



A Guide to Olympia Oyster Restoration and Conservation

APPENDIX 4 SITE EVALUATION TABLE: DETAILED EXPLANATION



APPENDIX 4 Site evaluation table detailed explanation.

How the table was created

Selection and weighting of oyster attributes

Oyster attributes were selected and weighted as discussed in this project's main document "A Guide to Olympia Oyster Restoration and Conservation." The relative importance of each of these variables was ranked qualitatively as very high, high, medium, or low, and assigned a numerical weight, or multiplier as discussed below.

Selection and weighting of environmental parameters

To create the site evaluation table, all environmental variables measured in the field were tested against all measured oyster variables in a series of bivariate regressions (see Appendix 2 for details of field monitoring). Data for both predictor and response variables were summarized for each of the seven quarters of our field studies, beginning in Spring 2012. Because many oyster variables are likely to be affected by season, we used quarter as a blocking factor. We looked for effects of environmental factors on oyster variables within the same quarter as well as offsetting quarters where it made sense to do so, *e.g.*, determining whether there were effects of spring or summer chlorophyll *a* (when brooding adults and/or larvae might be feeding) on recruitment in the fall. Environmental factors that these analyses determined had significant effects on oyster variables were included in the table. Some environmental factors were highly correlated. In these cases, we selected the factor with the greatest effect (largest R^2), after determining that including both factors did not improve the regression model.

In some cases, there were multiple approaches to quantifying environmental variables such as water and air temperature and salinity. For these variables we looked for statistically significant regressions across multiple summary measures including mean, maximum, minimum, and the number of days above or below certain thresholds. We selected the measure that had the greatest number of statistically significant relationships with multiple oyster response variables, selecting, for example, the number of days with average water temperatures above 12° C, because this had an effect on several measures of recruitment, growth, size, and adult oyster density. Three of our measured environmental variables -- water temperature, chlorophyll, and salinity -- were included in the table based on these analyses.

In addition, we included four factors that our laboratory experiments, the scientific literatures and/or longer term field data indicated have significant negative impacts on oysters: drill predation, low dissolved oxygen, high air temperatures, and extreme low salinity. Laboratory experiments focused on air and water temperatures, salinity, and dissolved oxygen. Details are available as Appendix 3 of this document. Oyster drills had been demonstrated to impact oyster populations in Tomales Bay CA (Kimbrow et al. 2009); work completed in San Francisco Bay just as this document was going to press indicates significant impacts of drills on oysters at one of our field sites, Eden Landing Ecological Reserve (Kiriakopolos et al. 2014). We used field data gathered during the course of this project to generate scores for the potential stressors of drill predation, low dissolved oxygen, and air temperatures, and longer-term data for scoring a site for the risk of very low salinity events (discussed below). None of these factors were statistically significant in terms of impacts on oyster attributes during the timeframe of this project, when all sites are considered in analysis. This is likely due to site-by-site differences in the presence and importance of stressors (i.e. very low DO occurring only in a subset of Elkhorn Slough sites) and/or differences in the time frame over which predictor variables were and potential response variables were measured. For example, sites with higher frequency of extreme low salinity events (based on long-term data) did very well in terms of oyster attributes during our study period, which was conducted during a drought. As another example, to generate a score for a site's risk of high air temperatures under future climate change scenarios, we used current temperatures, which are currently still below the levels expected to have deleterious effects on oysters, as a proxy for this future stress.

Generation of scores

To standardize across very different types of measurements, we converted our data into standardized scores. In most cases, we used a four-tiered scoring system, which generated scores of 0, 33, 67 or 100; in two cases we used five-tiers, generating scores of 0, 25, 50, 75, and 100. The bins were established in such a way as to give the greatest separation to the relative ranking of our sites, using the range of the measured variables during our study. In all cases, a score of 0 indicated the worst conditions for oysters and 100 the best. Each score was also weighted in terms of relative importance with a multiplier of 2 for very high (or 3 in the case of population estimate for generating the conservation value score, see Notes to the Site Evaluation Table in the main document), 1.5 for high, 1.25 for medium, and 1 for low. For oyster attributes, these ranks were made based on our own expert judgment; further studies of oyster population biology are needed

to generate data-based ranks. Weights for environmental parameters were based on the results of the field data and/or laboratory experiments.

In cases where data are missing, no score is entered, thus sites with fewer data points are ranked only on available data. While sites don't lose points for missing data, it should be noted that the validity of scores is less certain with less data.

We used averages of these weighted scores to create overall site scores under three potential scenarios: 1) restoration dependent on natural recruitment, 2) restoration with seeding and 3) conservation. For restoration dependent on natural recruitment, sites with no measured recruitment received a score of 0 regardless of scores on other oyster attributes or environmental parameters. For restoration with seeding, all oyster attributes and environmental parameters are used, and for sites that have 0 to less than 10 individuals, scores derived from existing oyster populations are deleted, scores related to recruitment are downweighted, and growth is weighted more highly. For the conservation value score, the order of magnitude population estimate is substituted for adult oyster density and is given the largest weight (a multiplier of 3); all other parameters are scored as for the first restoration score.

Key environmental parameters measured in field

Water temperature

Temperature is a major driver of virtually all oyster physiological processes, such as respiration, metabolism, filtration, and excretion (Hochachka and Somero 2002). Historical data and near-term models suggest that increased sea surface temperatures have occurred and will continue to occur in estuaries worldwide (Cloern et al. 2011). Effects of very warm water, alone and in combination with other potential stressors, were the subject of our laboratory experiments (Appendix 3), but we were also interested in whether current water temperatures at our sites were correlated with oyster performance.

Onset tidbit temperature loggers (www.onset.com) were deployed at each site at 0 m MLLW to measure water or air temperature (depending on tide height at any given timepoint) continuously every 15 min. Data were downloaded from loggers periodically and paired with tide level data for each site to parse out air or water temperatures for each site.

We ran linear regressions of the oyster demographic variables that we measured in the field on several measures of water temperature, using quarter as a blocking variable. Standard linear regressions on untransformed data were performed, except when the response variable was non-normally distributed – these were usually Poisson or negative binomially distributed – in which case we used generalized linear models with the appropriate link function (usually log). Our field data showed a strong relationship between warm water measured at a site and several oyster attributes, including growth rate, average size, recruitment rates, and adult density.

Many biological processes are driven by cumulative exposure to warmer temperatures (i.e. degree-days), usually above a given threshold. For example, threshold temperatures for onset of spawning are often cited in the literature (but appear to vary by location). Threshold temperatures for spawning and other important processes are not known for oysters in our study locations. To look for such a threshold, we calculated the length of exposure (days/year) each site had to water temperatures above each temperature level (in 1°C increments) in the range experienced at each site. We regressed each demographic variable on the number of days spent above each threshold temperature. We also explored relationships of oyster variables to maximum, mean, and minimum temperatures. Despite the very large number of regressions generated here, we did not correct regression p-values for multiple comparisons, as might be

done in statistical analyses of experiments, as our analysis was exploratory in nature. We were interested in the overall pattern of relationships, rather than testing a specific, falsifiable hypothesis.

After considering each possible threshold temperature across sites, we selected 12°C as the water temperature threshold that had the greatest number of significant relationships with the demographic variables we tested across all sites. This temperature may seem rather cool and not specifically tied to biologically relevant oyster physiology, but it acts as a single proxy measure that captures a number of temperature-related phenomena that are clearly relevant to oyster biology. The 12°C threshold correlated better with a larger range of oyster demographic measures than other standard measures of temperature such as the annual maximum, mean temperature, or temperature range.

The exposure of a site to warmer water temperatures was positively correlated with several measures of growth rate ($r^2 = 0.31$, $p < 0.0001$), average size ($r^2 = 0.19$, $p = 0.01$), and average quarterly recruit size ($r^2 = 0.56$, $p < 0.0001$). Here, we only show relationships between temperature and the mean of each demographic measure.

We weighted water temperature as medium importance given the wide range of effects that water temperature has on oyster demography, while also not having severe negative effects that might lead to a complete absence of oysters, at least over the range of temperatures observed.

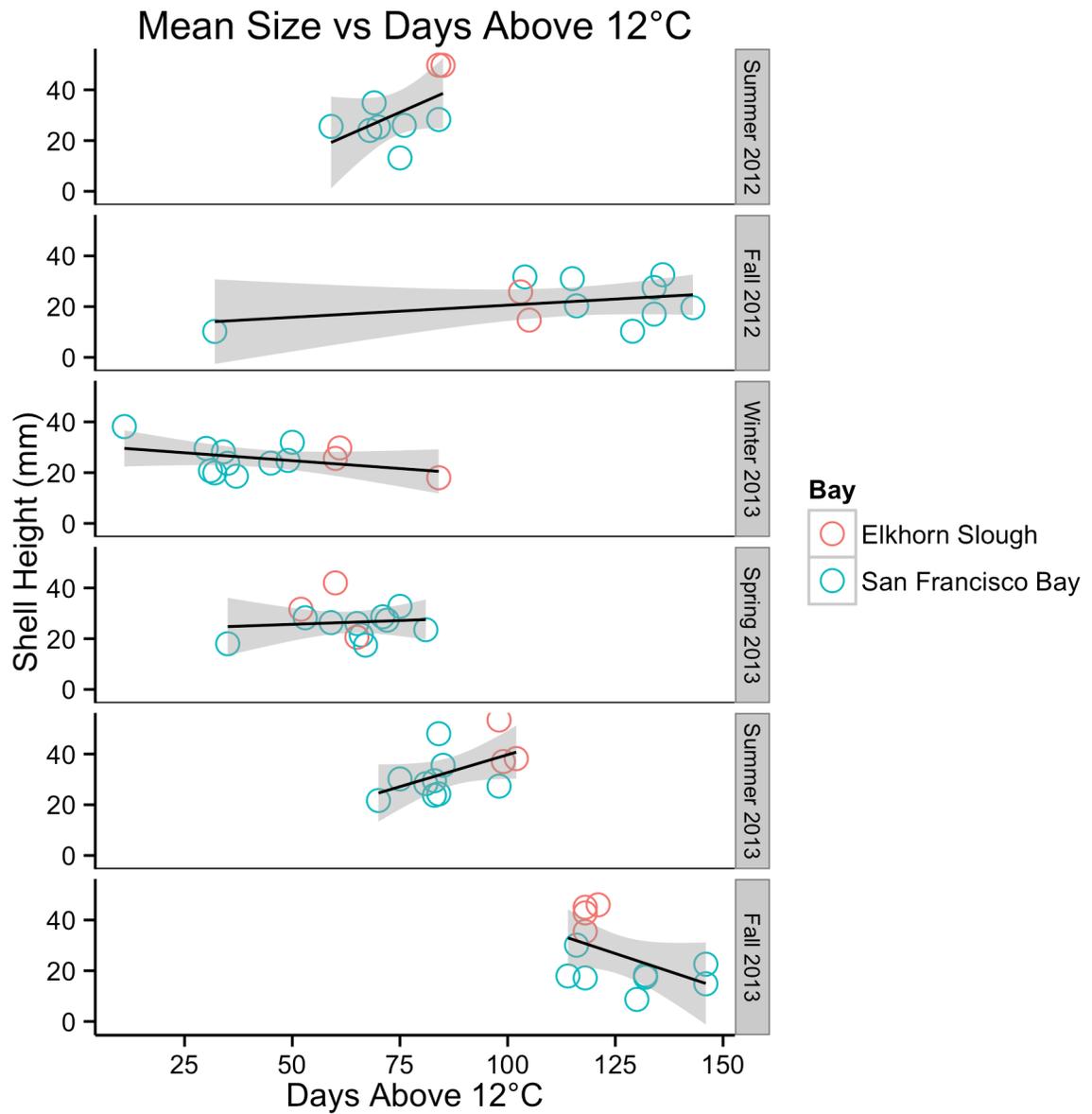


Figure 1: Mean oyster size (shell height) for each site at each timepoint, plotted against the number of days the site spent above 12°C water temperature during that quarter. Gray shading indicates 95% confidence band for regression line (in black). Note that relatively little recruitment was seen at Elkhorn Slough during this study.

Mean Quarterly Recruit Size vs Days Above 12°C

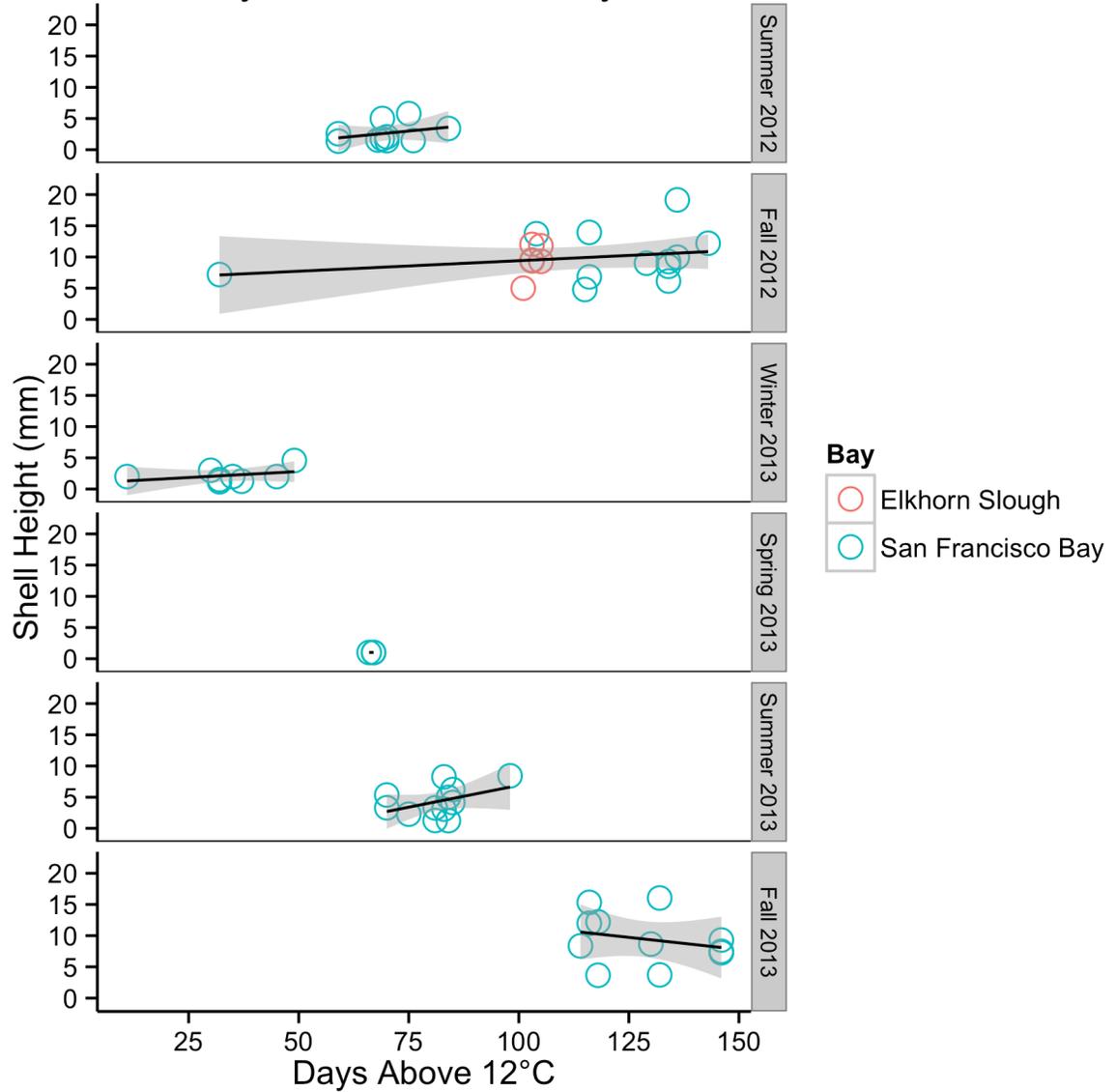


Figure 2: Mean quarterly recruit size for each site at each timepoint, plotted against the number of days the site spent above 12°C water temperature during that quarter. Regressions generally show positive relationships with days above 12°C, except Fall 2013. Gray shading indicates 95% confidence band for regression line (in black). Note that relatively little recruitment was seen at Elkhorn Slough during this study.

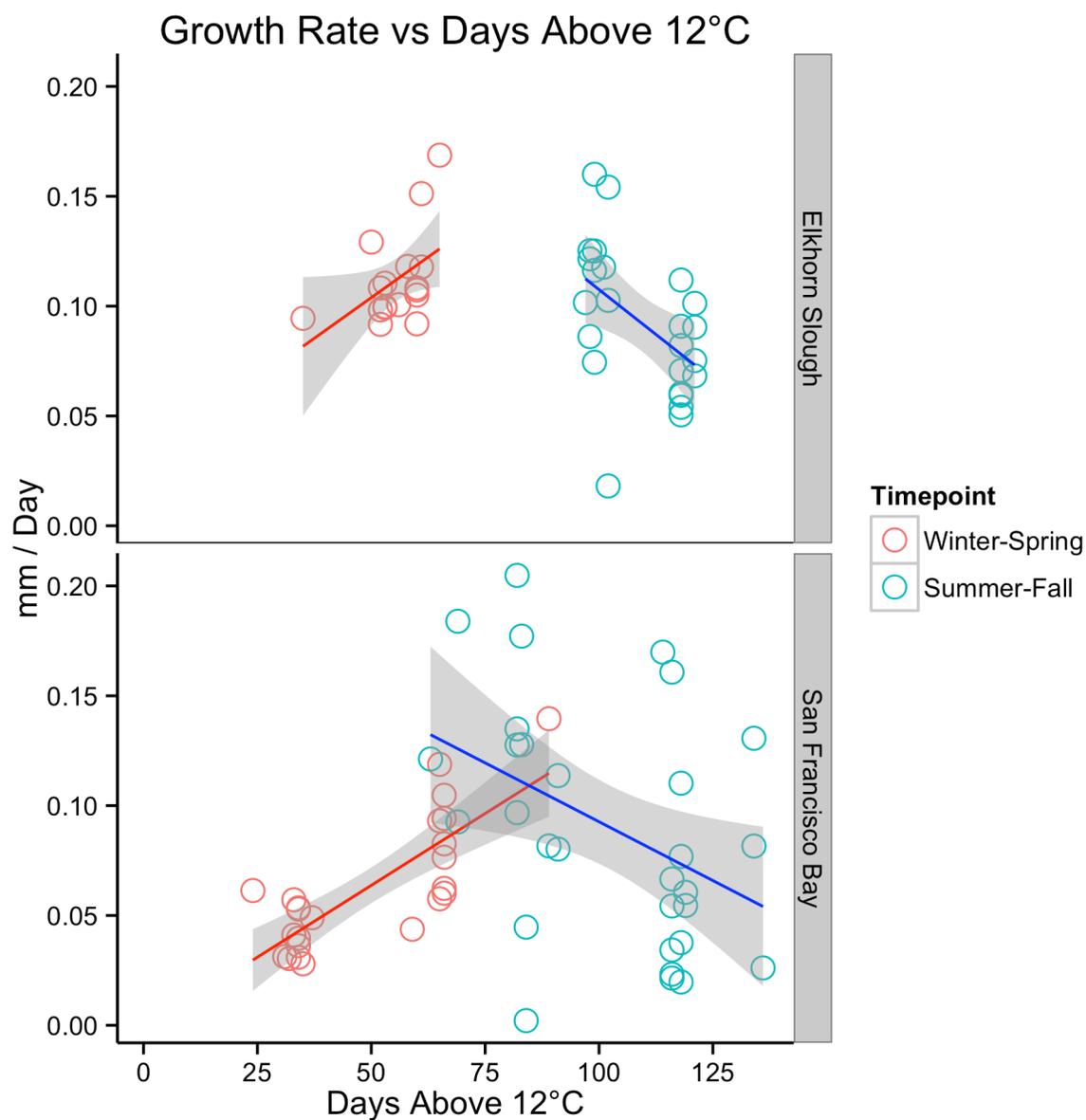
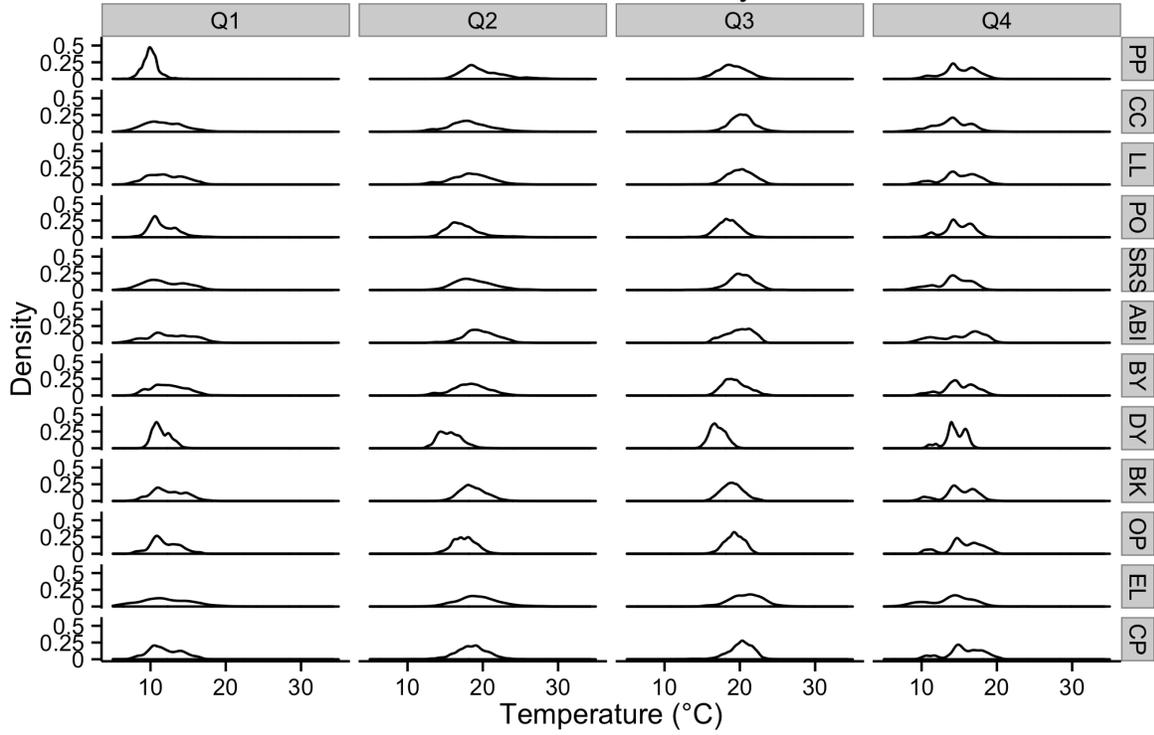


Figure 3: Growth rate at each site during 2013 plotted against the number of days the site spent above 12°C water temperature in that quarter. Regressions show positive relationships for both bays with days above 12°C for the combined summer-fall period (blue regression lines), and negative for winter-spring (red regression lines). Gray shading indicates 95% confidence band for regression line.

Water Temperature Probability Density San Francisco Bay



Elkhorn Slough

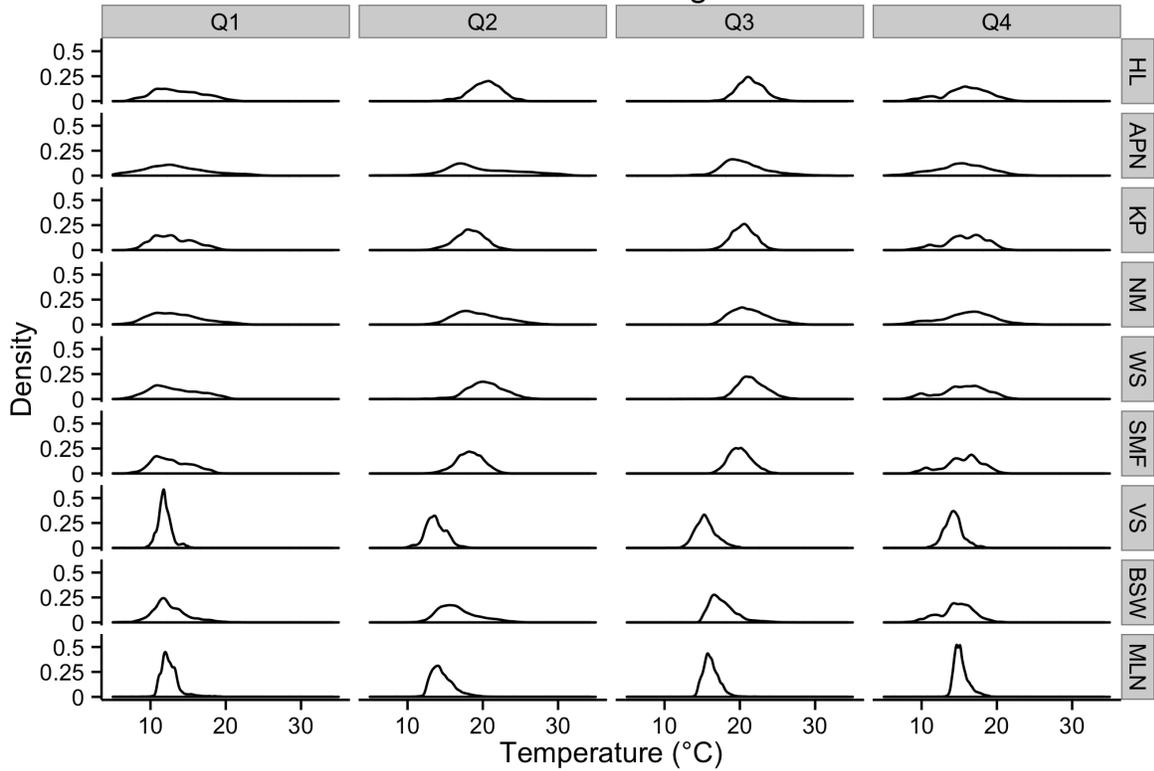


Figure 4. Probability density plots of water temperature at all sites in 2012 and 2013 by quarter (Jan-Mar, Apr-Jun, Jul-Sep, Oct-Dec). Sites are arranged from north to south for each bay. For each site, the line describes the relative amount of time the water at the site is at each temperature. These plots show the variation in the range of temperatures each site experienced during the study. Seasonal trends and geographic differences between sites are clearly visible. Site abbreviations are given in Appendix 2.

Chlorophyll a

We measured the photosynthetic pigment chlorophyll *a* (abbreviated chl *a*), which can be used as a proxy for the biomass of phytoplankton in the water sampled from a given site. This was measured monthly with one of two methods: directly via extraction from phytoplankton collected in bottled water samples as part of the long-term water quality monitoring program at Elkhorn Slough NERR, or indirectly with a handheld fluorometer using a calibration constant to equate these with direct measurements.

We found a strong and expected seasonal pattern with greater chl *a* scores (thus greater biomass of phytoplankton) in late spring and summer periods. There was a particularly strong bloom of phytoplankton during spring of 2012 at many sites. Sites with higher chl *a* included eutrophic areas with high residence time (Hudson Landing) or muted tidal exchange in ES and sites in SFB with higher residence time in the South Bay (Oyster Point, Coyote Point). The sites with the highest seasonal values included Oyster Point in SFB (25.3 ug/ml) and Hudson Landing in ES (>100 ug/ml). These were unusually high values and nearly all other sites had values between 3-10 ug/ml across all seasons.

We found that chlorophyll *a* was reasonably predictive of juvenile growth rates among a range of sites in 2013 (Summer) for which data were available and excluding one site with unusually high growth values (Fig. 5). Linear regression showed a positive but non-significant relationship ($y = 0.0064x + 0.0108$, $R^2 = 0.3379$, $p = 0.17$). This is likely due to the influence of other variables like water temperature that can strongly influence growth rates. We also found that chlorophyll *a* more strongly predicted recruitment rates across a range of sites in 2012 (Summer chl *a* and Fall recruitment) for which data were available excluding one site with unusually high recruitment (Fig. 6). Linear regression showed a positive and significant relationship between recruitment density and chlorophyll *a* ($y = 1.8159x - 10.197$, $R^2 = 0.5835$, $p = 0.01$). We think that phytoplankton biomass, as estimated by chl *a* values, had an important influence on these variables, but these effects must be considered in the context of other variables such as temperature, salinity and other influential variables.

For the Site Evaluation Table, we calculated the mean of the quarterly averages of measurements of chl *a* made in spring, summer and fall months, and bins were based on the

range of these averages. Chlorophyll *a* was ranked as being of medium importance relative to other environmental factors being considered.

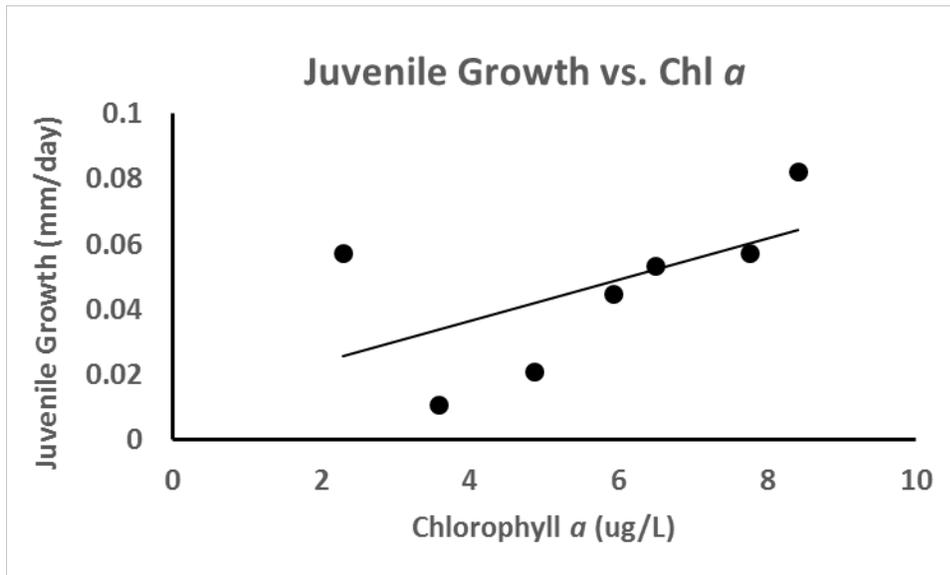


Figure 5. Juvenile oyster growth regressed on chlorophyll *a*.

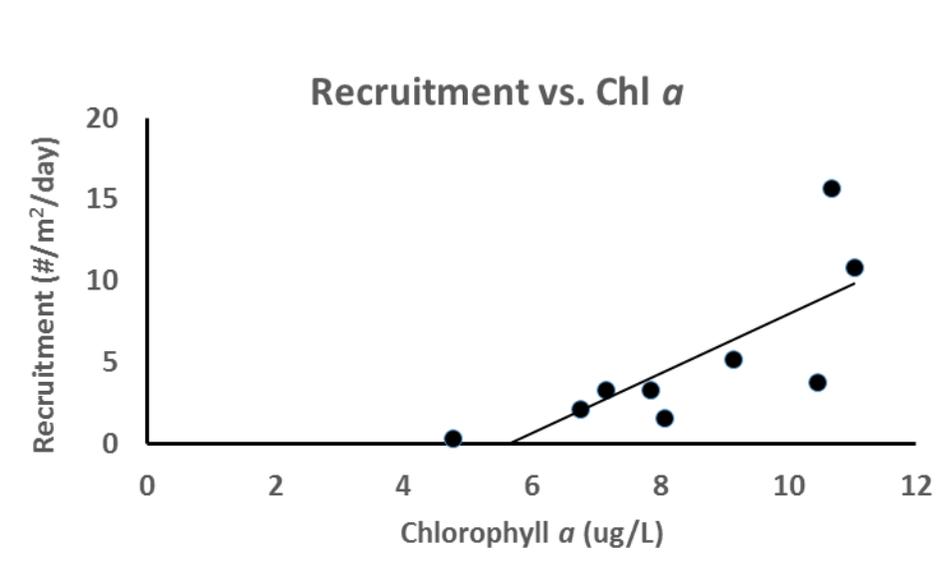


Figure 6. Oyster recruitment in Fall 2012 regressed on summer 2012 chlorophyll *a*.

Salinity range

Salinity places basic physiological constraints on all marine and estuarine organisms (Hochachka and Somero 2002), and is a fundamental determinant of where species can live in an estuary (Remane and Schlieper 1971). Although Olympia oysters tolerate a range of salinity levels, low salinity exposure is stressful and can cause death in severe cases (Gibson 1974). Our field data showed a strong correlation between exposure to lower salinity levels and several oyster attributes, including average size, recruitment rate, and growth. We have tried to capture the risk posed by extreme low salinity events separately (see section below on risk of extreme low salinity).

Our field data indicated that many oyster attributes were negatively correlated with the average percentage of days when salinity at a site was below 25 psu; these data are captured in the Site Evaluation Table in the “Salinity Range” row. Our field monitoring was conducted during a drought year. Salinity was below 25 psu 36% of the time at Loch Lomond Marina (SF Bay), but never dropped below 25 at several sites in both estuaries.

We initially used conductivity loggers from Onset Computer, but due to instrument failure and eventual recall by the manufacturer, we decided to use long-term monitoring data available from the USGS, NERR, and LOBO monitoring programs (MBARI 2013, NERRS 2014, USGS 2014).

Many biological processes in marine organisms can be affected by cumulative exposure to lower salinity levels (akin to degree-days for temperature), usually below a given threshold. Threshold salinity levels for spawning and other important processes are not known for oysters in our study locations. To look for such a threshold, we calculated the length of exposure (days/year) each site had to salinity below each salinity level (in 1 psu increments) in the range experienced at each site. We regressed each demographic variable on the number of days spent below each of these potential threshold salinity levels. We also explored relationships of oyster variables to maximum, mean, and minimum salinities. Despite the very large number of regressions generated here, we did not correct regression p-values for multiple comparisons, as might be done in statistical analyses of experiments, as our analysis was exploratory in nature. We

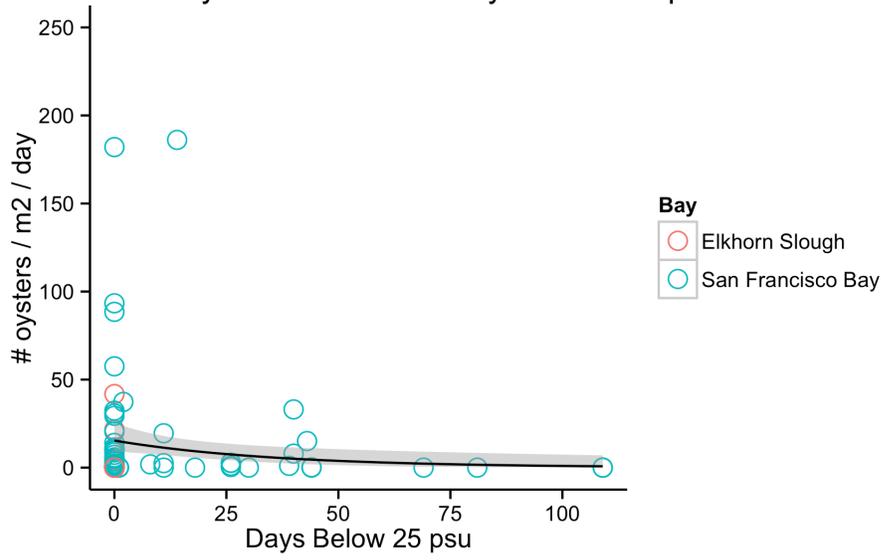
were interested in the overall pattern of relationships, rather than testing a specific, falsifiable hypothesis.

We ran linear regressions of the oyster demographic variables that we measured in the field on several measures of salinity, using quarter as a blocking variable. Standard linear regressions on untransformed data were performed, except when the response variable was non-normally distributed – usually Poisson or negative binomially distributed – in which case we used generalized linear models with the appropriate link function (usually log). Our field data showed a strong relationship between salinity levels measured at a site and several oyster attributes, including growth rate, average size, recruitment rates, and adult density (Fig. 7).

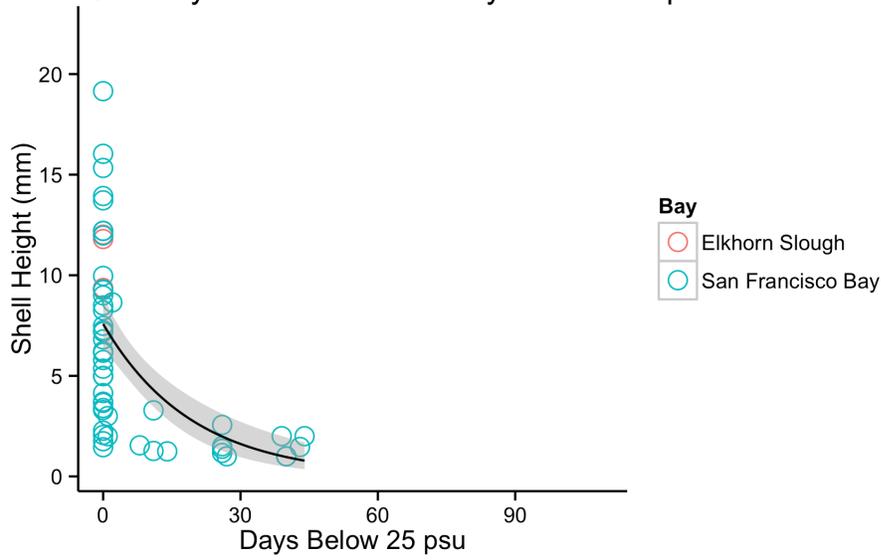
After considering each possible threshold salinity across sites, we selected 25 psu as the salinity threshold that had the greatest number of significant relationships with the demographic variables we tested across all sites. Similar to the warm water temperature variable, 25 psu may seem rather high and not specifically tied to biologically relevant oyster physiology, but it acts as a single proxy measure that captures a number of salinity-related phenomena that are clearly relevant to oyster biology. The 25 psu threshold correlated better with oyster demographic measures than other standard measures of salinity such as the annual (or quarterly) maximum, mean, or range.

The exposure of a site to salinities below 25 psu was positively correlated with mortality rate ($r^2 = 0.38$, $p = 0.042$) and negatively correlated with recruitment rate (pseudo- $r^2 = 0.17$, $p = 0.05$) and recruit size (pseudo- $r^2 = 0.63$, $p \ll 0.001$). We weighted salinity as having high importance given the number of oyster attributes it affects.

Mean Quarterly Recruitment vs Days Below 25 psu



Mean Quarterly Recruit Size vs. Days Below 25 psu



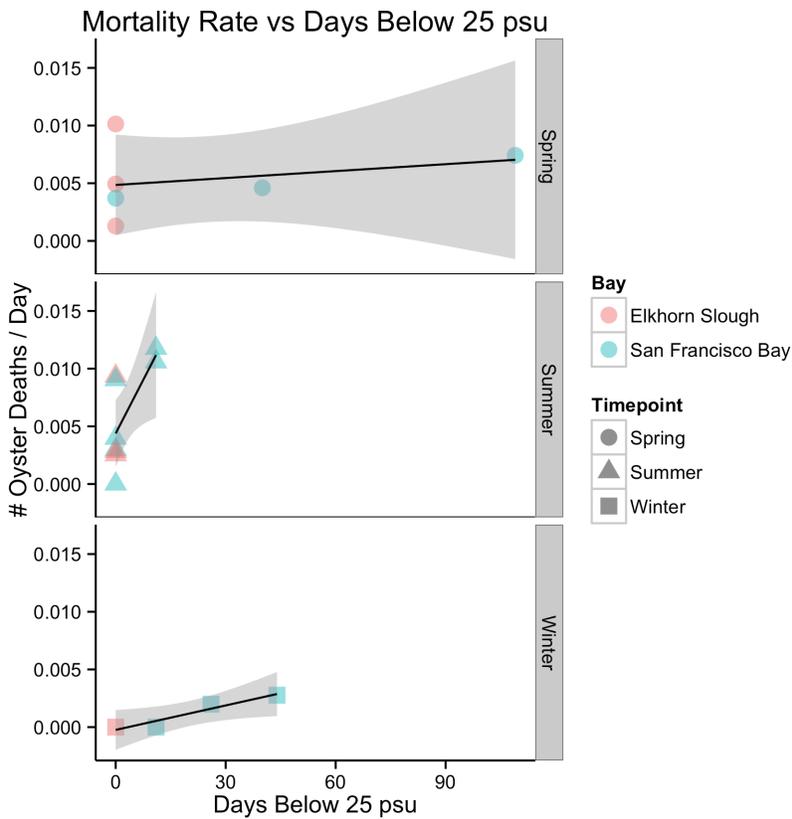
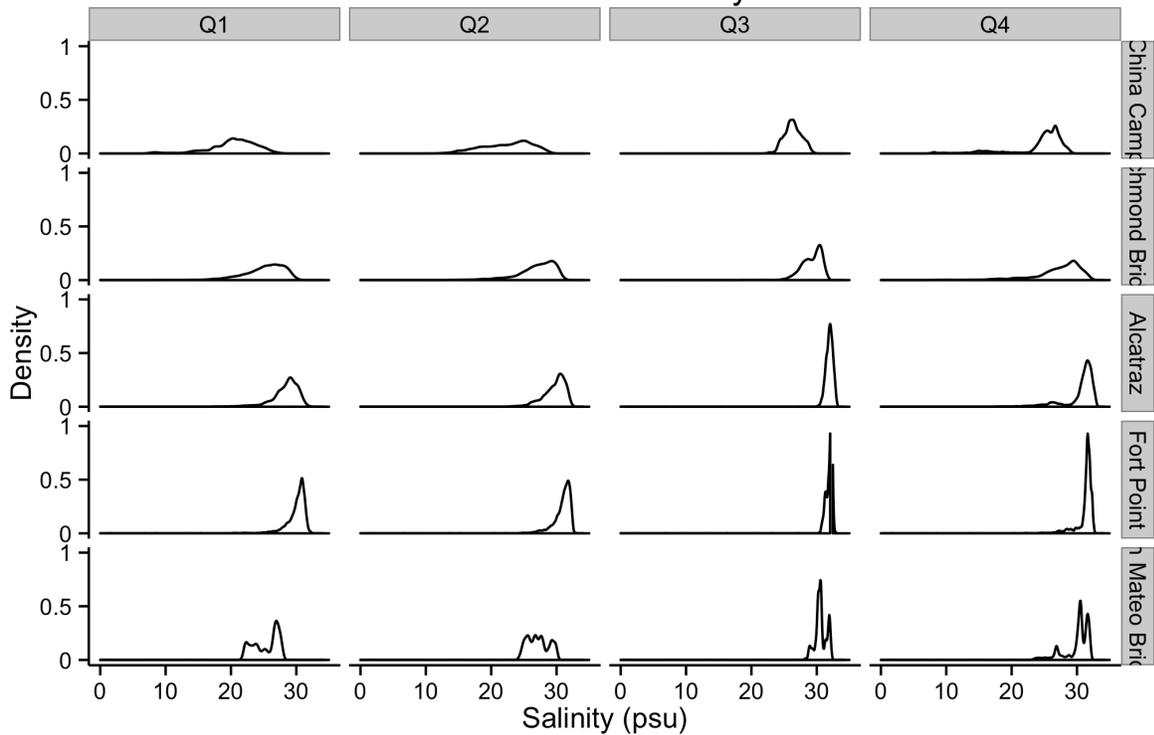


Figure 7: Oyster demographic variables (quarterly recruitment rate, quarterly recruit size, or mortality rate) measured at each site plotted against the number of days that site spent below 25 psu during the quarter of the measurement as estimated by continuous monitoring stations. Mortality was measured during 2013 only. Points are semitransparent to show overlap. Regression lines (black) are shown with 95% confidence intervals (gray band).

Salinity Probability Density San Francisco Bay



Elkhorn Slough

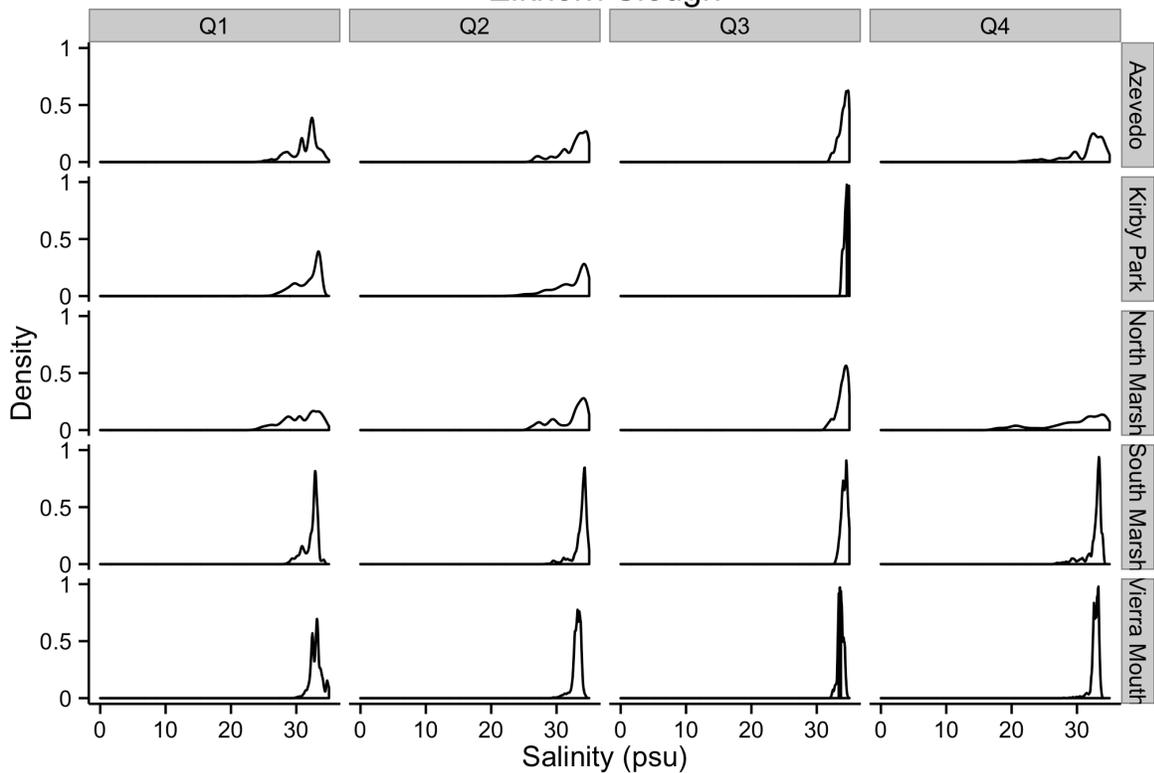


Figure 8. Probability density plots of salinity at continuous monitoring stations in 2012 and 2013 by quarter (Jan-Mar, Apr-Jun, Jul-Sep, Oct-Dec). Sites are arranged from north to south for each bay. For each site, the line describes the relative amount of time the water at the site is at each salinity level. These plots show the variation in the range of salinities each site experienced during the study. Seasonal trends and geographic differences between sites are clearly visible. Site abbreviations are given in Appendix 2. The sonde at Kirby Park was discontinued in fall 2012, so data represent 2012 only, and Q4 is not available.

Key stressors: combining laboratory and field data

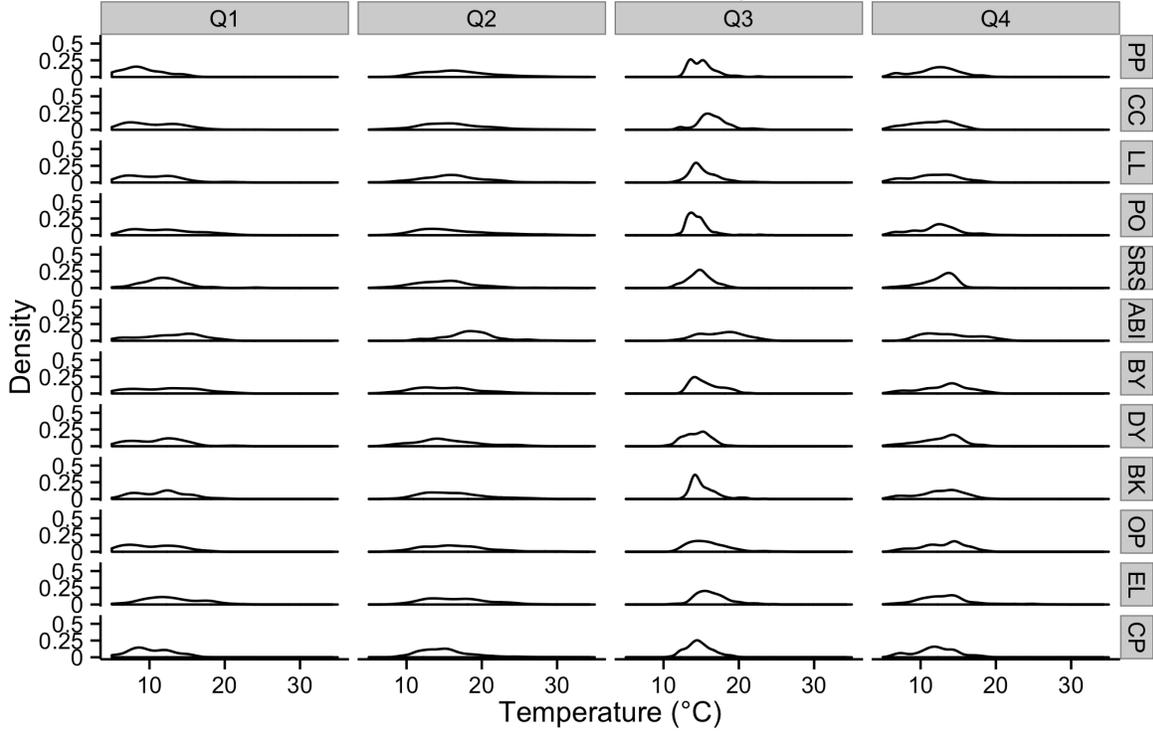
Risk of air temps above high threshold

Our laboratory experiments showed a significant effect of warm air temperatures (35°C and 40°C) on oyster survival, particularly when these temperatures followed exposure to low salinity (Appendix 3). The combination of low salinity and high air temperatures are experienced by oysters in the spring in Central California in years when late spring rains are followed by warm days and minus tides in the early to mid-morning.

Over the course of our study, sites rarely experienced air temperatures over 35°C at Mean Lower Low Water, which is where oysters are the most abundant. In Central California, minus tides in the spring are typically early in the morning, when the air is cooler. In the summer, low tides are later in the morning, but localized fog keeps air temperatures cool, particularly in the central portion of SF Bay and at Elkhorn Slough. Fall minus tides tend to occur in the late afternoon, just before sunset. However, higher air temperatures are expected under climate change scenarios.

Several sites of our sites experienced air temperatures of 30°C in May-early June. Sites at Elkhorn Slough, Berkeley Marina, and China Camp did not have any days over 30°C during our study period. Point Orient and our two South Bay sites on the west side of SF Bay, Oyster Point and Coyote Point had the highest number of days above 30°C (3, 4, and 4 respectively).

Air Temperature Probability Density San Francisco Bay



Elkhorn Slough

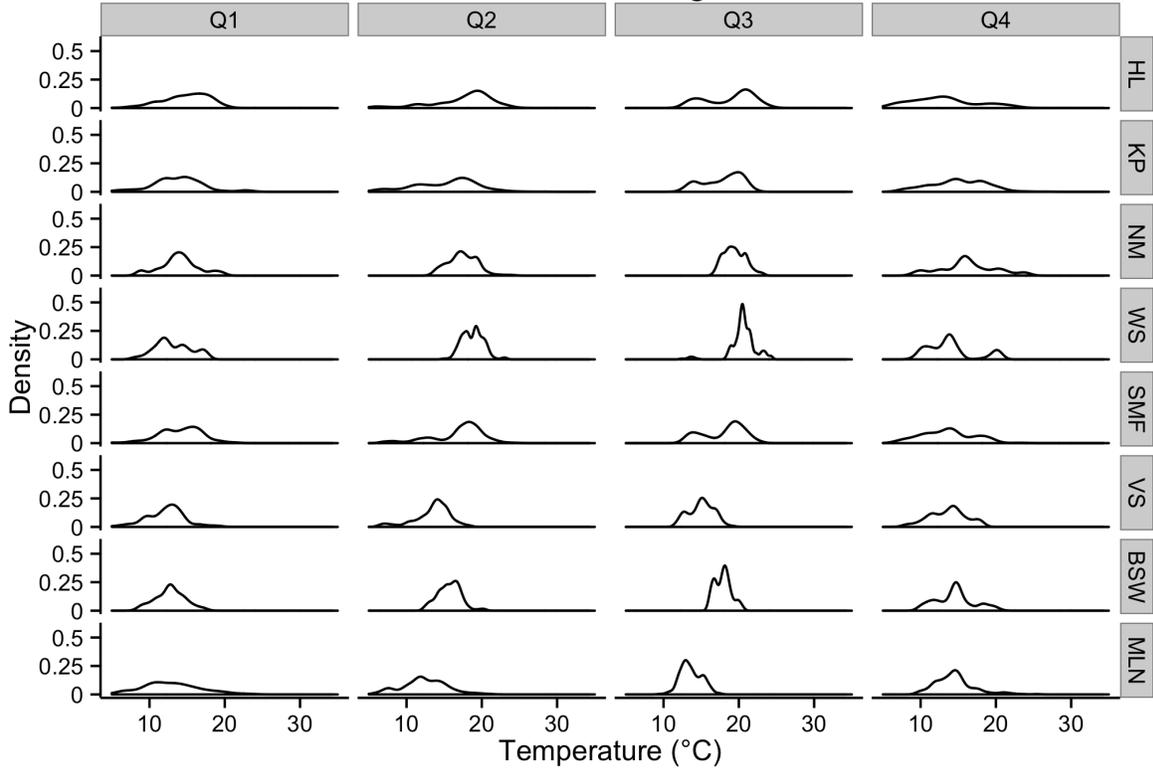


Figure 9: Probability density plots of air temperature at all sites in 2012 and 2013 by quarter (Jan-Mar, Apr-Jun, Jul-Sep, Oct-Dec). Sites are arranged from north to south for each bay. For each site, the line describes the relative amount of time the water at the site is at each temperature. These plots show the variation in the range of temperatures each site experienced during the study. Seasonal trends and geographic differences between sites are clearly visible. Site abbreviations are given in Appendix 2. Note that Azevedo Pond (APN) is not included here, as it is a muted site that is continuously submerged.

We suggest that sites that are already warmer are more vulnerable to increases in air temperature in the future. Our lab experiments indicated only moderately lower survival of oysters exposed to very high air temperatures alone; significantly more mortality occurred when oysters were already stressed by exposure to very low salinity. Given this, we assigned this parameter a moderate level of importance in the Site Evaluation Table (multiplier of 1.25). Effects on oysters were seen in our laboratory experiments after 3 days exposure to high air temperatures. We assumed a lesser effect over 1-2 days of such exposure, and a greater effect of 5 or more days and designated our bins for site scores accordingly.

Low dissolved oxygen

Our laboratory experiments indicated that low dissolved oxygen (DO) conditions, similar to that experienced currently in some locations at Elkhorn Slough, negatively affect oyster growth (Appendix 3). We did not see differences in survival in the low DO treatments, and differences in growth were partially offset by warmer water. Thus, we assigned this parameter a moderate level of importance in the Site Evaluation Table (multiplier of 1.25).

Previous field research at Elkhorn Slough has indicated an effect of low DO on oyster growth (Wasson et al. unpublished data) at sites where nighttime DO is very low. At these sites, DO is subsequently very high during the day. Researchers at Elkhorn Slough have examined the relationship between daytime DO measurements and nighttime hypoxia at six sites with continuous monitoring devices: the greater the variance from 100% saturation in daytime DO measurements observed, the greater the duration of nighttime hypoxia at that site (Hughes et al.

2011). For data collected as mg/L, this same relationship was found for the frequency with which measurements were greater than 11 mg/L and/or less than 7.5 mg/L. For this project, we had fewer samples, collected monthly, during daytime hours only. We used average deviation from 9.25 mg/L DO (100% saturation under typical estuarine conditions) as a proxy for nighttime hypoxia in our assessment. Most sites in SF Bay and two in Elkhorn Slough were less than 2 mg/L away from 9.25 on average; most Elkhorn Slough sites were within 2-2.9 mg/L on average. As expected, Azevedo Pond North, a shallow, tidally muted site near an agricultural field in Elkhorn Slough had the highest average deviation (4.5 mg/L) and was thus rated the lowest.

Risk of low salinity duration below threshold

Our field data from previous projects (CALFED) documented significant interannual variation in freshwater flow, with clear effects of severe low salinity exposure. Near complete mortality was observed at northern San Francisco Bay sites following a series of strong storms in March 2011 (Figure 10, Chang et al., unpublished data), while earlier surveys (CA Sea Grant) following severe storms in January 2006 showed strong evidence of a recent massive die-off in an area encompassing the 2011 die-offs (Zabin et al. 2010). Such events have not been observed in Elkhorn Slough due to the relatively short timespan of our observations and the likely very infrequent occurrences there, although occasional events with very high freshwater flow have been documented to cause complete mortality of estuarine fauna in similar low-inflow estuaries such as Newport Bay (MacGinitie 1939).

Multiple lab experiments support these field observations, with juvenile Olympia oysters suffering significant mortality when exposed to salinity levels below 10 psu for five or more days. Adult oysters showed a more complicated response, but low salinity levels generally caused increased mortality with longer exposure. However, there is evidence that among estuaries and even within San Francisco Bay, Olympia oysters from sites with different salinity regimes have divergent low salinity tolerances. In laboratory experiments, oysters from northern San Francisco Bay had higher survival in low salinity conditions than oysters from southern San Francisco Bay and nearby Tomales Bay (Bible, unpublished data).

We evaluated longer-term datasets from several monitoring programs to calculate the percentage of years in which salinity at our sites had dropped below 5 psu for 4 days or more. Most sites in the northern part of SF Bay had experienced such events in 25% of the years in the long-term data sets; while most mid-estuary sites at Elkhorn Slough and Central to South Bay sites in SF Bay had no years with these low salinity events. More severe flood years are predicted for the region under climate change scenarios.

Due to the significant risk posed by low salinity events, we rated this stressor as high importance in the Site Evaluation Table. Sites experiencing low salinity events in more than 25% of years were also observed to be those sites experiencing significant mortality (e.g. China Camp, San Francisco Bay, Figure 10) and were thus rated as our highest risk category. We then selected bins at lower risk categories according to observed mortality rates in San Francisco Bay during previous low salinity events.

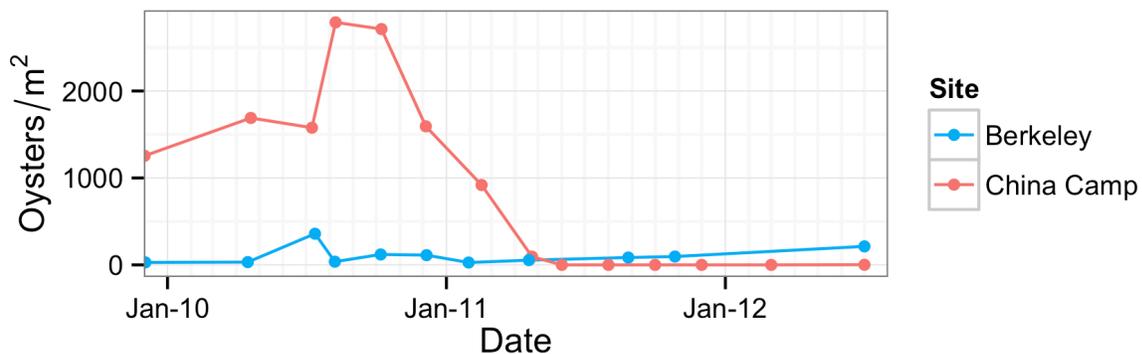


Figure 10. Oyster density at Berkeley and China Camp field sites in San Francisco Bay showing significant die-off following severe low salinity event in early 2011. Data from CALFED project population surveys performed every two months (Chang et al. unpublished data).

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