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Effects of Hypoxia on Fish Survival and Oyster Growth in a Highly Eutrophic Estuary

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Abstract Human land use activities around estuaries can result in high levels of eutrophication. At Elkhorn Slough estuary, a highly eutrophic California estuary, we investigated the effects of impaired water quality on two stress-tolerant estuarine species, a common fish, the staghorn sculpin, *Leptocottus armatus* and a foundational invertebrate, the Olympia oyster, *Ostrea lurida*. We caged the two indicator species at six wetlands with different levels of water quality impairment, four of which had restricted tidal flow. We also recorded water quality parameters simultaneously at all sites using YSI sondes, and sampled nutrients and chlorophyll-a monthly, building on the National Estuarine Research Reserve System-wide Monitoring Program. We found that the monitored environmental variables predicted ecological responses by the indicator species. In particular, we found that the duration and severity of hypoxia were negatively correlated with fish survival and oyster growth. Further, our results corroborate previous studies that artificial tidal restriction leads to increased hypoxia stress. We conclude that large diurnal fluctuations in dissolved oxygen and extended nighttime hypoxia can have lethal and sub-lethal effects even on stress-tolerant organisms in the estuary. While laboratory experiments have often shown such effects, it is relatively rare to demonstrate negative effects of oxygen variation with in situ experiments, which provide stakeholders with concrete evidence for impaired water quality at local wetlands. Tidally restricted sites, which experience the largest fluctuations in dissolved oxygen and longest periods of hypoxia, harbor conditions harmful to vertebrates and invertebrates in the estuary. Reversing the anthropogenically induced low oxygen levels, by restoring more natural tidal exchange and by decreasing agricultural runoff, could improve the survival and growth of important estuarine organisms.

Keywords Water quality · Oxygen · Anthropogenically altered · *Leptocottus* · *Ostrea*

The original version of this article was revised: The first sentence in the Discussion section of the original article was incomplete.

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Introduction

Estuaries are highly productive ecosystems and support distinctive communities of plants and animals (Beck et al. 2001, Cronin and Mansueti 1971). However, estuaries are highly altered by human activities (Edgar et al. 2000, Kennish 2001). Nutrient loading is one common stressor to estuaries and can result in eutrophication (Cloern 2001, Nixon 1995, Vitousek et al. 1997). Anthropogenic nutrient input to coastal waters has been suggested to be a contributing factor to water column hypoxia (Diaz and Rosenberg 2008, Turner and Rabalais 1994, Vitousek et al. 1997), particularly in areas with little circulation or extensive stratification (Caffrey et al. 2003, Rabalais et al. 2009, Vitousek et al. 1997). Hypoxia is well known to negatively impact many estuarine organisms (Diaz and Rosenberg 1995, Rabalais et al. 1994, Renaud 1986, Vaquer-Sunyer and Duarte 2008). These impacts can vary in severity from sub-lethal effects on movement, behavior, and reproduction to severe effects which can cause mortality or leave "dead zones" in large swaths of the ocean (Rabalais et al. 2002, Vaquer-Sunyer and Duarte 2008).

The Elkhorn Slough estuary in central California supports hundreds of fish, invertebrate, and bird species and hosts one of the most extensive salt marshes in the state outside of San Francisco Bay (Caffrey et al. 2002a). The estuary lies in an agricultural watershed and is subject to intense nutrient loading (Caffrey et al. 2002b, 2003, Hughes et al. 2011, 2013). Symptoms of eutrophication are spatially variable in the estuary, with low to moderate expression of eutrophication in strongly marine-influenced areas near the mouth of the estuary and high to hyper-eutrophic conditions in wetlands with long residence times, especially those behind water control structures (Hughes et al. 2011). Despite strong scientific evidence of water quality degradation, many people who visit the estuary for recreation—to kayak, birdwatch, or fish—are unaware of compromised water quality. The Central Coast Regional Water Quality Control Board (CCRWQCB) which develops and implements water quality regulations for the estuary suggests that beneficial uses of Elkhorn Slough are impaired by factors such as sedimentation/silt, pesticides, or low dissolved oxygen (Adams 2009). While CCRWQCB recognizes data on high nutrient concentrations and low-oxygen conditions are available and of concern, CCRWQCB has requested concrete evidence of negative effects of these conditions. The goal of our study was to examine how variation in water quality in contrasting wetlands affects two disparate estuarine indicator species in Elkhorn Slough estuary. We hypothesized that poor water quality would decrease invertebrate growth rates and increase fish mortality. While controlled laboratory experiments can yield cleaner results for assigning causality to particular variables, we chose to conduct in situ field experiments to determine how water quality conditions affect common organisms in the estuary. By demonstrating how wetland conditions affect local organisms, our experiments can be used to inform community stakeholders and regional policy. One example of such regional policy is the Water Quality Control Plan for the Central Coastal Basin—"The Basin Plan" (Saiz 2011) developed by CCRWQCB. The Basin Plan defines the need for developing total maximum daily loads (TMDLs) for nitrogen compounds and orthophosphate for the Central Coast Region. Our investigation is also an example of how biological studies can complement the National Estuarine Research Reserve System-wide Monitoring Program (NERR SWMP) (NERRS 2010) for water quality, in this case through linkages between NERR SWMP monitoring data and responses of biological indicators.

Materials and Methods

Study Sites

To test for the effects of varying water quality on growth and survival of key estuarine species, we conducted an in situ transplant experiment in Elkhorn Slough. The estuary has a main channel, and many adjacent side channels, some of which experience full tidal exchange while others have reduced tidal exchange due to dikes and water control structures. Two of the study sites had unrestricted tidal exchange (Fig. 1, F1, F2). Four of the sites (Fig. 1, R1–R4) had variable tidal exchange restricted by water control structures. The sites with restricted tidal exchange frequently experience large fluctuations in dissolved oxygen, especially in summer (Beck and Bruland 2000). Dissolved oxygen levels range from 18 mg/L (Beck and Bruland 2000) during the day when photosynthesis peaks to 0 mg/L at night, primarily between midnight and sunrise when both algae and consumers in the water column and benthos respire. The duration of extreme hypoxia (0–2.5 mg/L) can be up to 6 h, and the frequency can occur daily in the summer while more rarely in other seasons of the year.

Study Species

We chose two species that are common in estuarine systems along the west coast of the USA and that have high stress tolerances associated with estuarine species. The species used in this study were the staghorn sculpin, *Leptocottus armatus*, and the Olympia oyster, *Ostrea lurida*. Staghorn sculpin is the most common fish species found in Elkhorn Slough according to surveys from 1970 to 2010 (Hughes et al. 2012) and is distributed broadly in the estuary (Yoklavich et al. 2002); however, the frequency of detecting the species in surveys has been documented to drop by 31 % during hypoxic periods in the estuary (Hughes et al. 2012). Staghorn sculpin is tolerant because of its wide tolerance range of temperatures, salinities (Morris 1960, Yoklavich et al. 2002), and dissolved oxygen levels (Wagner 1990). The Olympia oyster, likewise, is widely tolerant to highly variable conditions in salinity, temperature, and dissolved oxygen levels (Baker 1995, Wasson et al. 2015). This native oyster was once common in the estuary, but now occurs in very low abundance, putting it at risk for local extinction (Wasson 2010).

Experimental Design and Monitoring

At each site, we deployed five staghorn sculpins and six Olympia oysters on July 18, 2012. Fish size was not measured since we expected a short experimental duration and no major changes in size. Oysters were measured (longest dimension quantified with calipers) prior to the start of the experiment. The fish were contained in plastic minnow traps (43 cm long,

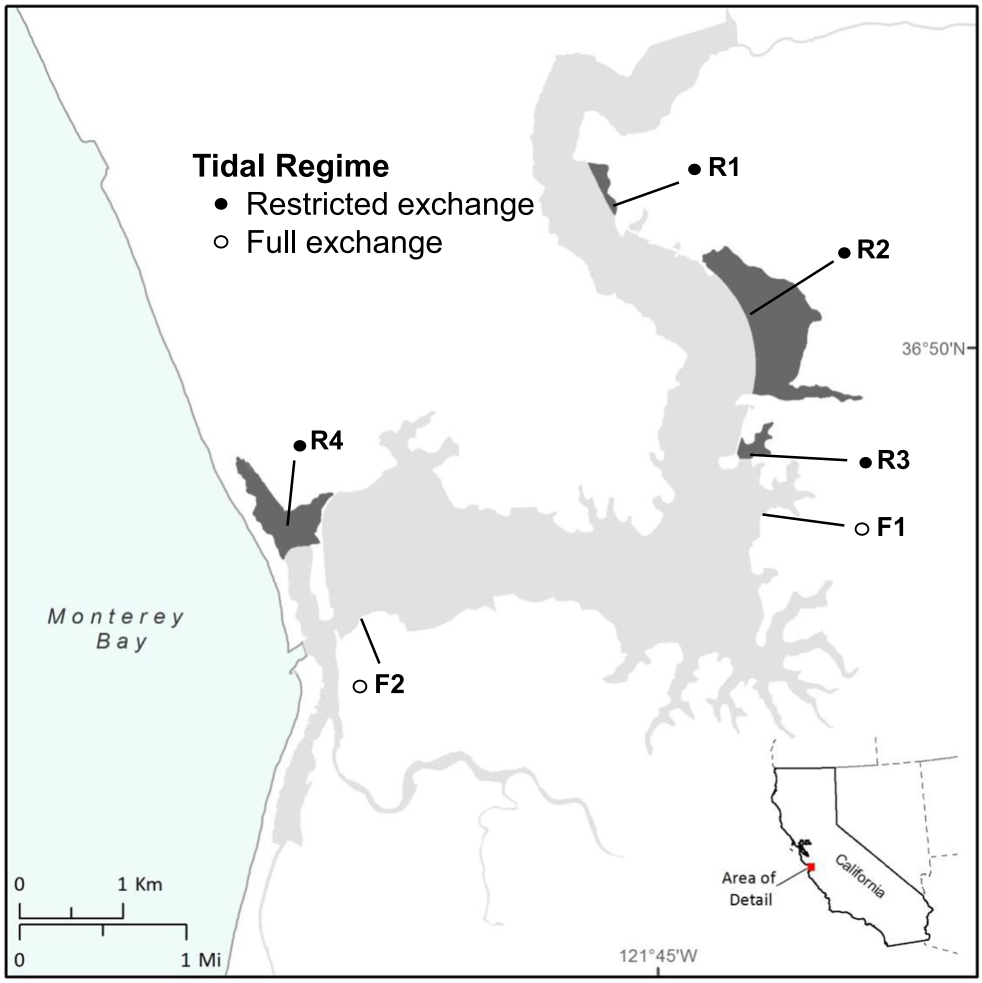

Fig. 1 Location of wetlands where water quality was related to success of indicator species in Elkhorn Slough

67 cm in diameter in the middle, and 52 cm in diameter at the ends) (Fig. 2a) with the openings plugged to prevent the fish from escaping, while at the same time preventing predation on the fish. We deployed one minnow trap cage per site. The oysters were contained in nylon mesh bags (17 × 23 cm) with an 8-mm mesh size (Fig. 2b). To distinguish individuals, each oyster was placed in a separate, numbered bag. The minnow traps and mesh bags were attached with nylon rope and a clip to a 3-m PVC pole, which was anchored to a cinderblock with two stainless steel bolts drilled through the cinderblock. We placed the fish and oysters in the very low intertidal, approximately 30 cm off the bottom of the seafloor at any given site. This was at least 30 cm below the mean lower low water, such that the cages and mesh bags were continuously submerged, even during low tide, during our experimental period. We fed the fish every other day by stocking traps with approximately 100 g fresh algae (*Ulva lactuca*) which contained the primary prey items of staghorn sculpins, i.e., isopods, amphipods, and polychaete worms (Fitch and Lavenberg 1975). The oysters were not fed because they had full access to estuarine water and associated phytoplankton. We checked fish and oyster survival every other day for 18 days, at which point we ended the fish component of the experiment because of the high mortality that had occurred (Fig. 2c–e). Thereafter, we checked oyster survival weekly until we ended the oyster experiment after 67 days, on September 24, 2012. At that point, we re-measured all the oysters to the nearest millimeter. To measure water quality parameters at each of the six study sites, we continuously deployed a YSI 6600 sonde and data logger (Xylem Analytics, Yellow Springs Instruments, Yellow Springs, OH, USA) (Fig. 2d) which recorded temperature, salinity, pH, dissolved oxygen, and turbidity every 15 min for 27 days, from July 18, 2012, to August 14, 2012. At one site, R4, data collection ended on August 1, 2012, due to sea otter disturbance of the sonde, but the data we collected from this site in previous and subsequent years suggest the 15-day period was representative. At four of the sites (F1, F2, R1, R2), the sonde was permanently deployed as a part of NERR SWMP monitoring, a National Estuarine Research Reserve System water quality monitoring program (NERRS 2010). We calculated daily means for temperature, salinity, pH, turbidity, and dissolved oxygen by averaging the 96 daily records (4 records per hour × 24 h) for each study site. Then, we calculated the overall mean for each site by averaging the 27 daily means for the duration of the study. We calculated tidal range by subtracting the minimum tidal

Fig. 2 Overview of sampling design: **a** cages used to confine staghorn sculpins; **b** bags used to hold Olympia oysters; **c** sculpin discovered to be dead at regular sampling check, **d** water quality sonde used at each site, and **e** field check of fish cage

height from the maximum tidal height during the experiment. In order to assess the duration of hypoxia at a site, we calculated the number of hours, within each 24-h period, where dissolved oxygen levels were less than 5.0 mg/L, and then averaged these 27 values to obtain a monthly mean. We recognize that the term "hypoxia" for coastal and estuarine organisms is defined by a range of thresholds (Vaquer-Sunyer and Duarte 2008) and that the absolute value of dissolved oxygen (DO) in milligrams per liter assigned to the term hypoxia depends on additional parameters such as temperature and salinity (Hofmann et al. 2011). Although a DO threshold of 2 mL O_2/L is generally accepted as a threshold for hypoxia in coastal systems (Diaz and Rosenberg 2008), which is equivalent to 2.8 mg/L (Diaz and Rosenberg 1995), there is no standard dissolved oxygen concentration threshold that can be universally applied across freshwater, estuarine, and marine systems (Rabalais et al. 2010). Previous studies have also shown that even slight decreases in oxygen concentrations have negative consequences for fish communities in estuaries (Levings 1980; Eby and Crowder 2002; Vaquer-Sunyer and Duarte 2008), including in Elkhorn Slough (Hughes et al. 2015). Finally, The Basin Plan (Saiz 2011), which is the Water Quality Control Plan for the Central Coastal Region, specifies that dissolved oxygen concentration in estuarine waters shall not be reduced below 5 mg/L at any time. In addition to the 5 mg/L threshold, we also calculated the 10th percentile of DO, which is a simple threshold measurement that incorporates both frequency and duration of the lowest DO and is a good proxy for hypoxia (Hughes et al. 2011, 2015). At each of the six study sites, we also collected monthly water grab samples. We collected one sample per month, for 3 months in summer 2012 at the six sites. We analyzed the 18 grab samples for nitrite, nitrate, phosphate, ammonia, and chlorophyll-a. All sample collections and analyses were conducted following the Standard Operating Procedures of the NERR system (NERRS 2012). Each of the nutrient concentrations (PO_4^-, NO_2^-, NO_3^-, NH_4^+, total dissolved N) and chlorophyll-a concentration were calculated as the mean concentration for the months July, August, and September 2012 to more comprehensively describe the site, instead of using only one value for the month of August.

Statistical Analyses

To understand how environmental parameters varied at sites with different fish and oyster responses, we employed several related multivariate statistical procedures, including nonmetric multidimensional scaling (nMDS), analysis of similarities (ANOSIM), and similarity percentages (SIMPER) with the program Primer v.6 (Clarke and Gorley 2006). Prior to analysis, we normalized the data for all the parameters shown in Table 1 and created a Euclidean distance matrix. ANOSIM was used to determine if environmental conditions differed significantly among sites with different fish/oyster responses. If ANOSIM revealed significant patterns, we used SIMPER to determine which parameters contributed most to the observed dissimilarities among groups (Clarke 1993).

We then used linear regression to investigate relationships between the key indicator species response (fish survival, oyster growth) and key environmental variables identified in the SIMPER (DO, temperature, and salinity). For model selection, we used a combination of P values (alpha set at 0.10) and Akaike information criterion (AIC) model selection (Burnham and Anderson 2002, Ettinger et al. 2011). We ran individual single linear regressions, which were compared to null models (no predictive parameters). We compared AIC scores to competing linear regression models to select the best-fit models, which were the models with the lowest AIC scores within 2 AIC units. Since we were comparing single-factor models, we did not use the AIC correction, i.e., AICc.

Table 1 Comparison of six study sites in terms of tidal regime, water quality, and response of indicator organisms

Site label	R1	R2	R3	R4	F1	F2
Site name	Azevedo Pond	North marsh	Whistlestop	Benneth slough	South marsh	Vierra
Tidal regime at site	Restricted	Restricted	Restricted	Restricted	Full	Full
Tidal range (m)	1.12	0.15	0.89	1.41	2.37	2.29
Temperature (°C)	19.8	20.7	21.4	17.7	19.1	15.1
Salinity (ppt)	35.1	34.7	34.8	33.3	34.3	33.6
pH	7.92	8.02	7.93	8.63	7.85	8.03
Tubidity (NTU)	6.6	8.5	8.0	11.0	13.9	5.1
Dissolved oxygen (mg/L)	5.29	4.68	5.60	8.80	6.03	7.59
Lowest oxygen 10th percentile DO (mg/L)	0.6	0.9	3.0	2.7	4.7	5.5
Hypoxia [h/day DO <5 mg/L]	12.5	12.3	10.9	5.0	4.1	0.9
Chlorophyll-*a* (μg/L)	3.82	7.67	2.49	3.85	5.17	2.96
PO_4^-(mg/L)	0.099	0.066	0.044	0.083	0.053	0.039
NO_2^-(mg/L)	0.0053	0.0025	0.0009	0.00304	0.005	0.0032
NO_3^-(mg/L)	0.003	0.029	0.004	0.175	0.046	0.074
NH_4^-(mg/L)	0.018	0.144	0.050	0.065	0.130	0.071
Total dissolved nitrogen (mg/L)	0.019	0.175	0.054	0.240	0.181	0.148
Fish survival (# days)	3	5.8	4.2	8	6.8	13
Fish survival category	Low	Low	Low	High	High	High
Oyster survival (# days)	60.3	62.7	6.58	50.3	65.8	50.3
Oyster growth category	High	Low	Low	Low	High	High
Oyster growth rate (mm/month)	0.287	−0.073	0.141	0.073	0.429	0.57

Further, we only selected models that had significant P values ($P < 0.10$). We used a conservative alpha because of the low replication within our in situ experiment. For AIC model selection, we used the MuMIn package in R v. 3.2.2 (R Core Team 2016).

Results

Tidal Range and Water Quality Differences Among Sites

There was high variation in water quality parameters, especially dissolved oxygen, among the six study sites (Table 1 and Fig. 3). Dissolved oxygen was lower at restricted sites. For example, DO often dropped below 1 mg/L and the duration of hypoxia was >10 h/day for most restricted sites. Also, temperatures were greatest at sites where tidal exchange was restricted (Table 1 and Fig. 3b). Other key water quality parameters, such as chlorophyll-a and nutrients, were highly variable (Table 1 and Fig. 3d), yet there were no discernable patterns between tidally restricted and unrestricted sites. The tidal range was much lower at the sites with water control structures. Some water quality parameters also showed strong variability among sites, while other parameters, such as salinity and pH, had relatively small differences among sites (Table 1, Fig. 3).

Fish Survival and Oyster Growth

At three sites where hypoxia was the most severe (R1–R3), all fish were dead after the first week (Table 1). In contrast, oyster survival did not differ markedly between sites (Table 1). However, the growth rate of oysters did show contrasts among sites where the lowest growth rates occurred at sites with the most severe hypoxia (Table 1). Note that the negative mean growth rate observed at one site is an artifact of measurement error but was not adjusted to zero because such error is part of the means for the other sites and cannot be corrected for those.

nMDS Analyses of Water Quality and Indicator Species Response

We divided the sites into two categories for fish survival: (1) "low" for the three sites where fish did not survive past the first week and (2) "high" for the other three sites. An ANOSIM revealed that sites with low vs. high fish survival differed in water quality and tidal range ($P = 0.1$, $R = 0.89$); given the very low sample size, this high R value combined with a marginally significant P value indicates significant differences (Fig. 4a). A SIMPER analysis revealed that the top contributor to the difference among sites with low vs. high survival was hypoxia (h/day DO <5 mg/L), accounting for 9.7 % of the difference. The next top five factors contributed fairly equally (between 8.1 and

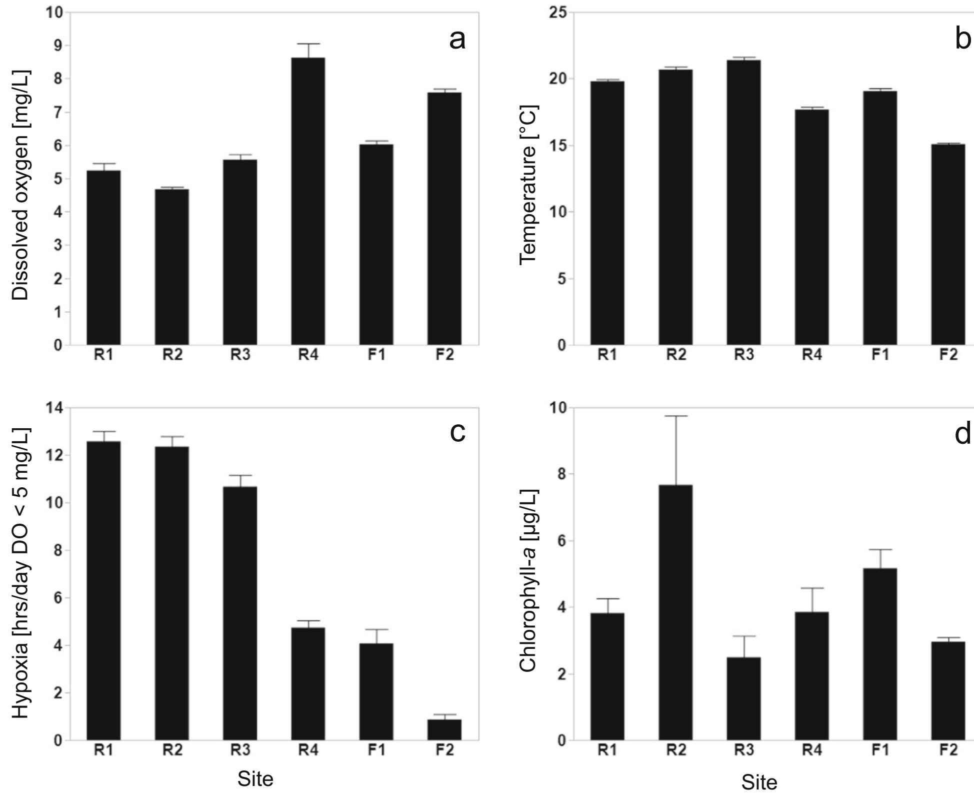

Fig. 3 Characterization of water quality at six study wetlands. **a** Daily mean dissolved oxygen concentration, **b** daily mean temperature, **c** daily mean hypoxia, and **d** monthly mean chlorophyll-*a* concentration. *Error bars* represent the standard error of daily averages over the 27-day monitoring period for panels **a**–**c**; for panel **d**, they represent the standard error of three monthly averages taken closest to the sampling period

8.8 %) to the difference: lowest oxygen (10th percentile DO) and temperature, which were lower at sites with high survival, and mean dissolved oxygen, mean salinity, and tidal range, which were higher at sites with high survival (Table 1).

We similarly divided the sites into two categories for the oyster growth rate: (1) "low" for the three sites with the lowest growth, which was near zero, and (2) "high" for the three sites with more substantial growth rates. An ANOSIM revealed no significant differences based on these categories. Restricted site R4, which clustered with the fully tidal sites (Fig. 4b), was a poor site for oyster growth but a good site for fish survival; conversely, restricted site R1 was a good site for oyster growth but a poor site for fish survival. These contrasts in the categories explain why fish survival showed a significant pattern in the ANOSIM but oyster growth did not.

Key Drivers of Fish Survival and Oyster Growth

Since the SIMPER analysis had revealed hypoxia duration to be the top contributor to differences in sites with low vs. high fish survival, we further examined the relationship between hypoxia, temperature, and salinity with indicator species responses. AIC model selection determined that hypoxia indices were significant predictors of both fish survival and oyster growth rates. For fish, hypoxia measured as mean daily hours of DO <5 mg/L was a significant ($P = 0.019$) predictor and declined with fish survival (Table 2, Fig. 5a). Further, temperature was also a significant predictor ($P = 0.014$) where increased temperature correlated with lower fish survival (Table 2, Fig. 5b). For oysters, temperature, hypoxia (h/day DO <5 mg/L), and lowest oxygen (10th percentile DO) were selected as best-fit models according to AIC

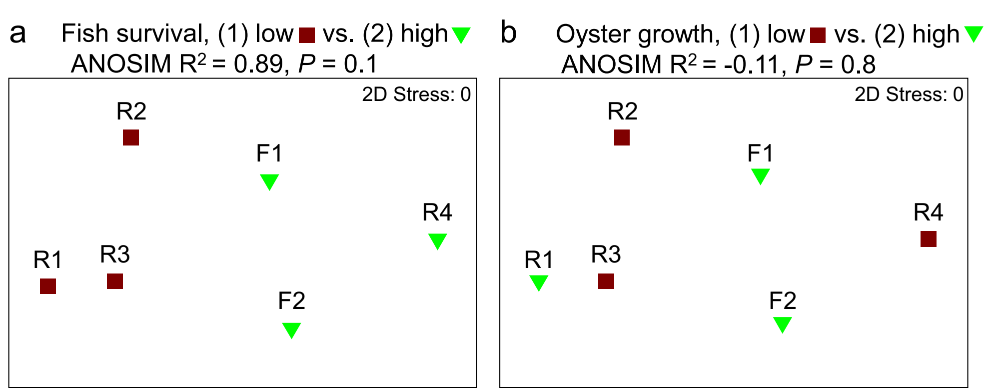

Fig. 4 Multivariate analysis of water quality differences between sites sorted by **a** fish survival categories and **b** oyster growth categories

Table 2 Model selection based on AIC and P values for both staghorn sculpin and Olympia oyster

Fish mortality				
Model	R^2	P	Slope	AIC
Null	NA	0.005	NA	16.02
Hypoxia (h/day DO < 5 mg/L)	*0.78*	*0.019*	*−0.632*	*8.88*[a]
Lowest oxygen (10th percentile DO)	0.58	0.079	1.363	12.83
Temperature	*0.82*	*0.014*	*−1.4329*	*7.89*[a]
Salinity	0.68	0.044	−4.183	11.19
Oyster growth				
Model	R^2	P	Slope	AIC
Null	NA	0.058	NA	−16.34
Hypoxia (h/day DO <5 mg/L)	0.46	0.14	−0.032	−18.01[a]
Lowest oxygen (10th percentile DO)	*0.55*	*0.09*	*0.09*	*−19.18*[a]
Temperature	0.45	0.144	−0.072	−17.94[a]
Salinity	Null	Null	Null	−14.67

Significant models ($P < 0.10$) are in italics

[a] Best-fit models

(Table 2); however, only the lowest oxygen was a significant predictor ($P = 0.090$) and that relationship was positive meaning sites with less severe hypoxia correlated with increased oyster growth (Fig. 5c).

The three sites with poor fish survival and the three sites with poor oyster growth were all restricted sites, even though they were not the same three sites (R1 was bad for fish, R4 bad for oysters, but not vice versa). Thus, tidal restriction was an important factor for both fish and oysters. Temperature was greater in tidally restricted sites (Table 1, Fig. 3), and moreover, hypoxia showed a strong negative relationship ($P < 0.022$, $R^2 = 0.71$ with tidal range (Fig. 5d).

Discussion

Negative Effects of Hypoxia on Estuarine Organisms

Our investigation revealed that water quality in four of the six wetlands examined was so degraded that it lead to negative responses even in estuarine indicator species known to be highly tolerant. Staghorn sculpin mortality was high in response to impaired water quality, with no fish remaining alive after the first week in three of the wetlands. Olympia oyster survival remained high in all wetlands, but growth was virtually zero in three of the wetlands, a striking sub-lethal response. Environmental conditions differed in a variety of ways in the wetlands, but the ANOSIM identified hypoxia as the best predictor of fish survival, and both fish survival and oyster growth showed clear relationships with hypoxia in linear regressions.

Fish are well known to be sensitive to oxygen conditions. Previous research has shown that fish biomass is strongly

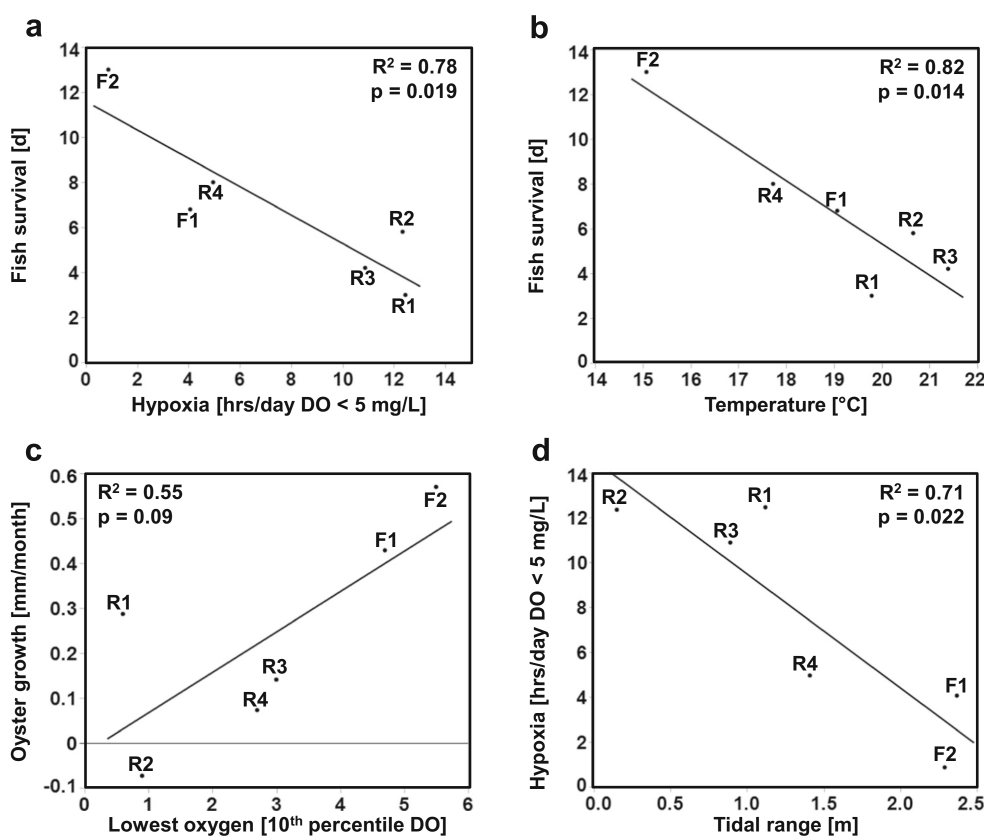

Fig. 5 Fish survival and oyster growth as a function of wetland tidal range and hypoxia: **a** fish survival vs. hypoxia duration (h/day DO <5 mg/L), **b** fish survival vs. temperature, **c** oyster growth rate vs. lowest oxygen (10th percentile DO), and **d** hypoxia duration vs. tidal range

correlated with hypoxia, in the Gulf of Mexico, near Marsh Island, LA (Renaud 1986), and fish were absent from hypoxic zones off the southwest Louisiana coast (Leming and Stuntz 1984). Furthermore, fish can increase their swimming speed in response to hypoxic conditions as an avoidance behavior, but when hypoxia increases, swimming speed drops and blood lactate levels indicate physiological stress (Herbert and Steffensen 2005). In addition to experiencing stress in low-oxygen conditions, Ross et al. (2001) found that common estuarine fish species experience stress in high-oxygen conditions, hyperoxia, which is also common in restricted areas of Elkhorn Slough (Hughes et al. 2011). Stress was quantified as elevated levels of antioxidants in tissues, which is a chemical defense against damaging reactive oxygen forms produced by aerobic metabolism in hyperoxic habitats (Ross et al. 2001). In our study, the fish were constrained from leaving the site due to caging. In many cases, this is not realistic; uncaged fish have the option to avoid mortality by leaving a site. However, water control structures (e.g., dikes, weirs, and culverts) can severely reduce fish passage and potentially subject mobile species to water quality conditions behind artificial structures. The caging experiment demonstrated that water quality conditions in these wetlands were lethal to the fish, making them unsuitable fish habitats. A recent investigation in Elkhorn Slough revealed that periods of low-dissolved-oxygen conditions in the entire estuary correlated with overall declines in fish diversity as well as flatfish abundance and offshore catch of flatfish in subsequent years (Hughes et al. 2015). Therefore, if water quality in the form of low dissolved oxygen is poor in the entire estuary then the value of the estuary for fish habitat is greatly compromised.

Olympia oyster responses to hypoxia were recently examined in a laboratory experiment (Cheng et al. 2015) motivated in part by the preliminary results of this caging experiment, and with diel-oxygen conditions based on Elkhorn Slough water quality data. The laboratory experiment provided a much clearer test of the role of oxygen levels than our field study, since all other parameters were held constant. The conclusion of this laboratory experiment was similar to that of our field experiment: oyster survival was high in simulated nighttime hypoxic conditions, but growth was reduced by up to 61 %. Oysters can avoid mortality under low-oxygen conditions by tightly closing their shell and decreasing their metabolism, but this also means that they cease feeding and thus decrease growth and their ultimate size (Widdows et al. 1989). Interestingly, we found no relationship between oyster growth and chlorophyll-a levels at these six wetlands, even though food availability is known to be important for oysters (Wasson et al. 2015). Furthermore, we found that colder rather than warmer sites enhanced oyster growth, even though laboratory experiments show greater growth at higher temperature (Cheng et al. 2015). This suggests that the negative effects of hypoxia outweigh the positive effects of greater chlorophyll-a levels and temperature at wetlands with restricted tidal exchange.

Management Implications and the Role of Tidal Restriction

Elkhorn Slough receives extremely high nutrient loads from surrounding agriculture and is consequently eutrophic (Hughes et al. 2011, 2013). However, at the level of individual sites, nutrient concentrations do not predict eutrophic conditions including hypoxia, while tidal range does (Hughes et al. 2011). In the current study, this pattern held—the fully tidal sites had dramatically less hypoxia than the restricted sites, but nutrient concentrations were not lower in the fully tidal sites (Table 1, Fig. 5d). The sites with restricted tidal exchange had less water movement, so oxygen-rich surface waters mix less with deeper waters. Furthermore, the restricted sites likely have increased oxygen consumption due to accumulation of macro-algal mats and other organic matter fueling microbial communities.

The three wetlands where no fish survived past the first week and the three wetlands where oyster growth virtually ceased were all tidally restricted sites (though in each case fish survival and oyster growth were fine in one restricted wetland, R4 and R1, respectively). Water control structures appear to degrade water quality conditions in ways that are harmful, yet differ in severity to fish and oysters. At least in theory, the proximate solution to these problems is local and straightforward: an increase in tidal exchange by opening tide gates or increasing culvert size or number will immediately improve habitat conditions for fish and oysters in these sites. Indeed, we have found in earlier investigations that overall biodiversity is enhanced with full tidal exchange (Ritter et al. 2008). In this study, the restricted site with the greatest tidal range (R4) also had the best oxygen conditions and fish survival, suggesting that water control structures can be managed to support healthy fish populations. In practice, changes in water control structure management can be difficult to implement due to concerns by adjacent landowners or costs of maintenance and repairs. Still, our results indicate that improving water quality in tidally restricted areas is an important local-scale mechanism for enhancing the habitat value of estuarine wetlands and can complement watershed-scale efforts to reduce nutrient loading.

The Value of In Situ Experiments and Monitoring

Controlled laboratory experiments are well suited to detecting the exact effects of particular factors, such as oxygen thresholds while holding other potentially confounding factors constant. In contrast, in situ experiments such as the ones we conducted cannot so clearly attribute causality, because sites differ in multiple ways. However, field experiments can be invaluable for illustrating how organisms respond to real conditions, including interactions between multiple stressors experienced in impaired habitats (Crain et al. 2008). For example, our results show that effects of hypoxia at wetlands with restricted exchange overwhelmed benefits of higher chlorophyll-a and temperature for oyster growth, the first such demonstration in the field for Olympia oysters.

Elkhorn Slough's water quality is considered impaired in a variety of ways (Caffrey et al. 2007, Hughes et al. 2011). The Central Coast Regional Water Quality Control Board is beginning a process of setting total maximum daily loads (TMDLs) for water quality stressors in the watershed. We have been informed by board staff that local water quality regulation requires local evidence of impairment of beneficial uses. While published literature from other systems is broadly incorporated into policy, studies from the local system are critical (Peter Von Langen, pers. comm.). Elkhorn Slough wetlands are beloved by local birdwatchers, hikers, fishermen, and kayakers (Kildow and Pendleton 2010). Our experience with these stakeholders is also that local studies with real examples prove very powerful in raising their concern about issues such as hypoxia, even though the problem is well characterized in the scientific literature. Our study provided very vivid evidence of impaired water quality in local wetlands by demonstrating that fish die within a week of being forced to endure conditions in three wetlands and oysters stop growing. Simple field experiments can thus provide concrete evidence of the negative effects of water quality degradation, inform regulators, and build stakeholder support for restoration or policy that improves water quality.

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