = 212.7				

DO Model				
<i>Source</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>P</i>
<i>DO</i>	0.234	0.0917	2.551	0.0107

Beach Seines:

Best-fit Model				
<i>Source</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>P</i>
<i>Salinity</i>	-0.104	0.052	-2.01	0.0445
<i>Temperature</i>	-0.324	0.155	-2.096	0.0361
<i>DO</i>	0.357	0.191	1.866	0.0621
<i>PDO</i>	0.968	0.401	2.418	0.0156
<i>AIC</i> = 81.17				

DO Model				
<i>Source</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>P</i>
<i>DO</i>	0.347	0.158	2.2	0.0278

Table 6. Logistic regression analysis of the presence/absence of flatfish during two 2005 surveys at 16 locations in Elkhorn Slough as a function of 10th percentile of DO from 1989-2011.

<i>Source</i>	<i>Estimate</i>	<i>Std. Error</i>	<i>z value</i>	<i>P</i>
<i>DO</i>	2.751	1.339	2.054	0.04

Table 7. Principal components loadings for stations where fish and water quality data were consistently collected. Monthly raw values for DO (mg/L), temperature (°C), and salinity (ppt) were used to calculate the PC scores. The variance explained for each PC was reported in parentheses.

	<i>PC1 (39.5%)</i>	<i>PC2 (33.5%)</i>
DO	-0.541	0.709
Temperature	0.542	0.708
Salinity	0.774	0

Table 8. Stepwise multiple regression analysis of the significant predictors of the annual 10th percentile of DO at two stations: (a) South Marsh (Figure 1, #10) in the central portion of the estuary and (b) Vierra Mouth (Figure 1, #14) in the lower portion of the estuary. The predictor factors used in the model were the annual means of salinity and temperature for each site, as well as the annual index values for ENSO, PDO, NPGO, and Monterey Bay upwelling. Factors with a P-value > 0.10 were removed from the final model.

(a) South Marsh (central estuary)

Model Summary				
<i>Model</i>	<i>R</i>	<i>R Square</i>	<i>Adjusted R Square</i>	<i>Std. Error of the Estimate</i>
1	0.817 ^a	0.667	0.641	0.3018
^a Predictors: Constant, SM MeanSal				

ANOVA ^a						
<i>Model</i>		<i>Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F</i>	<i>P</i>
1	Regression	2.372	1	2.372	26.042	0.000^b
	Residual	1.184	13	0.091		
	Total	3.556	14			
^a Dependent Variable: SM_10th_DO						
^b Predictors: Constant, SM_MeanSal						

(b) Vierra Mouth (lower estuary)

Model Summary				
<i>Model</i>	<i>R</i>	<i>R Square</i>	<i>Adjusted R Square</i>	<i>Std. Error of the Estimate</i>
1	0.908 ^a	0.825	0.8	0.10527
^a Predictors: Constant, Upwelling				

ANOVA ^a						
<i>Model</i>		<i>Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F</i>	<i>P</i>
1	Regression	0.365	1	0.365	32.918	0.001^b
	Residual	0.078	7	0.011		
	Total	0.442	8			
^a Dependent Variable: VM_10thDO						
^b Predictors: Constant, Upwelling						

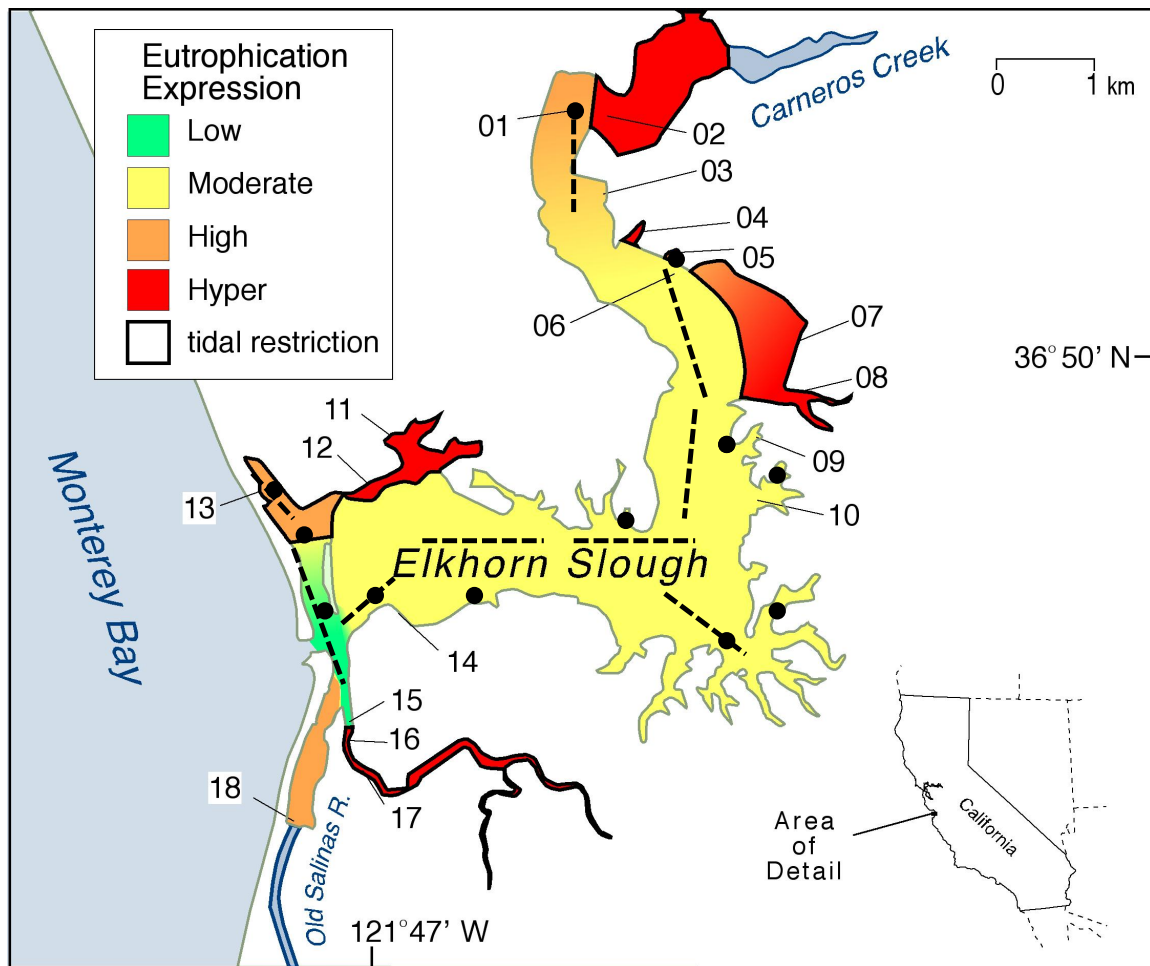


Figure 1. Extent of eutrophication in Elkhorn Slough from Hughes et al. (2011). The numbers are the ESNERR water quality monitoring stations as well as the 2005 slough-wide sampling stations from Ritter et al. (2008). Dots (•) indicate locations for historical beach seine surveys, and dashed lines (----) indicate approximate locations of historical trawl surveys.

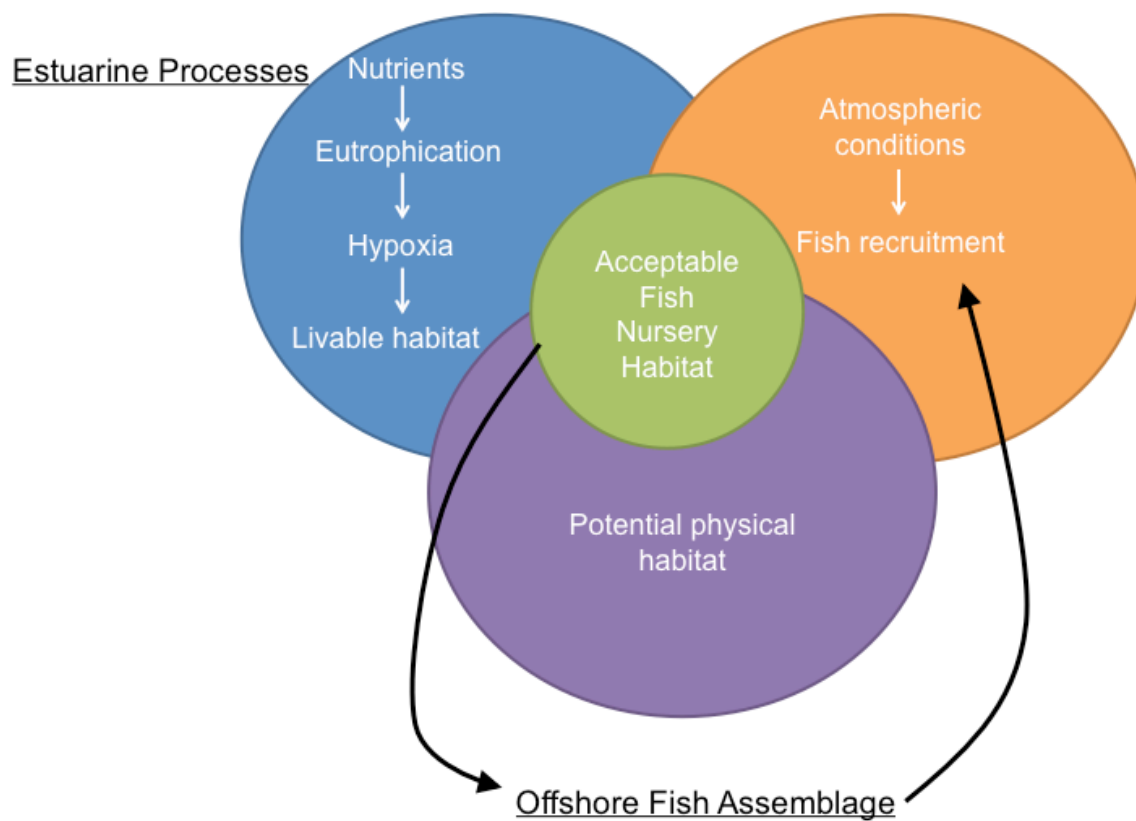


Figure 2. A generalized conceptual model of the synergistic processes affecting the estuarine fish assemblage and their relation to offshore fish assemblage.

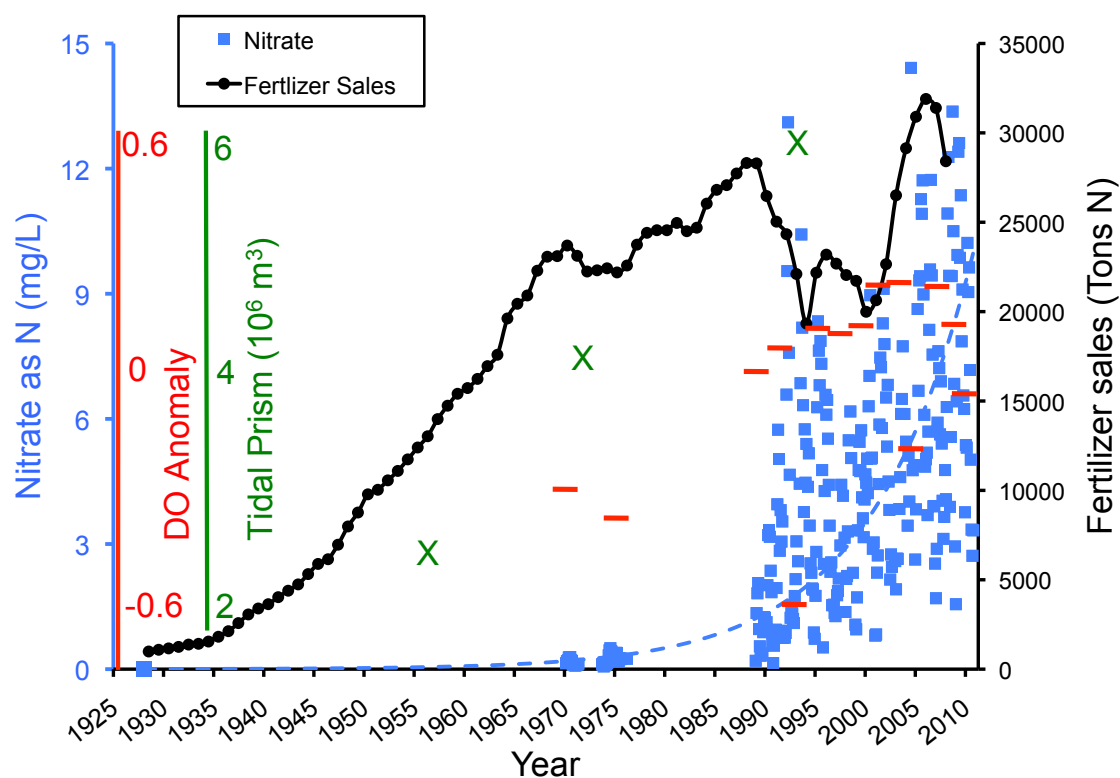
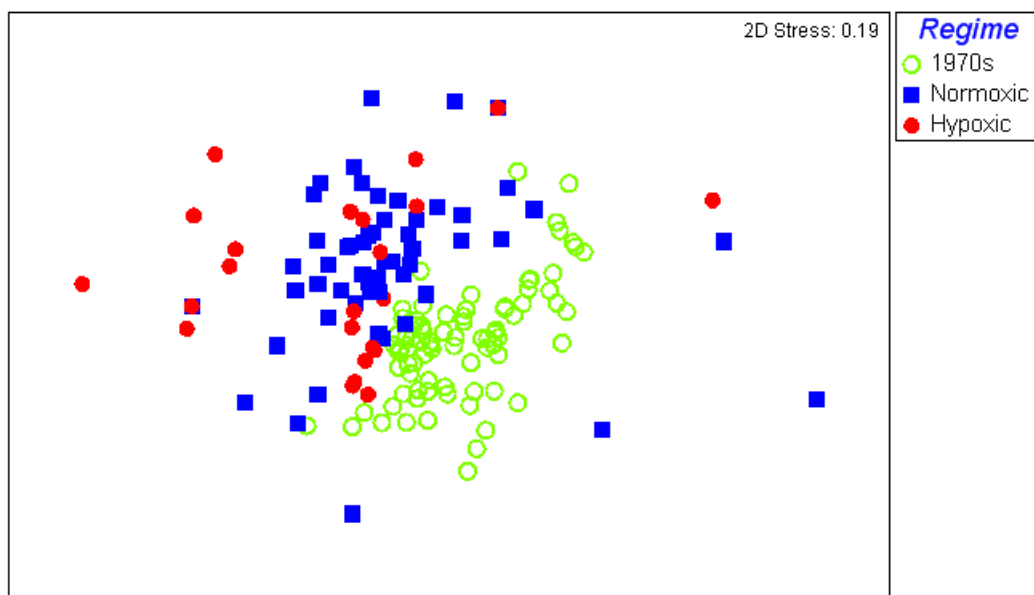


Figure 3. Threats to Elkhorn Slough as a function of fertilizer sales from the surrounding watershed (Monterey County), the three month moving average of nitrate in the lower slough, the two year running average of the DOA, and tidal prism estimated from three time periods.

(a) Otter trawls



(b) Beach Seines

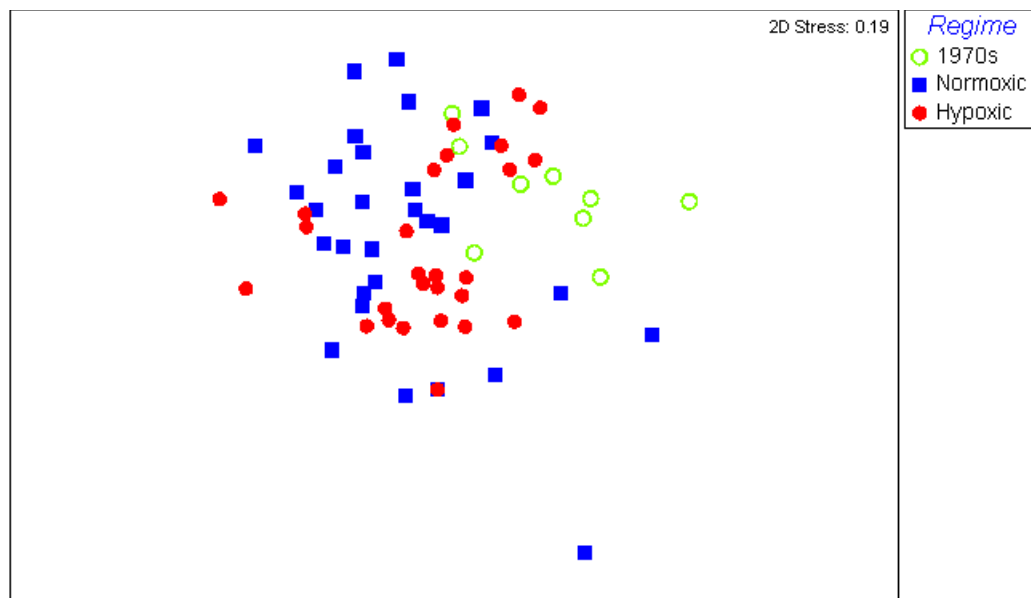
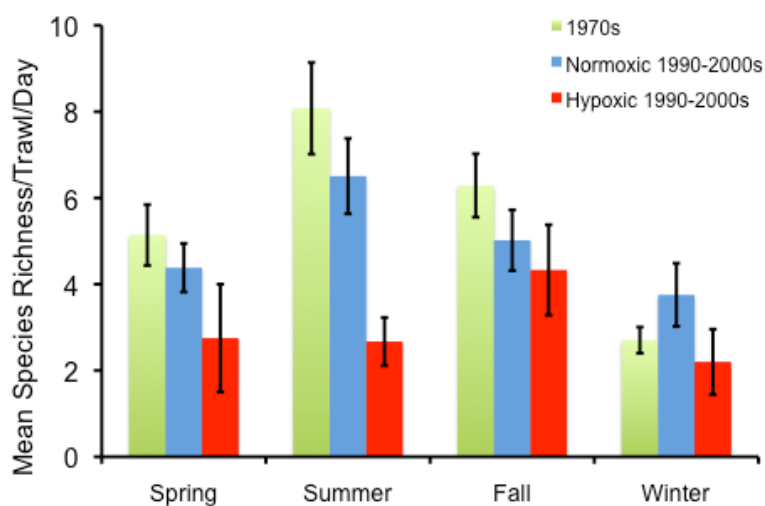


Figure 4. MDS plot showing differences in community structure between 1970s (hypoxic regime), and hypoxic and normoxic regimes from 1989-2010 for (a) otter trawls and (b) beach seines. Each point represents one sampling date, points closer in space have similar fish species composition. Points were labeled *a posteriori* by time period/hypoxic condition to illustrate differences in the fish assemblage.

(a) Otter trawls



(b) Beach seines

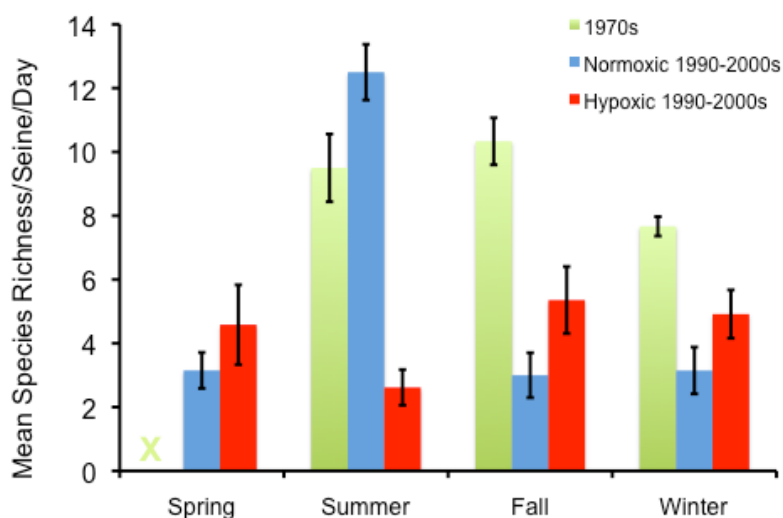
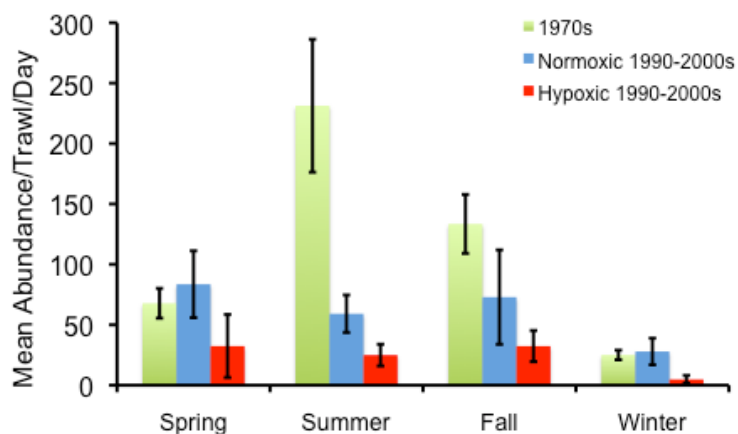


Figure 5. Comparison of fish diversity as a function of mean species richness/day for (a) otter trawls and (b) beach seines. See Table 3 for two-way ANOVA results. Note: there was no spring beach seine data from the 1970s. Error bars are $\pm 1SE$.

(a) Otter trawls



(b) Beach Seines

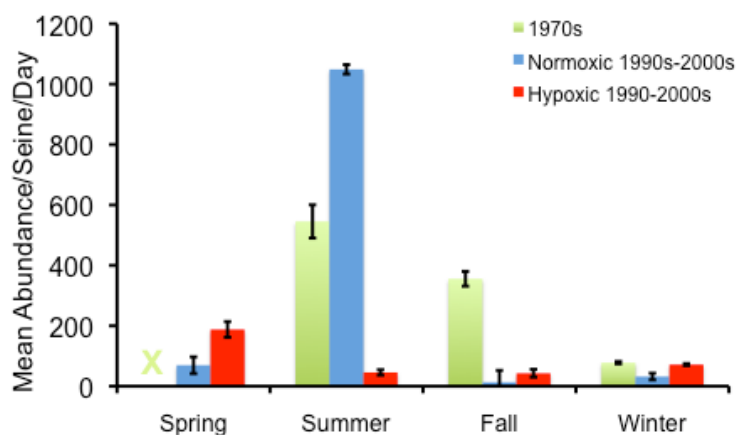


Figure 6. Comparison of fish abundance as a function of mean fish abundance/day for (a) otter trawls and (b) beach seines. See Table 4 for two-way ANOVA results. Note: there was no spring beach seine data from the 1970s. Error bars are $\pm 1SE$.

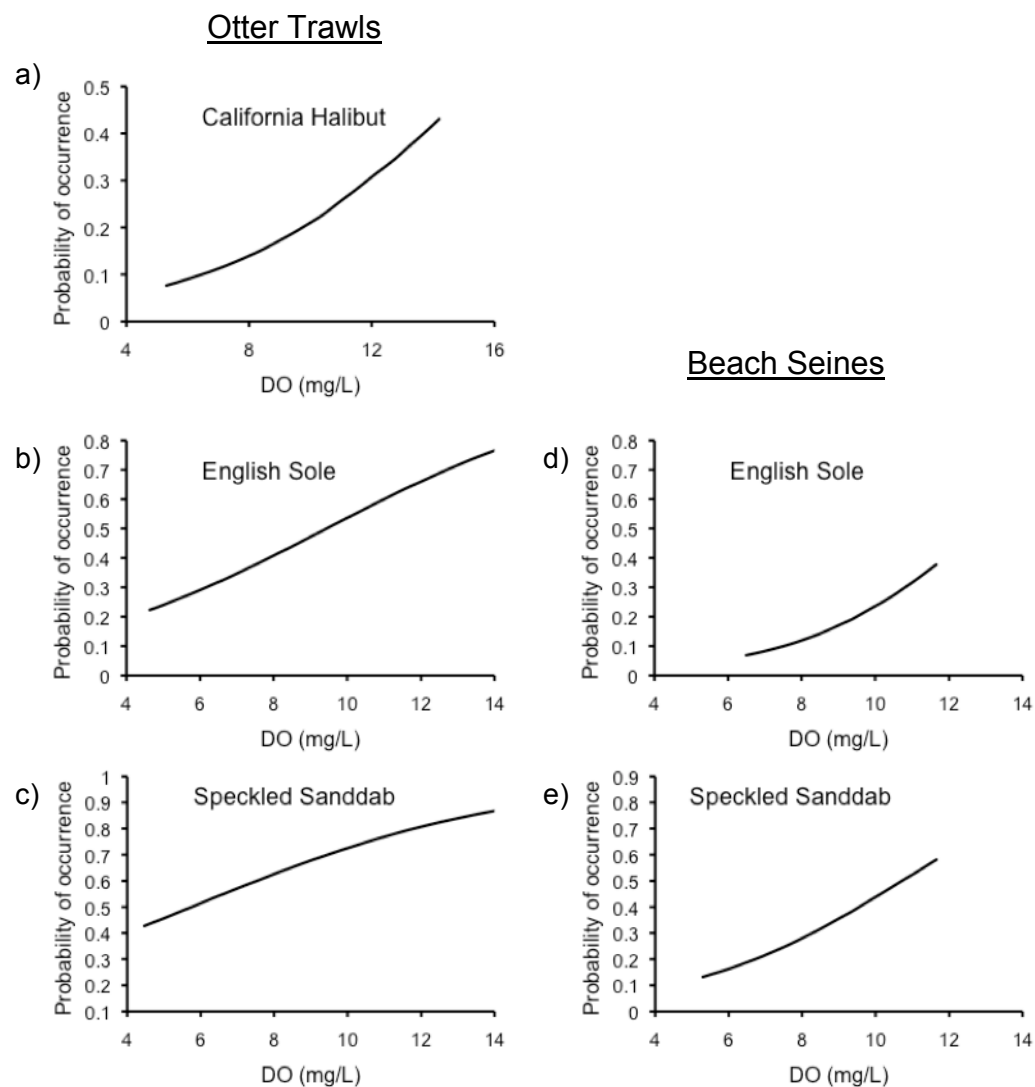


Figure 7. Logistic regression analysis of the predicted probability of presences for three species of flatfish as function of DO concentration (mg/L) for (a-c) otter trawls and (d-e) beach seines. California halibut did not have a significant relationship for beach seines. See Table 5 for logistic regression results.

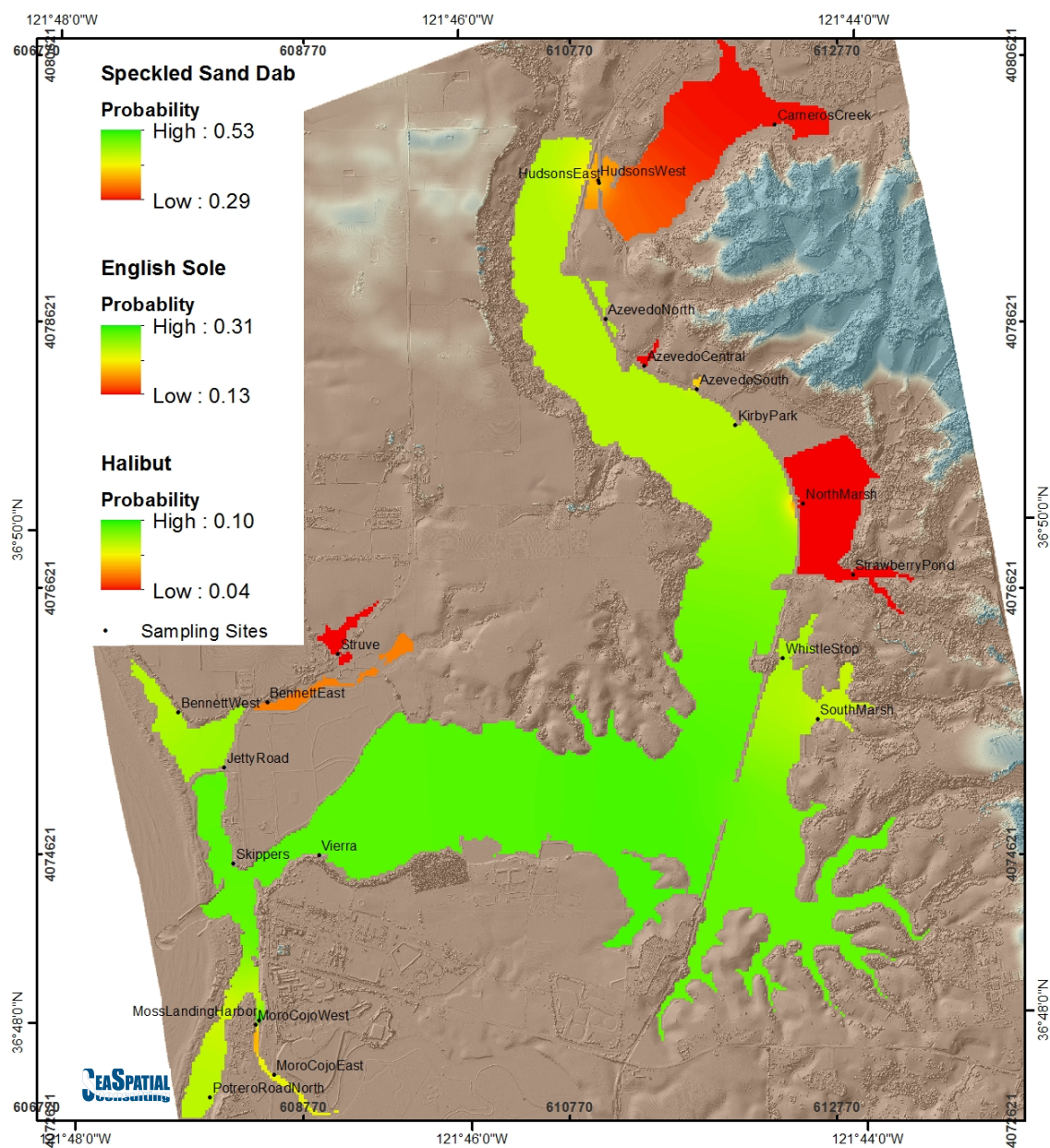


Figure 8. Predicted probabilities of presence of three flatfish species based on logistic regression analysis. Spatial probabilities were calculated based on the interpolated 10th percentile of DO (mg/L) from the 1988-2011 ESNERR water quality database. Probability scales for each species were adjusted to conform to the interpolated DO values.

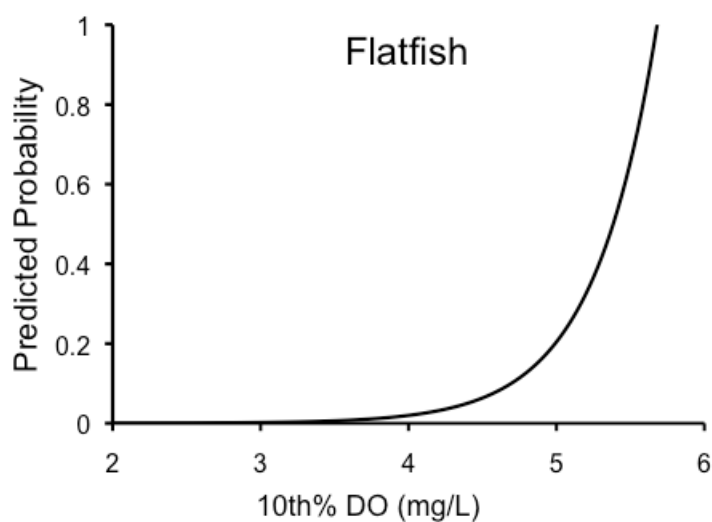


Figure 9. Logistic regression analysis of the predicted probability of flatfish occurrence during 2005 surveys at 16 locations (Figure 1) in Elkhorn Slough as a function of 10th percentile of DO from 1989-2011. See Table 6 for statistical results.

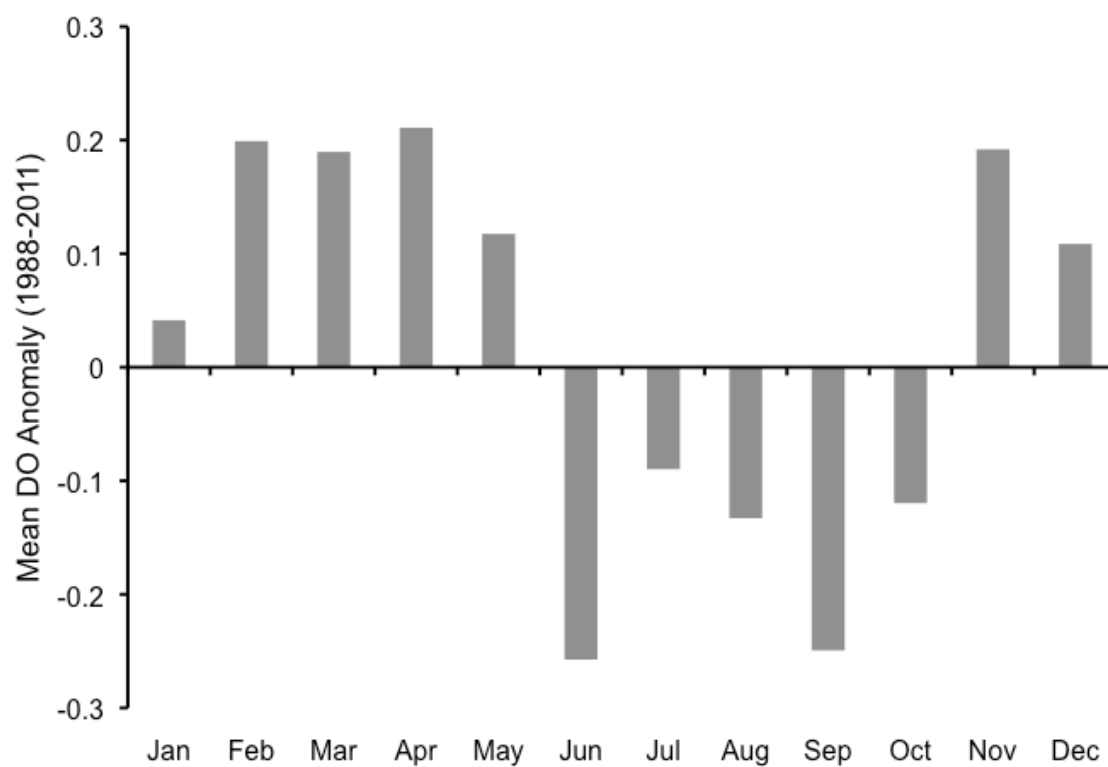


Figure 10. The mean monthly DOA for all water quality monitoring stations in Elkhorn Slough from 1988-2011.

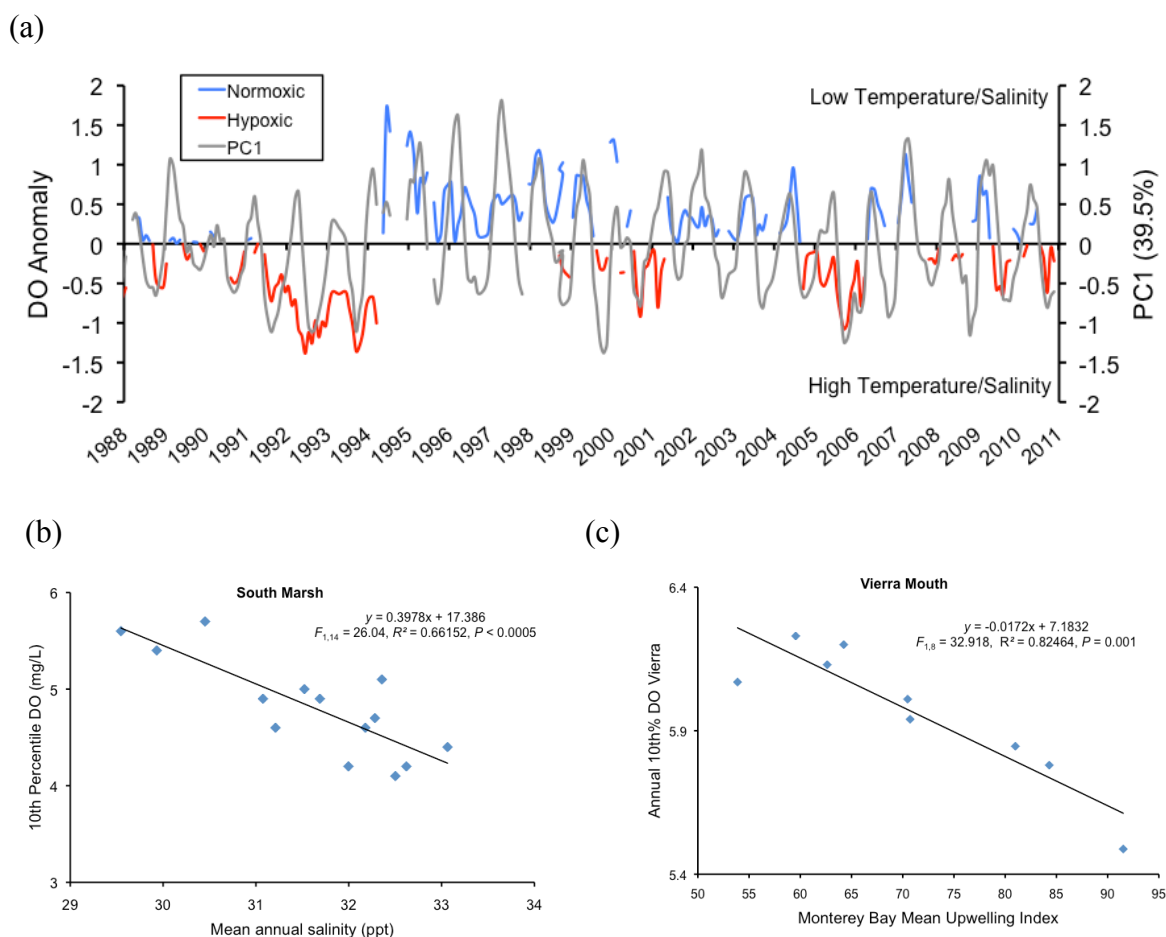


Figure 11. Key correlates with DO in Elkhorn Slough. (a) Time series of the three-month moving average of the DOA overlaid with annual PCA scores for salinity, temp, and DO (Table 7). The PC1 axis is labeled to indicate the correlation between hypoxia and temperature and salinity. (b) Relationship between the mean annual 10th percentile DO and salinity at SM. (c) Relationship between the mean annual 10th percentile DO and the mean annual upwelling index for Monterey Bay. Regression statistics reported in b-c were simple linear regressions and not the final stepwise regression model reported in Table 8.

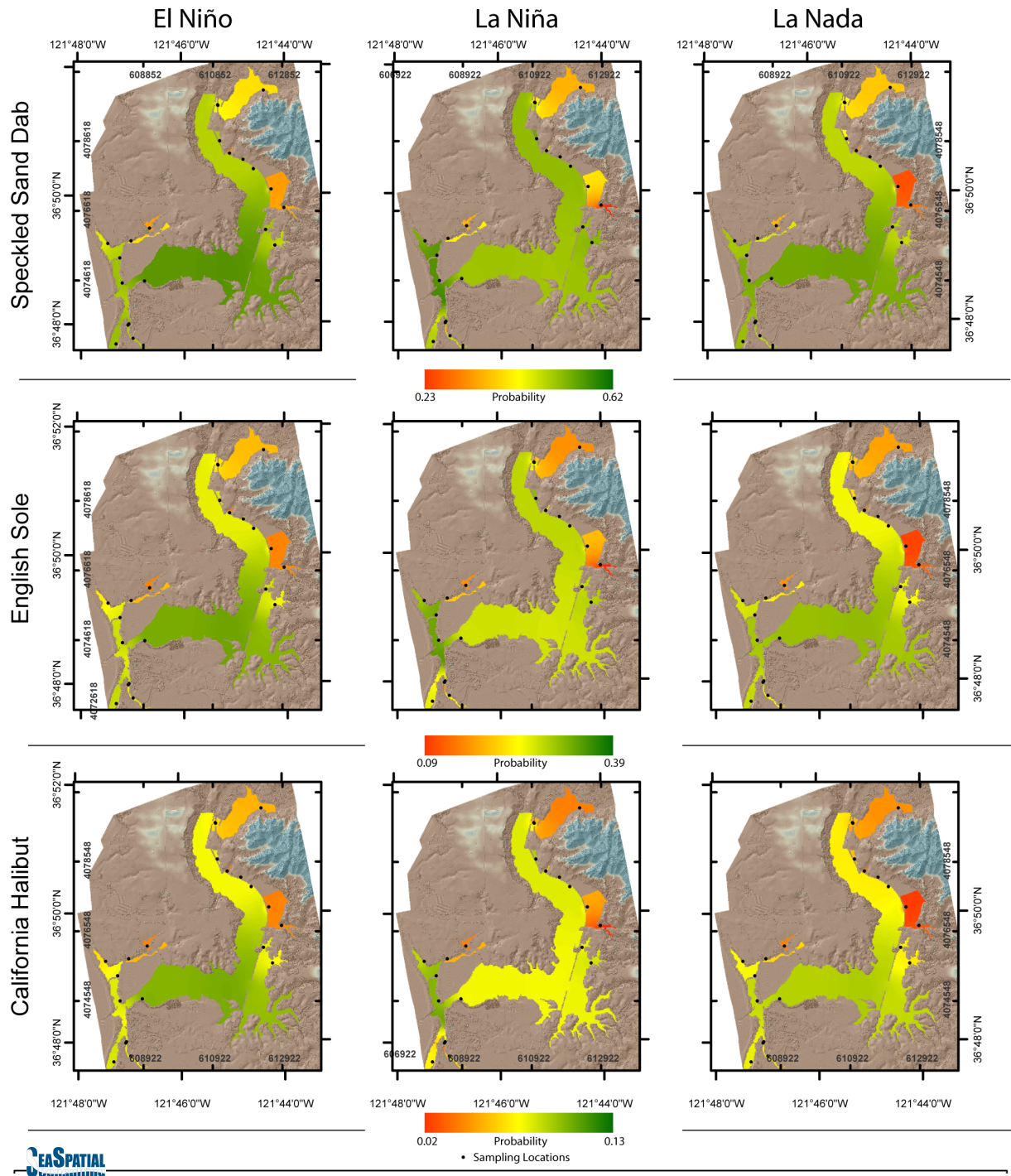


Figure 12. Predicted probabilities of presence of three flatfish species based on logistic regression analysis during different ENSO conditions. Spatial probabilities were calculated based on the interpolated 10th percentile of DO (mg/L) from the 1988-2011 ESNERR water quality database. Probability scales for each species were adjusted to conform to the interpolated DO values.

APPENDIX 1

Description of data sets

Elkhorn Slough flatfish data 1970-2010, Monterey Bay National Marine Sanctuary's (MBNMS) Sanctuary Integrated Monitoring Network (SIMoN), Nybakken et al. (1977), ESNERR

Fish data used in this study was primarily from the SIMoN database which was compiled from long-term Moss Landing Marine Labs (MLML) surveys, MLML and University of California Santa Cruz (UCSC) graduate theses, and various local scientists (i.e. UC Davis Granite Canyon Lab). The other data sets used for this study were sampled in similar locations using otter trawls and beach seines and included the following years 1974-76 (Nybakken et al. 1977), 2005 (Ritter et al. 2008), and 2009-2010 (ESNERR Fish monitoring program). These datasets did not include direct measurements of recruitment. However, it did include size class data collected seasonally that allowed us to indirectly determine recruitment. SIMoN dataset uses various gear types with beach seines and otter trawls being the most consistently caught over the entire time series. These are the gear types used in the analysis for this study.

Elkhorn Slough water quality and habitat data 1920s-2010, Elkhorn Slough National Estuarine Research Reserve (ESNERR)

ESNERR has been collecting data every 15 minutes since 1996, at one lower slough station and one middle slough station. Data measurements include: dissolved oxygen, temperature, salinity, pH, turbidity, and depth. Monthly water quality data, 1989-present, was collected at 24 stations around the estuary and surrounding watersheds. Data measurements include: nutrients (nitrate, phosphate, ammonia), chlorophyll *a*, dissolved oxygen, temperature, salinity, pH, turbidity, and depth. The primary data sets came from the following years: 1928 (MacGinitie 1935), 1970-71 (Smith 1973), 1974-76 Nybakken et al. (1977), 1977 (Central Coast Regional Water Quality Control Board), and 1988-present (ESNERR).

Monterey Bay oceanography 1928-2010, NOAA

Data sets from NOAA characterized oceanic and climatic conditions for El Niño Southern Oscillation Index (1), Pacific Decadal Oscillation (2), and local (Monterey Bay) upwelling indices (3), and North Pacific Gyre Oscillation (4), used as independent variables for explaining variation in flatfish populations.

(1) <http://www.esrl.noaa.gov/psd/enso/mei/table.html>

(2) <http://jisao.washington.edu/pdo/PDO.latest>

(3) http://www.pfeg.noaa.gov/products/PFEL/modeled/indices/upwelling/NA/upwell_menu_NA.html

(4) <http://www.o3d.org/npgo/npgo.php>

APPENDIX 2

Table 1. Species list of all fish species caught in otter trawls and beach seined during the 1970-2010 study period. Species are sorted by their rank abundance starting with the most abundant going down. Rarity was noted by R (< 1 % presence in total surveys) or C for common (> 1 % presence in total surveys). Loss of species since the 1970s or during 1990s-2000s Normoxia is noted with a “-“ and gain of species since the 1970s or during 1990s-2000s Normoxia is noted with a “+”, if the species was detected in both periods (i.e. both 1990s-2000s Normoxia and Hypoxia) then it was noted with a “+/-“.

Species Name	Common Name	Rank	Rare (<1% Presence)	Presence since 1970s	Normoxia Presence
<i>Leptocottus armatus</i>	Staghorn sculpin	1	C	+/-	+/-
<i>Cymatogaster aggregata</i>	Shiner surfperch	2	C	+/-	+/-
<i>Phanerodon furcatus</i>	White seaperch	3	C	+/-	+/-
<i>Platichthys stellatus</i>	Starry flounder	4	C	+/-	+/-
<i>Embiotoca jacksoni</i>	Black surfperch	5	C	+/-	+/-
<i>Citharichthys stigmaeus</i>	Speckled sanddab	6	C	+/-	+/-
<i>Atherinops affinis</i>	Topsmelt	7	C	+/-	+/-
<i>Hyperprosopon argenteum</i>	Walleye surfperch	8	C	+/-	+/-
<i>Parophrys vetulus</i>	English sole	9	C	+/-	+/-
<i>Scorpaenichthys marmoratus</i>	Cabazon	10	C	+/-	+/-
<i>Psettichthys melanostictus</i>	Sand sole	11	C	+/-	+
<i>Damalichthys vacca</i>	Pile surfperch	12	C	+/-	+/-
<i>Atherinopsis californiensis</i>	Jacksmelt	13	C	+/-	+/-
<i>Engraulis mordax</i>	Northern anchovy	14	C	+/-	+/-
<i>Paralichthys californicus</i>	California halibut	15	C	+/-	+/-
<i>Rhacochilus toxotes</i>	Rubberlip surfperch	16	C	+/-	+
<i>Clevelandia ios</i>	Arrow goby	17	C	+/-	+/-
<i>Clupea pallasii</i>	Pacific herring	18	C	+/-	+/-
<i>Triakis semifasciata</i>	Leopard shark	19	C	+/-	+/-
<i>Symphurus atricauda</i>	California tonguefish	20	C	+/-	+/-
<i>Myliobatis californica</i>	Bat ray	21	C	+/-	+/-
<i>Acanthogobius flavimanus</i>	Yellowfin goby	22	C	+/-	+/-
<i>Sebastes paucispinnis</i>	Bocaccio	23	C	+/-	+
<i>Genyonemus lineatus</i>	White croaker	24	C	-	N/A
<i>Sebastes mystinus</i>	Blue rockfish	25	C	-	N/A
<i>Porichthys notatus</i>	Plainfin midshipman	26	C	+/-	+/-
<i>Micrometrus minimus</i>	Dwarf perch	27	C	+/-	+/-
<i>Syngnathus leptorhynchus</i>	Bay pipefish	28	C	+/-	+/-
<i>Hexagrammos decagrammus</i>	Kelp greenling	29	C	-	N/A
<i>Gillichthys mirabilis</i>	Longjaw mudsucker	30	C	+/-	+
<i>Ophiodon elongatus</i>	Lingcod	31	C	+/-	+/-
<i>Citharichthys sordidus</i>	Pacific sanddab	32	C	+/-	-
<i>Hypsopsetta guttulata</i>	Diamond turbot	33	C	+/-	+/-

Appendix 2. Table 1 (continued)

Species Name	Common Name	Rank	Rare (<1% Presence)	Presence since 1970s	Normoxia Presence
	Threespine stickleback	34	C	+/-	+/-
<i>Gasterosteus aculeatus</i>	stickleback	34	C	+/-	+/-
<i>Sebastes auriculatus</i>	Brown rockfish	35	C	+/-	+/-
<i>Hyperprosopon ellipticum</i>	Silver surfperch	36	C	+/-	+/-
<i>Neoclinus uninotatus</i>	Onespot fringehead	37	C	+/-	+
<i>Rhinobatos productus</i>	Shovelnose guitarfish	38	C	+/-	+/-
<i>Mustelus californicus</i>	Grey smoothhound	39	C	+/-	+
<i>Lepidogobius lepidus</i>	Bay goby	40	C	+/-	+/-
<i>Hypsurus caryi</i>	Rainbow surfperch	41	C	+/-	+/-
<i>Roccus saxatilis</i>	Striped bass	42	C	-	N/A
<i>Platyrrhinoidis triseriata</i>	Thornback	43	C	+/-	+/-
<i>Mustelus henlei</i>	Brown smoothhound	44	C	+/-	+
	Kelp pipefish/ Great pipefish				N/A
<i>Syngnathus californiensis</i>	pipefish	45	C	-	
<i>Amphistichus argenteus</i>	Barred surfperch	46	C	-	N/A
<i>Pleuronichthys decurrens</i>	Curlfin sole	47	R	+/-	+
<i>Trachurus symmetricus</i>	Jack mackerel	48	R	-	N/A
<i>Dorosoma petenense</i>	Threadfin shad	49	R	+/-	+
<i>Amphistichus rhodoterus</i>	Redtail surfpech	50	R	-	N/A
<i>Gibbonsia metzi</i>	Striped kelpfish	51	R	-	N/A
<i>Girella nigricans</i>	Opaleye	52	R	+	+
<i>Spirinchus starksi</i>	Night smelt	53	R	-	N/A
<i>Amphistichus koelzi</i>	Calico surfperch	54	R	+/-	+
<i>Urobatis halleri</i>	Round stingray	55	R	+/-	+/-
<i>Merluccius productus</i>	Pacific hake	56	R	+/-	+
<i>Micrometrus aurora</i>	Reef perch	57	R	-	N/A
<i>Sardinops sagax</i>	Pacific sardine	58	R	+/-	-
<i>Sebastes carnatus</i>	Gopher rockfish	59	R	+/-	+
<i>Sebastes flavidus</i>	Yellowtail rockfish	60	R	+/-	+
<i>Hexagrammos superciliosus</i>	Greenling	61	R	+	+/-
<i>Seriphus politus</i>	Queenfish	62	R	-	N/A
	Slendernosed pipefish				N/A
<i>Syngnathus griseolineatus</i>	pipefish	63	R	-	
<i>Coryphopterus nicholsi</i>	Blackeye goby	64	R	-	N/A
<i>Gibbonsia montereyensis</i>	Scarlet kelpfish	65	R	+	-
<i>Glyptocephalus zachirus</i>	Rex sole	66	R	-	N/A
<i>Hippoglossus stenolepis</i>	Pacific halibut	67	R	-	N/A
<i>Lepidopsetta bilineata</i>	Rock sole	68	R	-	N/A
<i>Microstomus pacificus</i>	Dover sole	69	R	+/-	+
<i>Palometa simillina</i>	Pacific pompano	70	R	-	N/A
<i>Pleuronichthys verticalis</i>	Hornyhead turbot	71	R	-	N/A
<i>Sebastes caurinus</i>	Copper rockfish	72	R	-	N/A
<i>Sebastes goodei</i>	Chillipepper rockfish	73	R	-	N/A
<i>Tridentiger trigonocephalus</i>	Chameleon goby	74	R	+	+

Appendix 2. Table 1 (continued)

Species Name	Common Name	Rank	Rare (<1% Presence)	Presence since 1970s	Normoxia Presence
<i>Allosmerus elongatus</i>	Whitebait smelt	75	R	-	N/A
<i>Hippoglossina stomata</i>	Bigmouth sole	76	R	-	N/A
<i>Sebastes melanops</i>	Black rockfish	77	R	+	+
	Black-striped				N/A
<i>Sygnathus abaster</i>	pipefish	78	R	-	
<i>Leuresthes tenuis</i>	California grunion	79	R	+	+
<i>Zoarces</i> sp.	Eelpout	80	R	+	+
<i>Eucyclogobius newberryi</i>	Tidewater goby	81	R	-	N/A
<i>Sebastes rastrelliger</i>	Grass rockfish	82	R	+/-	+/-
<i>Cymatogaster gracilis</i>	Island surfperch	83	R	-	N/A
<i>Brachyistius frenatus</i>	Kelp perch	84	R	-	N/A
<i>Sebastes atrovirens</i>	Kelp rockfish	85	R	+	-
<i>Spirinchus thaleichthys</i>	Longfin smelt	86	R	+	+
<i>Sebastes serranoides</i>	Olive rockfish	87	R	-	N/A
	Red-eye round				-
<i>Etrumeus teres</i>	herring	88	R	+	
<i>Sebastes dalli</i>	Calico rockfish	89	R	-	N/A
<i>Pholis ornata</i>	Saddleback gunnel	90	R	-	N/A
<i>Artedius harringtoni</i>	Scalyhead sculpin	91	R	-	N/A
<i>Cottus asper</i>	Prickly sculpin	92	R	-	N/A
<i>Oxyjulus californica</i>	Senorita	93	R	+/-	N/A
Liparidae	Snailfish	94	R	+	+
<i>Pleuronichthys ritteri</i>	Spotted turbot	95	R	+	-
<i>Chilara taylori</i>	Spotted Cuskeel	96	R	+/-	+
<i>Xysteuryx liolepis</i>	Fantail sole	97	R	+	+