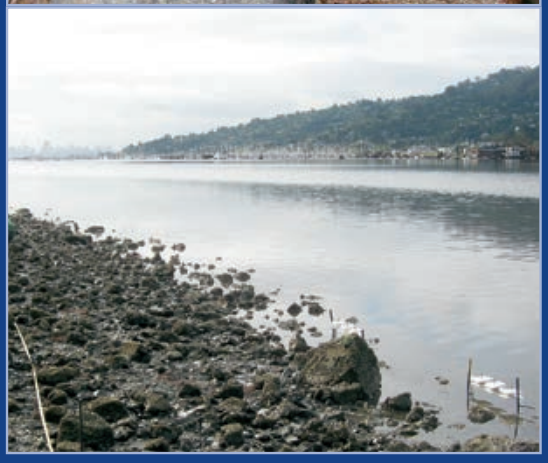

A Guide to Olympia Oyster Restoration and Conservation

APPENDIX 1
SUPPLEMENTAL INFORMATION
ABOUT PROJECT FUNDING
AND TEAM COMPOSITION

Coastal Conservancy

NOAA

Elkhorn Slough Foundation Since 1982

California Department of Fish & Wildlife

UC DAVIS

San Francisco State University

APPENDIX 1. Supplemental information about project funding and team composition.

This project was led by an interdisciplinary team from the San Francisco Bay and Elkhorn Slough National Estuarine Research Reserves, and was developed in partnership with the California State Coastal Conservancy and the University of California at Davis. The management questions addressed in the project arose directly from prior needs assessments of local and regional end-users: oyster restoration practitioners and the coastal decision-makers who set regional restoration policy and provide funding. Project staff consisted of program managers, applied scientists, graduate students and technicians, together having several decades of cumulative experience working at the interface of science and estuarine management. Core project staff and their roles and affiliations are as follows:

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Chris Knight, Kaylee Griffith, Kelly Laughlin, Phil Jones, John O'Brien, Carol Star, Doug Hooper, Miki Takada, Linda Rose, and Paul Stull. Additional field assistance was provided by Peter Deck, Blu Forman, Beth Gillepsie, Laura Gray, John Haskins, Zack Kaufman, Inger Marie Laursen, Daniel Vee Lewis, Laura Mehner, Alger Omongos, Amada Peters, Carrie Stevenson, and Dean Vila.

APPENDIX 3 Laboratory experiments: methods and results

Lab experiment 1: Oxygen, water temperature and salinity

Methods

We tested for multiple stressor effects by conducting laboratory experiments using newly settled oysters spawned from San Francisco Bay oyster broodstock. This experiment was conducted in three phases. In the first phase of the experiment, we exposed oysters to two temperatures (20° and 24° C) and three dissolved oxygen treatments (0.6 mg l^{-1}, 2.0 mg l^{-1}, and 7.0 mg l^{-1}) over a 14 day period. Importantly, we simulated hypoxia as it occurs in Elkhorn Slough, where hypoxia manifests at night for approximately eight hours and returns to normoxia during daytime photosynthesis (i.e. hypoxia in this case is diel-cycling). In the second phase of the experiment, we exposed oysters to a recovery period (86 days) of benign conditions. The third phase of the experiment exposed oysters to a low salinity challenge for eight days, in order to determine if salinity tolerance was related to early life history stress exposure and to identify critical low salinity thresholds. In total, we used 216 oyster tiles that contained 2,110 oysters.

To quantify the effect of environmental stressor treatments on oyster growth, we used linear mixed models (LMMs) with a Gaussian error distribution and identity link function. Oyster growth was analyzed at three time points: prior to the start of the experiment (day 0) to detect size bias among treatments, at the end of phase 1 (day 14) and after phase 2 (day 100). For phase 1 and 2, we used the fixed effects of temperature, dissolved oxygen, initial oyster size, and all interactions to predict oyster size. For these LMMs and all following analyses, we included the random effects of tank and tile nested within tank. To meet the normality and equal variance assumption, we graphically evaluated the data using probability plots and examined model predictions against residuals. Phase 1 data were natural log transformed because of increasing variation with decreasing dissolved oxygen content whereas phase 2 data were untransformed.

To measure the effect of environmental stressor treatments on oyster survival (a binary response), we used generalized linear mixed models or GLMMs with a binomial error distribution and a logit link function. All analyses and graphics were produced using R (Version 3.0.2) and the packages: “lme4”, “ggplot2” and “car”.

Results

In the first stage of the experiment, oysters did not exhibit any differences in mortality. In contrast, individuals increased growth in response to warmer temperatures (+28%), but exhibited reduced growth with decreased oxygen availability (-61%) (Figure 1). These effects were still apparent after 86 days of exposure to environmentally ideal conditions but the effect size was reduced (Figure 1). In the third phase of the experiment, we exposed these same oysters to low salinity stress after the 86 day recovery period. This delay in salinity stress simulates the fall seasonal period of high oxygen and high salinity conditions characteristic of San Francisco Bay and Elkhorn Slough. We exposed oysters to salinity treatments (33, 15, 10, and 5 psu). In this phase, survival under low salinity was reduced only in the most extreme treatment of 5 psu where oysters only exhibited 25% survival (Figure 2). Additionally, survival was unrelated to early life history exposure to temperature or dissolved oxygen (Figure 2).

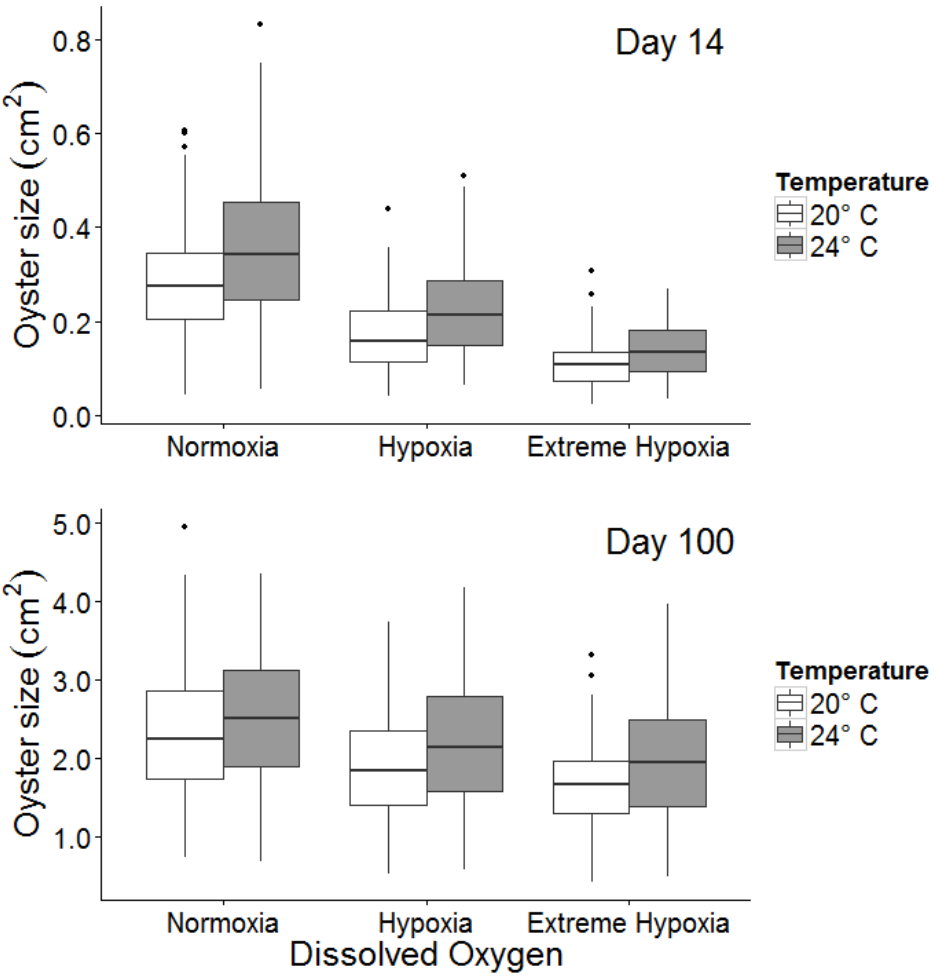

Figure 1. Boxplots (bold line is median, upper and lower portions of box refer to 25th and 75th percentiles of oyster size at the end of phase 1 (day 14) and phase 2 (day 100).

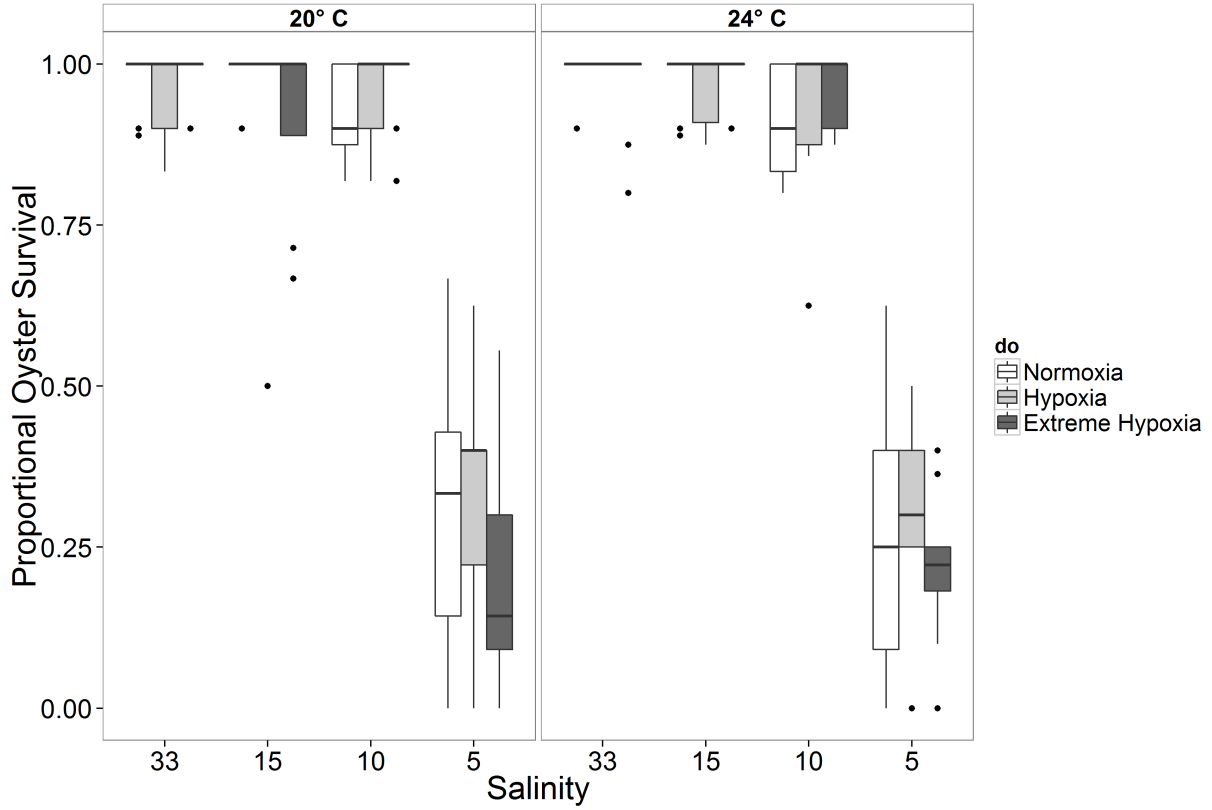

Figure 2. Boxplots of proportional oyster survival across salinity and early life history exposure.

Lab experiment 2: Salinity and air temperature

Methods

We performed an experiment assessing Olympia oyster responses to the combination of low salinity and high air temperature with different amounts of time between the two stressors. We mimicked natural conditions in San Francisco Bay by using 5-month old oysters (the youngest age at which oysters would be expected to experience these stressors in nature) and choosing stressor levels based on field measurements and previously determined thresholds. Oysters were exposed to 3 salinity levels (33, 10, and 5 psu) for 7 days, 3 air temperatures (18, 35, and 40°C, ramped over 3.5 hrs to mimic intertidal measurements) for 3 days in a row, and 3 different timings of the stressors (sequential, decoupled by 2 weeks, and decoupled by 4 weeks).

We collected adult oysters (n ≈ 100/site) from approximately 0 m above mean low low water at 6 sites in San Francisco Bay (Loch Lomond, Point Orient, Strawberry, Berkeley Marina, Candlestick, and Oyster Point). Oysters from each site were placed in a 100L culturing cylinder, kept at room temperature (19-24°C), and fed a daily diet of cultured *Isochrysis galbana* and Shellfish Diet (Reed Mariculture, Inc). High water temperatures and food levels were used to encourage larval release (Utting and Millican 1997, Jeffs et al. 2002). We collected larvae from each site and homogenized them in 100L culturing cylinders with 10 x10 cm PVC 'tiles' for settlement. After settlement, tiles were culled to approximately equal densities and transferred to a re-circulating seawater system in which all oysters experienced the same water temperature and food conditions for the growth phase of this experiment. Oysters experienced 3 months of subtidal conditions followed by one month of intertidal acclimation during which they were

ramped down to winter water temperatures and exposed to room-temperature air (18°C) biweekly for approximately 4.5 hrs for 3 days in a row.

After the acclimation period, tiles of oysters were randomly assigned to a treatment combination (salinity x air temperature x timing). Oysters in the 5 and 10psu treatments were ramped down from 33psu over the course of 4-5 days. Oysters were held at target salinities for 7 days. Those oysters in the sequential stress treatment were exposed to high air temperatures (35 or 40°C) for the last 3 days of their salinity trial. Oysters in the decoupled timing treatments were given either 2 or 4 weeks between the salinity trial and high air temperature exposure. Those oysters not experiencing high air temperature on a given day were given control (18°C) aerial exposure so that all oysters experienced air exposure at the same time. For air temperature exposure, tiles were hung from pieces of mesh in empty 20-gallon tanks within incubators. The incubators were programmed to mimic intertidal temperatures from our field measurements (ramping to a maximum temperature over the course of 3.5 hrs).

Results

Both low salinity and high air temperature resulted in oyster mortality. When low salinity and high air temperature treatments were sequential, the impact of low salinity and high air temperature combined was worse than the sum of the two stressors alone, thus a synergistic response. If oysters were given 2-4 weeks to recover from low salinity before high air temperature, survival was higher than if the stressors were sequential. At low salinity (5psu), survival was low (~45%) even at the most benign temperature (18°C) and was even lower (~5% and 0%) at 35°C and 40°C respectively. At medium salinity (10psu) and low air temperature (18°C), oyster survival was high (~95%), but when temperatures increased to 35 and 40°C survival decreased to ~68% and 0% respectively. At high salinity (33psu) only the highest air temperature (40°C) caused decreased survival (~80-90%) (Figure 3).

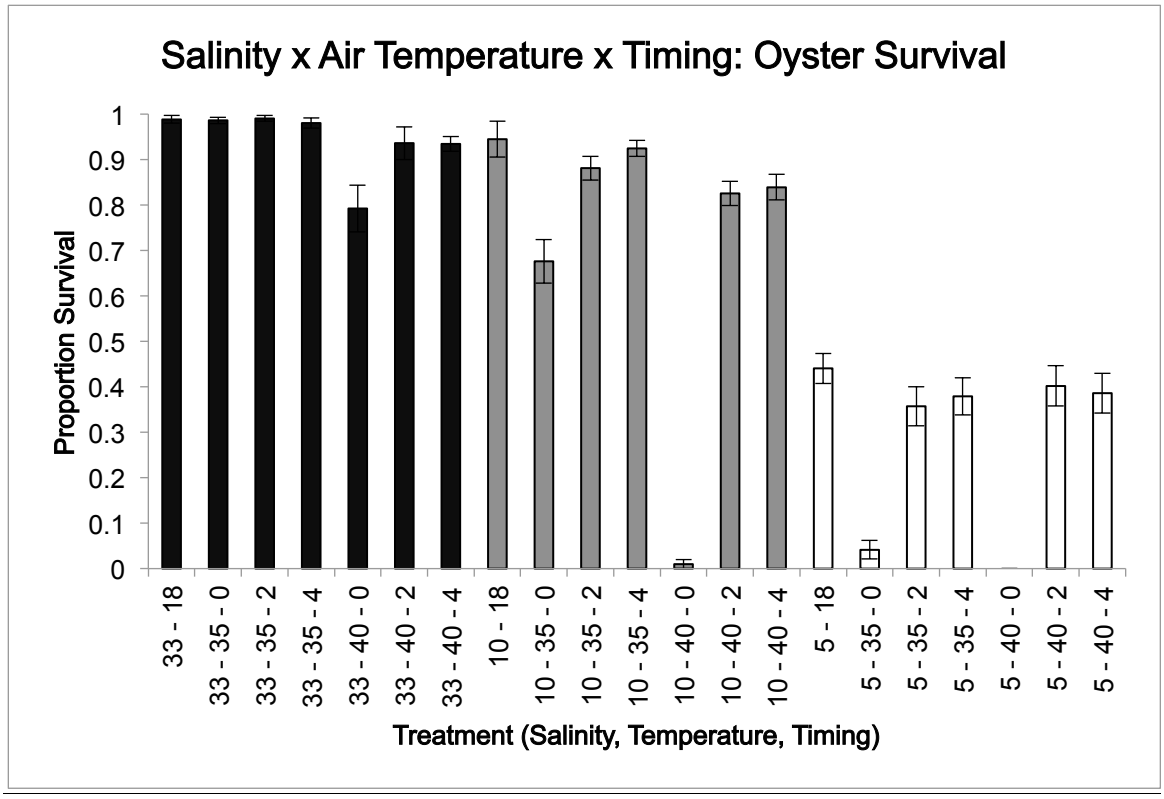

Figure 3: Proportion oyster survival in each treatment combination. Treatment combinations are salinity (psu), temperature (degrees C), and timing (# of weeks between salinity and temperature treatment).

Lab experiment 3: Salinity

Methods

We performed a laboratory experiment to test the effects of low salinity level and duration of exposure on the survival and feeding rates of adult Olympia oysters. We hand-collected oysters (>30 mm) in winter 2013 from two San Francisco Bay sites (Berkeley Marina (BK), Loch Lomond (LL)) that experience different salinity regimes throughout the year. Oysters were first acclimated in aerated holding tanks in a wet lab for one month before applying treatments. We chose treatment levels based on historical outflow volume and frequency in San Francisco Bay, using combinations of both current common outflow events and more extreme current outflow events that could become more common with climate change. Treatment combinations (collection site [site: BK, LL], salinity level [intensity: 0, 3, 6, 12, 30 psu], and duration of exposure [duration: 4, 14 days]) were randomly assigned to static mesocosm tanks (2 gallon volume) randomly distributed over four water tables, with each combination replicated three times and eight random oysters from the appropriate site assigned to each tank. Tanks were immersed in flow-through seawater baths to maintain temperatures approximating ambient conditions in northern San Francisco Bay. Oysters were fed daily with Shellfish Diet (Reed Mariculture, Inc.) and treatment water was mixed (± 0.05 psu of target level) and changed daily. After 4 or 14 days (depending on treatment assignment), oysters transitioned to a recovery phase in their treatment tanks with seawater (>25 psu) for a 60-day recovery period.

Oysters were examined for survival daily during the exposure and the first two weeks of the recovery period, and three times per week for the remainder of the recovery period. Oysters were removed from tanks when determined dead. During the experiment, we measured the percent change in chlorophyll *a* values (AquaFluor fluorometer, Turner Designs) from the time of feeding to the end of a 20-hour period (before water was changed and oysters were fed again). Food consumption was measured before exposure, after 1 day of exposure, at the end of each exposure, and after 10 days of recovery.

To test oyster response to low salinity level and duration of exposure, we examined survival at 60 days following exposure using a generalized linear mixed model with a binomial distribution and tank included as a random effect. Initial oyster size was initially used as a covariate, but resulted in a higher AIC value so was dropped from the model. To analyze feeding response, we used a fully crossed generalized linear model with a beta distribution and logit link function. We performed separate analyses for feeding rates at 20 hours exposure, at the end of exposure, and after 10 days of recovery (betareg package, betareg function). Treatment factors (site: BK or LL, duration: long or short, and intensity: 0, 3, 6, 12, or 30 psu) were treated as fixed effects in each analysis. We compared different treatment combinations for survival and feeding at the end of exposure and during recovery using Wald z pairwise comparison tests (multcomp package, glht function, Hothorn et al. 2008). All data were analyzed using R ver. 3.0.2.

Results

Oyster survival was not significantly reduced by a 4-day exposure to low salinity. However, after the 14-day exposure, oysters had lower survival at intermediate salinity (6 psu or 12 psu, depending on site) at both sites (Figure 4). During exposure, oysters generally did not feed at lower salinity levels, indicating oysters closed their shells to avoid low salinity. However, initial response to reduced salinity varied such that oysters stopped feeding sooner at the lowest salinity levels as compared to intermediate salinity levels. This initial exposure to reduced salinity water at intermediate levels, combined with extended closure during the longer 14-day exposure, likely contributes to the lower survival observed in those treatments. Results indicate that oysters are equipped to withstand shorter, more common low salinity events, but events that are more extreme in duration can significantly reduce survival.

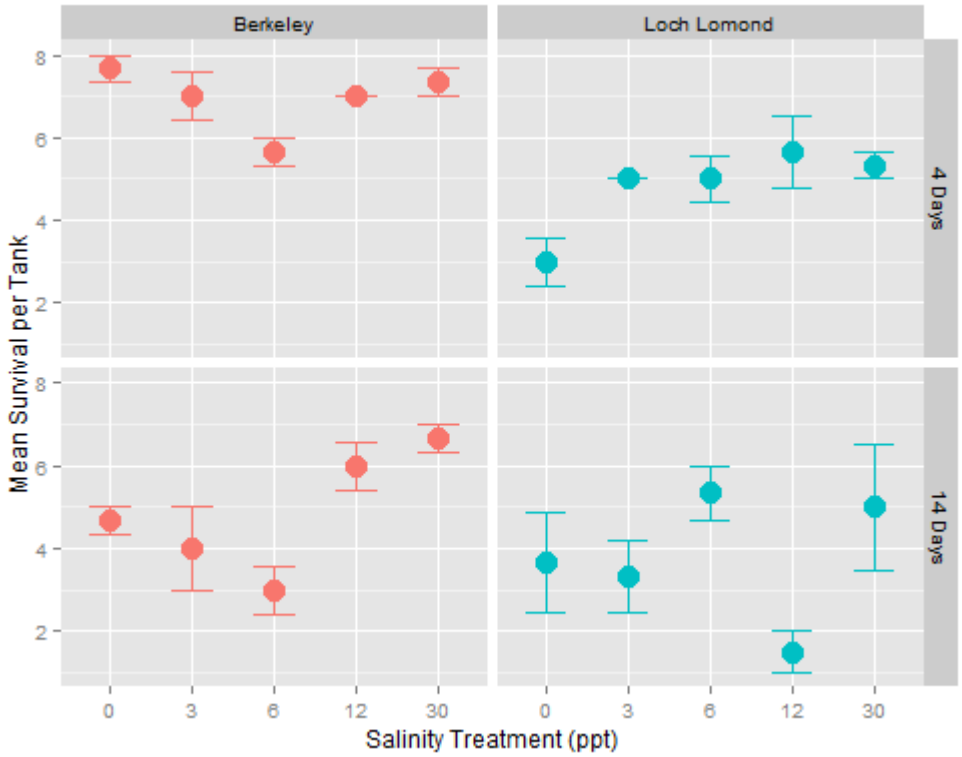

Figure 4: Average survival as a function of site (BK = red, LL = blue), salinity intensity, and duration, +/- standard error (a) BK short duration, (b) BK long duration, (c) LL short duration, (d) LL long duration

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